Hb J SICILIA: β 65 (E9) LYS→ASN, A BETA HOMOLOGUE OF Hb ZAMBIA

Giuseppe RICCO, Pier Giorgio PICH, Umberto MAZZA, Giacinto ROSSI*, Franco AJMAR**, Paolo ARESE‡ and Eugenio GALLO‡‡

Department of Special Medical Pathology and Clinical Methodology, University of Turin, Via Genova 3, 10100, Turin, Italy

Received 17 September 1973‡‡‡

1. Introduction

The present paper reports the observation of a new hemoglobin variant in a 28-year-old Sicilian woman living in Genoa. The electrophoresis position (pH 8.6) was typical for Hb J and the name of Hb J Sicilia is proposed for the variant. Its structural formula is $\alpha_2\beta_2$ 65 (E9) Lys→Asn. This mutation does not result in any difference in function. Hb J Sicilia can be considered as a homologue of Hb Zambia, in which alpha lysine 60 (E9) is replaced by asparagine. The proposita, heterozygous carrier of the abnormal hemoglobin, was also heterozygous for G 6-PD deficiency and displayed slight anemia. No other members of the family were examined.

2. Materials and methods

Routine blood studies were run. Hemoglobin electrophoresis was determined on cellulose acetate [1]. Red cell survival was evaluated with ^{51}Cr [2]. Chromatographic separation on a Sephadex DEAE A-50 column [3] was followed by vacuum concentration, incubation with parachloromercuribenzoate (PCMB) [4] and electrophoretic separation of the alpha and beta subunits. Globin obtained by cold precipitation with HCl–acetone from the abnormal fraction was digested with trypsin and fingerprinted [5,6]. Fingerprints were also obtained from the alpha and beta chains, following their separation with urea and β-mercaptoethanol [7] and amino-ethylation [8]. Each abnormal peptide was eluted with 6 N HCL and hydrolysed at 110°C for 20 hr prior to study in an Optica automatic amino-analyser. In addition,

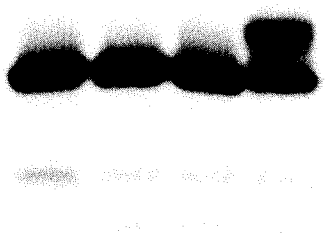


Fig. 1. Electrophoretic pattern of Hb J Sicilia (right). Cellulose acetate strip, glycine buffer, pH 8.6, ionic strength 0.04.

* Marxer s.p.A., Medical Department, 10010, Loranzè d'Ivrea, Turin.
** Hematology Department of the Institute of Internal Medicine, University, Viale Benedetto XV, n.6, 16132, Genoa.
‡ Institute of Biochemistry, University, Via Michelangelo 27, 10126, Turin.
‡‡ Institute of Medical Semeiotics of University, Strada San Vito 34, 10133, Turin.
‡‡‡ Delayed due to a technical fault.

North-Holland Publishing Company – Amsterdam

hydrolytic digestion of the abnormal peptides with carboxy-peptidase B [9], followed by high-voltage paper electrophoresis at pH 2.0 [5], examination with the amino-analyser and enzymatic analysis [10] was performed. Then, absorption spectra [11] of the abnormal hemoglobin, solubility of its reduced form [12], and molecule stability (heat and isopropylic alcohol tests)[13,14] were carried out.

3. Results and discussion

The hematological data included: Hb 11.6 g%, RBC 3.9 X $10^6\mu$l, PCV 38%, MCV 97 μm^3, MCHC 29.7 g/100 ml RBC, MCH 30.5 pg/RBC, reticulocytes 1.8%, mean red cell survival: T/2 = 18 days, plasma iron 115 γ%, total plasma bilirubin 1.3 mg%, G 6-PD 46 U/100 ml RBC. The abnormal hemoglobin concentration was 40% and Hb-A_2 was within the limits of normal (fig. 1). Separation of the alpha and beta subunits showed normal alpha and fast beta chains (fig. 2). The fingerprint from the undissociated globin (fig. 3) demonstrated the absence of β 8/9 and the presence of a histidine positive extrapeptide below β6. Each peptide of the so-called

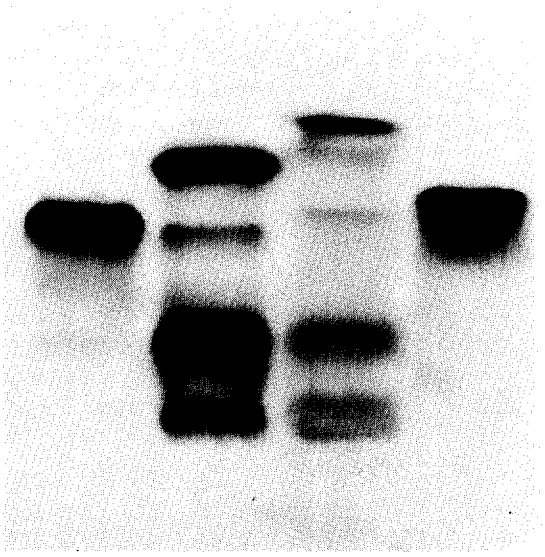


Fig. 2. Electrophoretic separation of alpha and beta subunits of hemoglobin by means of PCMB. Cellulose acetate strip, pH 8.6, ionic strength 0.04. From left to right: undissociated Hb-A, alpha and beta chains of Hb-A, alpha and beta chains of Hb J Sicilia, undissociated Hb J Sicilia.

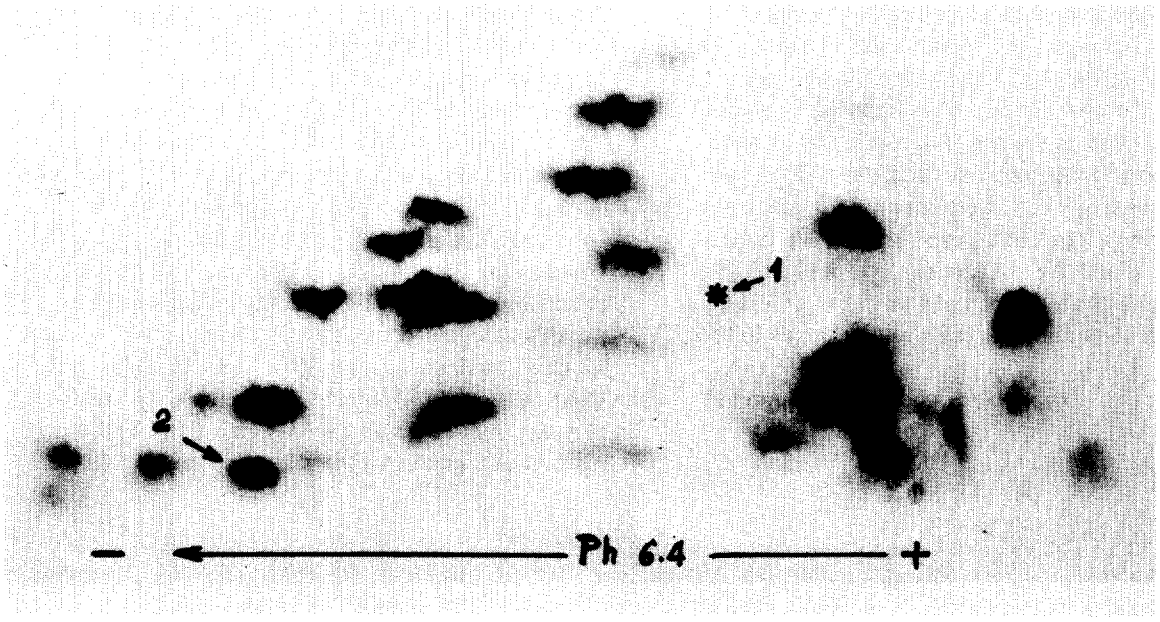


Fig. 3. Tryptic fingerprint, pH 6.4, of undissociated globin of Hb J Sicilia. 1 = absence of β 8/9.2 = new peptide. Note the small size of the last three peptides in the cathode group.

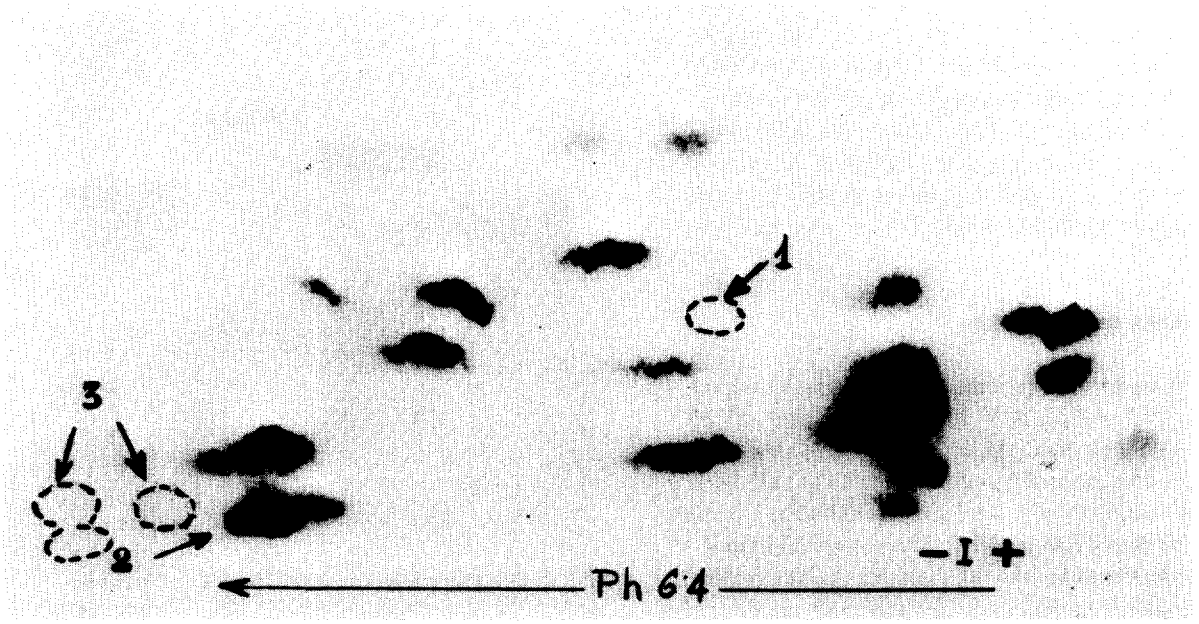
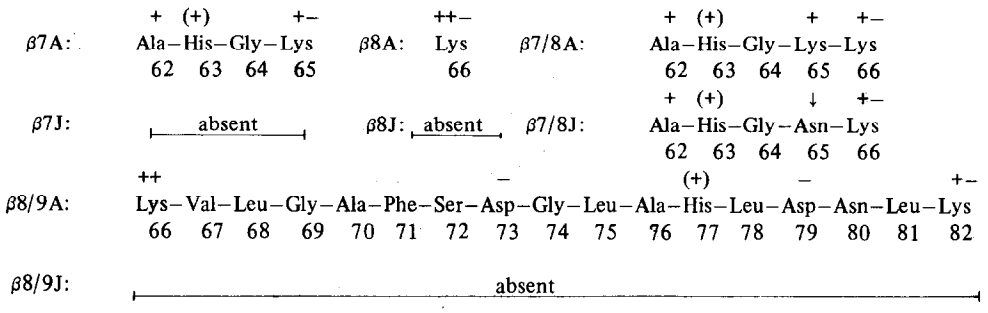


Fig. 4. Tryptic fingerprint, pH 6.4, of the separated and amino-ethylated beta chains of Hb J Sicilia. I and 3 = absence of: β 8/9, 7, 8 and 7/8. 2 = abnormal peptide.

cathode group (αβ7, 8 and 7/8) occupied less space than usual. Both αβ7 and αβ7/8 stained yellow-brown with ninhydrin, suggesting the presence of N-terminal glycine. The reduced size of these three peptides and the fact that glycine usually forms the N-terminal for α7 and α7/8, as opposed to alanine in β7 and β7/8, it seemed likely that the cathode group consisted of alpha peptides only. This was corroborated by the fingerprints for the alpha and beta subunits. The alpha map, in fact, was normal, while β7, 8 and 7/8 were missing, as well as β8/9 (fig. 4), and the extra-peptide was sited as already described. Its amino acid composition was as follows:

Residues:	Expected for Hb-A	Found (39.7 nmoles per residue)
Aspartic acid	–	0.98
Glycine	1	1.20
Alanine	1	1.01
Lysine	2	0.94
Histidine	1	0.88

This showed an abnormal β7/8 lacking a lysine and with an additional aspartic acid or asparagine. Asparagine is, of course, converted to aspartic acid during acid hydrolysis prior to amino acid analysis. In the present case, hemoglobin and abnormal peptide mobility pointed to a Lys→Asn mutation, with loss of a single positive charge. Genetic considerations also favoured this interpretation. Lys, Asn and Asp, in fact, are coded by the following triplets: Lys: AAA/AAG, Asn: AAU/AAC, Asp: GAU/GAC. Whereas the mutation Lys→Asn can be obtained by substitution of one base only, Lys→Asp would require two simultaneous substitutions. This can be regarded as virtually impossible [15]. Lastly, replacement at the β 65 (E9) level offers the only satisfactory explanation of all the observed data. The normal (A) and abnormal (J) sequences are as follows:



Loss of a positive charge explains reduced cathodic mobility of the abnormal peptide, while asparagine replacement of lysine gives a reason for the absence of β7, 8 and 8/9. An analogy may be drawn with Hb Zambia [16], whose extra-peptide has a very similar position.

To clinch the question, attention was given to the strictly biochemical aspects of the matter. The abnormal peptide was eluted from the fingerprint and digested with carboxy-peptidase B for 3 hr, while the α_2 normal peptide, composed by Thr–Asn–Val–Lys, was used as control and digested for 18 hr, because the position of asparagine requires a longer digestion. Hydrolysis was arrested by rapid vacuum drying and not by acetic acid treatment to prevent degradation of Asn to Asp as far as possible. Then, all specimens were subjected to both high voltage electrophoresis and amino-analyser examination; the latter technique proved unsatisfactory, since asparagine was not clearly visible even in α_2, while Asp was certainly present. It was therefore assumed that Asn degrades to Asp to a varying degree even during one or more phases of enzymatic hydrolysis or amino acid analysis.

Electrophoresis gave both non-significant findings and a number of runs marked by the contemporary presence, in the abnormal peptide, of two distinct spots at the Asn and control Asp levels respectively (fig. 5). This finding has no more than indicative value however, owing to the presence of incompletely digested residues.

Use was therefore made of an enzymatic technique. In a 4 cm cuvette containing 3.0 ml hydrolysed specimen dissolved in 0.1 M triethanolamine buffer, pH 7.6, 10 μmoles α-oxoglutarate, and 0.15 μmoles NADH, addition of malate dehydrogenase (110 units) and glutamate–oxaloacetate transaminase (8 units) allowed the assay of aspartate; subsequent addition of L-asparaginase (40 units) converted asparagine into aspartate and allowed its determination in the same cuvette. Equimolecular asparagine and aspartate were found in the abnormal peptides, while far less favourable asparagine–aspartate ratios were found in normal controls. These results allow the conclusion that aspartate is formed from asparagine during hydrolysis or analysis, and that the mutation is Lys→Asn. In spite of its clinical benignancy, it must not be forgotten that the Hb J Sicilia anomaly involves the presence of an abnormal amino acid two places after the distal histidine β63 (E7), i.e., very near the hemic pocket [17]. Assessment of its functional significance showed normal spectra for the Oxy, Deoxy, Meta and Cyanometa-derivatives. Heat lability and solubility of the reduced form were also within the limits of normal in both the purified fraction and the total hemolysates. It may therefore be assumed that the mutation has not significant effect on Hb function and is not responsible for any hematological abnormality.

Aknowledgements

Assistance received from Dr. S. Bianchi of the Marxer s.p.A., and Dr. G. Gaetani and Mr. G.B. Bruzzone of the Hematology Department, University of Genoa, is gratefully acknowledged.

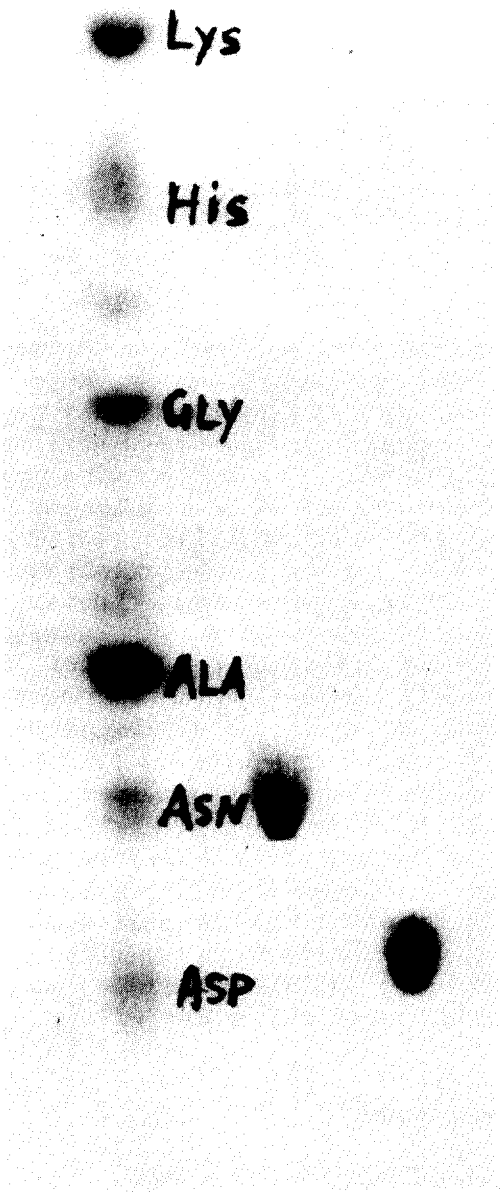


Fig. 5. High voltage paper electrophoresis, pH 2.0, of the abnormal peptide hydrolysed by means of carboxy-peptidase B. Two markers, Asn and Asp, have been added on the right. The supernumerary spots are probably due to undigested residues.

References

[1] Cimino, R. and Ventruto, V. (1967) Progresso Med. 23, 436.
[2] Mollison, P.L. and Veall, N. (1955) Brit. J. Haemat. 1, 62.
[3] Huisman, T.H.J. and Dozy, A.M. (1967) J. Chromatog. 19, 160.
[4] Rosemeyer, M.A. and Huhens, A.M. (1967) J. Mol. Biol. 25, 253.
[5] Sick, K., Beale, D., Irvine, D., Lehmann, H., Goodall, P.T. and McDougall, S. (1967) Biochim. Biophys. Acta, 140, 231.
[6] Beale, D. (1967) Biochem. J., 103, 129.
[7] Clegg, J.B., Noughton, M.A. and Weatherall, D.J. (1966) J. Mol. Biol., 19, 91.
[8] Raftery, M.A. and Cole, R.D. (1966) J. Biol. Chem., 241, 3457.
[9] Ambrosino, C., Cauvin, E., La Vecchia, L., Liberatori, J. and Michelin-Lausarot, P. (1969) Riv. Sci. (CNR, Roma), 39, 909.
[10] Bergmeyer, H.U. (1962) Methoden der enzymatischen Analyse, pp. 1654-1655, Verlag Chemie.
[11] Dubowski, K.M. (1964) Hemoglobin, Its precursors and metabolites, (Sundermann and Ass. eds) p. 49, Lippincott Co.
[12] Itano, H.A. (1963) Arch. Biochem. Biophys., 47, 148.
[13] Dacie, J.V., Grimes, A.J., Meisler, A., Steingold, L., Hemsted, E.H., Beaven, G.H. and White, J.C. (1964) Brit. J. Haemat., 10, 388.
[14] Carrell, R.W. and Kay, R. (1972) Brit. J. Haemat., 23, 615.
[15] Lehmann, H. and Huntsmann, R.G. (1966) Man's Haemoglobin, pp. 93-94, North Holland, Amsterdam.
[16] Barclay, G.P.T., Charlesworth, D. and Lehmann, H. (1969) Brit. Med. J., 4, 595.
[17] Perutz, M.F. and Lehmann, H. (1968) Nature, 219, 902.