UDC 616 (082) YU ISSN 0350-0071

AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE

RADOVI

KNJIGA LXXXVI

ODJELJENJE MEDICINSKIH NAUKA

Knjiga 24

SARAJEVO 1990.

FAMILY THROMBOCYTOPENIA ASSOCIATED WITH ABNORMAL HEMOGLOBIN — VARIANT STRUMICA (α_2^{112} His ⟶ Arg β_2)

DŽ. REZAKOVIĆ, S. BEKIĆ-KALUŽA, N. RADIČIĆ, S. DOBRAČA AND I. REZAKOVIĆ

Clinic for Hematology, Sarajevo
Institute for Clinical Biochemistry, Sarajevo
Internal Clinic for Gastroenterology, Sarajevo

UDC 616-056.7:616.15

Abstract. In the second generatino of a family from Sarajevo (SR of Bosnia and Herzegovina — Yugoslavia) thrombocytopenia was found in three members (a sister and two brothers), with prolonged time of hemorrhage, prolonged generation of thromboplastin with thrombocytes of the diseased, weak retraction of coagulum and shortened life of thrombocytes. There was no splenomegalia. In the same members of this family abnormal hemoglobin (Hb X) or variant STRUMICA was found, in addition to hemolysis, reticulocytosis, anem a of different degrees and elevated values of free bilirubin.

No chromosome alterations in the cariotype were found and the investigation of the HLA system showed no congruity amongst the HLA systems of the diseased.

Key words: Thrombocytopenia; Hemolysis; Reticulocytosis, Abn Hb STRUMICA.

INTRODUCTION

Different family diseases of thrombocytes are characteristic as to their number, production and biological capabilities, classified on the basis of clinical and laboratory findings. In the scope of these diseases, thrombocytopenia usually results either from reduced thrombocyte production or from shortened life of thrombocytes[3] (D o w t o n et al., 1985). As to their quantitative and qualitative alterations, familiy thrombocytopenia is a rather heterogenous disease and may be associated with a number of other diseases: leukemia when thrombocytopenia occurs as a pre-leukemic condition; carcinoma with different loccalities: mouth ca-

vity, larinx, stomach, lungs, prostate, neuroblastom; hemochromatosis, hydronephrosis, hemolitic anemia (Dawton et al., 1985; McKusick, 1966; Milnes et al., 1986), the immediate group of family thrombocytopenia includes: May-Hegglin anomaly, Wiskott-Aldrich syndrom, thrombocytopenia with megacaryocytopenia, Gray Thrombocyte Syndrom[1] (Bellucci et al., 1983). Family thrombocytopenias with or without antithrombocytic antibodies may occur in different ages, from childhood up to the third age (Bogart et al.). Alterations in chromosomes similar to those in Fanconi anemia are described in patients with thrombocytopenia from one family (Helmhorst et al., 1984)". while the results of the HLA system analyses in membres of a family with thrombocytopenia largely differ. Some investigators have found a certain congruity of HLA in the diseased family members, while other investigators found nothing of the sort in a family with thrombocytopenia. (Karpatkin et al., 1981; Muller et al., 1987; Milnes, et al., 1986; Helmhorst et al., 1984). Thrombocytopenias associated with structural or biochemical alterations of hemoglobin are very rare. Hutchison et al. (1984) found, in the members of a Glasgow family suffering from thrombocytopenia, abnormal hemoglobin variant Koln[12], while Thompson et al. (1977) in a family suffering from thorombocytopenia found elevated Hb A values and reduced activity of VIIIc factor.

THE STUDY OF THE FAMILY

Two generations of the same family were investigated: the first (I) and the second (II) one. The third generation (III), that is the children of the second (II) generation diseased parents were not investigated as the parents did not wish to cooperate.

The father of family (I-1, Fig. 1) died (the cause of death unknown to the family); the mother (I-2) has been suffering from a heart disease.

The propositus (II-1) states that she noticed as a child that she bruised easily, that »red spots« appered on her skin, bleeding after tooth-brushing, but real troubles started with first menstruations. Menstruations were excessive and prolonged. Spontaneous nose hemorrhages started to occur at that time. Her sclerae sometimes got yellowish but soon returned back to normal. At the age of 16 she underwent a detailed medical examination, when it was found that there was a shortage of blood platelets, which was probably the cause of hemorrhages. Since then she has been hospitalized in several clinics where the diagnosis was always: thrombocytopenia.

Physical examination shows no alterations, apart from a mild yellowish colour of the sclerae. The liver and the spleen are not enlarged, either percutorally or palpatorally, and do not pain.

The younger brother (II-2) had no troubles, either in his childhood or later. He only bruised rather easily and had prolonged hemorrhages after tooth-pulling. It was noticed that his sclerae were yellowish, but went normal from time to time. He was born with a shorter right leg.

He consumed large amounts of alcoholic drinks, though not regularly. On one occasion, when examined by a doctor, he was told that his blood »grease« content was heightened.

Physical examination: Sclerae mildly subicterical, liver is not enlarged but palpable, spleen is not palpable. Right leg shorter by about 2 cm. Other findings regular.

The elder brother (II-4), except for infantile diseases, does not recall having any serious health problems. It was noticed that his sclerae sometimes went »yellow« but soon turned back to normal. His gums often bled after teethbrushing. Lately he has been feeling a blunt pressure under his right ribs. He has never noticed any rash on his skin. He regularly consumes small amounts of strong alcoholic drinks. He easily gets tired with hard work.

Physical examination: Slight protrusion of both eye-balls. Sclerae slightly subicterical. Liver palpable, slightly enlarged. Spleen does not show enlargement either percutorally or palpationally.

The elder sister (II-6) has mostly had gall troubles. She had frequent fits of pain under right ribs. It was found that she had a gall-stone which was surgically removed. After several years the same problems reappeared and it has been found that she now has a gall-stone in her gall-canal. She has had no other problems. Menstruations regular and never excessive. She has had no nose hemorrhages or skin rash.

Physical examination: Apart for the scar in the area of the gall--bladder projection and slight sensitivity to palpation of the right hypohondrium, other physical findings are entirely within the normal range.

The investigated members of the family do not recall whether there was ony consanguinity in the previous generations.

INVESTIGATION METHODS

The number of erythrocytes and thrombocytes was made out by the calculator TECHNICON-766, the time of hemorrhage after the IVY method[22, 23], the coagulum retraction after the BIGS method, the thromboplastin generation test with thrombocytes of the diseased after the Bigs-Douglas metod, the thrombocytes adhesion after the BORCHREVINK method, capilary resistence after the Bumpel-Leed method, thrombocyte aggregation to colagen after the BORN method[43], antithrombocytic anty bodies after COOMBS direct-test method, life of thrombocytes Baldini method with Cr^{51}, thrombo-dynamo-elastography after the HARTERT method.[19] The promil of reticulocytes stained by brilliant-cresyl blue in 0.5% solution with absolute alcohol after the WOLFER method[22]. The test of erytrocytes resistence to hypotonic solutions of NaCl after the WINTROB method[22]. serum Fe after Stokey and Eisenwinter on the analyser RA—1000 TECHNICON; the VIIIc factor after the HARDISTI method[23], fibrinogen after the Shultz method in Nissel test-tubes[22].

The quantity of hemoglobin after the BURGER method calculated as mmol/1 per SI units[21], electrophoresis on colagen straps and on mul-

tipolar densimeter reader (chemogram-Milano); peptide division by the method of column chromatography on DOWEX 50—X4 after EFREMOV[5, 7, 8, 18].

RESULTS

Members of the J.. family were investigated in the first (I) and second (II) generation: the mother, two sisters, two brothers and their wifes (Fig. 1). The investigation of the family and check-up of the diseased have been carried out regularly over the last three years (since the end of 1984).

The study of thrombocytes

The propositus (II-1), the younger sister, constantly had reduced number of thrombocytes, exept when treated with corticosteroides, contraceptives and Fe medicaments when the thrombocytes grew in number and menstruations were rendered less abundant and regular. After the treatment the thrombocytes soon dropped below the value of 30 x 10'/1, the time of hemorrhage was very prolonged, coagulum retraction weak to very weak, the test of the generation of thromboplastin with thrombocytes very prolonged, maximum amplitude (a-mx) reduced, which pointed to a markedly pathological thermo-dynamo-elastographic curve, while the thrombocytes' life was as a rule shortened, both during the treatment and between the therapeutic sessions (Table 1, 2, 3, Fg. 3); thrombocyte adhesion poor, thrombocyte aggregation to colagen could not be acheived, probably due to a constantly low number of thrombocytes; direct COOMBS test positive, fibrinogen normal, VIIIc factor activity normal.

In the bone marrow smear obtained by megacaryocytosis puncture marked hypersegmentation of megacaryocyte nuclei with vacuolae in the megacaryocyte cytoplasma. In the peripheral blood smear no thrombocyte clusters were found, while frequent large thrombocytes were noticed. RUMPEL-LEED positive.

In younger brother (II-2) alterations in the thrombucyte number were moderate; moderate thrombocytopenia without oscillations; duration of hemorrhage slightly prolonged, coagulation retraction reduced, thromboplastin generation test with thrombocytes of the diseased moderately prolonged, life of thrombocytes shortened, adhesion weaker, aggregation of thrombocytes to colagen satisfactory, RUMPEL-LEED negative ,maximal amplitude (a-max) and thrombocyte potential index slightly dropped on the thrombo-dynamographic curve (Table 1, 2, and 3; Fig. 4); direct COOMBS test slightly positive; fibrinogen normal; VIIIc factor activity normal. In the bone marrow smear, obtained by puncture, no alterations are found, except for hypersegmentation of the megacaryocyte nuclei and presence of vacuolae in the cyoplasma. In the peripheral blood smear there are no thrombocyte clusters, though large thrombocytes are to be seen occasionally.

In the older brother (II-4) the aletrations in the number of thrombocytes and their function were also moderate but somewhat more pro-

nounced than in the younger brother (II-2). Moderate thrombocytopenia, duration of hemorrhage prolonged, coagulation retraction diminished, life of thrombocytes shortened, thromboplastin generation test with thrombocytes of the diseased prolonged, maximal amplitude (a-max) and thrombocytes potential index reduced (Table 1, 2, and 3; Fig. 5); thrombocyte adhesion poor; RUMPEL-LEED slightly positive, direct COOMBS test slightly positive; fibrinogen normal; factor VIIIc activity normal.

In the marrow smear, obtained by puncture, except hypersegmentation of nuclei in megacaryocytes, and the present vacuolae in the megacaryocyte sytoplasma, there are no other changes. In the peripheral blood smear there are no thrombocyte clusters but individual large thrombocytes are to be seen.

The Study of Erythrocytes

The propositus (II-1) had permanently reduced to very reduced number of erythrocytes. Microcytes prevailed in the peripheral blood smear, but anisocytosis and anisochromy were also pronounced. There were no spherocytes. In the periods of aggravation the serum Fe value dropped below 5.5 mol/L and medium erythrocyte volume below 73.4. Erythrocyte resistence in hypotonic NaCl solutions was reduced and the number of reticulocytes in promils markedly elevated. In the bone marrow smear obtained by puncture the percentage of acidophil erythroblasts was elevated (Table 4). HEINTZ bodies were not found. The patient was periodically treated by corticosteroids, contraceptives and Fe medicaments. Under such therapy the number of erythrocytes was rapidly normalized, and anisochromy and anisocytosis disappeared from the periphery. Microcytes were still present but in a lower percentage.

In the patient II-2 there was permanent moderate anemia, no spherocytes, moderate hywochromy, serum Fe was below 14 m/L. Erythrocyte resistence in hypotonic NaCl solutions was reduced and the reticulocytes promil elevated. (Table 4).

In the bone marrow smear obtained by puncture no particular alterations in the erythrocyte lineage are detected.

In the patient II-4 anemia was somewhat more pronounced than in II-2, with hypochromia but without microcytes and spherocytes. Individual macrocytes, were found. Reticulocyte promil markedly elevated, erythrocyte resistence in hyhotonic NaCl solution reduced (Table 4). Serum Fe was below 12 μmol/L, and in the bone marrow smear somewhat more pronounced acidophil erythroblastosis.

The Study of Hemoglobin

In all the three diseased members of the family (II-1, II-2 and II-4) the values of hemoglobin were reduced and, expressed in mol/L (SI units), parallel to the number of erythrocytes. Electrophoresis of hemoglobin showed a high percentage of Hb X in all the three diseased (Table 4). This unknown hemoglobin (Hb X), discovered by the method of electrophoresis, was identified by the method of column chromatography

Dowex 50-X4 as an abnormal hemoglobin — Strumica, where aminoacids in the alphaglobin chain had changed positions: α_2^{112} His ⟶ Arg β_2. This abnormal hemoglobin was identified by Prof. G. D. EFREMOV in the Reference Centtre for Hemoglobinotherapy, Skopje. (Fig. 6, 8, 9, 10, 11, 12).

In II-2 and II-4 free bilirubin was occasionally elevated, which coincided with hypercholic stools.

Both brothers (II-2 ind II-4) had permanent hyperlipidemia, which could be classified as Type IV after FREDERICKSON.

DISCUSSION

Data of findings given in the enclosed tables are the highest values recorded at times of compulsory check-ups. The other repeated findings, which refer to the investigated parameters, were as a rule lower.

Only the propositus (II-1) had a complete clinical picture of thrombocytopenia, with charcteristic clinical findings, confirmed by appropriate laboratory tests, while the other two patients (the brothers II-2 and II-4) did not have a pronounced clinical picture of thrombocytopenia. It was established only after certain laboratory tests that it was a case of a very mild thrombocytopenia: moderate drop of thrombocytes, poor coagulum retraction, thromboplastin generation with thrombocytes of the diseased prolonged, prolonged hemorrhage, corresponding to the number of thrombocytes in all the investigated patients. There is an opinion (THOMPSON et al., 1977)[25] that the duration of hemorrhage presents a direct biogical connection with the thrombocyte number andd functions.

The presence of the abnormal hemoglobin — variants STRUMICA did not result in any serious clinical alterations, though its percentage was rather elevated in all the patients investigated. There was an oscillating subicterus, a mild anemia (except in the case of the propositus) conditioned by the hemolitic component, which was confirmed by reduced resistence of erythrocytes and incidence of reticulocytosis, which was permanent.

The diseased sister (propositus II-1) was treated by the following therapy (due to hematologic crises resulting from long-lasting and excessive menstruations): Cortancyl 55 mg/die with gradual reduction down to 10 mg/die in the course of two months, additional therapy with contraceptives type »DIANA« between menstruations, and Fe drugs »TAREYFERON« perorally. During this first therapeutic course the patient had a very good response: there occured a complete clinical remission, menstruations were less excessive and lasted for about 6 days, the number of erythrocytes was normalized, though maximum thrombocyte number did not excede 50.000 mm'. Anemia was more of a hemorrhagic—ferroptive character and was probaby less affected by the hemolitic component.

Such a therapy was repeated depending on the clinical and laboratory findings of the patient. However, in the subsequent therapeutic courses the patient had increasing difficulties in using Cortancyl and Fe drugs. Comlications and troubles appeared: heightened blood pressure, hirsutism, gluteal streaks, heightened blood glucosis, nausea and stomach pains. Therapy with imunoglobins was not used since there was fear of infection: B virus or AIDS?

In connection with the afore stated, there are discussions about the justifiability of splenectomy as the only possible therapy to date.

In the diseased brothers (II-2 and II-4) no therapy was applied since there was no need to do so. However, they were warned that before any eventual surgical treatment they should first consult a hemostazeologist.

Another pathologic component in the investigated memebers of the family (II-1, II-2 and II-4) — the presence of the abnormal hemoglobin — variant STRUMICA — is like hemoglobinopathy a rare phenomenon and it seems that so far it has been described only in Yugoslavia (around Strumica, SR Macedonia) (NIAZ et al. 1975, EFREMOV et al. 1982, 1083, 1984[4, 5, 6]). It is a heterozygote phenomenon, where aminoacids are substituted in 112 positions in the alpha-globin chain:

$$(\alpha_2^{112}\ \text{His} \longrightarrow \text{Arg}\ \beta_2)$$

The only hematologic alterations detected earilier in the carriers of the abnormal hemoglobin — variant STRUMICA are moderate fragility of erythrocytes in the hypotonic NaCl solution and slightly elevated free bilirubin in the serum. No HEINTZ inclusions in erythrocytes were found.

In our patients (II-1, II-2 and II-4) we also found diminished erythrocyte resistence in hypotonic NaCl solutions, elevated but oscillating values of free bilirubin and pronounced reticulocytosis, which are all indicators of constant possibility of erythrocyte disintegration. To our knowledge, thrombocytopenia associated with abnormal hemoglobin variant STRUMICA, as described in three members of the same generation of a family, has not been reported in medical literature.

The analysis of this family imposes the following conclusions:

a. — Thrombocytopenia associated with abnormal hemoglobin, as a result of substituted positions of amino-acids in the alpha-globin chain, can be denoted as a syndrome.

b. — This syndrome may be hereditary.

c. — This heredity is probably autosomally dominant.

The second and the third statement undoubtedly demand further investigation.

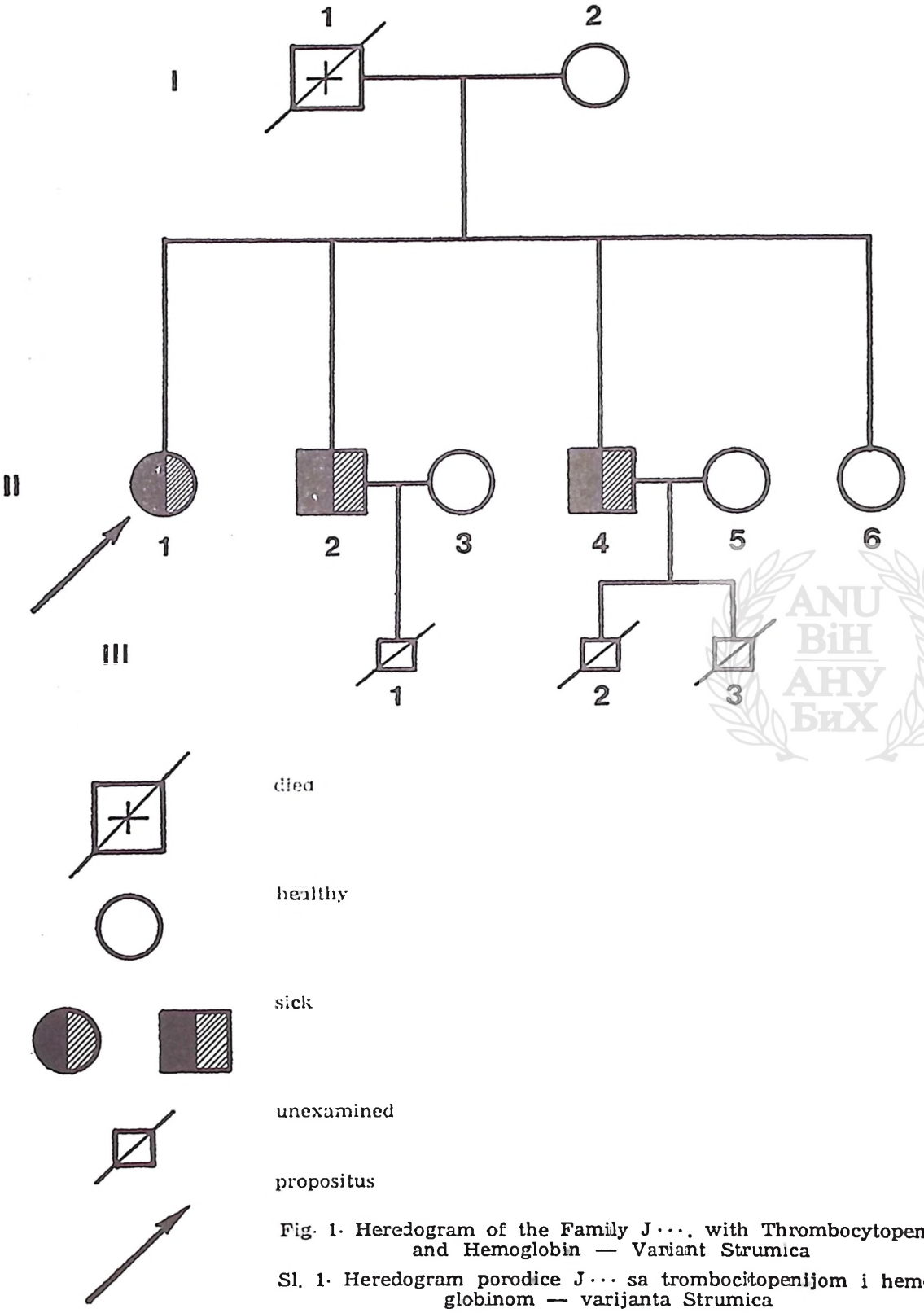


Fig. 1. Heredogram of the Family J···, with Thrombocytopenia and Hemoglobin — Variant Strumica

Sl. 1. Heredogram porodice J··· sa trombocitopenijom i hemoglobinom — varijanta Strumica

TABELA 1. — THROMBOCYTE ADHESION II—1, II—2, II—3 FEEBLE
ADHEZIJA TROMBOCITA II—1, II—2. II—3 : SLABA

Subject	Age	Thrombocytes (x 10^3/μl)	Time of hemorrhage	Coagulum retraction
II—1 ♀	27 g.	< 30	> 17 mn	very feeble
II—2 ♂	32 g.	< 110	> 14 mn	feeble
II—4 ♂	39 g.	< 100	> 15 mn	feeble

TABLE 2.
TABELA 2.

Subject	Age	TGT* with patients thrombocytes	Life of thrombocytes	
II—1 ♀	27 g.	prolonged	reduced	3 days
II—2 ♂	32 g.	prolonged	reduced	6 days
II—4 ♂	39 g.	prolonged	reduced	6 days

* Thromboplastin generation test
* Test generacije tromboplastina

TABLE 3.
TABELA 3.

Subject	Age	Thrombo-dynamo-elastography (blood)		
		» r + k «	a—mx	I T P*
II—1 ♀	27 g.	normal	< 30 mm	reduced
II—2 ♂	32 g.	normal	< 40 mm	reduced
II—4 ♂	39 g.	normal	< 40 mm	reduced

* Thrombodynamic potential index
* Indeks trombodinamskog potencijala

TABLE 4.
TABELA 4.

Subject	Age	erytrocytes (x10^6/μl)	reticulocytes (‰)	hemoglobin (mmol/L)	hemoglobin (%) HbA_0	HbX	HbA_2
II—1 ♀	27 g.	< 3,8	29/10^3 ERC*	< 7,0	72,5	24,9	2,6
II—2 ♂	32 g.	< 4,2	26/10^3 ERC	< 8,9	72,0	22,8	2,8
II—4 ♂	39 g.	< 4,0	28/10^3 ERC	< 8,8	69,9	27,6	2,5

* of the erythocytes
* eritrociti

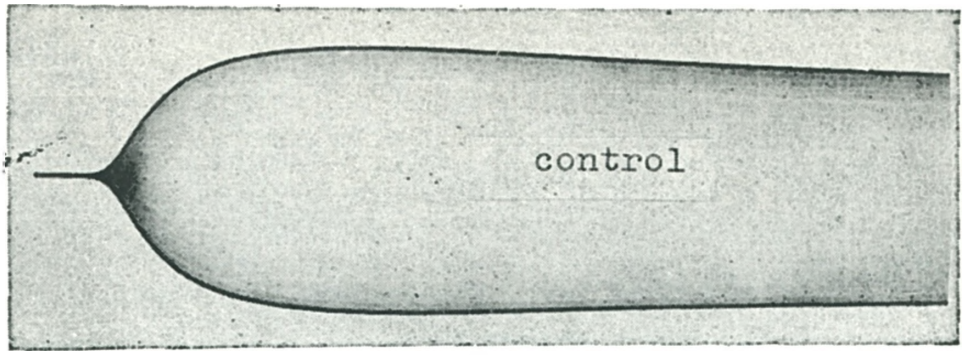
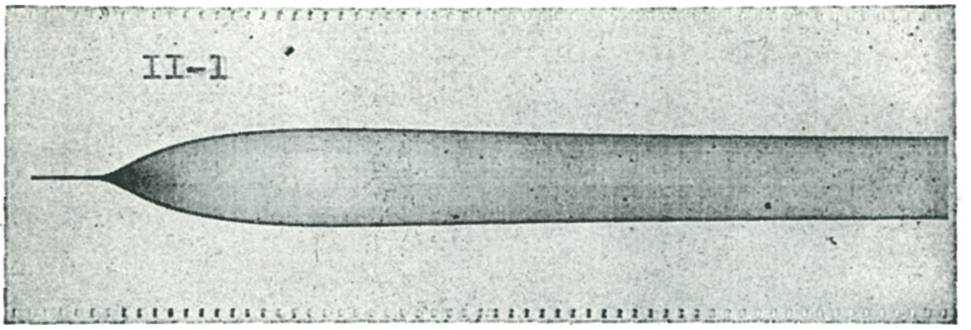
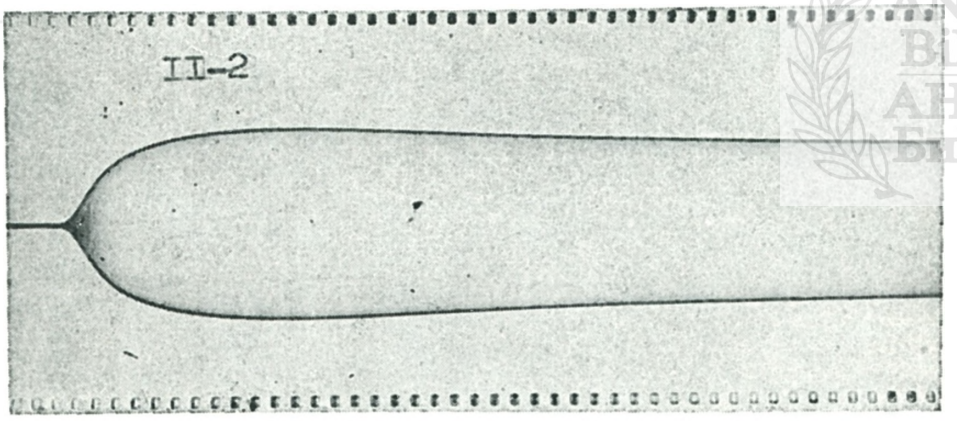


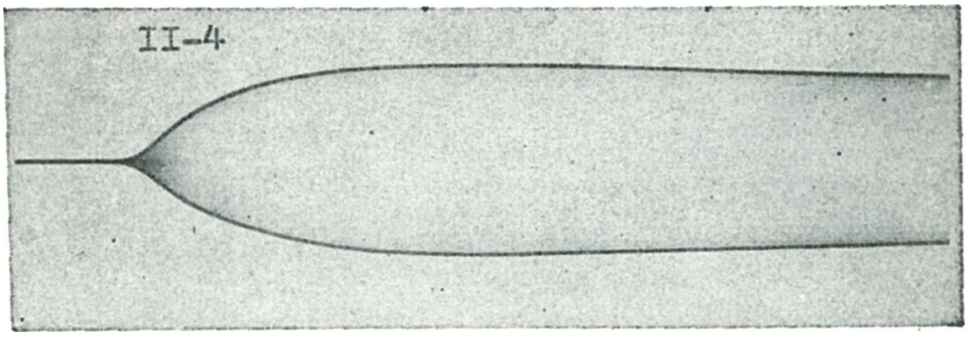
Fig. 2.
Sl. 2.



Fis. 3.
Sl. 3.



Fis. 4.
Sl. 4.



Fis. 5.
Sl. 5.

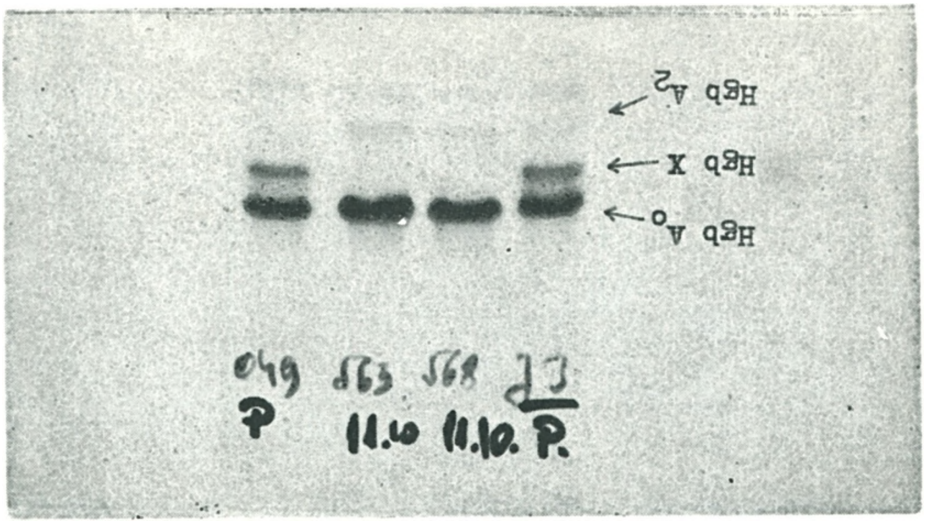


Fig. 6. Elektrophoresis of hemoglobin
Sl. 6. Elektroforeza hemoglobina

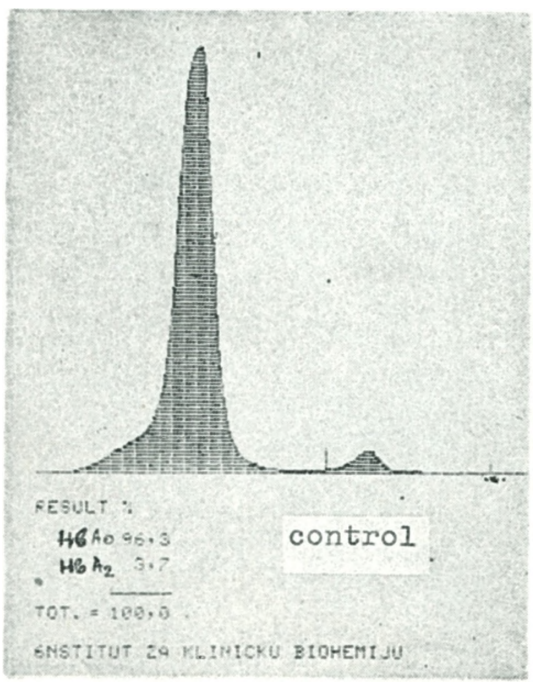


Fig. 7.
Sl. 7.

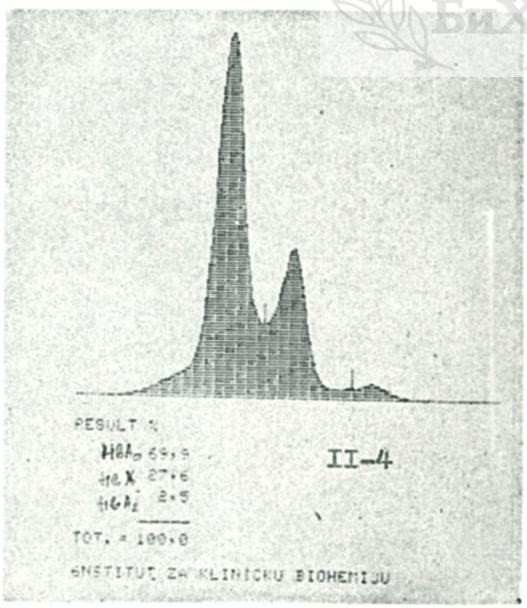


Fig. 8.
Sl. 8.

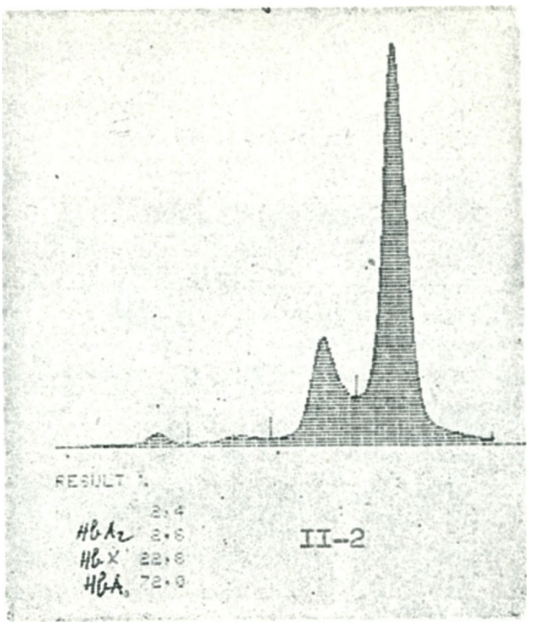


Fig. 9.

Sl. 9.

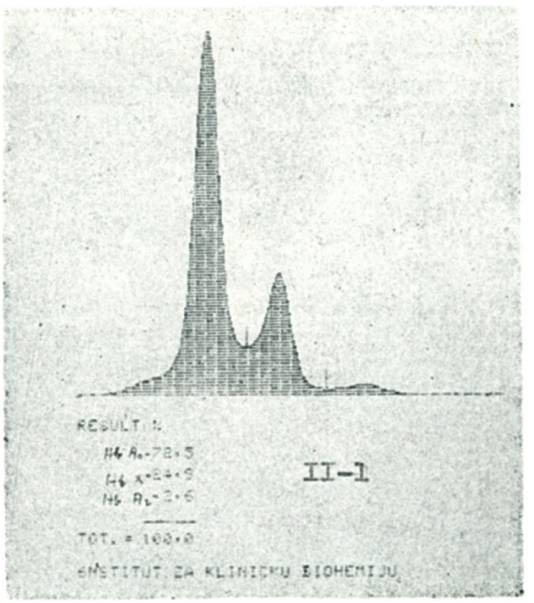


Fig. 10.

Sl. 10.

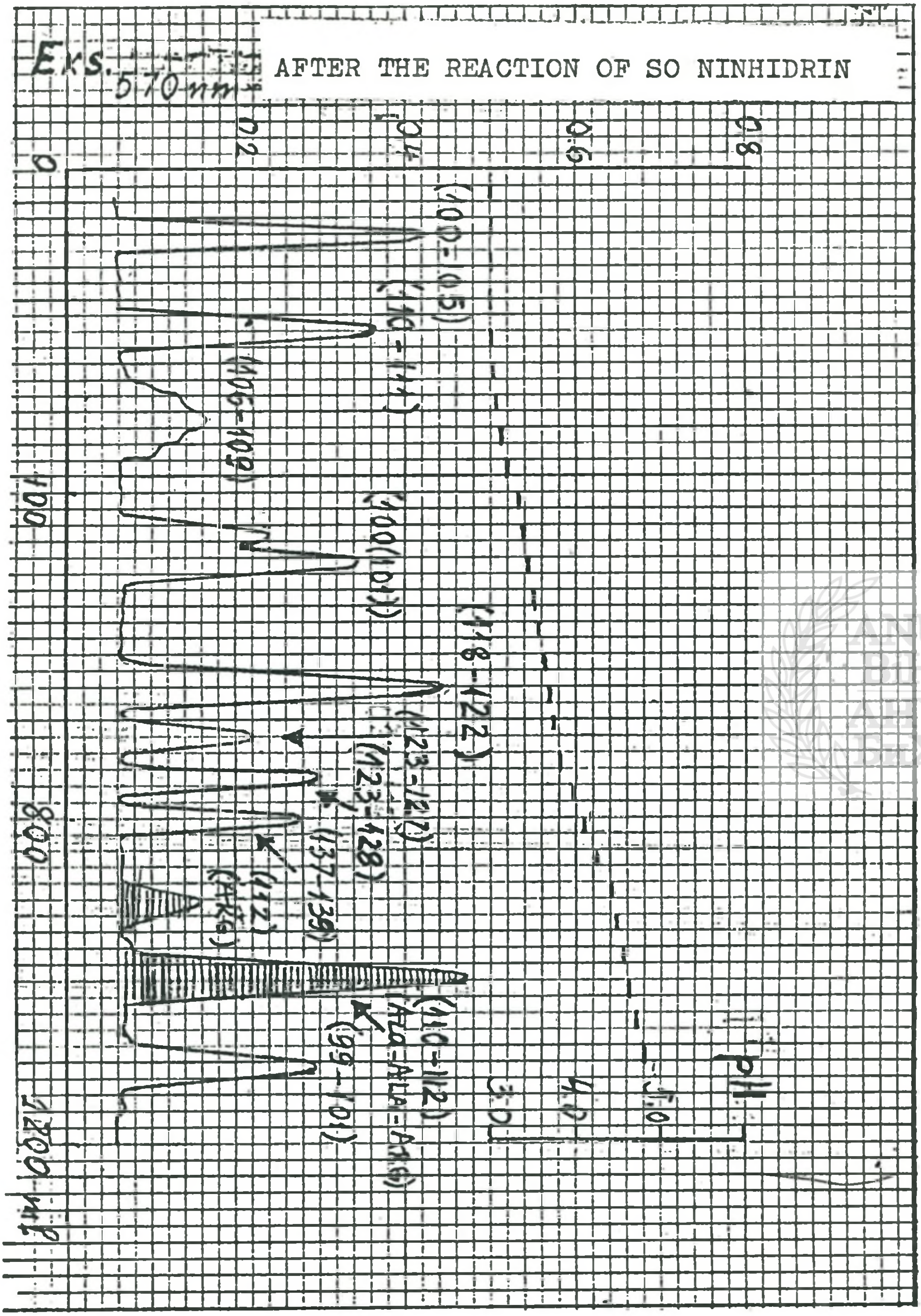


Fig. 11.

Sl. 11.

105 110 112 115

Leu, Leu, Val, Thr, Leu, Ala, Ala, His, Leu, Pro, Ala,

Normal sequence and position of amino-acids in the globin chain, Primary structure
Narmalan redosljed i pozicija aminokiselina u globinskom lancu: primarna struktura

105 110 112 115

Leu, Leu, Val, Thr, Leu, Ala, Ala, Arg, Leu, Pro, Ala,

Sequence and position of amino-acids in the α STRUMICA globin chain
Redosljed i pozicija aminokiselina u α Strumica globinskom lancu