A new case of haemoglobin Bucuresti in a Cuban family: further functional studies

Summary. A new case of haemoglobin Bucuresti β42 (CD1) Phe→Leu is described in a Cuban family.

The functional studies confirm the results already described—a low oxygen affinity and a decreased haem-haem interaction. In addition to this, the reactivity for 2, 3 diphosphoglycerate (2, 3 DPG) was shown to be normal. The instability is mostly due to a fast rate of haemichromes formation.

Haemoglobin Bucuresti β42 (CD1) Phe→Leu has been described independently in 1971 in Roumania (Bratu et al, 1971) and as haemoglobin Louisville in a Caucasian family (Keeling et al, 1971). We report here a Cuban case of this unstable mutant.

Case report

The diagnosis was made when the 25-year-old patient was admitted to the C. Finlay Hospital in La Habana for appendicitis. He had had five previous haemolytic crises during infectious processes, some of them with icterus and haemoglobin at 5 g/dl. A similar clinical feature of haemolytic anaemia of intermediate severity has been found in nine members of his family carrying the defect (Fig. 1). All have been splenectomized with variable results.

Methods

Structural studies. Specific precipitation of the abnormal β chain was obtained using parahydroxymercuribenzoate (PHMB) within 2h (PHMB/Hb molar ratio: 4/1) (Rosemeyer and Huehns, 1967). It was further purified by carboxymethylcellulose (CMC) chromatography (Clegg et al, 1966). The tryptic hydrolysate was fingerprinted on paper (Baglioni, 1961) or fractionated on Aminex A5 (Jones, 1964). The abnormal peptide was purified by anion exchange chromatography (Schroeder, 1967) and sequenced by the miniaturized Edman–dansyl method (Hartley, 1970).

Functional studies. Oxygen equilibrium curves were obtained by the method of Benesch et al (1965) as modified by Bellingham and Huehns (1968). 2, 3 DPG was assayed according to Beutler et al (1969). The haemolysate was stripped on Sephadex G 25.

Results

Haematological investigations. Haemoglobin was 9 g/dl; RBC, 4 × 10¹²/l; reticulocytes, 80 × 10⁹/l; osmotic resistance was normal; RBC survival was 6.2 d; bilirubin, 3.6 mg/l (61.6 μmol/l) (direct form, 1.3 mg/l [22.2 μmol/l]).

Structural studies. The abnormal component was estimated to be 30% by a kinetic stability test (Wajcman et al, 1973a).

The substitution was proved to be Phe→Leu in the βTpyV peptide by fingerprinting the tryptic digest of the β chain of the thermolabile fraction and analysing the spots.

For sequence studies, the PMB precipitate was used. It contained 90% pure β chain. A modified gradient (Brinshall et al, 1969) and a second purification gave a perfectly pure βTpyV which was further sequenced.

Functional studies. Even a few days after sampling in La Habana, the oxygen affinity, measured on intact RBC, was decreased (P₅₀ = 35 mm Hg [4.7 kPa], normal = 29.5 ± 1 mm Hg [4.0 ± 0.1 kPa]). The Hill coefficient was 2.0. The intracellular 2, 3 DPG was 13.3 μmol/g of Hb (normal = 14.7 ± 1). The Bohr effect in phosphate-free lysates was apparently normal at pHs ranging from 6.37 to 7.77. The effect of 2, 3 DPG is simi-
lar for Hb Bucuresti and Hb A (for details, see Fig. 2). The differential spectrum of oxidized Hb Bucuresti against oxidized Hb A at pH 6.5 shows a peak near 530 nm and a shoulder at 565 nm; both are characteristic of haemichromes.

Discussion
This is the third unrelated observation of Hb Bucuresti. This family had been previously known since 1941 and described as a case of thalassaemia in Cuba.

The inability to obtain the native abnormal component allowed functional studies only on the total haemolysate taking into account the percentage of the mutant. Our results confirm previous results, and, moreover, strongly favour the hypothesis of a normal reactivity for the DPG. The theoretical oxygen equilibrium curve for pure Hb Bucuresti is very close to what has been reported for Hb Hammersmith β 42 (CD1) Phe→Ser (Dacie et al., 1967; Wajcman et al., 1973b).

Though the β 42 residue acts as a spacer in the haem pocket, the haem loss was found to be negligible. The instability seems to be related to the fast rate of haemichrome formation, nevertheless much slower than in Hb Hammersmith. This might explain the better tolerance of Hb Bucuresti.

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