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Haemoglobin Porto Alegre in a Cuban family

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SUMMARY During a screening programme for abnormal haemoglobins in Habana, one case of Hb Porto Alegre was found in 23 000 cases analysed. The ability of this variant to polymerise *in vitro* and the absence of clinical features in the carriers have been confirmed. These observations are now explained by the findings of high levels of glutathione in the red cells of subjects heterozygous for Hb Porto Alegre: it is suggested that the increase of glutathione is responsible for the absence of *in vivo* polymerisation and accounts for the lack of clinical symptoms.

Haemoglobin Porto Alegre was first described in a Brazilian family (Tondo *et al.*, 1963). Two more cases of this abnormal haemoglobin have been found in the same country (Seid-Akhaven *et al.*, 1973) and one in Argentina (Peñalver *et al.*, 1971; Peñalver and de Miani, 1972).

Haemoglobin Porto Alegre presents one extra thiol group in the β-chain due to substitution of the serine in position nine for cysteine (Bonaventura and Riggs, 1967). This haemoglobin has the ability to polymerise spontaneously in the haemolysate, while *in vivo* it is present in the tetrameric form, which accounts for the lack of anaemia, erythrocyte morphological abnormalities, and clinical symptoms (Tondo *et al.*, 1963). The physicochemical properties of Hb Porto Alegre have been fully described (Tondo, 1972).

During the course of a screening for abnormal haemoglobins carried out on secondary school students of Havana City, one case of Hb Porto Alegre was found in 23 000 cases analysed. We report here the study of the family carrying this abnormal haemoglobin.

Subjects and methods

Routine haematological data were obtained by standard methods (Dacie and Lewis, 1966). Red blood cells were counted in a TOA automatic cell counter (Microcell Counter, Model CC-1002). Electrophoresis was carried out in polyacrylamide (Heredero *et al.*, 1974) and in starch gel (Smithies, 1959). Fetal haemoglobin was determined according to the method of Betke (Betke *et al.*, 1959) and HbA_2 according to Bernini (Bernini, 1969). Haemoglobin purification was obtained by DEAE-Sephadex chromatography (Jonxis and Huisman, 1968). Globin chain separation, aminoethylation, and trypsin digestion were carried out according to Clegg *et al.* (1966). Fingerprints of the aminoethylated abnormal β-chains were obtained as described by Baglioni (1951). Amino acid analysis was performed in a Carlo Erba Aminoacid Analyzer (Mod. 3A 47).

G6PD activity was determined by the method described in the WHO Report (1967). 6PGD glutathione peroxidase and glutathione reductase activity were determined as summarised in Yunis (1969). Glutathione levels were determined using the method of Beutler (1968).

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Results

The index case was found because of the appearance on polyacrylamide gel electrophoresis at *p*H 8·6 of a fresh haemolysate, of a large amount of a fast moving band similar to HbA_3. The case was a healthy 16-year-old boy, with no previous medical history. Physical examination did not show any abnormalities and the haematological values were within the normal range. All the other members of his family were studied: the father and 4 sibs were also found to be heterozygous for the abnormal haemoglobin. The pedigree is shown in Fig. 1 and the haematological data are summarised in Table 1.

Storage of the haemolysate at –20°C for a few days produced a slowband migrating between HbS and HbC on starch gel electrophoresis at *p*H 8·6 (Fig. 2).

Table 1 *Haematological data of family*

Family members and age (y)	*Hb type*	*Hb (g/dl)*	*RBC × 10⁶/ mm³*	*PCV %*	*=CV μ³*	*=CH pg*	*=CHC %*	*Ret. %*	*Glutathione mg GSH/ 100 ml cells*	*Glutathione reductose IU/g Hb*	*Glutathione peroxidase IU/g Hb*	*G6PD IU/g Hb*	*6PGD IU/g Hb*
I.1 50	A/PA	14.3	4.86	43	90	36	34	1.0	57	3.55	3.06	7.85	4.76
I.2 45	AA	13.6	4.73	38	81	27	34	1.5	17	2.01	3.28	5.80	4.50
II.1 30	A/PA	12.6	4.16	36	86	30	36	1.1	46	2.64	3.63	6.00	3.95
II.2 18	AA	14.5	4.87	43	90	30	34	1.8	19	3.40	3.78	4.85	6.00
II.3 6	A/PA	14.5	4.43	42	93	31	34	1.0	56	3.10	3.50	7.00	5.84
II.4 20	A/PA	13.2	4.78	40	85	28	34	0.8	42	3.91	5.18	6.95	6.30
II.5 23	AA	14.1	4.82	43	91	30	34	0.9	26	2.80	2.90	4.56	4.84
II.6 26	A/PA	15.6	4.95	46	96	33	34	1.0	51	2.65	4.06	6.94	5.60
II.7 27	A/PA	14.7	4.61	40	87	32	37	1.3	48	3.00	4.01	7.40	4.30

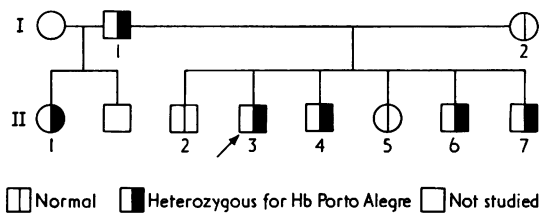

Fig. 1 *Family pedigree. The arrow indicates the propositus.*

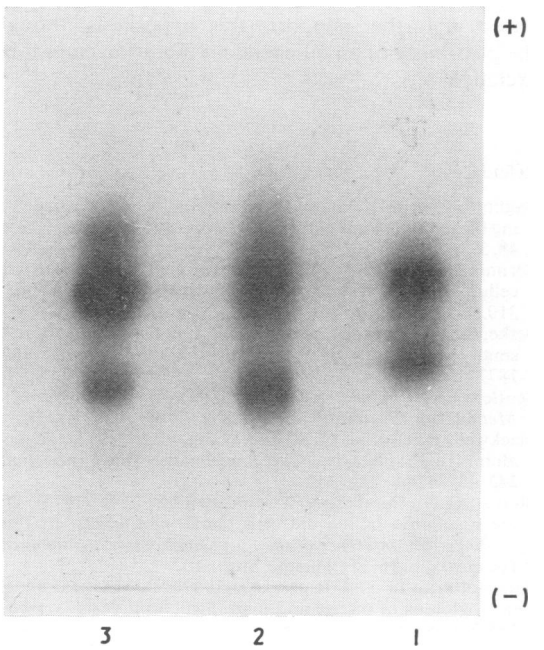

Fig. 2 *Electrophoresis of the carrier of Hb Porto Alegre at pH 8·6. (1) HbS heterozygote; (2) and (3) propositus.*

Its amount increased with time and its appearance could be prevented by addition of dithyothreitol to a freshly prepared haemolysate. This behaviour suggested that a spontaneous polymerisation caused by the formation of S–S bridges takes place in the haemolysate. The presence of such a polymer was confirmed by gel filtration on Sephacex G-100 and by ultracentrifugation. In both cases a molecular weight consistent with the presence of a haemoglobin octamer was obtained.

The abnormal haemoglobin was purified from a 25-day old haemolysate on a Sephadex G-100 column and on a DEAE-Sephadex column using a *p*H gradient: under this last condition the abnormal haemoglobin is eluted after HbA. The amount of the variant was found to be 37%.

It has been reported (Rucknagel *et al.*, 1964) that the β_2 tryptic peptide is absent in the fingerprint of the Hb Porto Alegre. In our case this peptide could not be located with ninhydrin staining, but after aminoethylation an abnormal peptide appeared in the position shown in Fig. 3. The composition of this peptide is given in Table 2. The abnormal haemoglobin was thus identified as Hb Porto Alegre ($\alpha_2\beta_2$ 9 Ser→Cys).

Discussion

Haemoglobin Porto Alegre is a clinical and electrophoretic silent mutant. Another variant behaving in the same way has been reported, Hb Ta-Li, another β-chain variant in which the presence of a cysteine in position 83 produces polymerisation on the haemoglobin in the haemolysates (Blackwell *et al.*, 1971). In

Table 2 *Amino acid composition of abnormal β-II peptide*

Amino acid	*β_2 Residues expected*	*β_2 Residues observed*
Thr	1	0·95
Ser	1	—
Gly	1	1·05
Ala	2	2·08
Val	1	1·21
Leu	1	0·97
Lys	1	0·98
S-Ae-Cys	0	0·94
Trp	1	*

* The peptide stained positively for tryptophane.

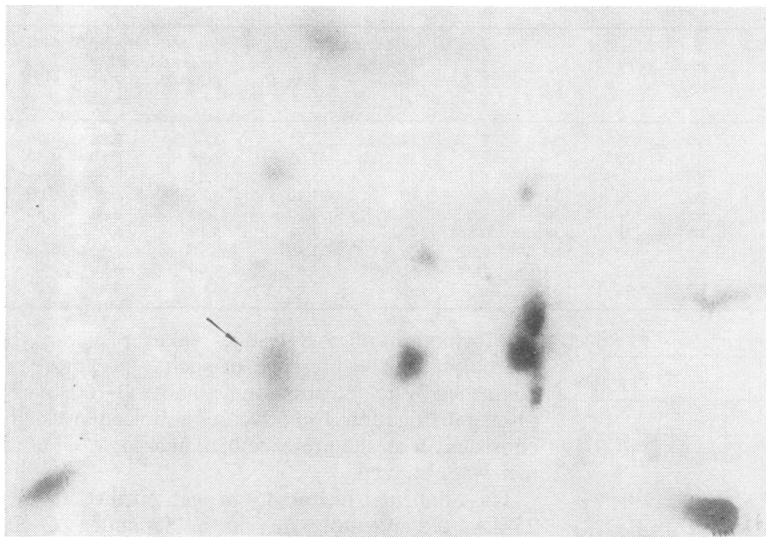

Fig. 3 *Fingerprint of aminoethylated β-chain of Hb Porto Alegre. The arrow indicates the abnormal peptide.*

both cases there are no pathological consequences and no haematological abnormalities, probably because both variants are kept in the normal tetrameric form in the erythrocytes. It would be interesting to know whether cells carrying Hb Porto Alegre require a special metabolism to maintain the extra thiol groups of the variant in the reduced form: our results indicate that these cells contain higher amounts of glutathione (Table 1). In fact, glutathione levels are twice those of a normal subject, which seems to correspond to the twofold increase of -SH groups (4 in Hb Porto Alegre compared to 2 in HbA). Glutathione peroxidase and reductase, as well as G6PD and 6PGD activities were found to be normal (Table 1).

It is believed that at least one of the functions of glutathione is to protect haemoglobin as well as other thiol containing proteins from denaturation under oxidative stress (Srivastava and Beutler, 1970). Independently of the different mechanism proposed to explain this function (Blume *et al.*, 1973), our findings suggest that higher levels of glutathione are needed in the presence of unusually high number of -SH groups. Thus it seems reasonable to propose that the increased levels of glutathione in the red cells of Hb Porto Alegre carriers is the result of the need to maintain the abnormal haemoglobin in the tetrameric functional form, though the biochemical mechanism responsible for this high concentration is not clear. Experiments are under way to clarify this point.

To explain the findings of 3 probably unrelated cases of Hb Porto Alegre, 2 in Brazil and 1 in Argentina, it has been suggested that the gene responsible for this variant could be relatively common in Portugal and in Spain (Seid-Akhaven *et al.*, 1973). The finding of a new case in a Cuban family of Spanish ancestry, further supports this hypothesis, though the possibility of an independent mutation cannot be excluded.

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