

Adenosine Deaminase Activity IN β – Thalassaemia

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Abstract

The activity of adenosine deaminase enzyme has been investigated in normal and thalassaemic sera. The data obtained reflect an elevation of the enzyme activity in thalassaemic samples compared with those of healthy normal objects. The study was carried out with (6.5)PH value and 37C as maximum temperature. This study was comprehensive to the electrophoretic behavior of the hemolysate serum in a step of electrophoresis analysis of the thalassaemic and normal hemoglobin.

Introduction

The major function of blood cells is to carry blood gases. Erythrocytes are the principal agent that transport oxygen from the lungs to the respiring tissues and also transport most of the carbon dioxide from the tissues back to the lungs, these functions are accomplished mainly by the protein hemoglobin, which is ideally suited to carry oxygen and also in a great help in the transport of carbon dioxide. The hemoglobin molecule is a complex structure made up of four subunits. Stated another way, hemoglobin as a tetramer made up of four monomers, as shown in fig (1). Each monomer consists of a heme and a globin unit. The globin units are made up of polypeptide chains of two distinct types. In human adults one type is referred to as the alpha-chain. The other two are called beta-chains (1). Hereditary anemia's include disorders of the structure or

Synthesis of hemoglobin; deficiencies of enzymes that provide the red cell with energy or protect it from chemical damage; and abnormalities of the protein of the red cell's membrane (2). Inherited diseases of hemoglobin, hemoglobinopathies, are the most imported (3). The structure of the hemoglobin (Hb) changes during development. By the 12th week embryonic hemoglobin is replaced by the adult fetal hemoglobin (HbF), which is slowly replaced after birth by the adult hemoglobin; HbA1 and HbA2. Each type of hemoglobin consist of peptide chains (4). The most imported of these is HbA1, which account for about 97% of the total HbA [1]. HbA has the structure $\alpha_2\beta_2$ (namely, two α chains plus two β chains), HbA2 has $\alpha_2\delta_2$ and HbF $\alpha_2\gamma_2$ (4). The schedule of synthesis of the various types of normal globin chain during prenatal and postnatal life are given in fig (2). The most important of which are the sickling disorders and thalassaemia (hereditary defects of the synthesis of either the α or β globin chains (5).

Thalassaemia

The term thalassaemia comes from the Greek word "Thals" meaning sea, and "emia" which is stand for blood. This compound word came into being because the anemia was originally found only in people living in areas bordering the Mediterranean sea (1). Thalassaemia syndromes are a heterogeneous and complex group of inherited diseases they share certain clinical manifestations such as a reduced Mean Corpuscular (MCHC), infective hematopoieses and accelerated hemolysis. These symptoms are the result of abnormal hemoglobinosynthesis. However, thalassaemia differ from other microcytic hypochromic anemia in that the anemia is not caused by abnormalities in synthesis (5).

Because α -chain are shared by both fetal and adult hemoglobin, mutations of the α -globin genes affected hemoglobin production in both fetal and adult life; diseases that are due to defective β -globin production are only manifest a birth when HbA replaced HbF(6).

Geographical Distribution

The syndromes is broadly distributed throughout parts of Africa, the Middle East, the India, and South Asia , and occurs sporadically in all

racial groups, it is thought to be common because carries have been protected against malaria (3). The last report of Kurdistan thalassaemia Society indicated that the total number of thalassaemic patients in Kurdistan region reached to 810 patients, distributed in Erbil 310 patients represented 38.27%, in Sulaimania 270 patients represented 33.33%, and in Duhok were 230 represented 28.39 % (7). One – seventh of the Cyprounian people, one – twelfth of the Greece people and in Asian and in all middle East, also Indian, Pakistan, the south of China, Hong Kong, Vietnam; in all these regions the disorder changes from one – tenth to one – fifty, in Africa and the east of India there are one – fifty are defected by this syndrome, between Britannian there are one per thousand carriers of thalassaemia, (7). Number of thalassaemic carriers reached 2500-5000 person in USA (8), in Saudi Arabia reached 3.5%, Yaman 2.4%, Emarat 1.7%, and in Amman 2.4% (9). Ramot and Abrahamor (10). did a statistical study and concluded that this syndrome is present widely between Indian and Kurdistan Jews after studying of 85% of these cases. Fig (3), shows the geographical distribution of the disease in the world which consist of all regions mentioned before.

Classification of Thalassaemia

In each if the thalassaemias the principal biochemical manifestation is a partial or complete (always selective) deficiency in the production of one of the globin chains, Normal adult hemoglobin contains two α and two β chains. In healthy red blood cell precursors, the α and β globin chains are produced in thoroughly equal amounts. In the thalassaemias production of one of these globin chains is deficient, but the formation of the other chains is not affected. Consequently less than the normal amounts of adult hemoglobin is produced, and the erythroblast will have an excess of the unaffected globin chains. Since the two main types adult globin chains are α and β globins, thalassaemias may be divided into two main categories according to the globin chains that is deficient – in α – thalassaemia and β – thalassaemia. Each of these categories include a number of variant that differ in the severity of the anemia they produces (1).

α – Thalassaemia

In this group of disease, the deficiency is in the synthesis of α – globin. The β – globin are not affected and are produced at their normal rate. The human genome contains two sets of two α – genes that is, two α – genes on the chromosome inherited from the father, and two α – genes on the chromosome inherited from the mother. All four genes are normally expressed in developing red cells, and suppression of all four genes is needed to completely suppress α – chain synthesis. The normal mechanism of suppression of α -genes is by deletion(11). The abnormally long α – globin molecules, which replaced normal α – globin are present only in small amounts. The small amount of α – globin results either from decreased rate of synthesis or more likely, from increased rate of breakdown (6).

 β – Thalassaemia

Most globin structure are made up of about 150 amino acid residues, but the number varies for each globin chain. For instance, the human α – chain globin has 141 amino acids residues, and the β – and γ – chains each have 146 amino acid residues (1). β – thalassaemia are characterized by a deficiency in β – globin genes, total or partial suppression of either or both β – genes will also of the results in low hemoglobin production (12). The underlying mechanisms causing β – thalassaemia are completely different from those causing α thalassaemia. Alpha – thalassaemia are the result of gene deletion, whereas β – thalassaemia are caused by deficiencies in the regulation of β – genes (6). Amino acids composition of β – chain of one HbA molecule are explained in fig. (4)(13).

Causes of β – Thalassaemia

More and more evidence is becoming available showing that β – thalassaemia is usually caused by mutations affecting the normal transcription of β – globin chain. A number of patients with severe β – thalassaemia produce normal β – globin but in abnormally small quantities. This type of β – thalassaemia is referred to as β – thalassaemia. The anemia associated with this type of thalassaemia may range from

mild to sever, depending on how much β – globins made. Stated another way, the degree of anemia depends on how severely the regulation of β – globin gene transcription is disturbed. In another patients with β – thalassaemia, the transcription process is so disturbed that no viable messenger RNA (mRNA) is produced, this type of thalassaemia is known as β – thalassaemia (9).

Classification of β – Thalassaemia

Customarily the following three different forms of β – thalassaemia are recognized :

- 1- β – thalassaemia major (β – thalassaemia)
In this form of disorder, β – globin chains are not produced, resulting in a complete absence of HbA1.
- 2- β – thalassaemia intermedia (β – thalassaemia)
In this condition some β – chains are produced, resulting in varying amount of HbA1.
- 3- β – thalassaemia minor (heterozygous β – thalassaemia)
In this condition at least one normal β – globin gene is present. Thus considerable amount of HbA1 are being produced [14]. In those instance in which very few or no β – globin are produced, very little HbA1 will be present; these conditions are always associated with sever anemia, the resulting anemia is also known as Cooly's anemia or Mediterranean anemia.

Treatment of β – Thalassaemia

In general there are several ways for treatment of β – thalassaemia:

Blood transfusion,
Desfiral and Vit C,
Folic Acid,
Splenectomy,
Bone Marrow Transplantation,
Gene Therapy.

Adenosine Deaminase Activity in Thalassaemia Blood

Several numbers of enzymes are made up from the liver. Many authors has been investigated the level of ADA in different tissues (15-

17). The liver defects as a result of iron overload, because of continuous hemoglobin of the blood, resulting in non – regular production of enzymes such as 5– Nucleotidase and other related enzymes. Adenosine deaminase hydrolyze adenosine and produce inosine and ammonia which is the basic material by which the activity of the enzyme after converting it to a stable color solution can be measured .This enzyme , by indirect way, its activity is correlated with that of 5 – Nucleotidase because of their involvement in the degradation of nucleotides and nucleosides. Amount of ammonia produces as a result of hydrolysis of adenosine has been measured and indicate the activity of enzyme.

Materials and Methods

Chemicals

The following chemicals were used and purchased from BDII, Analar, and firms. These were of high analysis grade unless otherwise specified.

Na₂HPO₄, NaH₂PO₄ . 2H₂O, Phenol, NaCl, NaOH, Adenosine, Ammonium sulphate.

Equipments

Electrophoresis (Shandon type), Beckman Acta M (UV, Visible-near IR research spectrophotometer).

Samples

Normal blood samples (100 samples) were collected using veinopuncture from healthy volunteers. Thalassaemic samples (100 samples) were obtained from patients with β – thalassaemia major supported from Children hospital (Sulaimania city). Sera were isolated after lefting the blood at room temperature for about one hour (after coagulation). About 3 to 4 ml serum were collected which is used on the same day of the experiment.

Experimental

Estimation of ADA enzyme activity

Enzyme activity were estimated for both normal and thalassaemic sera. Table (1) represents the added volume of the reagents in μL (18).

Calculations

Enzyme activity of ADA is calculated using the following equation:

$$\text{ADA activity(U/L)} = \frac{\text{AT} - \text{AC}}{\text{AS} - \text{AB}} \times 50$$

The activity expressed in IU/ L. which is defined as that release of one μM of ammonia per minute.

These activities were reflects highest PH value fig (5) and highest Temp fig.(6) in thalassaemic samples than those in control one.

Hemoglobin Electrophoresis

Electrophoresis of hemoglobin, a conjugated protein, is based on the fact that various proteins diluted with buffer in an electric field between two electrodes can be separated into two layered bands because of their varying migration speed. The migration speed of a protein depends mainly on the charge carried by the protein molecule at the PH of the buffer and to some extent on the supporting medium (agar gel, cellulose, starch gel, paper etc.). In the case of hemoglobin the migration speed

Depends on the amino acid composition of the peptide chains; therefore, various hemoglobin differ in the speed they travel. The purpose of Hb electrophoresis for thalassaemia blood is to determined various globin present in the hemoglobin. As mentioned above, thalassaemic hemoglobin consist mainly of HbF and small amount of HbA, instead of large amount of HbA;(HbA1 and HbA2), therefore, in electrophoresis we may obtain different part differ in its migration. Fig (7). shows the obtained electrophoretic pattern for normal and thalassaemic hemoglobin. Two different separation types can be seen using Titan III - cellulose acetate plate soak in buffer. The hemolysate blood were prepared by collecting 0.5 ml EDTA- anti coagulated blood. Ten ml of normal saline were added then centrifuged for 3 min and discard the saline sol. A six parts hemolysate reagent to one part were packed and the red cells were

washed. 5 μ L. of the hemolysate and also 5 μ L. of the normal hemoglobin were applied to the electrophoresis using 450volt for 15 min.

Results and Discussion

The study has been concentrated on the estimation of the ADA – enzyme activity in thalassaemic and compared with those of normal objects table (2). The data obtained were reflects an elevation in the ADA activity in thalassaemic patient serum (35 + 0.15 – 45.2 + 0.23 IU/L) when compared with normal serum (20. 11 IU/L). In addition, the study were also contributes the investigation optimum conditions and the effect of the factors influencing the ADA activity. Those factors like PH and Temp. were studied and the data obtained reflects maximum activity of ADA at PH 6.5 and at highest Temp of 37.5C. fig. (5&6).

The author suggested the following explanation for the data obtained. Firstly, as it was known that liver organ is the important organ in the body due to its responsibility of protein synthesis and other related cell constituents metabolism. Therefore, enzymes in general as they are proteinic in nature their destruction in the gene expression of the cell, which is responsibility of the nucleic acids DNA and RNA, will results in disturbances in protein biosynthesis, i.e, enzyme structure. Thus, as thalassaemia is a type of an anemic disease (inherited type), and it cause is the destruction occurs in the hemoglobin contents, a type of protein structure, so, the author suggested the study of the other liver protein disturbances due to thalassaemia disorder, i.e., studying other liver enzymes damages. Of these enzymes was the ADA enzyme which is catalyzed the degradation of adenosine as a substrate and produced from the degradation of the nucleic acids by mean of 5-Nucleotidase. The highest activity of the ADA in thalassaemic serum compared with that of normal serum can be explained to be due to the liver cells damage occurs. Therefore, the liver constituent were excreted out of the liver and released in circulation and thus its level was elevated in serum.

Discussing figures 5 and 6, we found that the activity were prohibited and this can be explained to be due to the denaturation occurs in the α – Helix of the proteinic structure of the enzyme and exactly when the temperature elevated.

References

- 1-Haen, P.J. (1995)Principle of Hematology pp 131-138, Wm.C., Brown Communications, Inc. USA.
- 2-Rashid, S.J. (1997) An MSc theis. University of Salahaddin.
- 3-Weatherall, D.J. (1985) Ann. NY. Acad. Soc .
- 4-Tillyer, M.I. and Tillyer, C.R. (1994) J. Clin. Path. UK. 47(3): 205-208.
- 5-Schwartz, S.H. (1970) Am. J. Dis. Child.20(2): 358
- 6- Weatherall, D.J. (1997) B.M.J.314: 492-496
- 7-Kurdistan Thalassaemia Society , pp 40 ,(1999).
- 8-United Kingdom Thalassaemia Society, 107 Nitragal London, 8(7): (1999).
- 9-Carols, M.M.; Faibank, V.F.; Bugert, E.O.;Johne, S.; Stephan, S.and Elliot C , (1986) Am. J. Med. 292(3): 147-151.
- 10-Nita, M.;Vasantha, S. and Joseph, W.T.P. (1986).Saude Med. J. 10: 62-64.
- 11Ramot, B.; Abrahamar, A. Frayer, Z. and Gafin, D. (1974). British J. Haematol.10: 155-158.
- 12-Arjona, S.N.;Eloy, J.M.; Gu, L.H.; Smetanina, N.S.and Husman, T.H. (1996) British J Hematol. Jun 2:93(4): 841-844
- 13-Heilmeyer, L.; Wincklemann, G. and Eutick, B. (1968) Grundress der Pathophysiologie des Bultes Gustav Fischer Verlag
- 14-Devlin, T.M. (1993) Text book of biochemistry with clinical correlation. 3rd, Willy-Liss Inc, USA.
- 15-Al-Dahan, J.F. (1996). an MSc thesis, College of Education, Univ. Tikrit,
- 16-Al-Falahi, Y.S. (1978).an MSC thesis, College of Science, Univ. Baghdad,
- 17- Hassan ,H. G. and Hikmat ,G. (2002)Zanco J, 14(2): 53- 59.
- 18-Galanti, B. and Guist, G. (1968)Mal. Inf. Parass, 20: 982

Table(1)ADA activity measurement procedure

No	Solution	Test	Control	Standrd	Blank
1	Phpsphate buffer	-	-	-	1000
2	Adenosine Sol	1000	1000	-	-
3	Ammonium standerd Sol	-	-	1000	-
4	Serum	50	-	-	-
5	Distled water	-	-	50	50
Tubes shaken very well and covered with rubber stopper, incubated at 37C for 60 min.					
6	Phenol-Sodium nitroprosside Sol.	3000	3000	3000	3000
7	Serum	-	50	-	-
8	Sodium hypochlorite	3000	3000	3000	3000
Shaken , incubate at 37C on water bath for 30 min. Absorbancy read at 630 nm.					

Table(2) Serum ADA activity in normal and thalasaemic patient at 6.5 pH value and 37C Temp

Factor	Normal SADA unit u/l	ThalasseamicSADAunit4/2
-	18 – 20	35 – 45
pH 6.5	20	45
Temp 37C	19.25	34.11

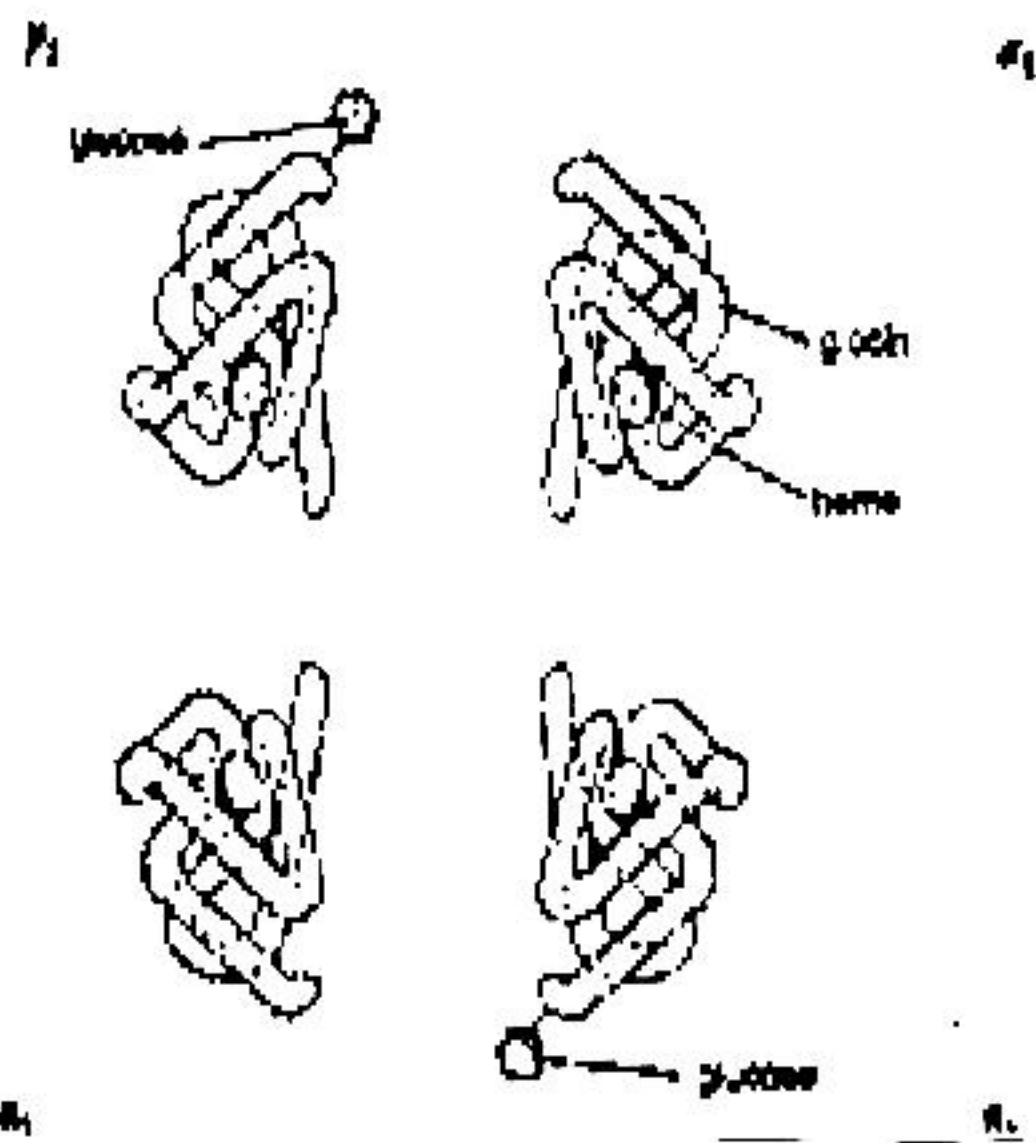


Fig .(1)quaternary structure of haemoglobin

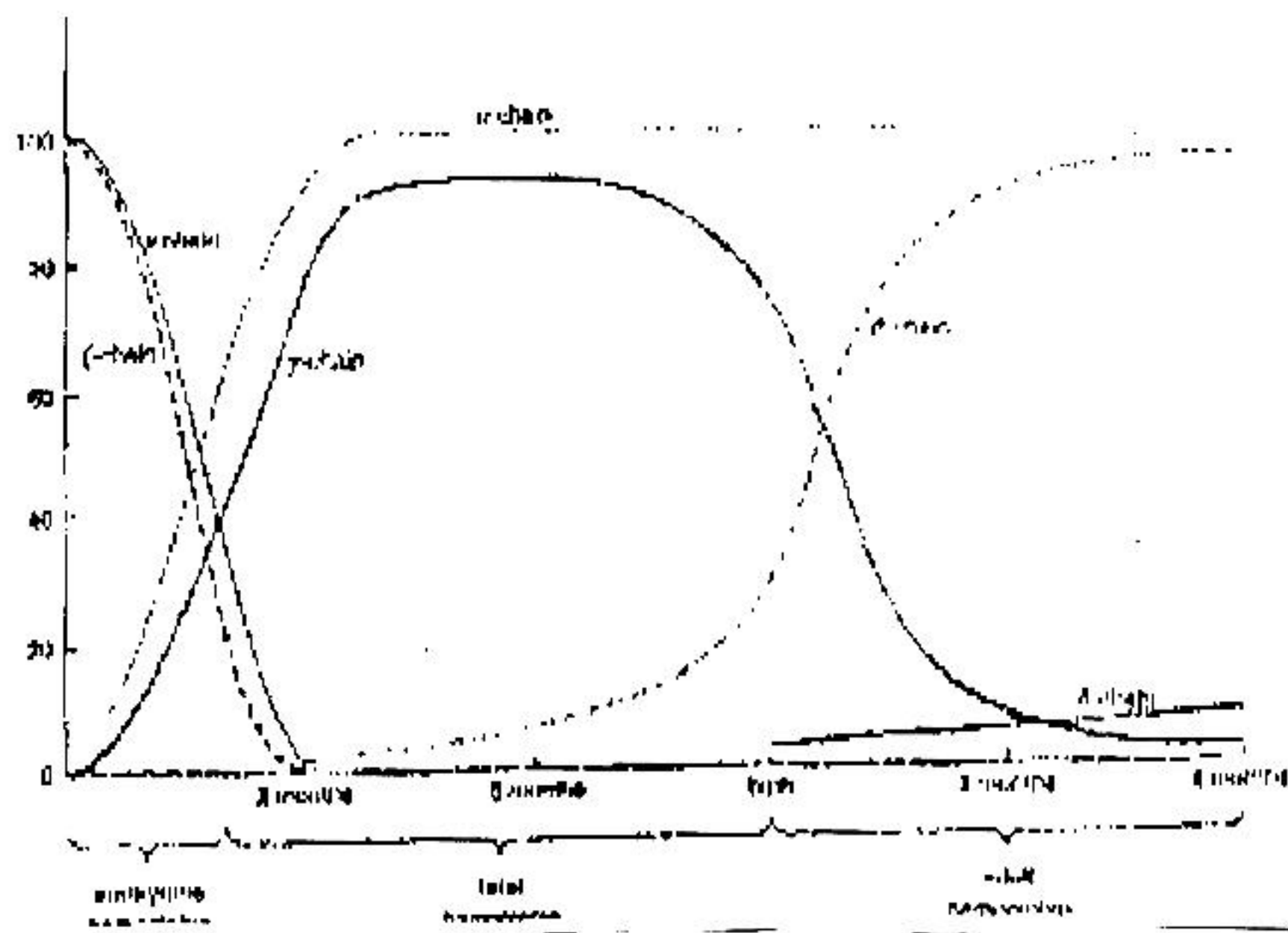


Fig .(2)The relative rates of globin chain synthesis

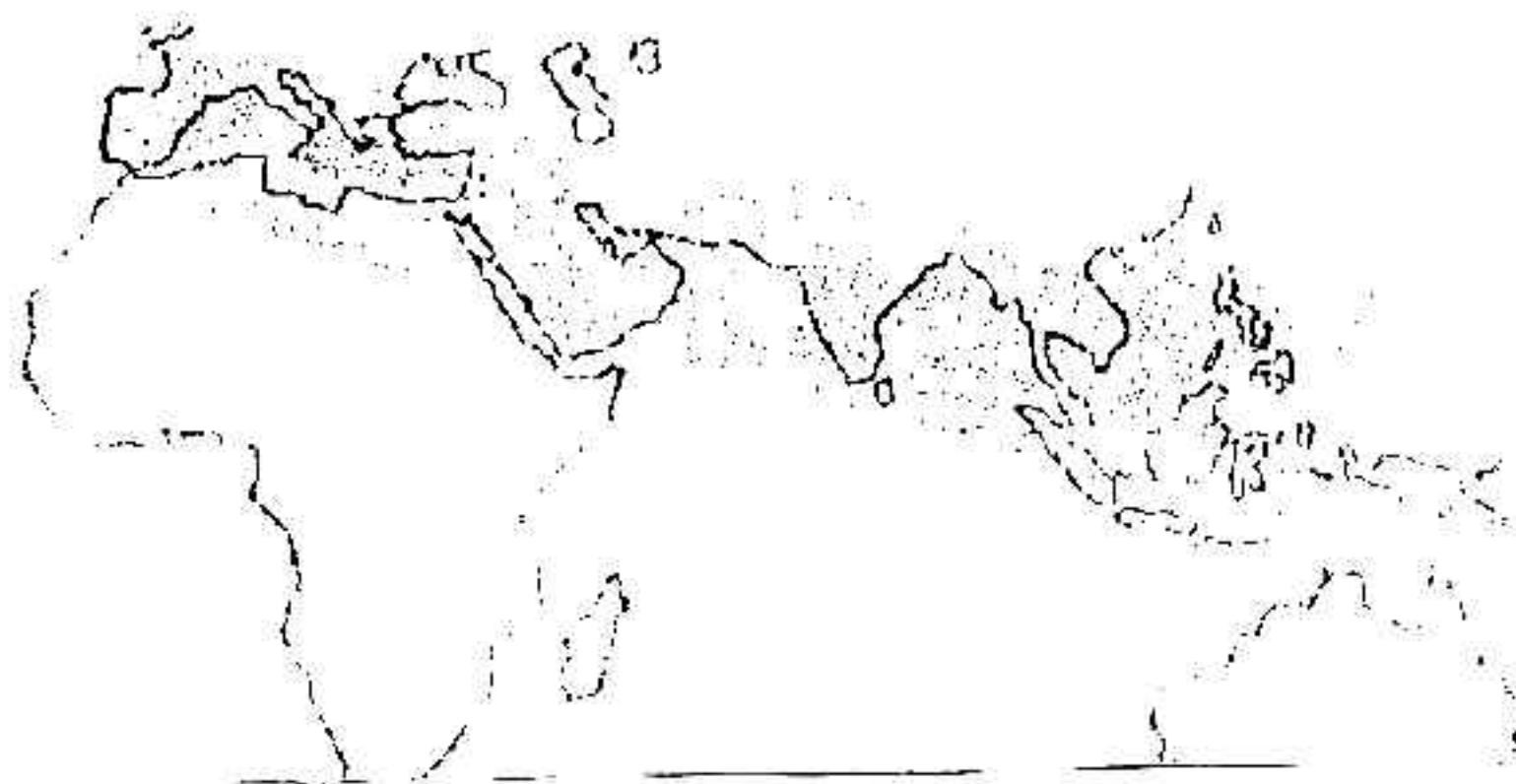


Fig.(3)Geographical distribution of thalassemia(weathorall1985)

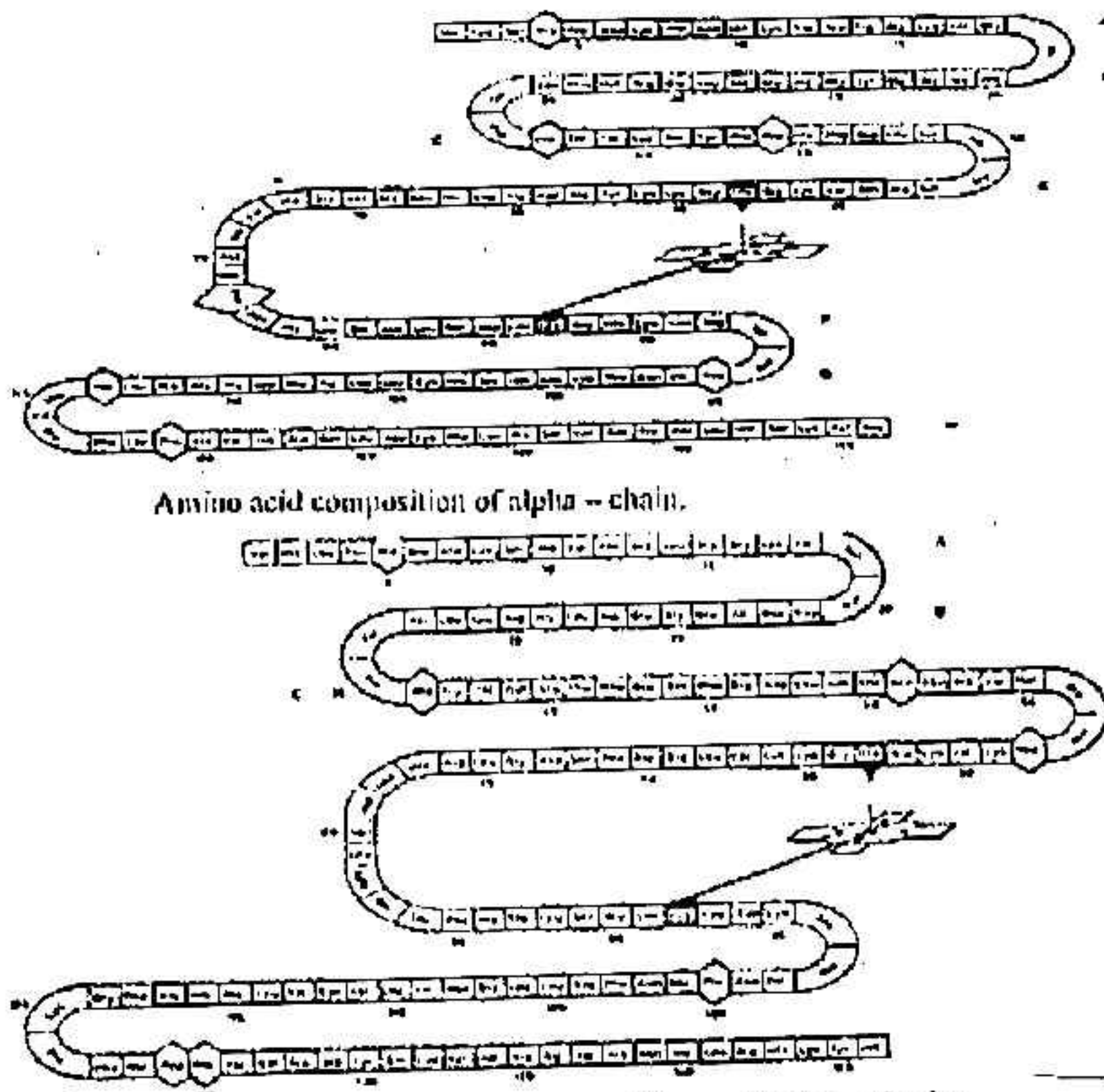


Fig.(4)Amino acid composition of beta- chain

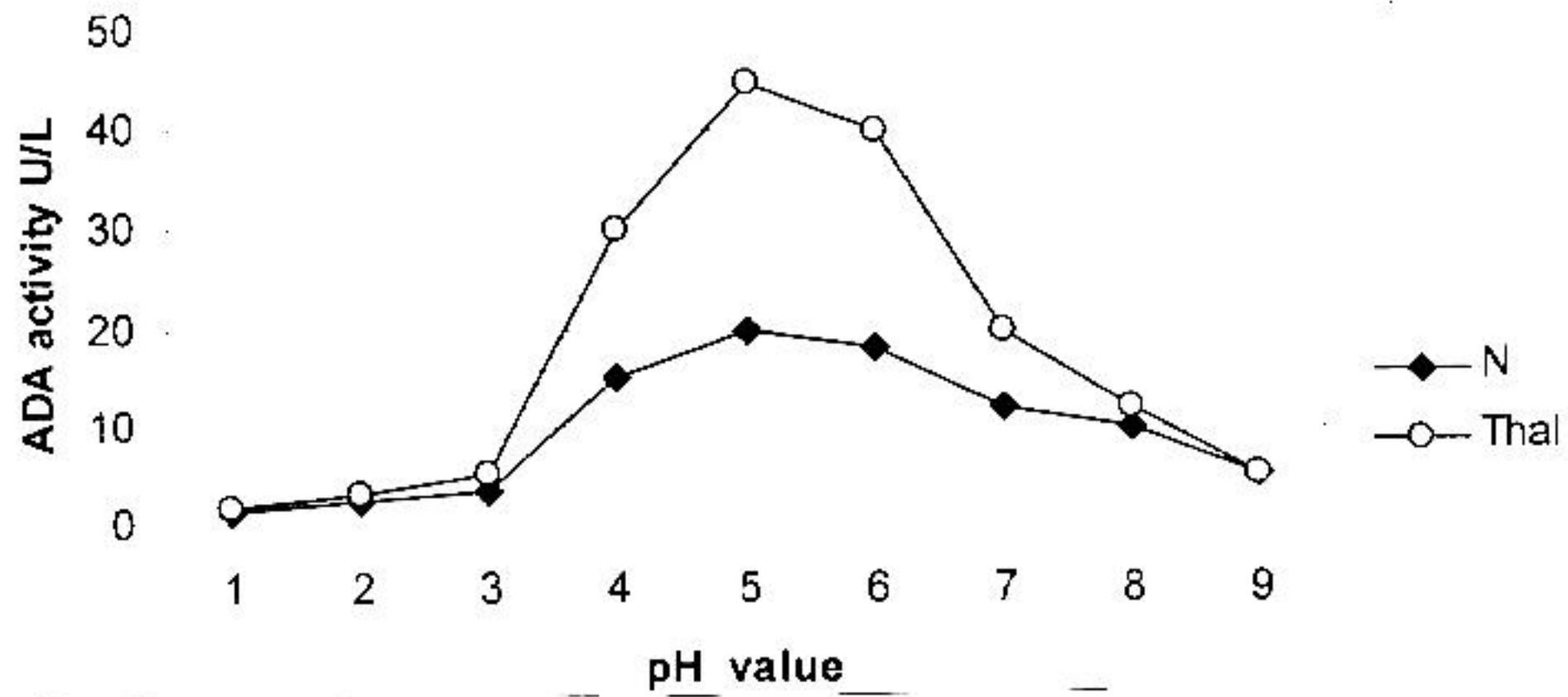


Fig .(5) pH Effect on ADA activity

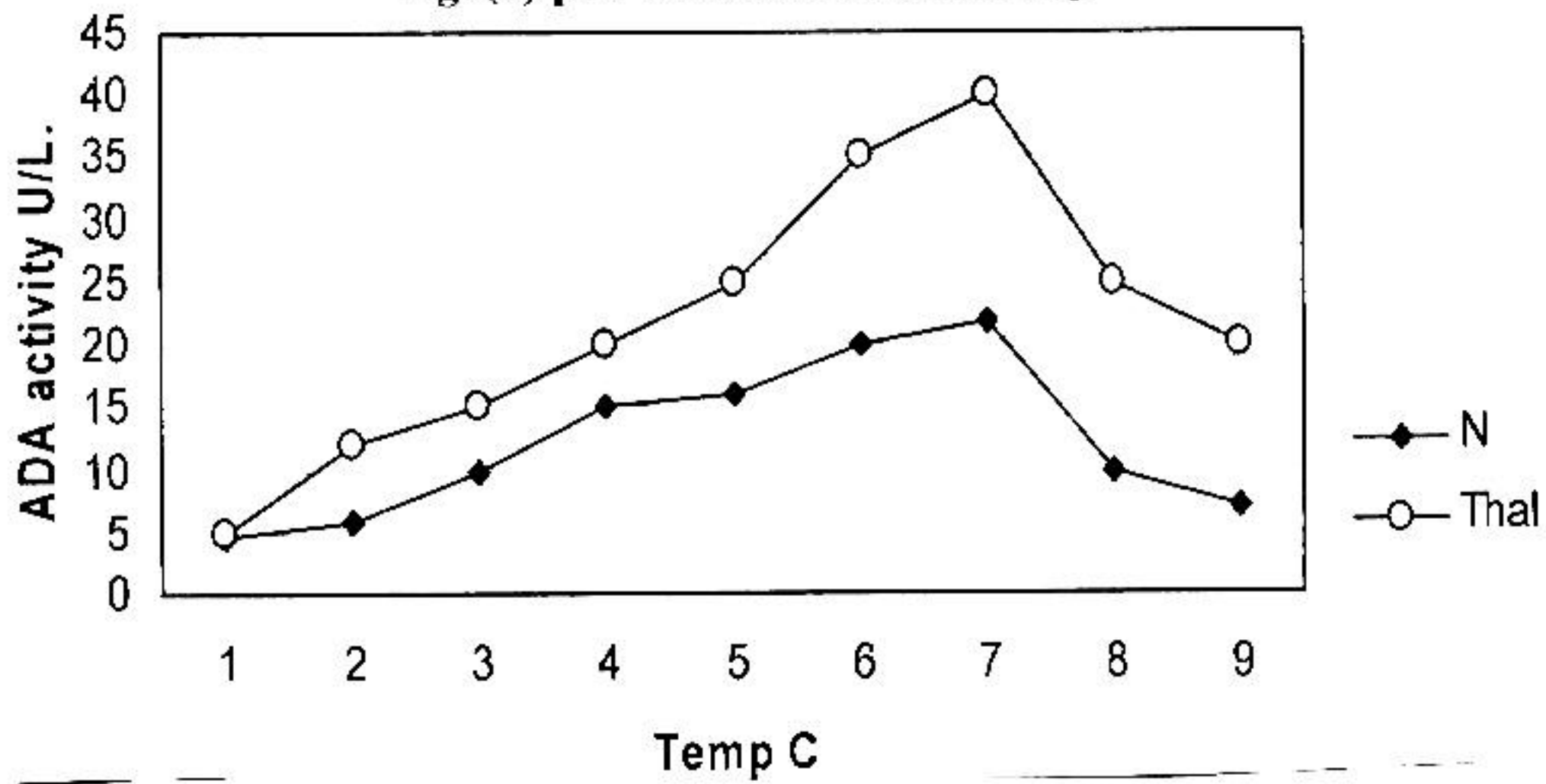


Fig .(6) Temperature Effect on ADA activity.



Fig .(7) Electrophoresis of β -Thalassemia- Hb on cellulose acetate plate

نشاط أنزيم الادي أمينيز في مصول أنيميا البحر الابيض المتوسط (بيتا - ثلاسيما)

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الخلاصة

لقد بحثت مستويات نشاط أنزيم الادي أمينيز في مصول دم المصابين بداء البحر الابيض المتوسط و قورنت النتائج بمثيلاتها من دماء الاصحاء . أثبتت النتائج أن مستويات الانزيم ترتفع عند المصابين مقارنة بنشاطه عند الاصحاء. أجريت الاختبارات في درجة الاس الامثل و درجة الحرارة المثلى . لقد تناولت الدراسة كذلك تحليل هيموغلوبين الحالتين وذلك بأتباع طريقة الترحيل الكهربائي .