Int. J. Biochem. Vol. 12, pp. 791 to 793

0020-711X/80/1101-0791$02.00/0

THE MEASUREMENT OF ERYTHROCYTE UROPORPHYRINOGEN I SYNTHASE IN THE DIAGNOSIS OF LATENT AND ACUTE INTERMITTENT PORPHYRIA

DAVID BROCKLEHURST, EVA A. WIDER DE XIFRA and ALCIRA BATLLE
Area Department of Clinical Chemistry, Doncaster Royal Infirmary, Doncaster, England and Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP), Facultad de Ciencias Exactas y Naturales, University of Buenos Aires and Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET) Ciudad Universitaria, Pabellón II—4 to Piso-Nuñez, 1428, Buenos Aires, Argentina

Abstract—1. This paper confirms the increase in sensitivity obtained for erythrocyte UIS measurement by pre-incubation of the red cells in a 0.2% Triton-X 100 solution containing 1 mmol/l $ZnSO_4$ and dithiothreitol as described by Piepkorn *et al.* (1978).

2. To achieve optimal precision in this assay a substrate concentration of delta aminolaevulinic acid (ALA) of 1000 nmol/ml is required. Interpretation of the results obtained by this method is discussed and its use in the detection of both latent and acute intermittent porphyria is demonstrated. Comparative studies were carried out by using the Batlle *et al.* (1978) method and a modification employing ALA as substrate.

INTRODUCTION

The introduction of simpler methods for the measurement of erythrocyte uroporphyrinogen I synthase (UIS) (EC 4.3.1.8) (Granick *et al.*, 1972; Peterson L. R. *et al.*, 1976; Piepkorn *et al.*, 1978; Batlle *et al.*, 1978) has made it possible to detect both the latent and acute forms of Acute Intermittent Porphyria (AIP). This short communication discusses some of the problems inherent in the use of delta aminolaevulinic acid (ALA) as substrate for the assay of UIS and demonstrates its use in the detection of both latent and acute forms of AIP.

METHODS

The methods of Peterson *et al* (1976) and Piepkorn *et al.* (1978) were used to assay UIS activity. These methods are performed identically except for a pre-incubation of the erythrocytes in a 0.2% Triton-X 100 solution containing 1 mmol/l $ZnSO_4$ and diethiothreitol (DTT) in the Piepkorn procedure. Comparative studies were also performed using both the method of Batlle *et al.* (1978) and a modification as follows: 0.5 ml of red blood cells (hemolized and diluted) are preincubated at 37°C for 30 min with 1 ml of Triton-$ZnSO_4$-DTT, Piepkorn solution, 0.2 ml (100 μmol) of 0.5 M phosphate buffer pH 7.4 and 0.25 ml of 0.3 M NaCl + 0.06 m Mg_2Cl. Then, 5 μmol of ALA are added in a final volume of 2 ml. Incubations are carried out for 2 hr at 37 and 45°C. The reaction is stopped by adding 1 ml of 15% TCA and porphyrins measured in the supernatant as described (Batlle *et al.*, 1978).

RESULTS AND DISCUSSION

A comparison of erythrocyte UIS activity obtained by Peterson *et al.* (1976) and the modified Piepkorn *et al.* (1978) method at increasing substrate concentration in one patient is shown in Fig. 1.

Using the Batlle *et al.* (1978) method and its modification as described here, comparative determinations were carried out in normal subjects and porphyric patients, results obtained are shown in Table 1.

The pedigree of a family in which AIP has been established in three generations by the measurement of UIS activity using the method of Peterson *et al.* (1976) is shown in Fig. 2. The pedigree of a second family in which AIP has been diagnosed in four members, by using the method of Batlle *et al.* (1978) is illustrated in Fig. 3.

The original method of Peterson *et al.* (1976) which used ALA as substrate has subsequently been shown to give low results in patients with lead intoxication (Piepkorn *et al.*, 1978). These workers have overcome this problem by preincubation of the erythrocytes for 30 mins at 37°C prior to assay in a 0.2% Triton-X 100 solution containing 1 mmol/l $ZnSO_4$ and DTT. This paper confirms the increase in sensitivity achieved by this procedure in the order of between 4 and 7 nmol/porphyrin formed/ml/hr and demonstrates that the optimum substrate concentration for good precision is achieved at 1000 nmol/ml although 70% of maxi-

Table 1. Uroporphyrinogen I synthetase activity in different subjects

	Enzymic activity (units/ml RBC)			
	PBG-ase		UIS	
Subject	(1)	(2)	(1)	(2)
Control	24.04	22.50	56.30	55.90
Lead-intox.	27.18	26.80	57.07	57.30
AIP	12.60	12.50	27.50	26.95
PCT	26.40	26.90	59.25	60.03

(1) Activity data obtained using the modification of the Batlle *et al.* (1978) procedure as described in Methods.
(2) Activity values obtained using the method of Batlle *et al.* (1978).

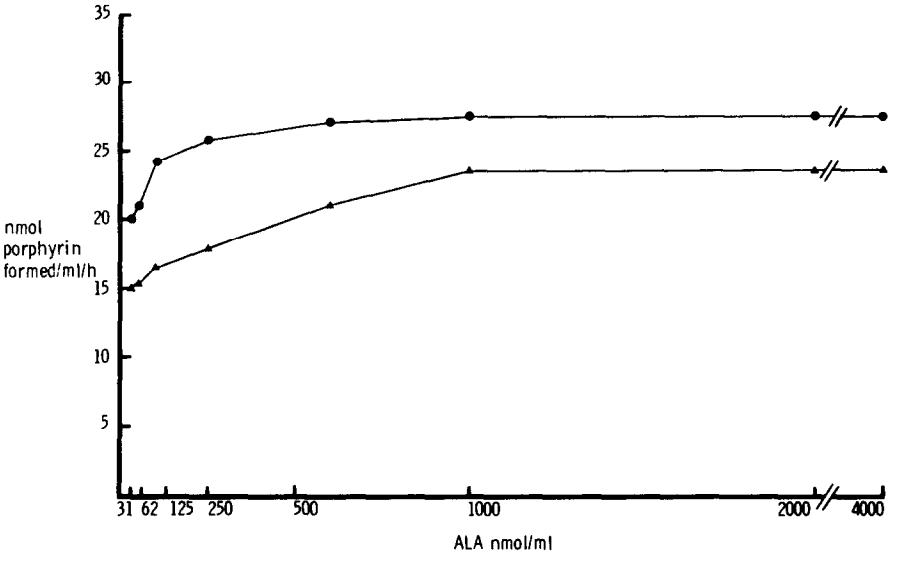

Fig. 1. Uroporphyrinogen I synthase activity at increasing ALA substrate concentration. ▲——▲ Erythrocyte UIS activity measured by the method of Peterson *et al.* (1976). ●——● Erythrocyte UIS activity measured by the method of Piepkorn *et al.* (1978).

mum activity can still be measured at 32 nmol/ml substrate concentration (Fig. 1). It should be noted that there is greater difficulty in achieving the protein-free filtrate with the Piepkorn procedure and longer centrifugation times are required following TCA precipitation. The use of ALA instead of, the more labile and expensive, porphobilinogen (PBG) as substrate for erythrocyte UIS measurement assumes the erythrocyte aminolaevulinic acid dehydrase (ALA-D) to be present in excess in all patients. This may not be so in patients with low levels of this enzyme, and therefore false low values would result in these cases.

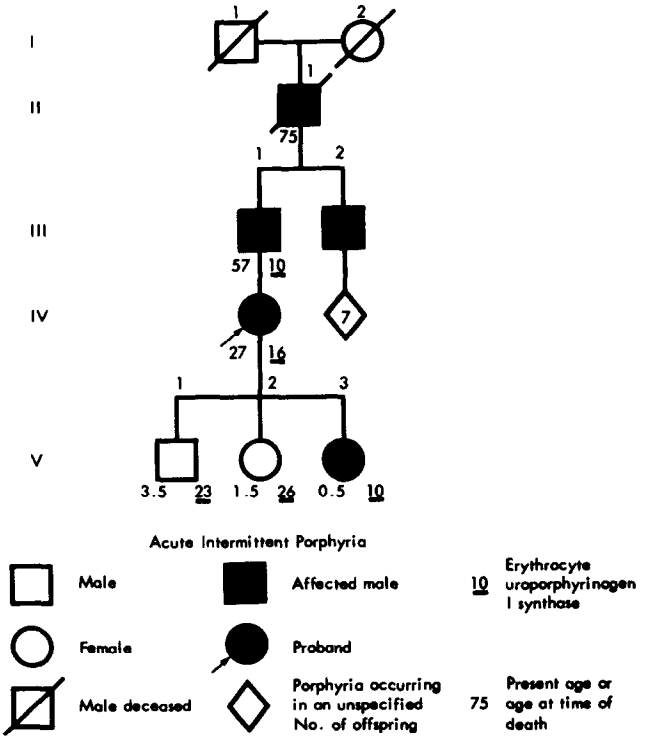

Fig. 2. Pedigree of a family in which AIP has been established in three generations by UIS activity assayed by the method of Peterson *et al.* (1976).

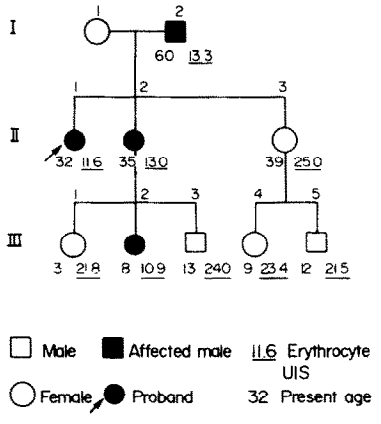

Fig. 3. Pedigree of a family in which AIP has been diagnosed in three generations by UIS activity assayed by the method of Batlle *et al.* (1978).

FAMILY HISTORY

The initial diagnosis of overt AIP in the first family was made by conventional urine testing for porphobilinogen on a "port wine" coloured urine passed by the proband (IV.I) during her second pregnancy. She underwent tubal ligation after this pregnancy but unfortunately, as it turned out, she recanalised. Six months after her third pregnancy the proband, her parents and her children were assayed for erythrocyte UIS activity. Her father (III.I) and her third child had significantly low levels of erythrocyte UIS activity, although her level was within the lower part of the statistical reference range. This latter finding has been described previously by Astrup (1978) using the method of Granick *et al.* (1972) which uses PBG as substrate and by Peterson using ALA as substrate. It may be due to a shortening of the mean average age of the erythrocytes under test or the degree of primary genetic defect which will vary from one family to another. It is therefore important wherever possible to compare sibs within the same family as suggested by Astrup (1978) before a diagnosis is made.

The importance of early diagnosis of this condition in its latent form cannot be stressed too strongly. In this first family, the proband's grandfather had undergone a number of unnecessary abdominal operations, and her father had managed by a process of trial and error to avoid "things which upset him". It is to be hoped that his grandchild will not have to undergo unnecessary surgery and her "trial and error" will be limited to substances not already recorded to cause problems in these patients.

The diagnosis of overt AIP in the second family was made on the basis of ALA, PBG and porphyrins levels in the urine and reduced activity of porphobilinogenase (PBGase) and UIS in blood (Wider de Xifra *et al.*, 1980). The proband (II.1), a 32 year old woman, was treated with high carbohydrate therapy when first admitted to the hospital; she responded well. Six months later, she felt the signs of one of her acute attacks, then she was put under folic acid therapy (Wider de Xifra *et al.*, 1980), to which both symptomatic and biochemical improvement immediately followed. By then her parents, two sisters, three nieces and two nephews were examined for PBGase and UIS activities, none of them had any clinical symptoms nor were ALA, PBG and porphyrins urinary levels abnormal. Her father (I.2), one of her sisters (II.2) and one of her nieces (III.2) had definitely low levels of erythrocyte UIS activity, indicating that they are all latent cases of AIP. In conclusion the method of Peterson *et al.* (1976) has been shown to successfully diagnose latent as well as the overt forms of AIP (Fig. 2). The same results have been obtained by using the procedure of Batlle *et al.* (1978) and its modification, employing ALA as substrate, as described here (Fig. 3).

REFERENCES

ASTRUP E. G. (1978) Family studies on the activity of uroporphyrinogen I synthase in diagnosis of acute intermittent porphyria. *Clin. Sci. Mol. Med.* **54**, 251–256.

BATLLE A. M. DEL C., WIDER DE XIFRA E. A. & STELLA A. M. (1978) A simple method for measuring erythrocyte porphobilinogenase and its use in the diagnosis of Acute Intermittent Porphyria. *Int. J. Biochem.* **9**, 871–876.

WIDER DE XIFRA E. A., BATLLE A. M. DEL C., STELLA A. M. & MALAMUD S. (1980) Acute intermittent porphyria—another approach to therapy. *Int. J. Biochem* **12**, 819–822.

GRANICK S., SASSA S., GRANICK J. L., LEVERE R. D. & KAPPAS A. (1972) Assays for porphyrins, delta aminolaevulinic acid dehydratase and porphyrinogen synthetase in microlitre samples of whole blood: applications to metabolic defects involving the heme pathway. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2381–2385.

PETERSON L. R., HAMERNYIK P., BIRD T. D. & LABBE R. F. (1976) Erythrocyte uroporphyrinogen I synthase activity in diagnosis of acute intermittent porphyria. *Clin. Chem.* **22**, 1835–1840.

PIEPKORN M. W., HAMERNYIK P. & LABBE R. F. (1978) A modified erythrocyte uroporphyrinogen I synthase assay and its clinical interpretation. *Clin. Chem.* **24**, 1751–1755.