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# Simultaneous occurrence of Hb C trait and polycythaemia vera*

by

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**Abstract:** A slow abnormal haemoglobin was found in a 27 year-old Negro man who had polycythaemia vera. Chemical and structural analysis showed it to be Hb C. The oxygen affinity showed a normal $P_{50}$ value. Clinical and haematological investigations are described and discussed.

The association between erythrocytosis and variant haemoglobin was first described in 1966 by Charache *et al.* Since then a further 24 stable high affinity haemoglobin variants associated with erythrocytosis have been described (Stephens, 1977).

The majority of abnormal haemoglobin which has been found to have altered oxygen binding properties has structural changes that affect one or more of the following parts of the molecule: 1) haeme pocket; 2) haeme contact; 3) alfa-beta subunit contact; 4) 2,3 DPG binding site; 5) group involved in the Bohr effect; or 6) integrity of helical structure. However, the association between erythrocytosis and Hb C has not been described in the literature. Thus, we report the determination of primary structure and physico-chemical properties of the Hb C trait found in association with polycythaemia vera in a 27 year-old Negro man.

## MATERIAL AND METHODS

Routine haematological studies followed standard methods. The red cell haemolysates were prepared by the method of Drabkin (1946) and then adjusted to a final concentration of 10 g/100 ml. Cellulose acetate electrophoresis was carried out in a Beckman Microzone apparatus using a Tris–EDTA-borate buffer pH 8.6 (Rozmon *et al.*, 1963), and agar gel electrophoresis was also employed using citrate

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buffer pH 6.2 (Robinson *et al.*, 1957). The relative proportions of the haemoglobin components were estimated by densitometry in a Carl Zeiss densitometer. Foetal haemoglobin determination was performed by two-minute alkaline resistance (Betke *et al.*, 1966). The haemoglobin variant was isolated by paper electrophoresis (Cradock-Watson *et al.*, 1959). The red band containing the haemoglobin was cut out, eluted and the eluate resubmitted to electrophoresis in order to separate the variant from traces of Hb A. For studies of primary structure, the globin was prepared by acid acetone precipitation at -20 C (Anson and Mirsky, 1930).

The abnormal beta chain was separated by column chromatography on CM-cellulose using an ionic gradient of 8 M urea (Clegg *et al.*, 1966). The abnormal beta chain was digested with trypsin-TPCK at 37 C for 2 hours, and the resulting soluble peptides were fingerprinted using high voltage (55 volts per cm) at pH 6.4, and ascending chromatography for 18 hours (Baglioni and Ingram, 1960). Diagnostic fingerprints were stained with 0.2 per cent (w/v) ninhydrin in acetone and with colour reagents specific for peptides containing divalent sulfur, histidine, tyrosine and tryptophan (Smith, 1969) and arginine (Yamanada and Itano, 1966).

For amino acid composition studies, relevant peptides were eluted and hydrolysed with constant boiling 6 N HCl in sealed and evacuated tubes at 108 C for 18 hours. After the removal of excess HCl in vacuum, the amino acid compositions of dried hydrolysates were determined by a Locarte amino acid analyser (Spackman *et al.*, 1955).

Studies of oxygen affinity on whole haemolysates were performed by the descontinuous spectrophotometric methods (Bellinghan and Huehns, 1968).

The patient, S. L. a 27 year-old Negro, male, was hospitalised in June 1973 for investigation of erythrocytosis associated with an abnormal haemoglobin similar to Hb C. There was no suggestive history of jaundice. Clinical investigation, including pulmonary function and cardiac output, were normal. Routine haematological studies showed the following: haemoglobin, 20 g/100 ml; red cells, 9,26 million/$\mu$l: haematocrit, 71 per cent; M. C. V., 76.6 $\mu^3$; M. C. H. C., 28.1 per cent; M. C. H., 21.5 pg; white cells, 12,700/$\mu$l; erythrocyte sedimentation rate, 0.5 mm/hour.

The blood film showed mild anisopoikilocytosis, microcytosis, slight hypochromia and increased number of platelets.

## RESULTS

The haemoglobin electrophoresis at pH 8.6 on cellulose acetate revealed an abnormal haemoglobin component moving in the position of Hb C. This fraction was shown to be 48 per cent of the total by quantitative estimation in the densitometer. On agar gel electrophoresis at pH 6.2, the abnormal haemoglobin had a slower mobility than Hb A. Alkali resistant haemoglobin was 0.75 per cent of the total.

Peptide maps of the tryptic digestion of the globin showed absence of the normal peptide $\beta$Tp I and the presence of two "new" peptides: one, $\beta$Tp Ia, positive with specific histidine stain was towards the cathode, near to the normal $\beta$Tp XIV, and its amino acid analysis was composed of the residues 1 to 6, revealing it to be the substitution in the 6th position of beta chain of glutamic acid by lysine; $\beta$Tp Ib, composed of the residues 7 and 8, was in the neutral zone, with very slow ascending chromatography below the normal $\beta$Tp I (Fig. 1).

Oxygen affinity, performed by Prof. H. Lehmann in Cambridge, was normal ($P_{50}$ - 2.8 kPa).

## DISCUSSION

The finding in our patient of a type of abnormal haemoglobin, electrophoretically similar to Hb C, in association with erythrocytosis, was investigated by fingerprints of peptides which showed chromatographic and electrophoretic abnormalities typical of peptide βTp I. This peptide, in Hb A, is in the neutral zone and it is positive with histidine stain. By comparison, the fingerprints of the abnormal haemoglobins with those of Hb A showed that the spots representing βTp Ia and βTp Ib, were similar to the Hb C pattern. These peptides, submitted to amino acid analysis revealed a substitution of glutamic acid by lysine in the 6th position of the beta chain. This mutation does not alter the physical properties of the molecule because it occurs on the external surface. Normal oxygen affinity confirmed that the erythrocytosis was not due to the Hb C. The absence of abnormalities in pulmonary function and cardiac output also showed that it was not due to secondary causes. Haematological studies revealed that it was compatible with polycythaemia vera associated with Hb C trait.

## RESUMEN

Una hemoglobina lenta anormal fue hallada en un hombre negro que tenía policitemia vera. Los análisis químicos y estructurales mostraron que se trataba de Hb C. La afinidad por el oxígeno presentó un valor normal de $P_{50}$. Se describe y discute los aspectos clínicos y hematológicos.

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Fig. 1. Peptide map of the tryptic digest of the $\beta^C$ polypeptide chain of the patient with Hb AC trait and polycythaemia.
1- $\beta$Tp I normal missing; 2- $\beta$Tp Ia (residues 1-6) histidine positive and 3- $\beta$Tp Ib (residues 7-8).


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