ACTA MEDICA IRANICA
Vol.XX. 1977. P. 1-8

HAEMOGLOBIN M $_{Boston}$ IN AN IRANIAN FAMILY

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Abstract

Haemoglobin M_{Boston} is described in a 19 year old Iranian male, his father and three out of his five brothers and sisters which were cyanotic from the birth. The presence of haemoglobin M was established after strach-gel electrophoresis of the ferricyanide treated haemolysate at pH 7.1 and by spectroscopic examination of the purified abnormal haemoglobin at pH 6.5.

Introduction

When the pressure of oxygen falls, oxyhaemoglobin is converted to reduced haemoglobin and oxygen is released and donated to the tissues. The iron atom is in the ferrous state (Fe++) in oxyhaemoglabin, being able of transporting and releasing oxygen to the tissues. When the iron atom of the haem group becomes oxidised to ferric state (Fe+++), Methaemoglobin is formed which is unable to carry oxygen. The presence of the methaemoglobin gives a brown colour to the blood and produce cyanosis of the skin and mucosa.

In the normal circumstances, methaemoglobin formation occurs at a rate of 3% a day, this process is compensated by a more rapid reduction phenomena which converts the methaemoglobin to oxyhaemoglobin and maintains the iron atom in it's ferrous state., Nearly most of the methaemoglobin reducing activity is attributed to a red cell's enzyme known as NADH-methaemoglobin reductase or Diaphorase. Ascorbic

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acid and glutathione are also potent methaemoglobin reducing agents.

The presence of methaemoglobin in a patient is due to three major causes:

1. Excessive methaemoglobin formation (intoxication by nitrite and other chemicals).
2. Decreased reduction and conversion of methaemoglobin to oxyhaemoglobin (diaphorase deficiency).
3. Abnormality of the globin moiety of haemoglobin molecule which converts the haem iron to it's ferric state, resulting in an abnormal methaemoglobin or haemoglobin M.

Aquired methaemoglobinemias are mostly classified in the first category, while hereditary methaemoglobinemias are due mostly to a genetic enzymatic defect of the diaphorase or, the presence of haemoglobin M in the patient's red cells, causing hereditary cyanosis.

Among a large varieties of abnormal haemoglobin discovered, haemoglobin M are of prime interrest causing hereditary methaemoglobinemia and as a good example of a molecular disease. Most of these haemoglobins M variants present the substitution of a tyrosine residue for the distal or proximal histidine in their α or β chains; haemoglobin M_{Boston} α 58 His–Tyr (3), haemoglobin M-Saskatoon β63 His→Tyr(3), haemoglobin M_{Iwate} α87 His → Tyr (7), and haemoglobin $M_{Hyde-Park}$ β92 His – Tyr (5).

In addition to these 4 haemoglobins M, there are 2 other haemoglobins variants presenting methaemoglobinemia which fits in haemoglobin M category, haemoglobin Milwaukee β67 Val →Glu (3) and haemoglobin Saint-Etienne or Istambul β92 His Gln (1).,.,

Most cases of haemoglobin M observed throughout of the world is haemoglobin M-Boston. The chemical structure of this haemoglobin was first investigated by Park Gerald and Efron (3)., In this article we describe haemoglobin M-Boston in members of an Iranian family which were cyanotic from the birth.

Case History

The propositus was a 19 year old male admitted because of

cyanosis markedly seen in the lips, nail beds, ears and malar eminences, presenting the typical "Lavender blue" colour described by Horlein and Weber. The onset of cyanosis was told to be few days after birth. The cyanosis was also detected in his father, brother and his 2 sisters.

In physical examinations, cyanosis was the only prominent manifestation, no abnormality was found in the cardiovascular or respiratory systems. Spleen and liver were in normal sizes. The result of the routine haematological examinations were; RBC 6'000'000, WBC 7'000/mm^3, Hb 16gms/100ml, haematocrait 52%, MCV 87 um^3, MCH 27 pg, MCHC 31%, red cell morphology was normal, no inclusion bodies was found in the red cells after incubation in Brilliant Cresyl Blue.

Methods and Results

Haemolysate was prepared by washing the red cells 3 times with 0.9% saline and lysed by addition of 1 volume of distilled water and 0.5 volume of toluene followed by centrifugation. Haemolysate of the patient had a chocolate brown colour. Electrophoresis of the haemolysate at alkaline pH on cellulose acetate and in the starch-gel with Tris-EDTA-Borate buffer showed a normal pattern. Starch-gel electrophoresis of the ferricyanide treated haemolysate at pH 7.1 (6), revealed presence of 2 major haemoglobin fractions (Fig.1). The most anodal fraction had a chocolate brown colour as compared with the ferricyanide treated normal haemolyate., Haemoglobin M fraction was purified by strach block electrophoresis of the ferricyanide treated haemolysate with phosphate buffer pH 7.1,0.05M (4), Spectroscopic examination of the purified haemoglobin M as well as normal haemoglobin was carried out at pH 6.5 (4), using Unicam SP 800 recording spectrophotometer. Figure 2 is a composite tracing of the absorption curves of the purified haemoglobin M (solod line) and from haemoglobin A (interrupted line)., Haemoglobin M shows the characteristic absorption peak at 495 and 602 nm instead of 502 and 632 nm, in acid methaemoglobin A.

Structural studies of the purified haemoglobin M was carried out according to the following steps; Globin was prepared by the treatment of purified haemoglobin M with 2% HC1 in acetone at -20°C, precipi-

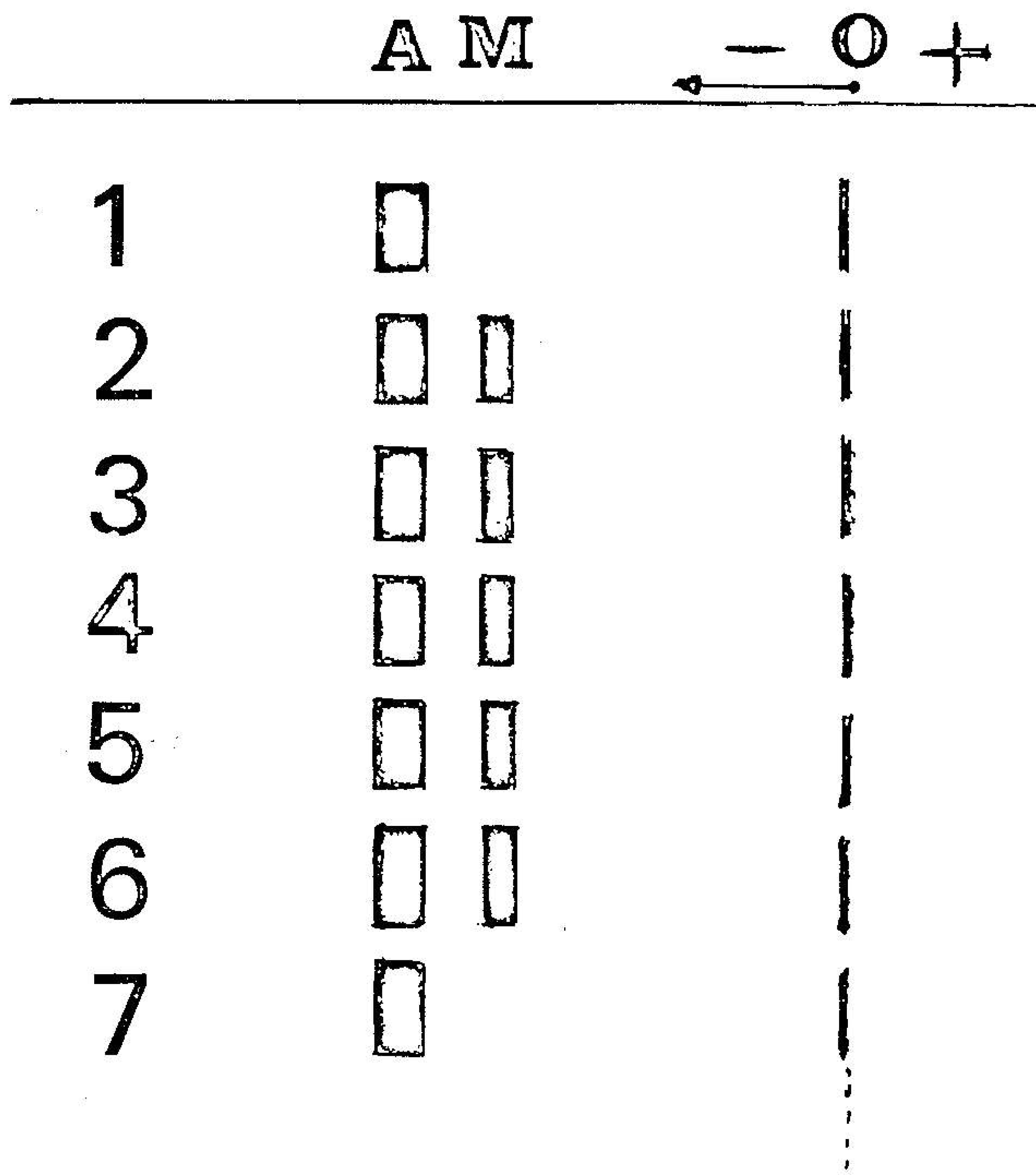

Fig. 1. Tracing of the starch-gel electrophores of the ferricyanide treated haemolysates at PH 7.1 (cathode to the left)
1. normal haemolysate, 2-propositus, 3-father, 4,5 & 6-brother and sisters of the propositus, 7-mother

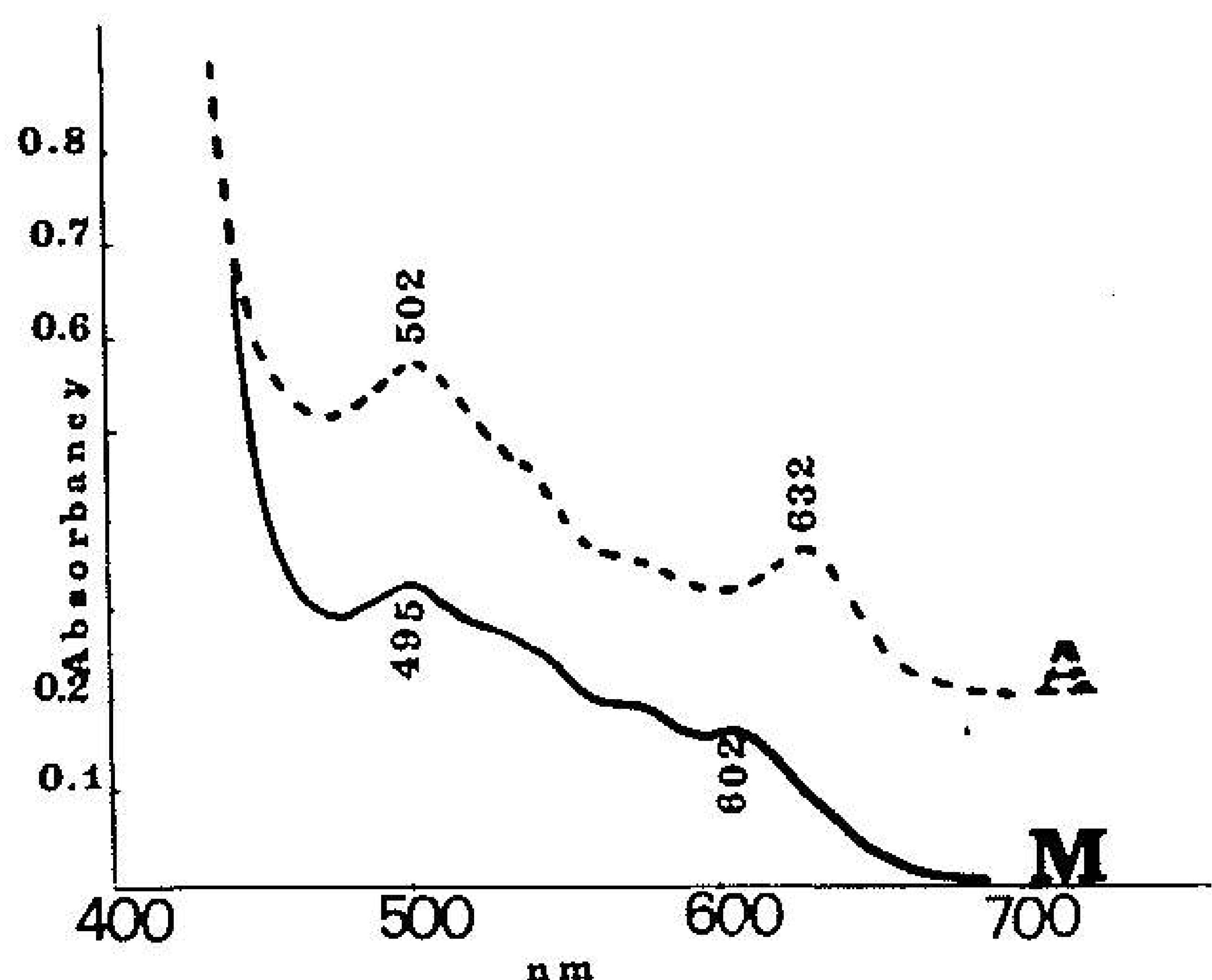

Fig.2 .Absorbtion curves of the ferricyanide treated haemoglobin A and purified haemoglobin M .

tates was washed with cold acetone and freeze dried. Peptide chains of the globin were prepared by an standard technique (2), except that the aminoethylation of the separated chains was omitted. Tryptic digestion and fingerprinting of the α and β -chains was carried out as previously described (2).

Figure 3 shows the fingerprints of the α -chains of haemoglobin M as well as haemoglobin A. It can be seen that the peptide α Tp 7 and α Tp 7-8 are missing from their usual positions 1, 2 and shifted upward and towards the anode 3,4. Both new peptides were negative for histidine staining and positive for tyrosine.

The new peptide α Tp 7 of haemoglobin M was eluted from 4 preparative fingerprints with 6N HC1 and hydrolysed for 20 hours at 110°C.

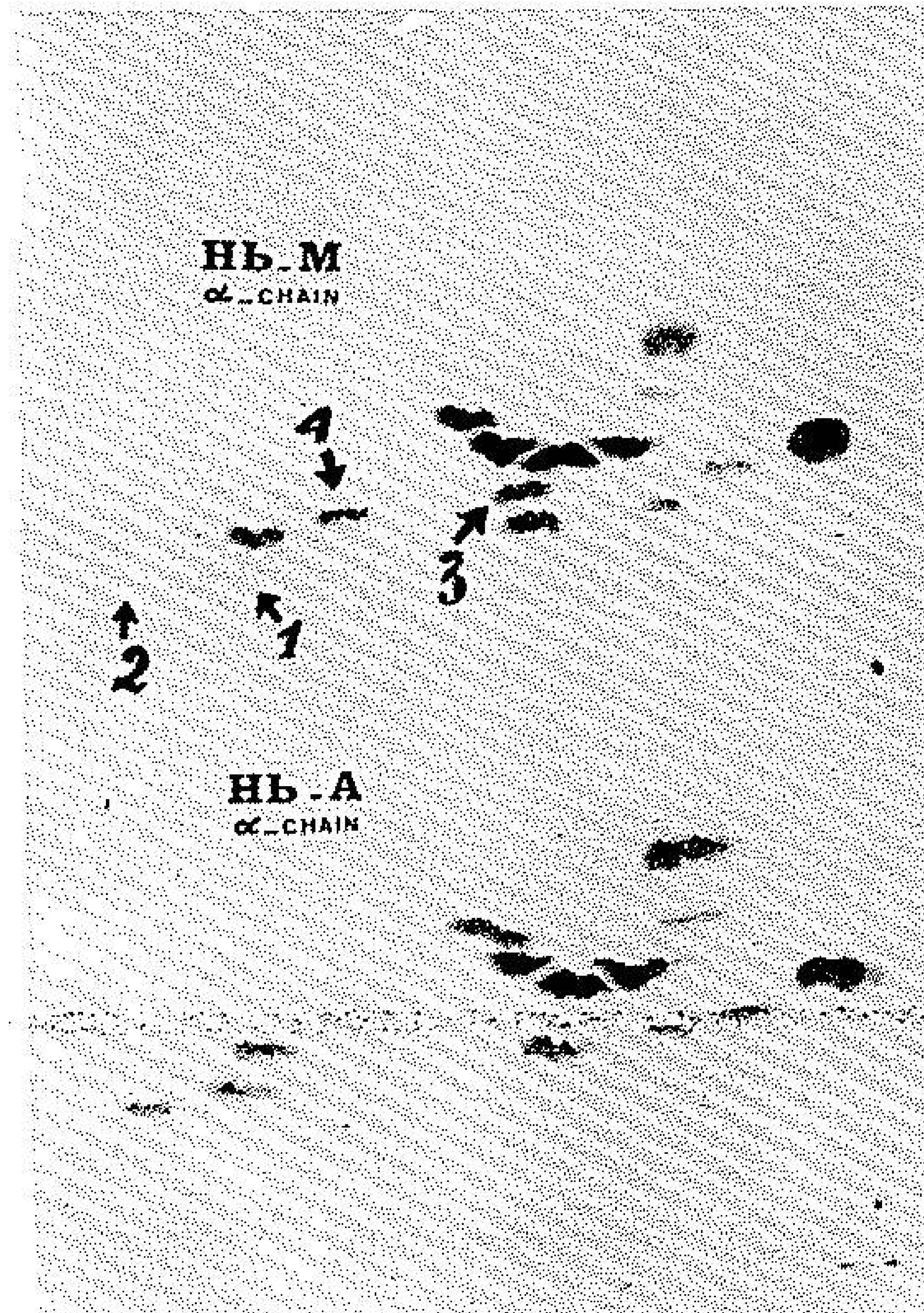

Fig 3. Fingerprints Of TheSoluble Tryptic Peptides Ofthe -ChainOf HbM (above),and of normal -chains(below)
172,are the normal postions of pepties TP and 7-8 374,are new peptides TP 7and TP 7-8, both staining for tyrosine but not for not histidine.

After removal of HC1 in a vaccuum desicator, the amino acid composition of this peptide was determined on an automatic amino acid analyser.

The amino acid analysis of the peptide α Tp 7 of haemoglobin M revealed that it consists of 1 Gly, 1 Ala, 1 Tyr, and 1 Lys, suggesting of the substitution of distal histidine by tyrosine.

Acknowledgement

This work was supported by research grants from the University of Tehran and the Ministry of Science and Higher Education of Iran.

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