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Increased protoporphyrin in erythrocytes in a child with acute intermittent porphyria

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SUMMARY A child is described with acute intermittent porphyria but having an erythropoietic component, with disordered metabolism of porphyrins in bone marrow.

Many cases of porphyria are encountered which do not fall into the present classifications. The justification for classifying porphyrias into erythropoietic and hepatic forms, based on the site of the metabolic abnormality, was put in doubt when disorders of porphyrin biosynthesis were found to occur in both bone marrow and liver in the same case (Schmid *et al.*, 1954; Scholnick *et al.*, 1971; Nicholson *et al.*, 1973; Wolff *et al.*, 1975).

The case presented here is one of acute intermittent porphyria according to the biochemical and genetic findings. Nevertheless, large amounts of protoporphyrin were present in the erythrocytes, which is not described in acute intermittent porphyria.

Case report

A 6-month-old girl was admitted with convulsions. She was seriously ill, unconscious, heart rate 200/min, and with hepatosplenomegaly. Her psychomotor development was retarded, with weakness especially of the lower extremities, and she was unable to hold up her head. Bilateral congenital cataracts were present. From birth she had been passing pink urine.

From the age of 6 months until her death at 22 months she was admitted several times with convulsions, and on different occasions subdural haematoma, urinary tract infection, and pneumonia were found. It was observed that phenobarbitone administed for controlling convulsions led to increased convulsions, with deterioration of her general condition, and to more obvious pink colouration of the urine. Similar effects were produced by infections.

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A mild hypochromic anaemia was noted (Hb 9·6–11 g/dl); reticulocytes ranged from 10 to 25%; serum iron 120 μg/100 ml (21·5 μmol/l). Hypersensitivity to light or other skin changes were never observed. Examination of the teeth at 10 months showed no discoloration or fluorescence.

Methods

Protoporphyrin in the erythrocytes was determined by the method of Heilmeyer (1964), urinary and faecal porphyrins by the method of Rimington (1961), porphobilinogen and δ-aminolaevulinic acid in urine by the method of Mauzerall and Granick (1956). Porphyrins and their precursors in the subdural fluid were estimated as in urine. Esterification and separation of methyl esters of porphyrins in stool and urine were carried out by the methods described by Doss (1969). Jensen's (1963) method was used for separation of I and III coproporphyrin isomers. The activity of the uroporphyrinogen synthetase was determined according to Magnussen *et al.* (1974).

Results

A raised level of urinary porphobilinogen was found (Table 1) and indicated a diagnosis of acute intermittent porphyria. An increased level of uroporphyrin was found and accounted for 82% of all porphyrins excreted in the urine (Table 2). The proportion of isomer-III of coproporphyrin may be related to acute intermittent porphyria (AIP), though this proportion was below that typically found in AIP.

With a view to confirming the diagnosis of AIP, the family was studied (Table 3; Fig.). The results indicate that AIP was inherited from the mother. This was confirmed by observing that in the mother's

Table 1 *Porphyrin precursors: porphobilinogen (Pbg), δ-aminolaevulinic acid (ALA), and porphyrins in urine*

Age (m)	*Pbg (mg/24 h)*	*ALA (mg/24 h)*	*Coproporphyrin (μg/24 h)*	*Uroporphyrin (μg/24 h)*	*Urine (ml/24 h)*
6	10·1	2·5	31·2	86·2	400
9	5·4	0·5	29·6	261·5	450
11·5	22·2	2·5	39·0	175·0	770
12	21·8	2·6	35·4	154·0	240
13	20·1	4·6	49·0	185·0	325
Normal (Käser *et al.*, 1963)	0·075–0·37 ±0·055	0·125–0·7 ±0·092 SD			
Normal (/l) (Beauvais *et al.*, 1976)	⩽2	⩽3	<250	0	
Normal* (/l) (authors)	1·14 ±0·67 SD	2·42 ±1·5 SD	<100	<35	

*Authors' normal values established in 20 healthy children aged 1–2 years.

Table 2 *Urinary porphyrins determined by separation of their methyl esters and thin-layer chromatography*

Porphyrin type	*μg/24 h*	*%*	*% of isomer-III*
4 COOH	6·0	5·63	62·4
5 COOH	6·8	6·38	
6 COOH	1·0	0·94	
7 COOH	5·3	4·98	
8 COOH	87·4	82·06	

Table 3 *Urine investigations in the family*

	Pbg (mg/24 h)	*ALA (mg/24 h)*	*Copro-porphyrin (μg/24 h)*	*Uropor-phyrin (μg/24 h)*
Mother				
1	4·0	5·3	221·0	26·0
2	14·9	2·1	287·0	187·0
3	3·4	2·6	166·0	0
4	4·4	5·0	196·9	0
Mother's sister I	1·3	4·1	63·7	0
Mother's sister II				
1	5·0	2·8	294·0	0
2	2·6	4·0	258·0	0
Mother's brother				
1	2·4	2·4	226·0	1·9
2	3·2	4·4	138·0	7·6
Grandmother (maternal)	5·0	4·2	206·0	17·0
Father				
1	0	3·9	63·0	0
2	6·2	0·7	106·0	49·0
3	1·8	2·6	125·0	0
Grandfather (paternal)				
1	2·1	1·9	131·0	0
2	3·2	4·7	147·0	0

erythrocytes the activity of urosynthetase was decreased; 14·7 nmol porphyrins produced per ml erythrocytes per hour. The high level of porphobilinogen (Pbg) found on a single occasion in the father, and the slightly raised Pbg level in the grandfather are difficult to explain, and unfortunately these relatives did not allow further studies for urosynthetase activity. No clinical manifestations of porphyria were noted in any members of the family.

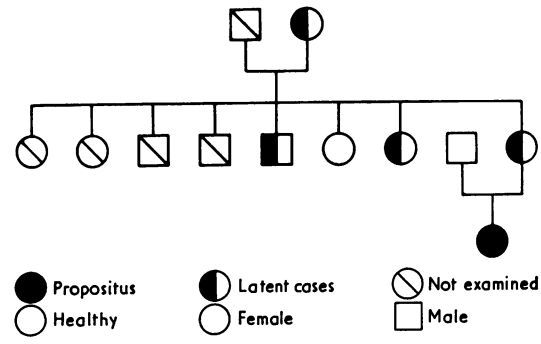

Fig. *Family tree. Note: 4 sibs of the mother died in early childhood and were not examined.*

The level of protoporphyrin was much raised in the patient's erythrocytes (Table 4), while the level of coproporphyrin was only slightly so (Table 5). In the earlier stages of the disease no abnormality was observed in faecal excretion of porphyrins, but later it increased to 55·7 μg/g dry weight. Coproporphyrin accounted for 69% of all porphyrins excreted, and it was present mainly in the form of isomer-I. In

Table 4 *Coproporphyrins and protoporphyrins in erythrocytes and faeces of patient*

Age (m)	*Erythrocytes (μg/100 ml)*		*Faeces (μg/g of dry weight)*	
	Copro	*Proto*	*Copro*	*Proto*
6			7·7	2·3
9	50·2	1123·0		
11·5	15·8	872·6	13·8	5·7
Normal	0·5–2·7	22–175*	0–14	5–51†

*Aldrich *et al.* (1955). †Chisholm (1964).

Table 5 *Faecal porphyrins separated by thin-layer chromatography in patient at 16 months*

Porphyrin type	*μg/g dry weight*	*%*	*% of isomer-III*
2 COOH	5·52	6·83	
4 COOH	55·7	68·9	7·8
5 COOH	trace		
6 COOH	trace		
7 COOH	trace		
8 COOH	13·1	16·21	
X	6·5	8·04	

healthy subjects coproporphyrin isomer-III accounts for about 90% of the excreted coproporphyrin. No rise in porphyrin content of erythrocytes and faeces was observed in the members of the family investigated. In the cerebrospinal fluid (Table 6) obtained from the subdural hygroma, slight amounts of porphyrin precursors, and a high level of uroporphyrin were found.

Table 6 *Porphyrins and their precursors in subdural fluid in patient at 9 months*

Pbg (mg/l)	*ALA (mg/l)*	*Coproporphyrin (μg/l)*	*Uroporphyrin (μg/l)*
2·02	0·24	21·7	374·4

Discussion

The diagnosis of AIP, rarely seen in small children (Beauvais *et al.*, 1976), was based on clinical suspicions and the results of biochemical and genetic investigations. This diagnosis was suggested by convulsions, paresis of muscles, tachycardia, pink urine, and worsening of the general condition with passage of darker urine after phenobarbitone and during infections. But the increased level of protoporphyrin in erythrocytes and the presence of coproporphyrin isomer-I in the faeces are not known to occur in AIP. Various forms of porphyria with atypical biochemical patterns have been reported by Hofstad *et al.* (1973), Rimington and With (1973), Eriksen and Eriksen (1974), and Tschudy (1974), but we know of no case with the findings of ours. A large increase in protoporphyrin in the erythrocytes and the presence of coproporphyrin isomer-I may suggest disturbances of porphyrin synthesis in bone marrow paralleling similar disturbances of synthesis in the liver. It has been suggested that porphyrin metabolism may be disturbed in both bone marrow and liver in erythropoietic porphyria, but in this condition increased urinary porphobilinogen excretion has never been observed (Gray *et al.*, 1964; Goldberg, 1968; Scholnick *et al.*, 1971; Nicholson *et al.*, 1973; Wolff *et al.*, 1975).

The high uroporphyrin content of the subdural fluid remains to be explained. Raised levels of porphyrin precursors in the cerebrospinal fluid have been observed during attacks of AIP (Bonkowsky *et al.*, 1971) and increased levels of porphyrins in porphyria cutanea tarda (Filippini and Simmler, 1973).

The result in our case suggest that there was disordered porphyrin synthesis in the bone marrow together with the characteristic abnormalities found in acute intermittent porphyria.

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