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HEMOGLOBIN J BANGKOK : STRUCTURAL IDENTIFICATION AND IN COMBINATION WITH HEMOGLOBIN E

ฮีโมโกลบิน เจ บางกอก : การวิเคราะห์โครงสร้าง และการรวมกับฮีโมโกลบิน อี

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ABSTRACT

A fast hemoglobin was found on a routine study of hemoglobin starch gel electrophoresis in two unrelated families of Thai ancestry. Three heterozygotes for the fast variant including one who was detected in cord blood study, and one individual with a double heterozygosity for the fast variant and hemoglobin E were examined. With the exception of a subject with heterozygous state for the fast variant being anemic due to advanced state of Hodgkin's disease, the others including the double heterozygote appeared

to be a symptomatic and had normal hematologic data. The quantitation of the fast variant in the newborn and adult heterozygotes and in the double heterozygote were 5.26, 51-65, and 69.5% respectively. The fast variant from two families was structurally characterized. An abnormal globin chain was isolated on CM cellulose chromatography in 8 M urea. Tryptic peptide mapping of the abnormal chain revealed that the altered peptide was the peptide corresponding to residues 41-59 of β chain. Peptide map of trypsin and cyanogen bromide cleavage of the abnormal β chain suggested that the amino acid substitution was in the peptide containing residues 56-59. The amino acid analysis of the abnormal peptide indicated that glycine residue 56 was replaced by aspartic acid, which is identical to the previous report as Hb J Bangkok.

บทคัดย่อ

ได้ตรวจพบฮีโมโกลบินชนิดวิ่งเร็วในการศึกษา **Starch gel electrophoresis** ในคนไทยสองครอบครัว สามรายพบว่าเป็น **Heterozygote** ของฮีโมโกลบินชนิดวิ่งเร็ว ซึ่งหนึ่งในสามรายได้ตรวจพบจากเลือดสายสะดือ อีกหนึ่งรายพบว่าเป็น **Double heterozygote** ของฮีโมโกลบินชนิดวิ่งเร็วและฮีโมโกลบิน อี ในหนึ่งรายของ **Heterozygote** ของฮีโมโกลบินชนิดวิ่งเร็ว ตรวจพบว่าซีดเนื่องจากระยะสุดท้ายของโรค **Hodgkin** ส่วนรายอื่น ๆ รวมทั้ง **Double heterozygote** ไม่พบอาการผิดปกติ และผลการตรวจทางโลหิตวิทยาปรากฏว่าเป็นปรกติ การตรวจหาปริมาณของฮีโมโกลบินชนิดวิ่งเร็วในทารกแรกเกิด และผู้ใหญ่ที่เป็น **Heterozygote** และ **Double heterozygote** ได้ ๕.๒๖, ๕๑–๖๕ และ ๖๙.๕% ตามลำดับ ฮีโมโกลบินชนิดวิ่งเร็วที่ตรวจพบในสองครอบครัวได้ถูกนำมาวิเคราะห์โครงสร้าง ได้แยกสายโกลบินที่ผิดปรกติโดย **CM cellulose chromatography** ผลของ **Tryptic peptide mapping** ของสายโกลบินที่ผิดปรกติ แสดงว่า **Peptide** ที่ผิดปรกติคือ **Peptide** ที่มีกรดอะมิโนตำแหน่งที่ ๔๑–๕๙ ของสายโกลบิน เบต้า **Peptide map** ที่ย่อยด้วย **Trypsin** และ **Cyanogen bromide** ของสายโกลบินที่ผิดปรกติ แสดงว่า ความผิดปรกติของการเปลี่ยนแปลงกรดอะมิโนเกิดใน **Peptide** ที่มีกรดอะมิโนตำแหน่งที่ ๕๖–๕๙ การศึกษาวิเคราะห์กรดอะมิโนของ **Peptide** ที่ผิดปรกติบ่งว่า **Glycine** ที่ตำแหน่ง ๕๖ ถูกแทนที่โดย **Aspartic acid** ซึ่งจะเหมือนกับฮีโมโกลบิน เจ บางกอก ที่เคยรายงานไว้ก่อนแล้ว

INTRODUCTION

Abnormal hemoglobins are prevalent in Thailand.[21] Hemoglobin (Hb) E, a variant with glutamic acid residue 26 of β chain being replaced by lyscine,[10] and Hb Thai (Constant Spring), a peculiar variant with 31 amino acid residues elongated from the C-terminal of normal α -chain, [5,20] are frequent with an insidence of 13 and 4% respectively.[20,21] In addition to the two common variants, a number of rare abnormal hemoglobins is also documented.[14,15] This communication presents a structural identification of a rare, fast hemoglobin in two unrelated families of Thai ancestry. The hemoglobin variant from both families was found to have the amino acid substitution at glycine residue 56 of β chain being replaced by aspartic acid, which is identical to Hb J Bangkok.[4,16] One subject with a double heterozygosity for Hb J Bangkok and Hb E is also described.

MATERIALS AND METHODS

Subjects

Family T.K., the propositus, a Thai boy from the North-East of Thailand suffering from advanced state of Hodgkin's disease, was encountered to have a fast hemoglobin on a routine screening of starch gel electrophoresis. His father was also examined.

Family F.K., the propositus, a neonate of Thai ancestry born at Siriraj Hospital, was found to have a fast hemoglobin variant which migrated slightly behind Hb Bart's in cord blood study. Parents were also examined.

Hematologic studies

A venous blood was collected in EDTA. Hemoglobin concentration, red cell count, packed red cell volume and mean corpuscular volume, were determined by an electronic Coulter hemoglobinometer and Coulter counter, Model ZF with Coulter MCV/Hct accessory. Red cell osmotic fragility was recorded by Danon's fragiligraph.[6] Hemoglobin starch gel electrophoresis in Tris-EDTA-borate buffer at pH 8.6[7] was performed. Alkali resistant hemoglobin was measured according to Singer et al.[17] The quantitation of Hb A_2 and abnormal hemoglobin components were determined by the elution technic of cellulose acetate electrophoresis.[19]

Structural characterization

Globin, prepared from the whole hemolysate was separated by CM cellulose chromatography in 8 M urea.[4] The abnormal globin chain was digested with trypsin. The tryptic peptide was then treated with cyanogen bromide as described by Gross and Witkop.[8] Peptide mapping was performed by high voltage electrophoresis at pH 6.4 and descending chromatography according to Pootrakul and Dixon.[12] The abnormal peptide was isolated and purified by preparative paper electrophoresis and chromatography. The unstained peptide was eluted with 5.7 NHCl and hydrolyzed under vacuum at 105°C for 18 h. The hydrolysate was analyzed on the Hitachi, KLA-3B, amino acid analyzer.

RESULTS

Hematologic data of the subjects in two families are summarised in Table 1. The propositus of Family T.K. was anemic which was believed as a result of advanced state of Hodgkin's disease. He had 51.8% the fast hemoglobin component in addition to Hb A and A_2 (Fig. 1). His father was healthy and had normal hematologic data, but hemoglobin types comprised of two major components, 30.43% Hb E and 69.57% the fast variant (Fig. 1). Therefore, the subject represents a double heterozygosity for Hb E and the fast variant. In family F.K., the propositus, a newborn and parents had normal hematologic data except the presence of 5.26% and 65.8% of a fast variant, which electrophoreticly migrated just behind Hb Bart's in the cord blood of the newborn and in the hemolysate of the father respectively.

Globin prepared from the whole hemolysate of the double heterozygote for Hb E and the fast variant was fractionated by CM cellulose chromatography in 8 M urea with sodium salt gradient and three major globin chains were observed. The first globin chain was eluted at lower ionic strengths than the normal β chain, this was believed to correspond to the fast β chain (β^J). Based on the ionic strength elution pattern of chromatography, the second and third globin chains represented the abnormal β chain of Hb E and the normal α chain respectively. No normal β globin chain was observed. The tryptic peptide map of the fast β chain as compared with the normal β chain revealed that the peptide corresponding to β^A TpV (tryptic peptide No. 5 containing amino acid residues 41-59 of normal β chain) was missing but a new peptide β^J TpV having more negative charge was illustrated (Fig. 2). Since the β TpV contains methionine, the tryptic peptide from a fresh preparation was then treated with cyanogen bromide in order to cleave the β TpV into two peptides namely an acidic peptide CNBrI corresponding to residues 41-55 and a basic peptide CNBrII corresponding to residues 56-59 (Fig. 3). The peptide map of trypsin and cyanogen bromide cleavage of normal β chain (Fig. 4) revealed that the peptide β^A TpV disappeared and a basic peptide CNBrII-A was observed. The acidic peptide CNBrI-A is not illustrated in the map, this is believed to run off the paper in high voltage electrophoresis since it contains three acidic amino acids (Fig. 3). The peptide CNBrII-A was isolated and studied the amino acid analysis. The amino acid composition of the peptide CNBrII-A as shown in Table 2 was compatible with the peptide residue 56-59 of β chain (Fig. 3). The peptide map of trypsin and cyanogen bromide cleavage of the fast β chain revealed that the peptide CNBrII-J contained more negative charge than the corresponding peptide CNBrII-A (Fig. 4). The amino acid composition of the peptide CNBrII-J (Table 2) indicated that glycine residue was missing but an additional aspartic acid or asparagine residue was observed. It is most likely that glycine is replaced by aspartic acid rather than asparagine since the latter substitution would not be expected to change the charge of the altered peptide in the peptide map at pH 6.4. In conclusion, the structural identification indicated that glycine residue 56 of β chain is replaced by aspartic acid (Fig. 3) which is identical to the previous report as Hb J Bangkok.

Globin from the whole hemolysate of the father in Family F.K. was also carried out for structural identification. The result was also identical to the Hb J Bangkok.

DISCUSSION

Thorup and co-working[18] first described Hb J in an American family. Upon electrophoresis at alkali pH, the variant migrates faster than Hb A but slower than Hb Bart's. Based on the identical properties of electrophoresis and chromatography to the original one, 25 Hb J variants with different amino acid substitutions, 16 in α chain and 9 in β chain, have now been documented in various ethnics.[11] In Thailand, Pootrakul and colleagues[16] described Hb J Bangkok in a Thai family from the North-East of the country. Blackwell and co-workers[1] also reported Hb J Korat in a Thai family found during a survey study in the North-East provinces. Subsequently, the structural identification of the Hb J Bangkok and Hb J Korat was found to be identical and that glycine residue 56 of β chain is replaced by aspartic acid.[2]

In this communication, two unrelated families of Thai ancestry were encountered to have a fast hemoglobin variant which had electrophoretic mobility like Hb J. Since the Hb J is rare and a number of Hb J variants have now been described, the structural characterization of the fast variant in two families was carried out. From tryptic peptide mapping, the mutation occurred at β^J TpV, a methionyl peptide containing 19 amino acid residues. Cyanogen bromide cleaved at the methionine residue of the β^J TpV into two peptides; CNBrI-J and CNBrII-J (Fig. 3). This led to easily isolate the short peptide CNBrII-J. The result of amino acid analysis of CNBrII-J in the variant from two families indicated that the amino acid substitution was identical to Hb J Bangkok.

In addition to the designation as Hb J Bangkok, the variant with amino, acid substitution as $\alpha_2\beta_2$ $^{56Gly\rightarrow Asp}$ has also been described as Hb J Korat[1], J Meinung[2], and J Manado.[3] This variant so far is observed in Thai,[1,16] Chinese[2, 13] Indonesian[3], and American Negro[9]. The available hematologic data of a heterozygous state including in this communication appear to be normal except the presence of around 50-60% of the fast pigment. In most heterozygotes for β chain variants, the abnormal pigments generally constitute around 30-40% of the total hemoglobin. Since the Hb J is a fast hemoglobin, Hb A_3 is certainly included in the abnormal component. However the amount of Hb J variant is significantly greater than that of other β chain variants. The explanation of the increased amount of Hb J is not known.

During the survey study in the North-East of Thailand Blackwell and co-workers[1] reported a family of Hb J in combination with Hb E but the hematologic data of the double heterozygotes were not described. In this paper, a subject with double heterozygosity for Hb J Bangkok and Hb E was healthy and had normal hematologic findings with hemoglobin constitution of 69.57% Hb J and 30.43% Hb E. Recently two American Negroes with double heterozygosity for Hb J Bangkok and Hb S also appeared to be normal and had around 59% Hb J.

Since the glycine residue 56 of β chain corresponding to D7 (amino acid residue 7 of helical segment D) of β chain, which locates at the surface of the hemoglobin molecule, the replacement by aspartic acid would not be expected to disturb the stability of the hemoglobin molecule. This is consistent with no clinical and hematological abnormalities in a heterozygous state for Hb J Bangkok.

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Table 1. A summary of hematologic data of Hb J Bangkok in two Thai families.

	Age	Hb (g%)	RBC (mil/mm³)	PCV (%)	MCV (μ³)	MCH (γγ)	MCHC (%)	RBC Morphology	Osm. frag.	Hb type	Alk. denat (%)	% hemoglobin A_2 E	% hemoglobin J	Designation
Family T.K.														
Propositus*	13	8.7	3.16	29	93	27	30	N	N	A_2+A+J	0.44	2.88	51.80	Hb J trait
Father	40	15.5	5.38	45	84	29	34	N	N	E+J	0.76	30.43	69.57	Double heterozygote for Hb E and Hb J
Family F.K.														
Propositus	Cord blood	15.8	5.72	51	89	27	31	N	N	FA+J	77.96	–	5.26	Hb J trait
Father	36	15.4	5.90	48	81	26	32	N	N	A_2+A+J	0.22	3.20	65.80	Hb J triat
Mother	32	11.5	4.37	36	82	26	32	N	N	A_2+A	0.20	3.50	–	Normal

Hb = hemoglobin concentration, RBC = red blood cells, PCV = packed cell volume,
MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin,
MCHC = mean corpuscular hemoglobin concentration, Osm. frag. = osmotic fragility.
Alk. denat = alkali denaturation hemoglobin, N = Normal

*With advanced state of Hodgkin's disease.

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Table 2. Amino acid composition of peptides CNBrII-A and CNBrII-J.

Amino acids	CNBrII-A		CNBrII-J	
	μ mole	residue	μ mole	residue
Lys	0.430	0.97 (1)	0.412	0.88 (1)
Asp	0.480	**1.08 (1)**	1.048	**2.23 (2)**
Pro	0.424	0.95 (1)	0.417	0.99 (1)
Gly	0.447	**1.00 (1)**	0.00	**0.00 (0)**

Numbers in parentheses represent amino acid residues, deduced from the molar ratio, in the analyzed peptides.

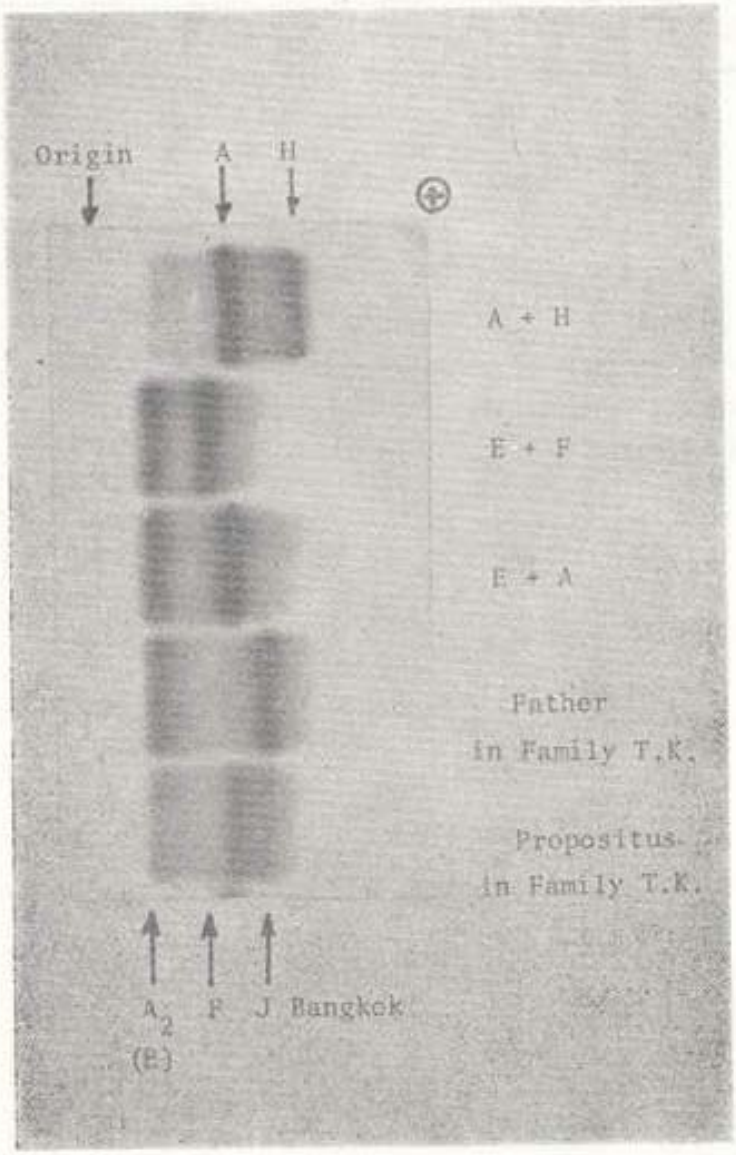

Fig. 1 **Hemoglobin starch gel electrophoresis in Tris-EDTA-borate buffer, pH 8.6 stained with orthodianisidine.**

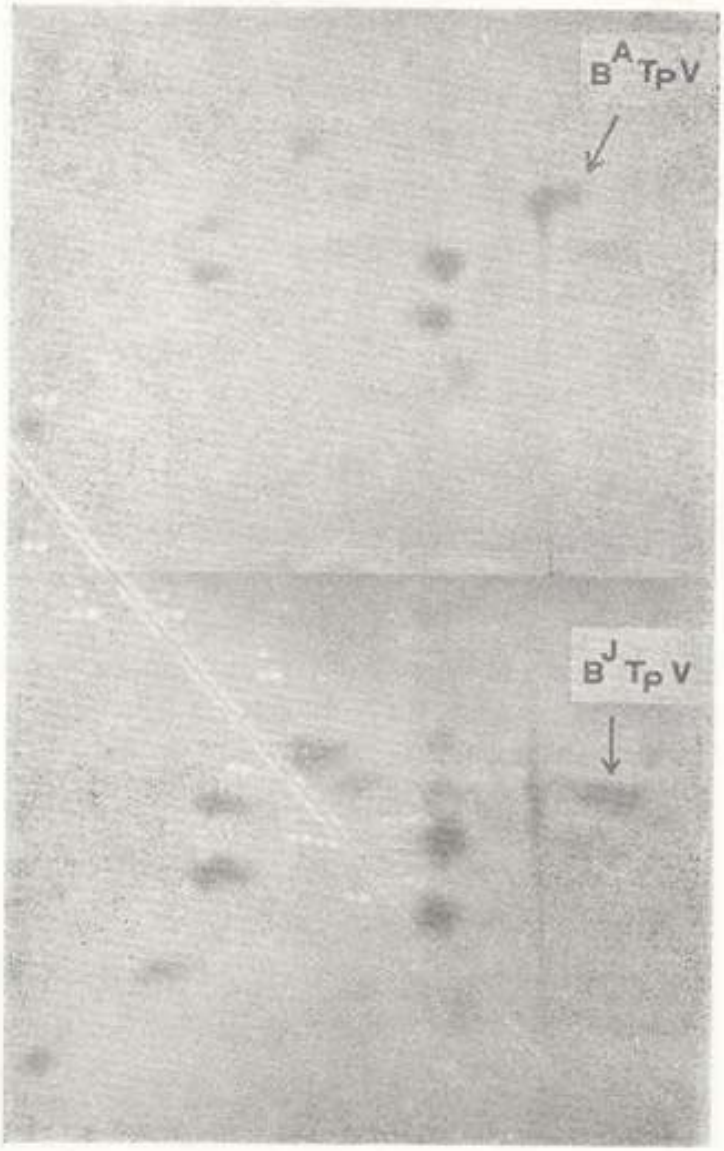

Fig. 2 Tryptic peptide maps of normal β chain (β^A) and the fast β chain (β^J), stained with ninhydrin. β^A TpV and β^J TpV represent the tryptic peptide No. 5 of the normal and abnormal β chain respectively.

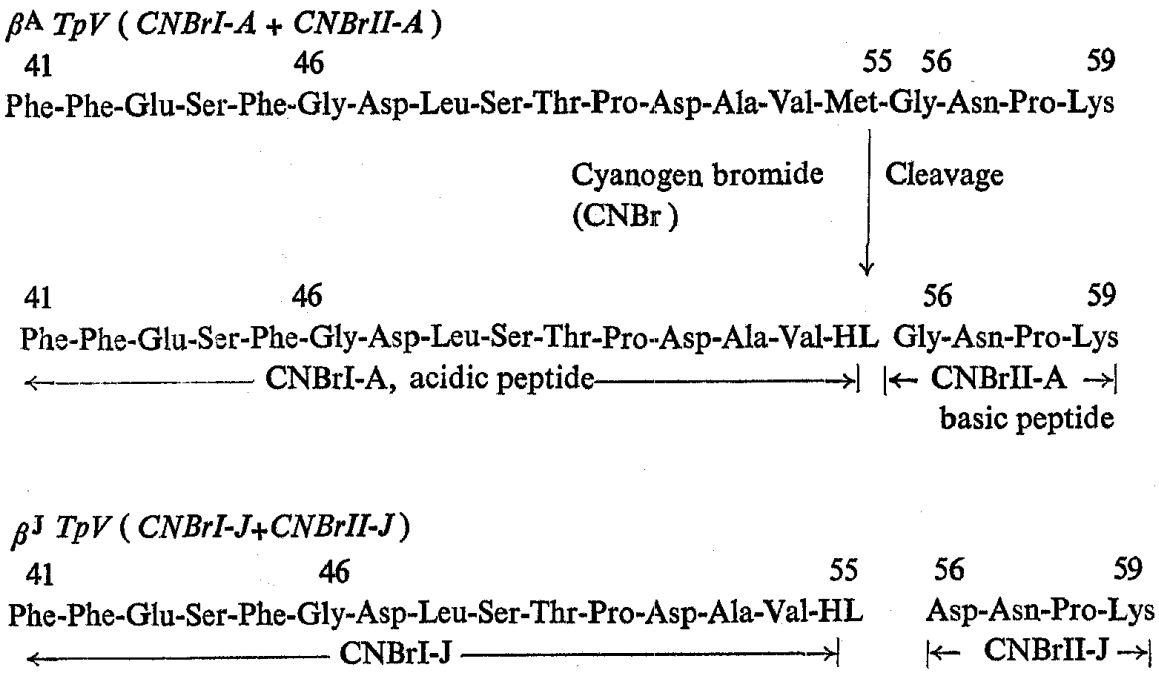

Fig. 3 A diagram of amino acid sequence of β^A TpV with cyanogen bromide cleavage and the amino acid sequence of CNBrll-J of Hb J Bangkok.
HL = homoserine Lactone.

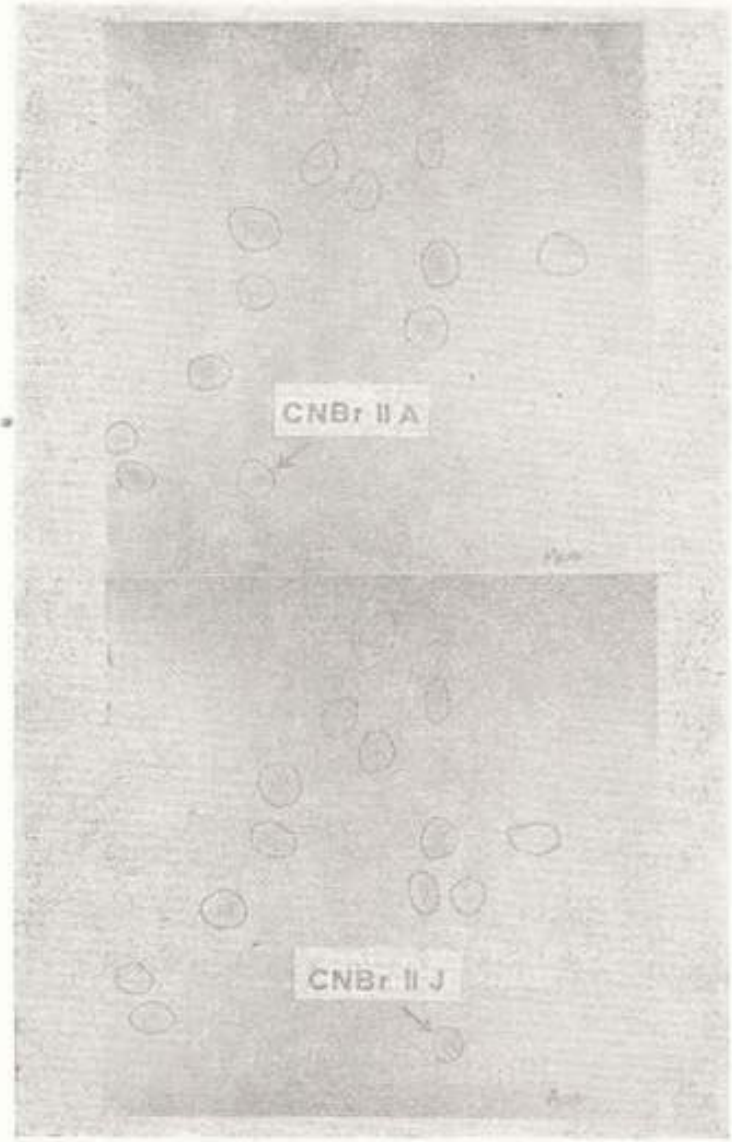

Fig. 4 Peptide maps of trypsin and cyanogen bromide cleavage of normal β chain and the fast β chain. CNBrII-A and CNBrII-J represent peptide residues 56-59 of the normal and abnormal β chain respectively.