# THE INHIBITION OF THE DENATURATION OF METHEMOGLOBIN BY THE ACTION OF SUGARS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Leon Lack

1948

This is to certify that the

thesis entitled

The Inhibition of the Denaturation of Methemoglobin by the action of Sugars

presented by

Leon Lack

has been accepted towards fulfillment
of the requirements for

M. S. degree in Chemistry

C. D. Ball

Major professor

Date May 14, 1948

M-795

# THE INHIBITION OF THE DENATURATION OF METHEMOGLOBIN BY THE ACTION OF SUGARS

by

Leon Lack

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

MICHIGAN STATE COLLEGE

Department of Chemistry

1948

TABLE OF CONTENTS

6/7/43
g-

## ACKNOWLEDGMENT

I wish to express my deepest appreciation to Professor Charles D. Ball for his invaluable guidance and assistance.

# INTRODUCTION

It is likely that few groups of biological substances have been more intensively studied than the blood pigments and their derivatives. Their physiological importance is apparent, and this has stimulated the vast amount of research which has been going on since 1674 when Mayou demonstrated that blood would yield a quantity of oxygen in excess of the amount one could attribute to a physical solution of the gas. He called it, "nitro aerial particles" because this substance had the same property as saltpeter, which burned combustibles in the absence of air. It is remarkable that he associated this substance in the blood with other types of combustion, and attributed body heat to this phenomenon (1).

An important aspect of these studies has concerned itself with denaturation of the protein moities of the pigments. This is understandable since denaturation studies of proteins have a great deal of significance due to the fact that many pathological conditions and death of tissue are frequently associated with denaturation of some of the proteins involved.

The purpose of this work is to determine whether the denaturation of methemoglobin is inhibited by various reducing sugars.

Previous workers have shown that some proteins are stabilized by sugars and mannitol and it was their work which indicated the plausibility of investigating the effect of sugars on methemoglobin.

## DEFINITIONS

Due to the fact that many of the blood pigments and their derivatives were recognized as discrete entities long before their chemical natures were determined, a very unsystematic nomenclature has arisen. In order to avoid any confusion of terminology in this discussion, I would like to set down a table of definitions. The following terms are those advanced by Pauling and Coryell (2).

Ferriheme: Ferric porphyrin complex.

Ferriheme chloride: (hemin) Ferriheme plus chloride ions.

Ferrohemochomogen: (hemochromogen) Complex of ferroheme and one or two nitrogenous substances, such as pyridine, nicotine, ammonia, etc.

Ferrihemochromogen: (para hematin) A complex of ferriheme and certain nitrogenous substances.

Ferrohemoglobin: (hemoglobin) A complex of ferroheme and native globin.

Oxyhemoglobin: The complex of ferrohemoglobin and oxygen.

Ferrihemoglobin: (methemoglobin) The complex of ferriheme and native globin.

Globin ferrihemochromogen: (or cathemoglobin) A complex of ferriheme and denatured globin.

The term denaturation is used in the sense of Neurath's definition: "Denaturation is any non-proteolytic modification of the unique structure of a native protein giving rise to definite changes in chemical, physical or biological properties" (3).

# HISTORICAL

The historical discussion divides itself logically into two parts, the effects of sugars on the stabilization of proteins, and the chemical properties of the protein portion of methemoglobin and its prosthetic group.

## A. The Protective Action of Sugars On Proteins.

Beillinson (4) found that serum albumin and egg albumin were protected against heat coagulation by sucrose. He was able to obtain complete protection of both these albumins when their solutions were saturated with sucrose. Under the foregoing conditions serum albumin was heat stable at 62°C., and egg albumin showed no coagulation at 75°C.

Duddles (5) has shown that glucose, fructose, sucrose, mannose, and mannitol inhibit the coagulation of egg albumin. At saturation with glucose and fructose, the egg albumin solution is completely thermostable. The procedure employed by Duddles was to add the sugar to the protein solution buffered at a pH of 4.5. After heating at 70°C. for 10 minutes, the sugar protein mixture was filtered and the nitrogen of the filtrate was determined. The extent of

coagulation is indicated inversely by the amount of nitrogen in the filtrate.

Newton and Brown (6) working with plant saps found that glucose and fructose prevented denaturation of plant sap proteins which were subjected to freezing.

Ball, Hardt, and Duddles (7) found that D(+)glucose, D(-)fructose, D(+)mannose, L(-)arabinose, D(+)xylose and D(+)mannitol inhibited the appearance of sulfhydryl groups in egg albumin which had been subjected to heat treatment. Hardt, Huddleson, and Ball (8) working with blood plasma proteins showed that D(+)glucose, and to a lesser extent D(+)galactose, L(+)arabinose, sucrose, D(-)fructose, lactose, L(+)mannitol, and D(+)xylose aided these proteins in maintaining a normal electrophoretic pattern after being heated.

B. The Chemical Nature of Methemoglobin.

Methemoglobin or ferrihemoglobin is produced by oxidizing hemoglobin or oxyhemoglobin. The $E_o$ for the hemoglobin-methomeglobin redox system is + 150 millivolts at pH of 7.0(9).

Ozone, iodine, chlorates, ferricyanide, hydroxylamine, hydroquinone, p. amino phenol, formaldehyde, hydrogen peroxide, pyrogallol, sodium nitrite, atmospheric oxygen, and permanganate, to some extent can act as chemical agents in the formation of methemoglobin.

Stadie (10) has shown that pneumococcus both in vivo and in vitro can form methemoglobin. Barnard (11) has produced methemoglobin by anodic oxidation.

Until recently methemoglobin was considered an abnormal constituent of blood. Its appearance in considerable amounts is caused by either poisoning by some of the agents listed above or as a result of infection by pneumococcus. Paul and Kemp (12) discovered methemoglobin to be present in small amounts in normal human blood samples.

Unlike ferrohemoglobin, methemoglobin does not combine with carbon monoxide or oxygen. It does react with cyanides, sulfides, peroxides, fluorides, nitrites and salicylates. These reactions are discussed in the following paragraphs.

1. Reaction with Cyanides.

In 1901, Von Zeynek (13) reported a series of experiments which indicated that hydrocyanic acid will react only with methemoglobin. It was shown that no compound results when oxyhemoglobin or hemoglobin is exposed to the cyanide. There exists a stochiometric ratio of 1:1 between the cyanide radical and the ferric atom in cyanomethemoglobin. The globin portion of the molecule did not react with the hydrocyanic acid and as such no denaturation was reported.

2. Reactions of methemoglobin with Sulfides.

Keilin (14) reported that methemoglobin formed a definite compound with hydrogen sulfide analagous to cyanomethemoglobin. This reaction is reversible for on treatment with lead acetate the sulfomethemoglobin compound forms methemoglobin. One molecule of hydrogen sulfide is required for each iron atom in the methemoglobin molecule in order to complete the reaction.

3. Reactions with Peroxides.

Keilin and Hartree (15) discovered that hydrogen peroxide and ethyl hydrogen peroxide react with methemoglobin to produce a red compound. This new substance has two characteristic absorption bands at 589 mμ and 545 mμ. There is a stoichiometric relationship between the iron and the peroxide molecules in a ratio of 1:1. This reaction is completely reversible and on standing methemoglobin reforms.

4. Reaction with Fluorides.

Ville and Derrien (16) found that sodium fluoride modified the spectrum of methemoglobin. The band at 633 m disappeared and a new band at 612 m appeared. This reaction is reversible but no data is given as to the quantitative relationship between the fluoride ion and methemoglobin.

5. Reactions with Nitrites.

Barnard (17) investigating the effects of nitrite ions on methemoglobin and ferrohemoglobin found that nitrite combines with the prosthetic group of methemoglobin to alter its redox characteristics. In addition, nitrite combines with the globin portion of methemoglobin. The complete nature of the reaction was not explained but the globin portion of the particle was denatured.

In the above reaction there is no one to one stoichiometric relationship between nitrite and iron. The absence of this ratio and the presence of denaturation of the globin particle are significant. However, in the case of cyanide, peroxide, and sulfides there is no evidence of the actual denaturation of the globin particle.

6. Reaction of Sodium Salicylate with Methemoglobin.

Anson and Mirsky (18) have shown that sodium salicylate reacts with methemoglobin to denature it and keep it in solution. This reaction is reversible. Dialysis of the

sodium salicylate from solution restores the original methemoglobin. Salicylate of lower concentrations not sufficient to denature the methemoglobin completely, produces an equilibrium mixture of native and denatured methemoglobin.

The substance resulting from the reaction of methemoglobin and salicylate has the typical spectrum of the parahematin type which Keilin (19) has shown to be the same spectra as the complex of ferriheme and denatured globin. This type of spectra has no absorption in the yellow or red. This serves to differentiate the parahematin from alkaline methemoglobin and neutral or acid methemoglobin. Alkaline methemoglobin has a band in the yellow and none in the red, whereas, acid and neutral methemoglobin have no band in the yellow but one in the red. Furthermore, in parahematin there is an increase in absorption in the green and it is this increase that lends itself to quantitative colorimetric analysis.

Methemoglobin treated with salicylate has two more properties which are characteristic of denatured proteins:

a. Decrease in Solubility.

Anson and Mirsky (18) added 1 ml. of 1% methemoglobin to 1 ml. of 1 M. salicylate. They then found that

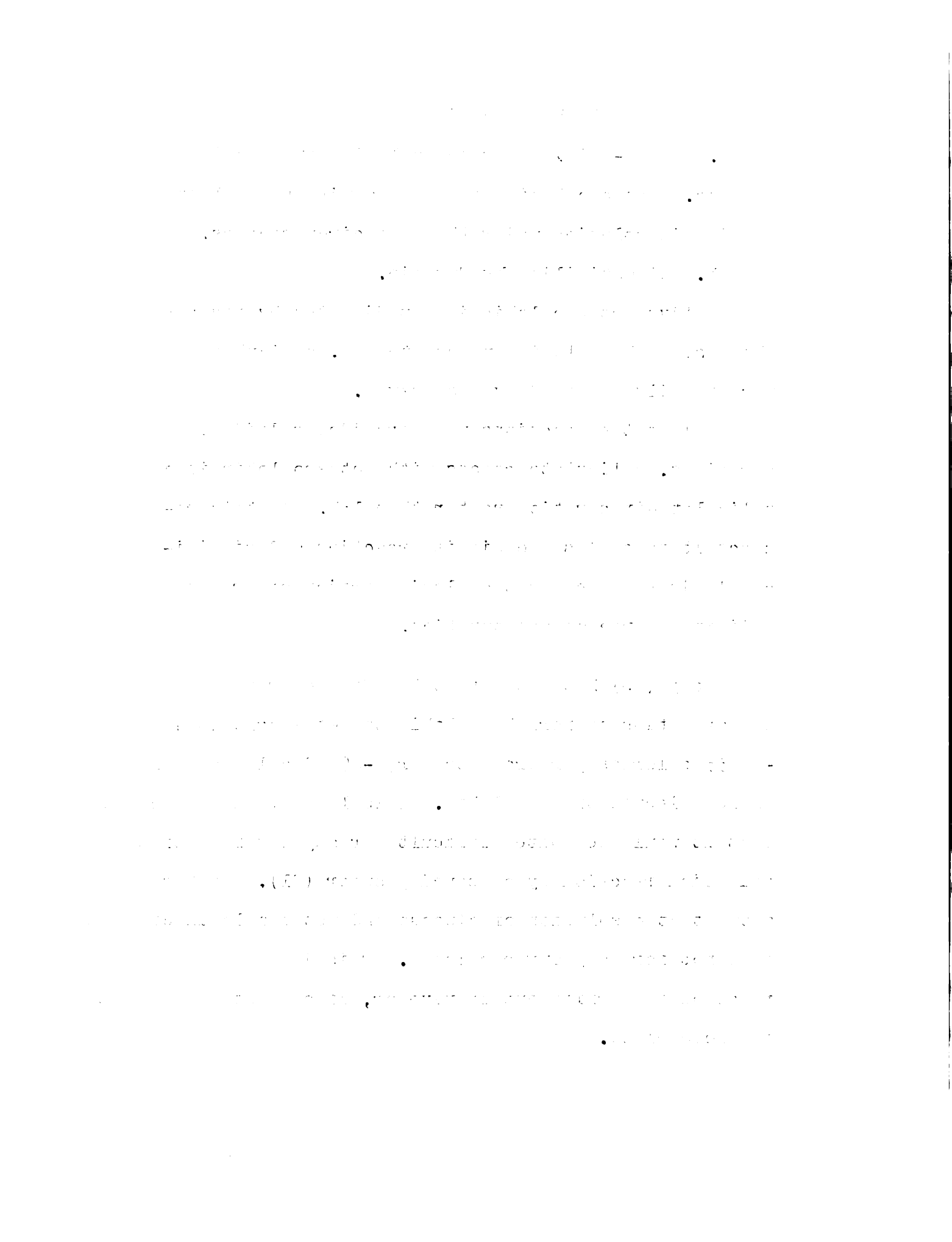
the protein could be precipitated by the addition of 18 ml. of one-third saturated solution of ammonium sulfate. Methemoglobin not treated with salicylate remains in solution under the same circumstances.

b. Digestibility by Trypsin.

Native methemoglobin is not digested by trypsin whereas, methemoglobin exposed to 3 N. salicylate is very readily attacked by the enzyme.

Unlike the reactions with cyanide, sulfides, and peroxides, salicylate reacts with methemoglobin in a salicylate-iron ratio greater than 1:1. In this respect it resembles the nitrite reaction and significantly in these cases the globin portion undergoes a definite degree of denaturation.

Levy (20) has shown that the free energy change in the oxidation of ferrohemoglobin to methemoglobin is -22455 calories per iron atom or, -89820 calories per whole molecule of hemoglobin. This large value indicates that it would be quite difficult for any reversal of the oxidation reaction by a reducing sugar (21). He has shown that a solution of glucose and methemoglobin crystals does not form any oxyhemoglobin. This is not so if the reaction is carried out in vivo or, if one uses a solution of whole blood.



Brooks (22) has demonstrated that the methemoglobin produced in rabbits with sodium nitrite is converted in vivo to oxyhemoglobin by glucose injection. Furthermore, rabbits pre-treated with glucose do not show methemoglobin in the blood after injection with sodium nitrite or phenyl hydroxylamine, both methemoglobin producers.

Warburg and Christian (23) investigating the question why glucose was oxidized by methemoglobin in vivo but not in vitro found that hemolyzed erythrocytes do not oxidize glucose on the addition of methemoglobin. However, when glucose-6-monophosphate is added to a solution of hemolyzed red blood cells and methemoglobin, the glucose-6-monophosphate undergoes oxidation. On treatment with aluminum hydroxide and centrifuging the solution of hemolyzed blood cells will not oxidize glucose-6-monophosphate in the presence of methemoglobin.

It is apparent that for the oxidized ferrohemoglobin to be reduced by the reducing sugar some complex of respiratory enzymes is necessary.

# EXPERIMENTAL

## 1. Preparation of Methemoglobin.

Methemoglobin was prepared by the oxidation of dog oxyhemoglobin obtained by the method outlined by Haliburton (24). The 300 ml. of dog's blood was defibrinated by whipping. This was centrifuged and the erythrocytes removed. These red blood cells were centrifuged with physiological saline solution. The corpuscles were then suspended in 2 volumes of water, saturated with ethyl ether, at $0^{\circ}$ centrigrade. Three drops of a 1.8% solution of sodium acid sulfate was then added to precipitate the cell stroma, which is removed by centrifuging. The clear red fluid is cooled to $0^{\circ}C$. This solution was permitted to stand at $5.0^{\circ}C$. for 24 hours.

The crystals were filtered at $0^{\circ}C$. and redissolved in 100 ml. of water. These were recrystallized as before and dried in vacuo over sulfuric acid.

The use of potassium ferricyanide was precluded since the resulting ferrocyanide ions - which cannot be dialysed (11) - would be present in an undetermi-

nable amounts. This would invalidate any spectrophotometric determinations.

Drabkin and Austin (25) reported that dog oxyhemoglobin passes into the ferric state on exposure to air and it was this observation that was utilized in this work.

After five days of exposure to air the dried oxyhemoglobin crystals assumed a brown color and gave the absorption spectrum as shown in Figure I.

The bands at 500 mμ, 540 mμ, 580 mμ, and 630 mμ are typical for dog methemoglobin. Furthermore, the addition of dry potassium cyanide turned the solution of these crystals, a deep red, a fact giving a further indication of methemoglobin (26). The dried methemoglobin was stored thereafter in the refrigerator.

2. Materials and Apparatus.

The sugars used in these inhibition experiments were Pfanstiehl C.P. The sodium salicylate was Merck's C.P. which gave the necessary clear solution after filtration. The absorption spectra and colorimetric determinations were made with a Beckman Quartz Spectrophotometer, Model D.U. The spectrophotometer cells were of 0.999 cm. thickness made of Corex glass. Their structural tolerances were of sufficiently small magni-

tude to make them fall far below the limits of precision of the spectrophotometer itself. Therefore, the cells can be considered in effect "identical" or interchangeable.

3. Procedure.

Solutions of methemoglobin were freshly made for each run in order to prevent any possible error due to changes that the protein might undergo if permitted to remain in solution for any length of time.

These solutions were made up by weighing out 0.1g of methemoglobin and dissolving it in 9.9 ml. of a solution buffered at pH of 7.0. The solution was filtered and used immediately. This filtration prevented the reproducibility of the concentration of methemoglobin. The sodium salicylate made up to 1.0 Molar was also prepared anew for each determination.

The experimental solutions were made in the following manners:

One ml. of the stock solution of methemoglobin was placed in a 10 ml. volumetric flask. To make up a solution of x tenth Molar salicylate and y Molar sugar, x ml. of 1 Molar salicylate were added to this flask together with 5 ml. of 2 y Molar sugar. The whole was made up to 10 ml..

The same procedure was used in making up the controls (the methemoglobin and salicylate without the sugar) as

well as all the blanks. Controls or blanks were solutions containing everything that the pigment containing solutions had with the exception of the pigment itself. Thus, in order to obtain the absorption spectrum of methemoglobin itself, the blank solution contained water and buffer in concentrations similar to those of the buffered methemoglobin solution. To obtain the spectra of methemoglobin in salicylate solution the blank contained the buffer and salicylate in the same concentrations as those of the methemoglobin solutions. Similarly, the inhibited solutions required blanks to be made up with the proper concentrations of sugar salicylate and buffer. These control blanks permitted compensation for absorption that might be due to the solvent and substances other than the pigment. These precautions were necessary because these substances although colorless, do absorb some visible light in amounts that vary with wave length.

Determinations were made of the protective properties of the following sugars:

D(+)glucose, D(+)galactose, D(-)fructose, D(-)ribose. The concentrations of sugars were 1 Molar, 2 Molar, and 80% saturation for glucose, fructose, and galactose. Ribose was studied only at one Molar concentrations.

The concentration of the salicylate used was 0.2 and 0.3 Molar. Higher concentrations, with more complete

denaturation of the methemoglobin were avoided since the original investigators found that colorimetric determinations were less accurate at higher concentrations of salicylate (18).

Duplicate determinations were made throughout the experimental work.

Calculation of denaturation:

The extent of denaturation in both the methemoglobin - salicylate solutions and the sugar-methemoglobin-salicylate solutions were made on the basis of absorption in the green specifically at 560 mμ. At complete denaturation the globin ferrihemochromogen absorbs light of 560 mμ 1.6 times as much as does the native protein. This amount was obtained by determining the absorption at 560 mμ with successively higher concentrations of salicylate until there was no further increase in absorption. These results are given in Table I.

Since the optical density is proportional to the concentration of a substance, the following equations hold:

Let R1 be the optical density of the unchanged methemoglobin solution.

Let R2 be the optical density of the solution containing native and denatured methemoglobin.

Let x be the fraction of native methemoglobin undergoing denaturation.

$$\text{Then } \frac{1}{(1-x + 1.60x)} = \frac{R1}{R2}$$

$$\frac{1}{1 + 0.6x} = \frac{R1}{R2}$$

$$1 + 0.6x = \frac{R1}{R2}$$

$$0.6x = \frac{R2}{R1} - 1 = \frac{R2}{R1} - \frac{R1}{R1}$$

$$0.6x = \frac{R2 - R1}{R1}$$

$$x = \frac{R2 - R1}{0.6\ R1}$$

By substitution of the proper colorimetric readings into the last equation the percent denaturation of any dog methemoglobin solution containing salicylate can be determined.

In order to exclude the possibility that salicylate and the sugar interacted the specific rotation of the sugars in the presence of salicylate was observed over a long time interval, Table II. Since there was no change in specific rotation with time, chemical interaction of these substances is not indicated. The initial drop in rotation might be due to the salt effect.

The absorption spectra of methemoglobin in the presence of the sugars but in the absence of salicylate was measured. A time study to determine whether the exposure of the protein pigment to the sugar over a time period affected the protective properties of the sugars was also made.

## EXPERIMENTAL RESULTS AND DISCUSSION

When 1 M. sodium salicylate was dissolved in 2 M. solutions of the sugars, the specific constant rotations did not change over a period of time as shown in Table II. The initial decrease in rotation may be attributed to a salt effect caused by the sodium salicylate. The failure of the solutions of sugar to change their rotations over the time periods, indicates that there is no interaction between the two solutes. This shows that the effects that the sugars may have on the spectra of the blood pigments cannot be attributed to the inactivation by chemical means of the denaturant.

The protective properties of D(+)glucose, D(-)fructose, D(-)ribose, and D(+)galactose are shown in Tables III, IV, V, and VI. The changes in absorption spectra of the native methemoglobin by sodium salicylate and the mitigation of these changes are shown in Figures II-XVI.

The absorption spectra show that in all cases the sugars inhibit the absorption in the green and also cross over to inhibit the transmittance in the red at 630 mμ.

The quantitative determinations - summarized in Tables III, IV, V, and VI show that the order of protective capacis: glucose, galactose, ribose, and fructose.

Furthermore, for a given concentration of salicylate

inhibition increases with concentration of sugar whereas, for a given concentration of sugar denaturation increases with concentration of denaturant.

Table VII shows that the inhibition does not vary with the time that the sugar was exposed to the protein.

Table VIII shows that the exposure of the sugar to the blood pigment does not alter its spectral properties.

The foregoing results indicate that there are no indications of chemical combination of the native methemoglobin with the sugar.

The data showing no change in the absorption spectra of methemoglobin after exposure to the sugars as well as the data showing that inhibition of the denaturation did not vary with time of exposure of the protein to the sugar are sufficiently positive indications that the mode of protection does not involve the initial formation of a sugar-methemoglobin. Hardt, Huddleson and Ball (8) in their work with bovine plasma report that, "ageing in the presence of d-glucose did not alter the electrophoretic pattern of the bovine plasma". Levy (20) aged D(+)glucose in the presence of methemoglobin and also reported no change in the protein.

However, a consideration of the absorption curves appended to this work, gives a strong indication of the mode of action of the sugars. If the inhibition involved a mere shifting of the equilibrium, Native Protein $\rightleftharpoons$ Denatured

Protein, to the left all the ordinates of the curve showing inhibition would lie part way between the ordinates of native proteins curve and those of the denatured protein. However, we find that this is not always the case and that there are points which can be called aberrational which lie either above the other two curves or below them. These unique points show that another compound is present and, therefore, the following hypothesis is in order. Anson and Mirsky (18) have maintained that the process of denaturation involves a number of activated intermediates. In the present work, there is a strong possibility that these intermediary reactive compounds have combined with the sugars to form new compounds which have driven the aforementioned equilibrium to the left and as such have indicated inhibition.

This hypothesis suggests a possible explanation of the mechanism for the formation of glyco proteins - namely, that whatever enzyme or a complex of enzymes are involved in the process there is a partial modification of the protein which makes it more susceptible to linkage with sugars. This modification - which can occur during the enzyme - substrate combination - can have a qualitative similarity to partial denaturation.

While the work reported above merely suggests such a possibility, there are sufficient positive factors to justify further investigation.

TABLE I

Data Showing the Increase in Absorption at 560 mμ of Mhb with Concentration of Sodium Salicylate.

| Molarity of Sodium Salicylate | Optical Density at 560 mμ |
|---|---|
| 0.0 | .2236 |
| 0.2 | .2825 |
| 0.3 | .3120 |
| 0.4 | .3300 |
| 0.5 | .3600 |
| 0.6 | .3600 |

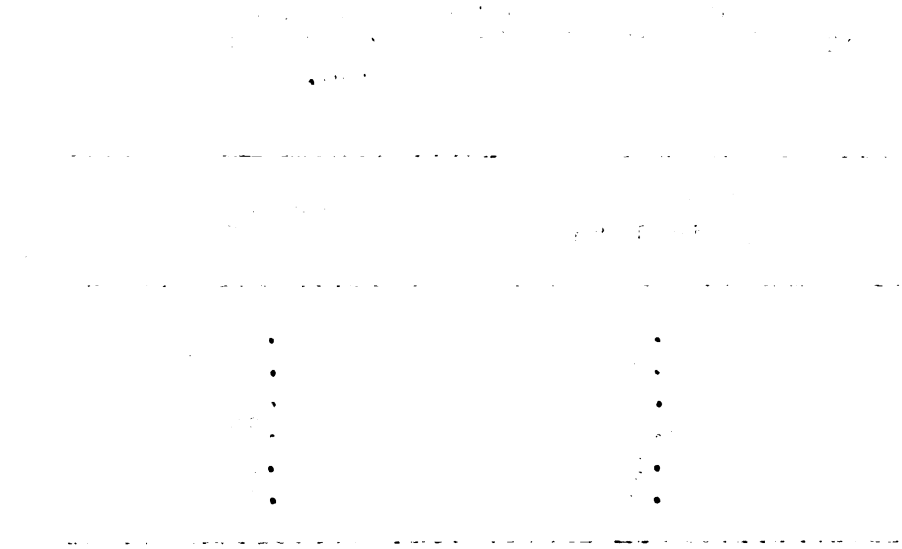


TABLE II

Specific Rotation of Sugars at 2 M. Concentration in the Presence of 0.3 M. Sodium Salicylate.

| Sugar | Constant Specific Rotation of Sugar | Time (hours) of Contact of Sugar with Salicylate | Constant Specific Rotation |
|---|---|---|---|
| D(+)Glucose | + 52.2 | 0 | + 51.8 |
| | + 52.2 | 1 | + 51.8 |
| | + 52.2 | 2 | + 51.8 |
| | + 52.2 | 3 | + 51.8 |
| | + 52.2 | 4 | + 51.8 |
| | + 52.2 | 5 | + 51.8 |
| | + 52.2 | 24 | + 51.8 |
| D(+)Galactose | + 80.5 | 0 | + 79.5 |
| | + 80.5 | 1 | + 79.5 |
| | + 80.5 | 2 | + 79.5 |
| | + 80.5 | 3 | + 79.5 |
| | + 80.5 | 4 | + 79.5 |
| | + 80.5 | 5 | + 79.5 |
| | + 80.5 | 24 | + 79.5 |
| D(-)Ribose | - 23.7 | 0 | - 22.1 |
| | - 23.7 | 1 | - 22.1 |
| | - 23.7 | 2 | - 22.1 |
| | - 23.7 | 3 | - 22.1 |
| | - 23.7 | 4 | - 22.1 |
| | - 23.7 | 5 | - 22.1 |
| | - 23.7 | 24 | - 22.1 |
| D(-)Fructose | - 92.2 | 0 | - 89.6 |
| | - 92.2 | 1 | - 89.6 |
| | - 92.2 | 2 | - 89.6 |
| | - 92.2 | 3 | - 89.6 |
| | - 92.2 | 4 | - 89.6 |
| | - 92.2 | 5 | - 89.6 |
| | - 92.2 | 24 | - 89.6 |

TABLE VI

Data Showing Inhibition Properties of D(-)Ribose

| Concentration of Denaturant Molarity | Concentration of Inhibiting Agent Molarity | Optical Density of Mhb | Optical Density of Mhb + Salicylate | Optical Density of Mhb + Salicylate + Sugar | %Denaturation | %Inhibition |
|---|---|---|---|---|---|---|
| 0.2 | - | .251 | .313 | - | 41.3 | - |
| 0.2 | 1.0 | .251 | - | .290 | 26.0 | 37.1 |
| 0.3 | - | .248 | .350 | - | 68.7 | - |
| 0.3 | 1.0 | .248 | - | .329 | 48.3 | 29.9 |

TABLE VII

Data Showing Inhibiting Effects of Ageing Methemoglobin in Presence of Sugars.
Concentration of Sugar 1 M.
Concentration of Sodium Salicylate .2 M.

| Time of Contact of Sugar with Mhb (hours) | %Inhibition Due to Glucose | %Inhibition Due to Galactose | %Inhibition Due to Fructose | %Inhibition Due to Ribose |
|---|---|---|---|---|
| 0 | 47.6 | 47.2 | 23.9 | 36.6 |
| 1 | 47.7 | 47.3 | 23.9 | 36.8 |
| 2 | 47.7 | 47.3 | 24.0 | 36.8 |
| 6 | 47.5 | 47.3 | 23.9 | 36.8 |

## TABLE VIII

The Effect of Sugar on the Absorption Spectra of Methemoglobin after Exposure of the Protein to the Sugar for 6 hours.

| Wave Length mμ | %Trans-mittance Mhb | %Trans-mittance Mhb + 3 M. D(+)Glucose | %Trans-mittance Mhb + 3 M. D(-)Fructose | %Trans-mittance Mhb + 3 M. D(+)Galactose | %Trans-mittance Mhb + 3 M. D(-)Ribose |
|---|---|---|---|---|---|
| 440 | 10.5 | 10.4 | 10.5 | 10.6 | 10.6 |
| 450 | 21.0 | 21.3 | 21.2 | 21.3 | 21.2 |
| 460 | 27.9 | 27.9 | 27.8 | 27.8 | 27.8 |
| 470 | 33.0 | 33.2 | 33.1 | 33.1 | 33.2 |
| 480 | 36.5 | 36.4 | 36.5 | 36.5 | 36.5 |
| 490 | 35.8 | 35.5 | 35.5 | 35.7 | 35.6 |
| 500 | 35.4 | 35.3 | 35.2 | 35.3 | 35.3 |
| 510 | 36.2 | 36.3 | 36.2 | 36.2 | 36.3 |
| 520 | 37.8 | 37.5 | 37.8 | 37.8 | 37.8 |
| 530 | 36.5 | 36.5 | 36.5 | 36.4 | 36.4 |
| 540 | 33.2 | 33.2 | 33.1 | 33.2 | 33.2 |
| 550 | 38.5 | 38.3 | 38.3 | 38.3 | 38.3 |
| 560 | 46.5 | 46.5 | 46.5 | 46.5 | 46.5 |
| 570 | 44.5 | 44.4 | 44.4 | 44.4 | 44.5 |
| 580 | 40.5 | 40.3 | 40.5 | 40.5 | 40.4 |
| 590 | 51.5 | 51.4 | 51.4 | 51.5 | 51.6 |
| 600 | 64.5 | 64.4 | 64.3 | 64.5 | 64.5 |
| 610 | 66.5 | 66.5 | 66.5 | 66.5 | 66.5 |
| 620 | 66.4 | 66.3 | 66.4 | 66.4 | 66.4 |
| 630 | 65.5 | 65.4 | 65.4 | 65.4 | 65.4 |
| 640 | 67.5 | 67.4 | 67.4 | 67.4 | 67.3 |
| 650 | 75.0 | 75.0 | 75.1 | 75.2 | 75.1 |
| 660 | 83.5 | 83.5 | 83.4 | 83.5 | 83.5 |
| 670 | 87.0 | 87.1 | 87.2 | 81.0 | 87.0 |
| 680 | 90.0 | 90.1 | 90.3 | 90.0 | 90.0 |
| 690 | 90.5 | 90.5 | 90.5 | 90.4 | 90.6 |
| 700 | 91.0 | 92.0 | 91.0 | 91.8 | 91.8 |

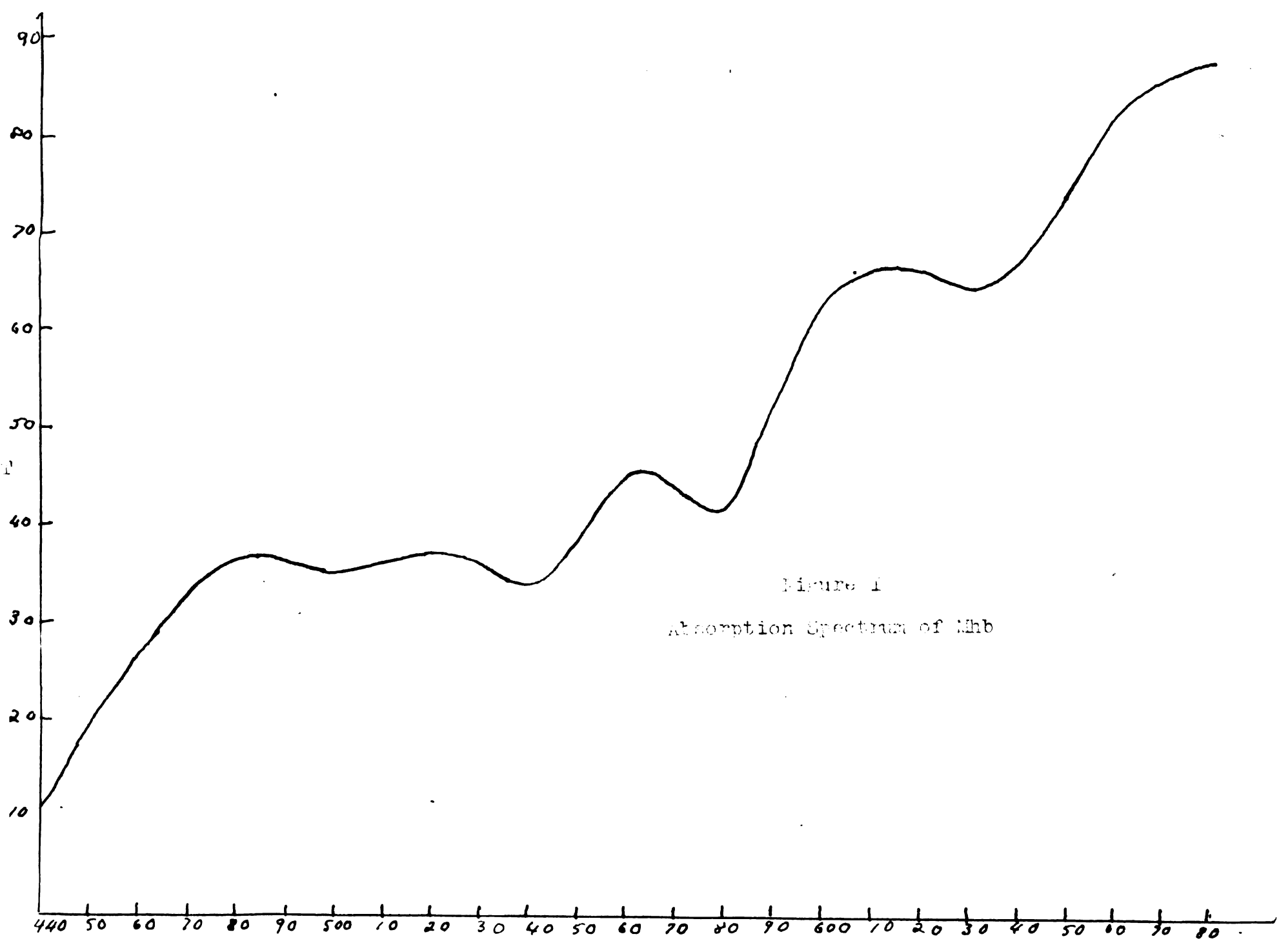



Figure 1

Absorption Spectrum of Mhb

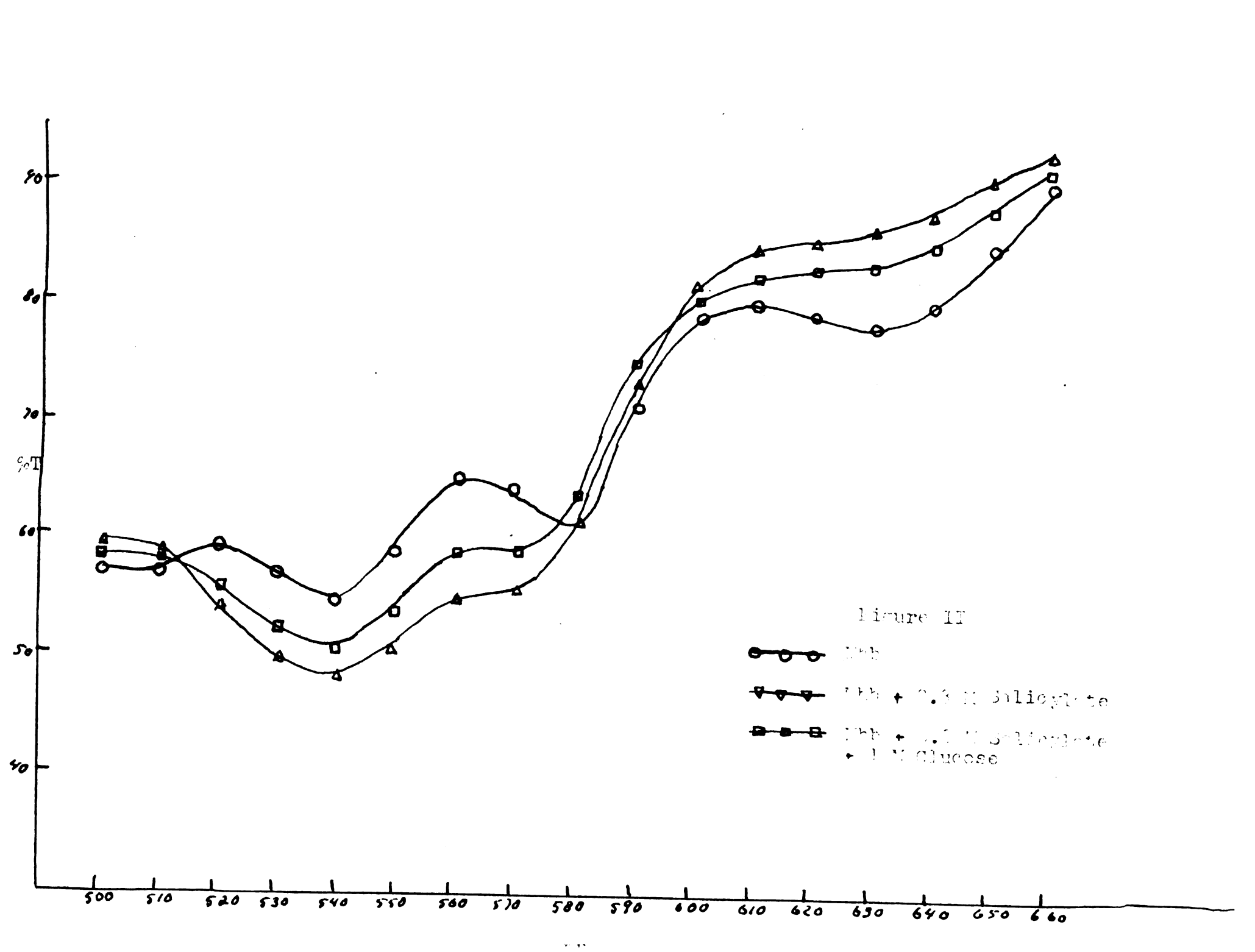
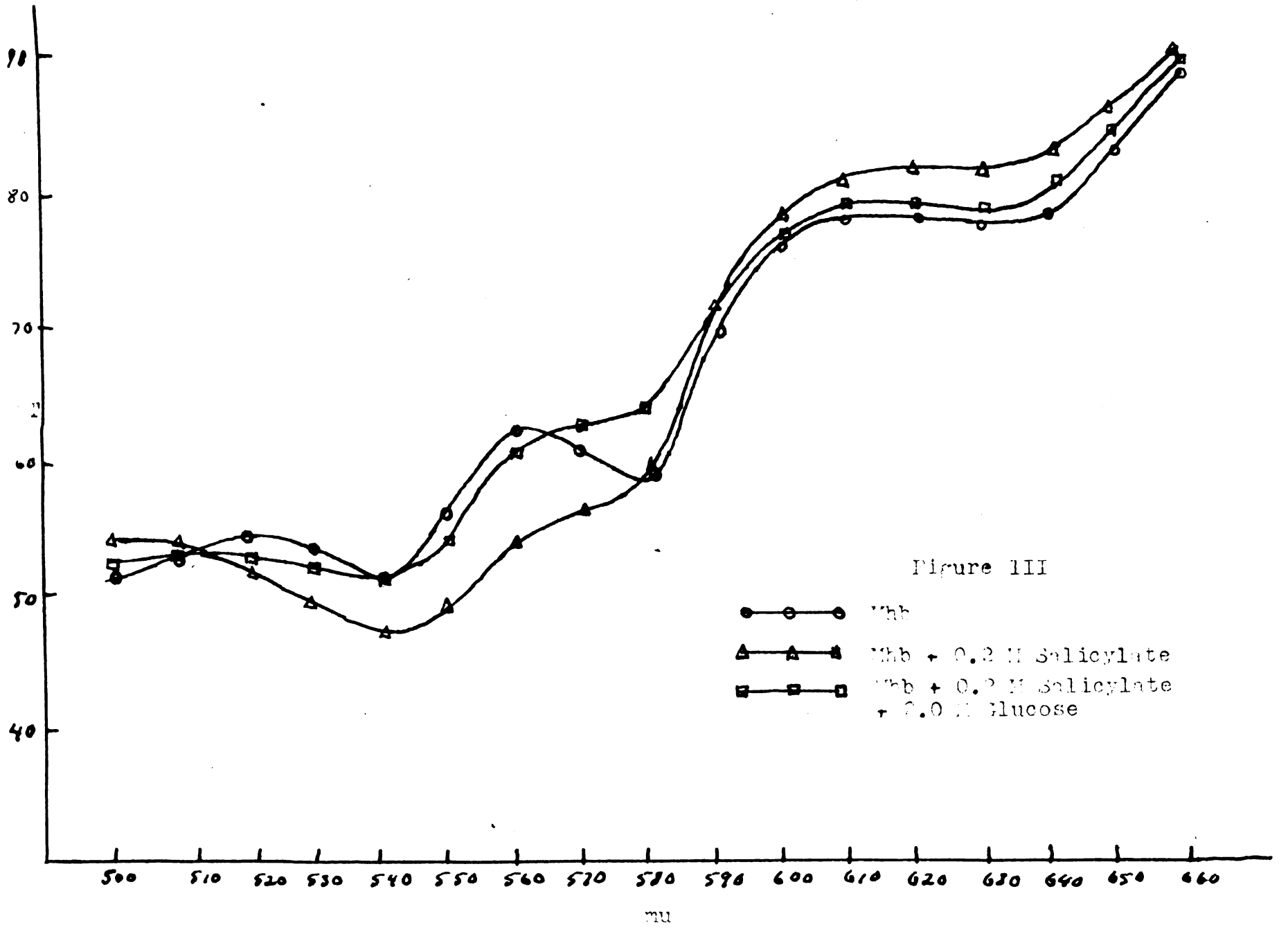
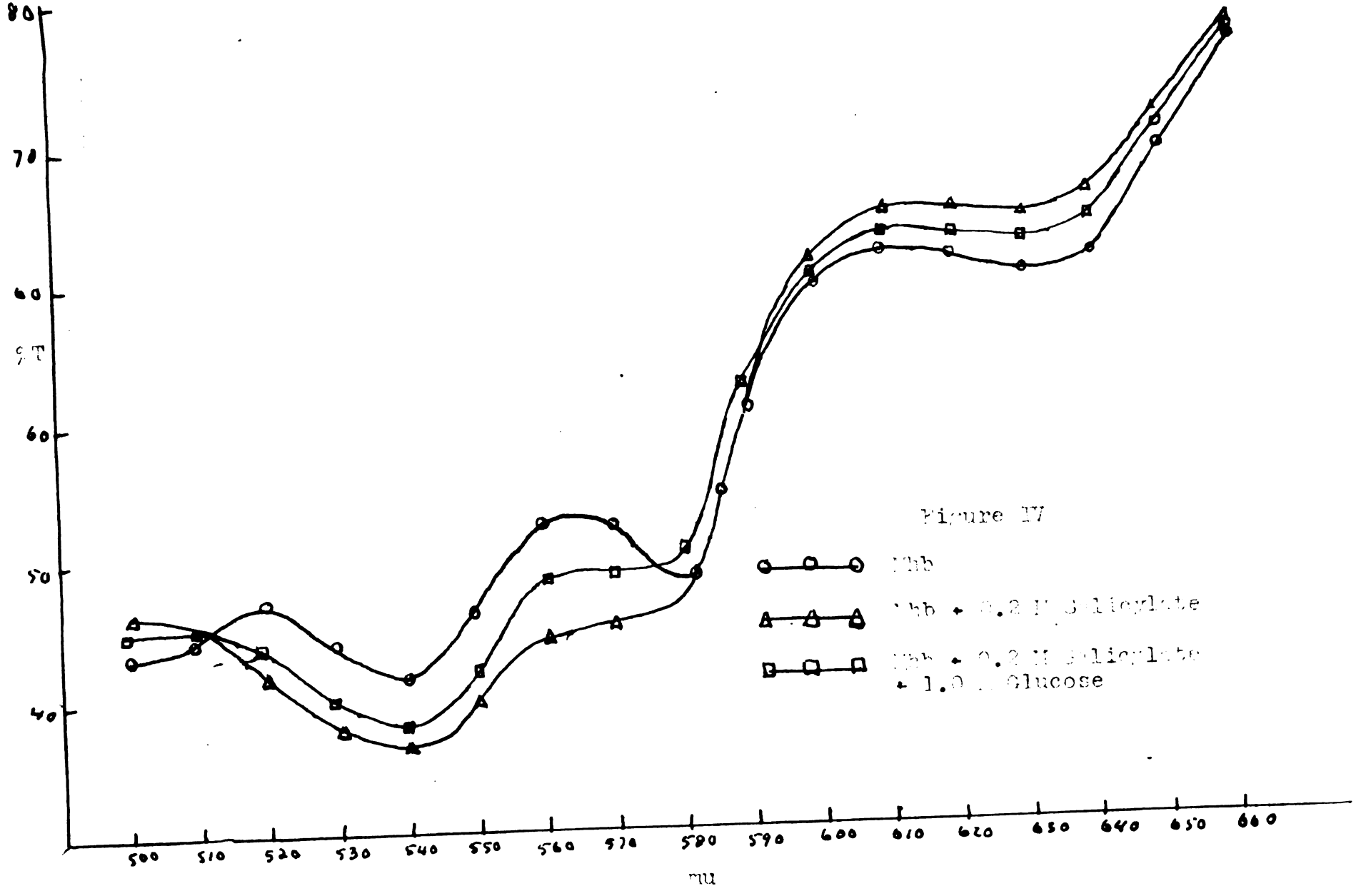

90
80
70
%T
60
50
40
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660

Figure II


90
80
70
60
50
40
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
mu
Figure III
Hb
Hb + 0.2 M Salicylate
Hb + 0.2 M Salicylate
+ 0.0 M Glucose


80
70
60
%T
50
40
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
mu
Figure IV
Hb
Hb + 0.2 M Salicylate
Hb + 0.2 M Salicylate
+ 1.0 M Glucose

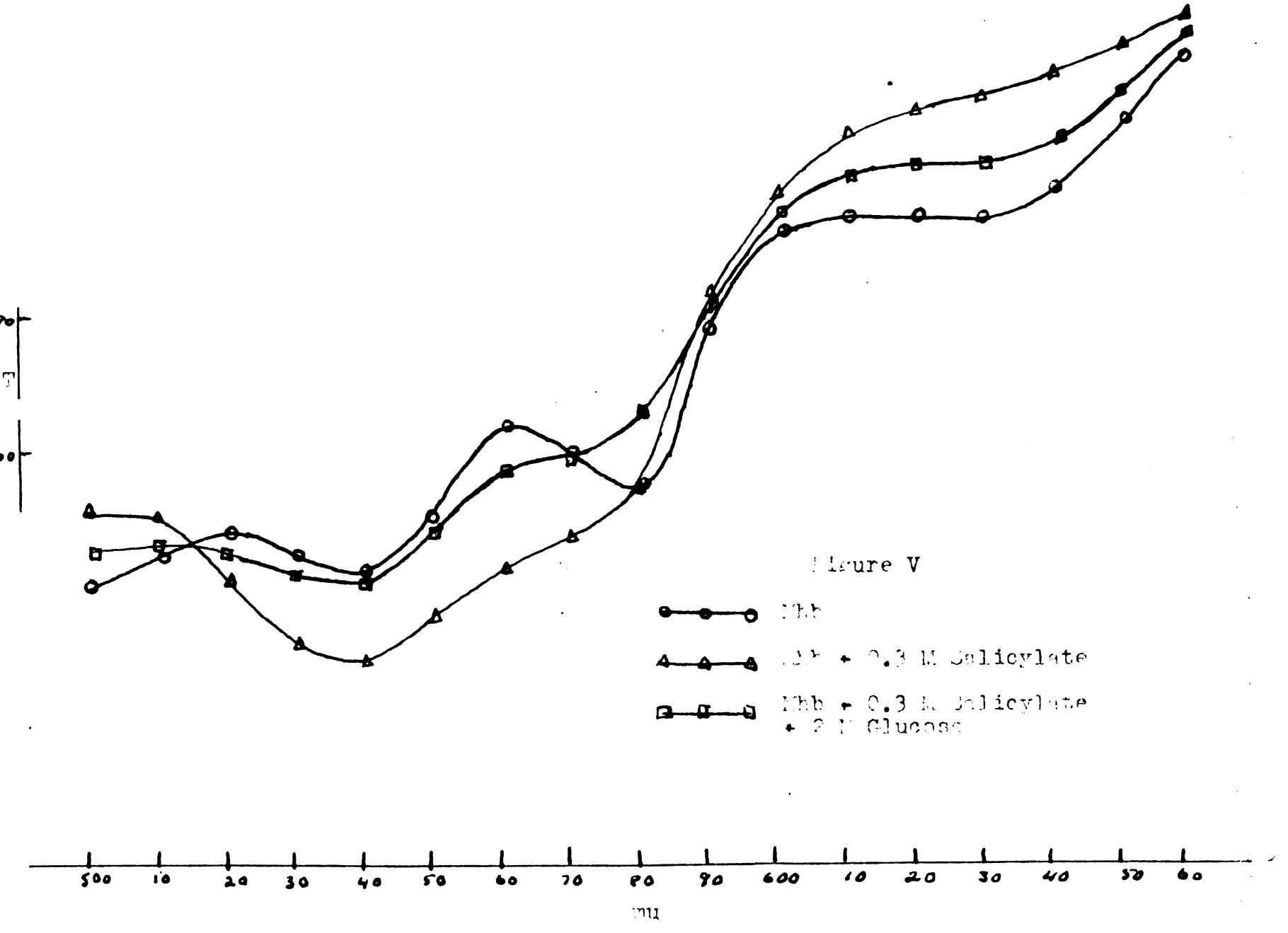



Figure V

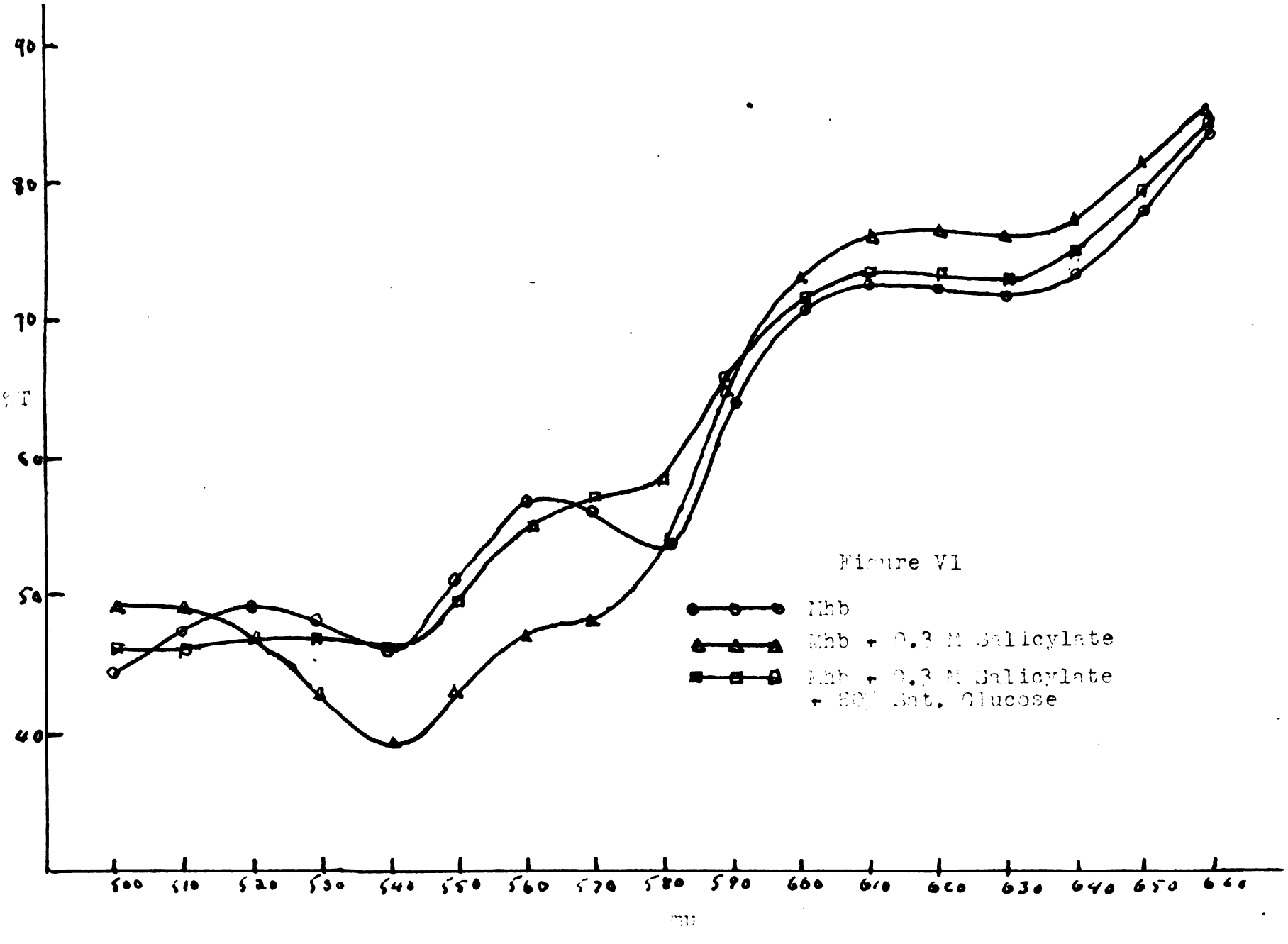

90
80
70
60
50
40
%T
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
mμ
Mhb
Mhb + 0.3 M Salicylate
Mhb + 0.3 M Salicylate
+ 80% Int. Glucose

Figure VI

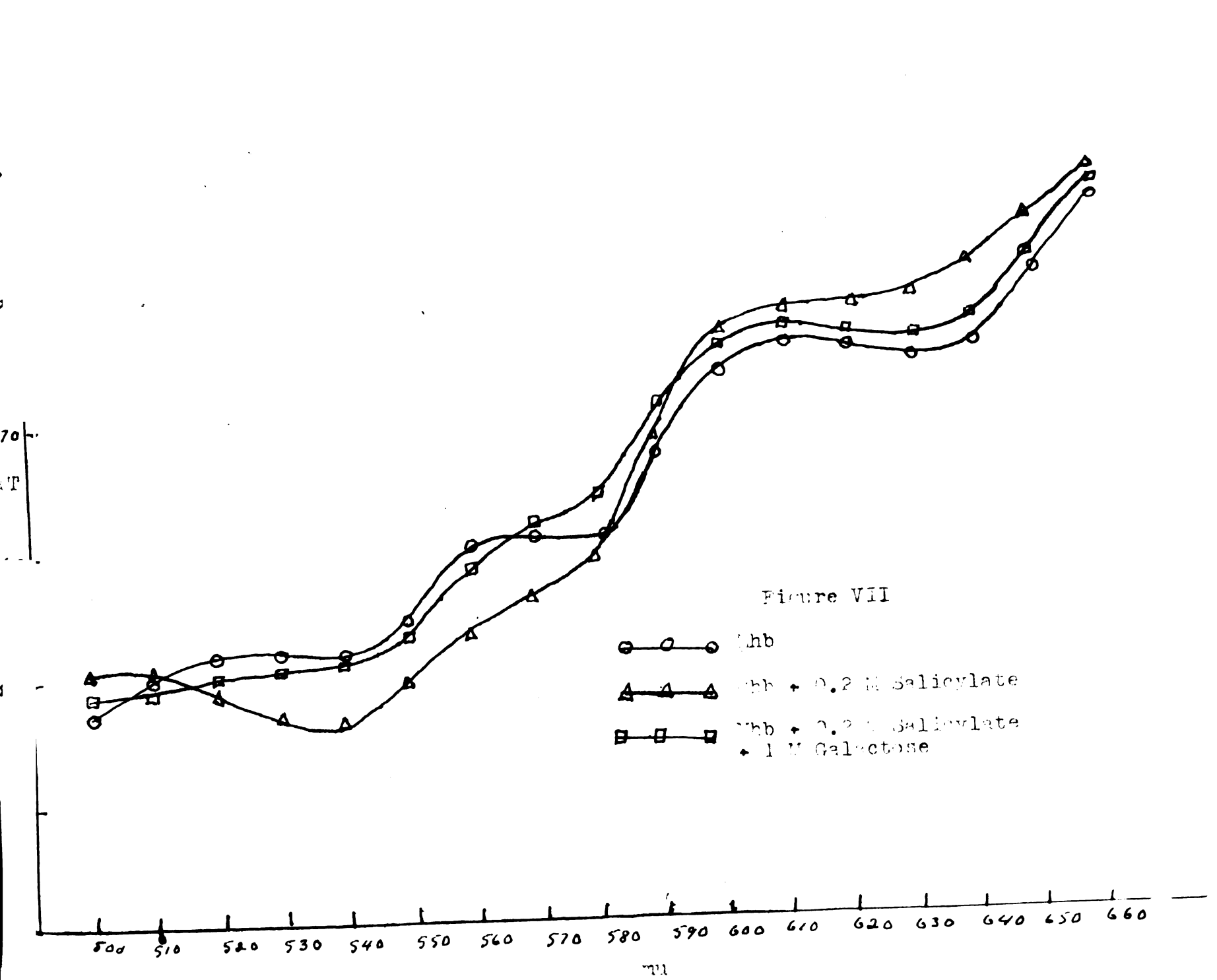

70
T
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
mμ

Figure VII

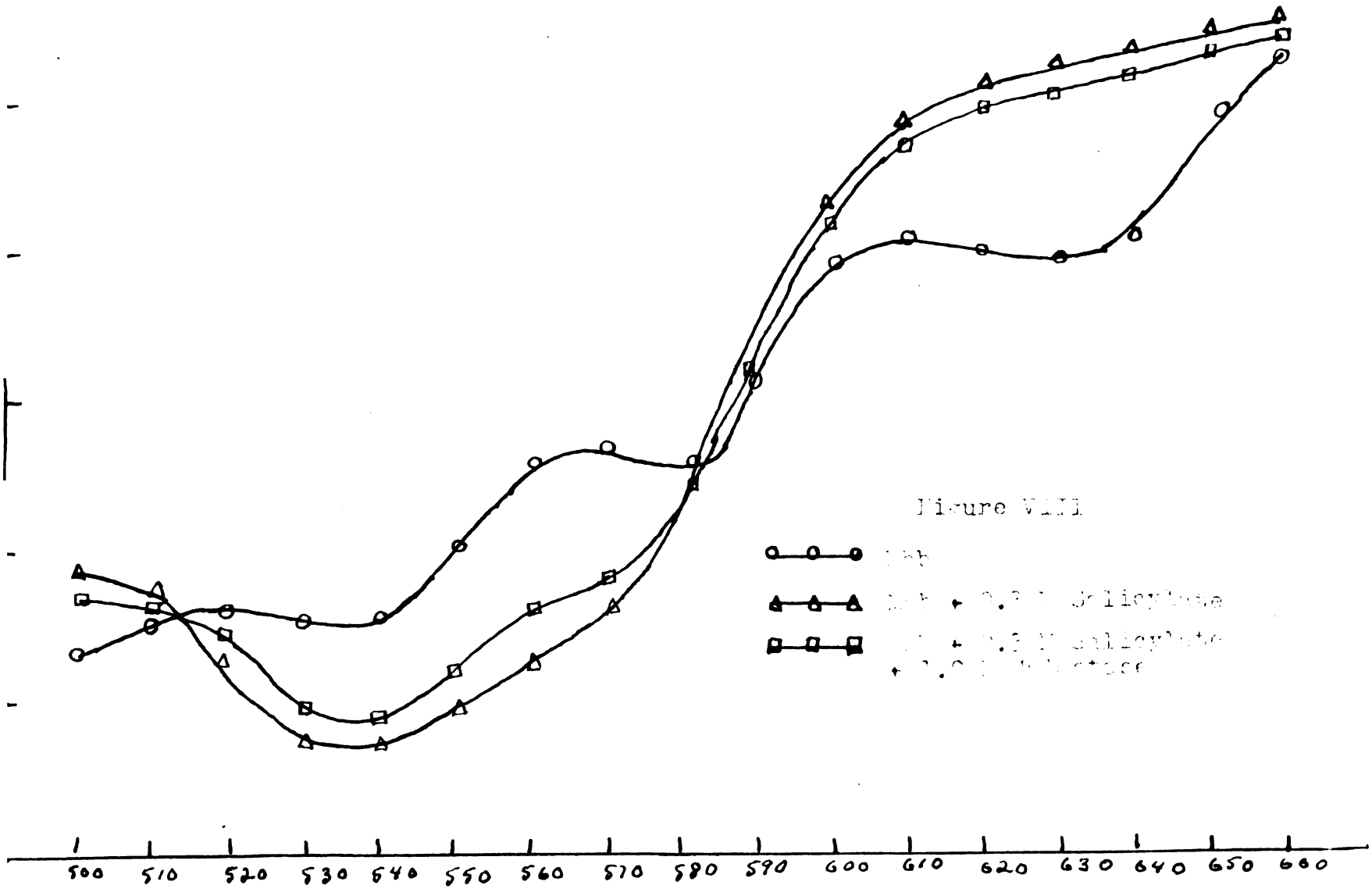

500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660

Figure VIII

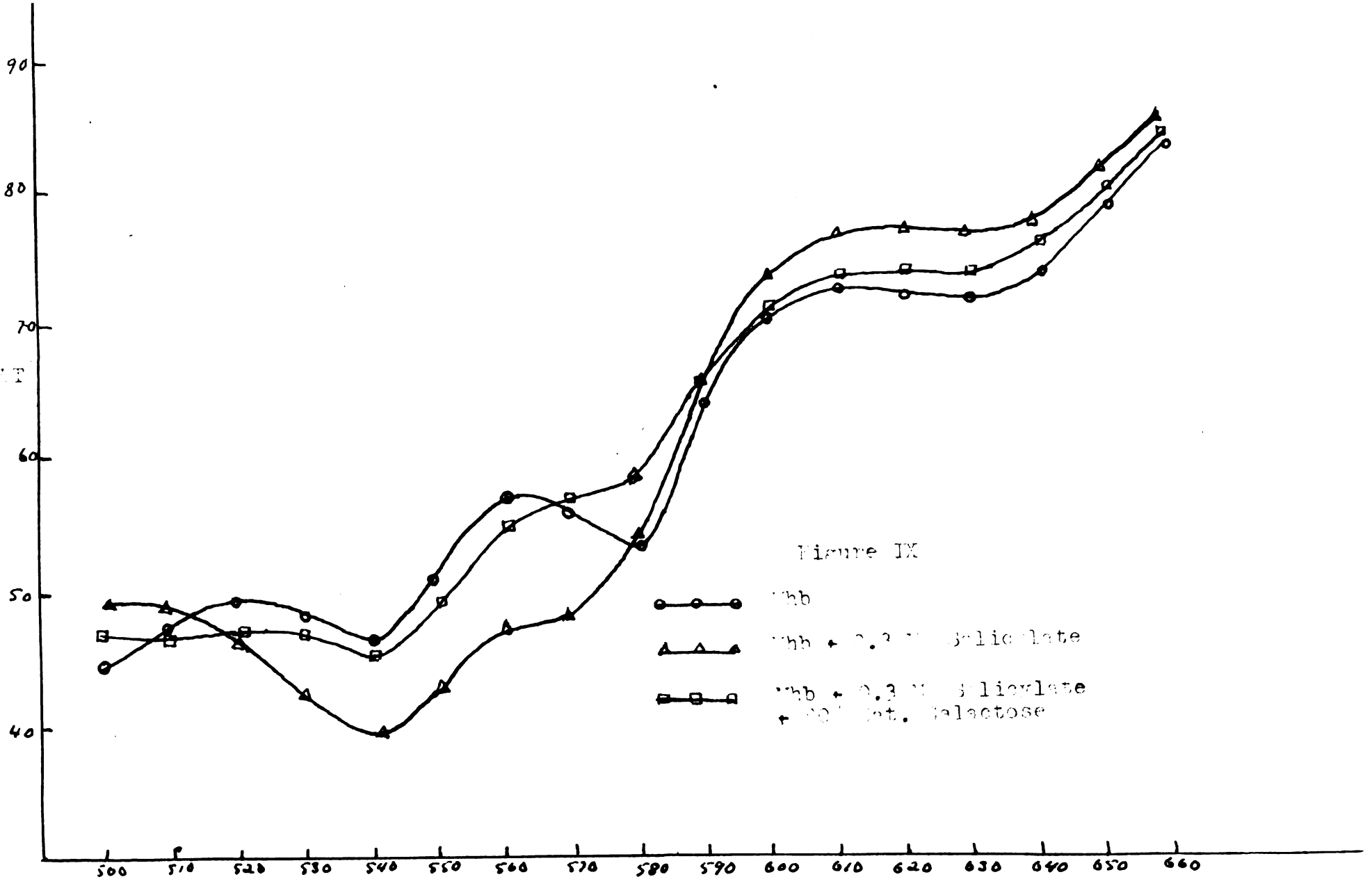

90
80
70
60
50
40
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660

Figure IX

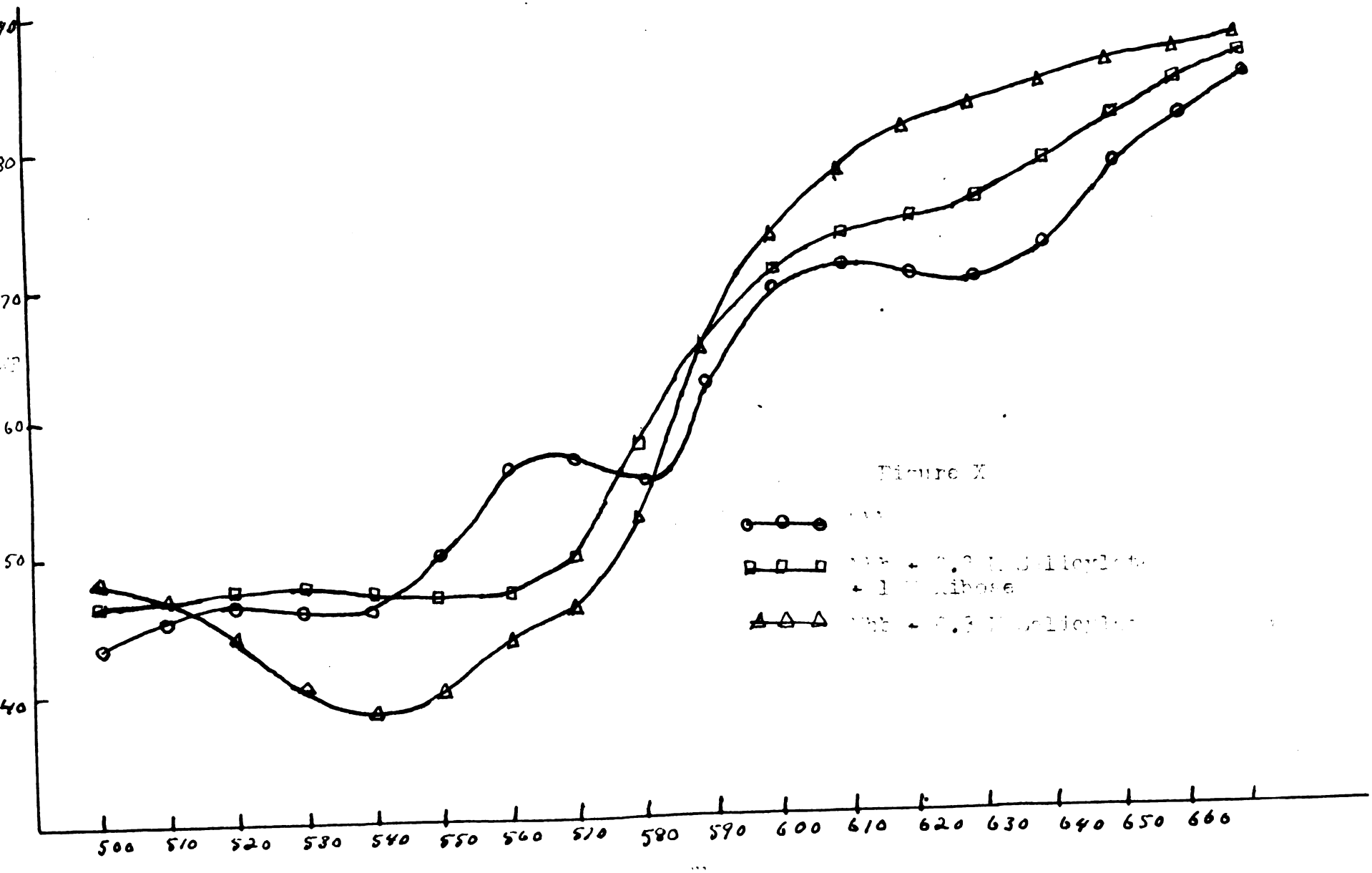

80
70
60
50
40
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660

Figure X

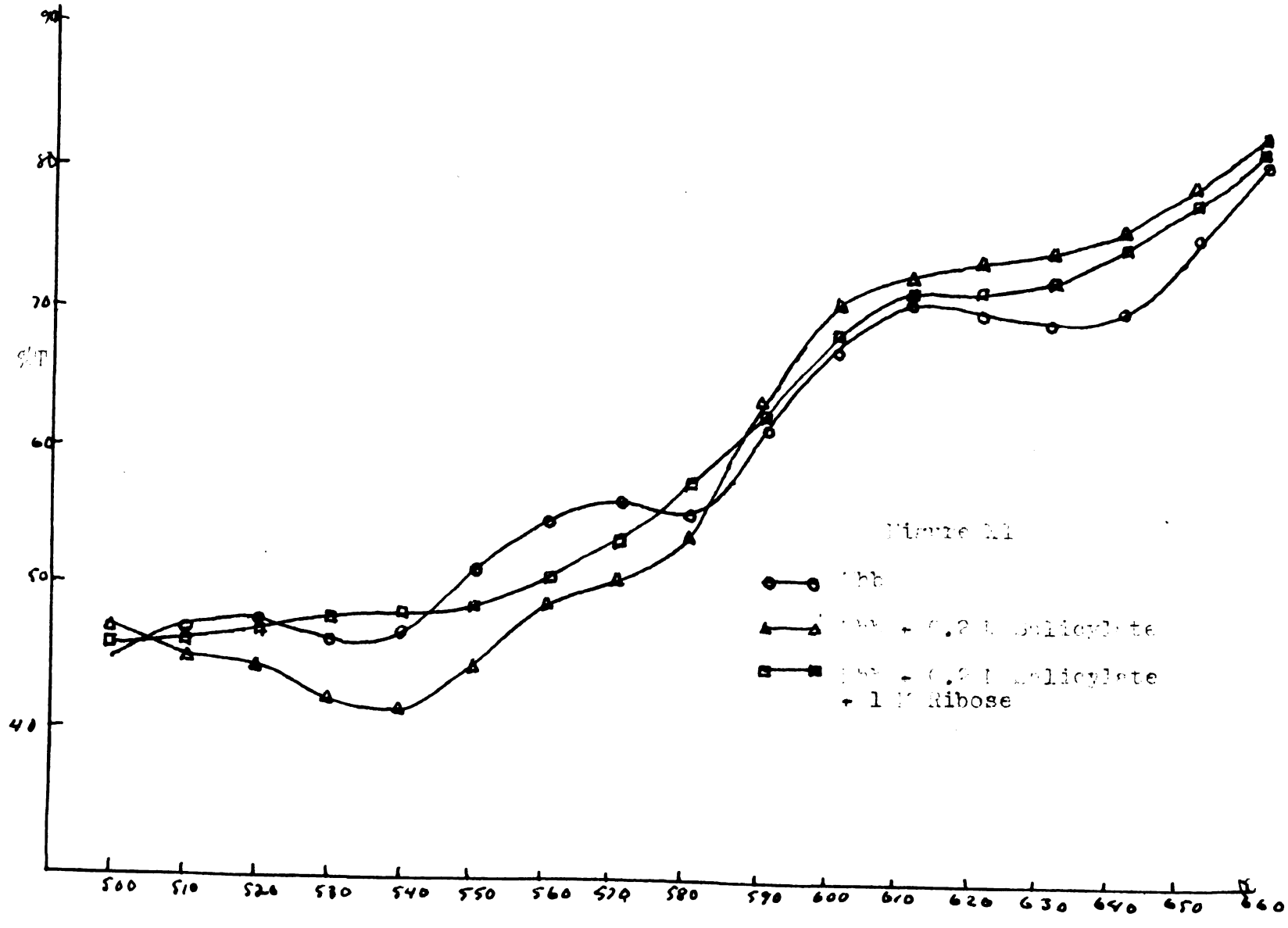

90
80
70
%T
60
50
40
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
nm

Figure 11

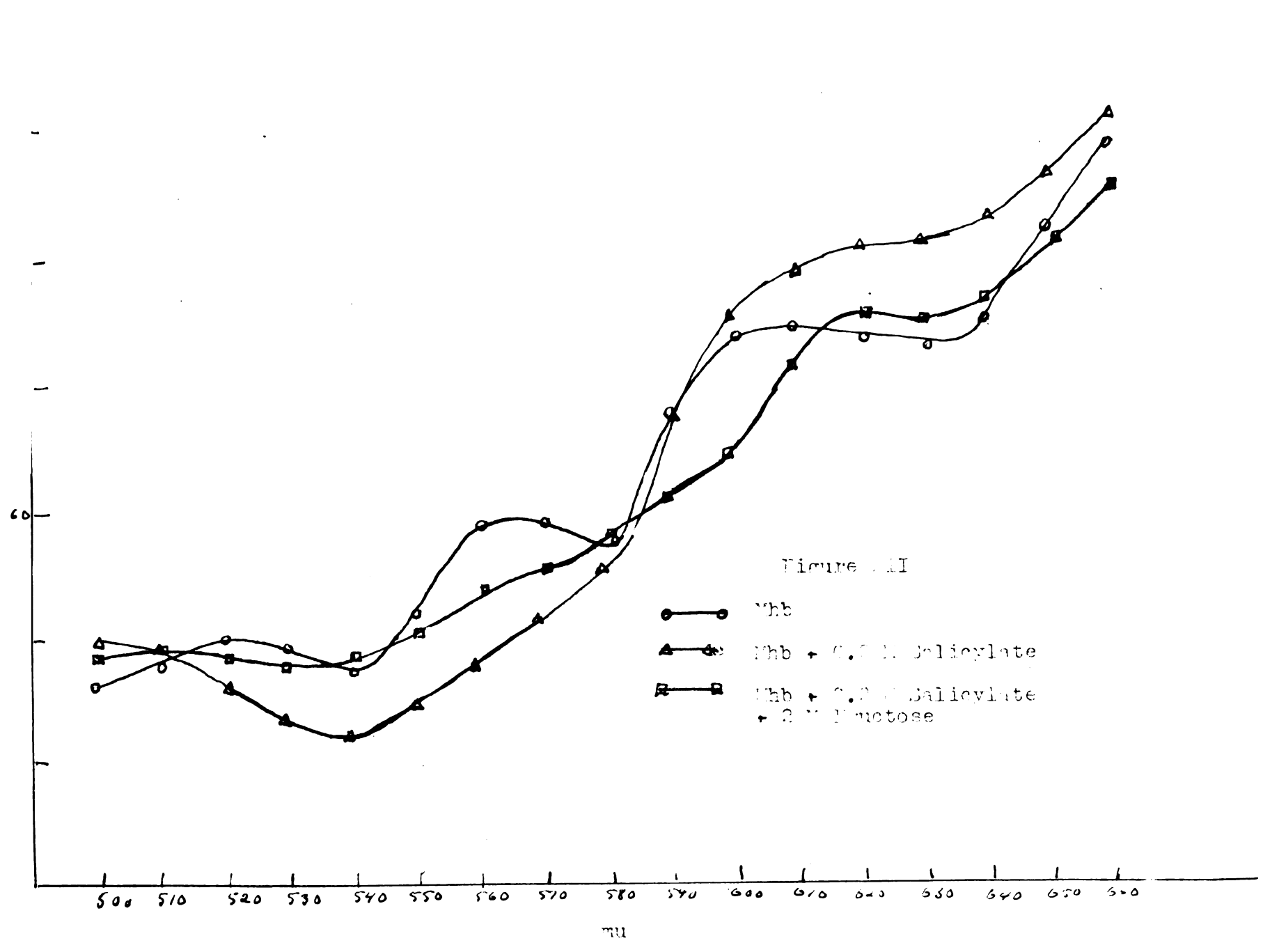
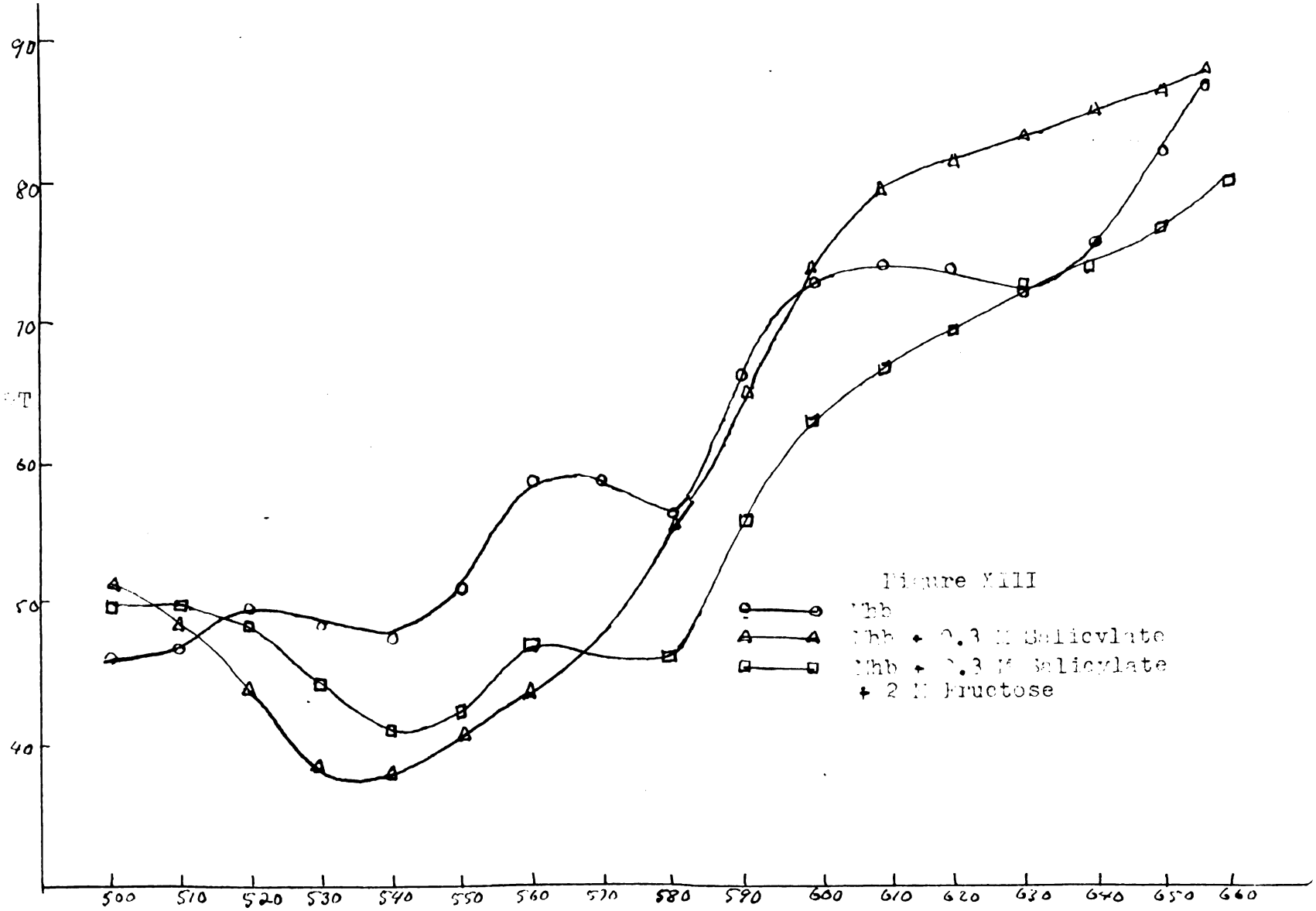

60
500 510 520 530 540 550 560 570 580 590 600 610 620 630 640 650 660
mμ
Hhb
Hhb + C.[illegible] M Salicylate
Hhb + C.[illegible] M Salicylate
+ 2 M Fructose

Figure [illegible]I


90
80
70
60
50
40
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
Figure XIII
Hbb
Hbb + 0.3 M Salicylate
Hbb + 0.3 M Salicylate
+ 2 M Fructose

## CONCLUSIONS

1. Sodium salicylate - a chemical denaturant of methemoglobin - does not react chemically with D(+) glucose, D (+) galactose, D(-) ribose, D(-) fructose.

2. These sugars inhibit the denaturation of methemoglobin by sodium salicylate.

3. These same sugars do not combine chemically with the native protein.

4. The order of protective capacity of the sugars studied is: D(+) glucose, D(+) galactose, D(-) ribose, D(-) fructose.

5. There are indications that the above sugars combined with an activated intermediate of the methemoglobin.

BIBLIOGRAPHY

1. Stillman, H. The Story of Early Chemistry. D. Appelton and Company, New York. (1924).

2. Pauling, L. Coryell, C.D. Magnetic properties and structure of hemoglobin oxyhemoglobin and carbonmonoxyhemoglobin. Proc. Nat'l Acad. Sci. 22, 210, (1936).

3. Neurath, H., Greenstein, J.P., Putnam, F.W., and Erikson, J.O. The chemistry of protein denaturation. Chem. Reviews 34, 157 (1944).

4. Beilinnson, A. Thermostabilisation der Eiweisslosungen mit Rohrzucker und Glycerin. Biochem. Z 213, 399, (1929).

5. Duddles, W. J. The effect of sugars and mannitol on the coagulation of egg albumin. Thesis for the degree of M.S., Michigan State College, East Lansing, Michigan. (1932).

6. Newton, Brown. Frost precipitation of proteins of plant juice. Can. J. Research. 5, 87, (1931).

7. Ball, C. D., Hardt, C. K., and Duddles, W.J. The influence of sugars on the formation of sulfhydryl groups in heat denaturation and heat coagulation of egg albumin. J. Biol. Chem. 151, 163, (1943).

8. Hardt, C.R., Huddleson, F., and Ball, C.D. An electrophoretic analysis of changes produced in blood serum and plasma proteins by heat in the presence of sugars. J. Biol. Chem. 163, 211, (1946).

9. Conant, J. B., Pappenheimer, A.M. A redetermination of the oxidation potential of the hemoglobin-methemoglobin system. J. Biol. Chem. 98, 57, (1932).

10. Stadie, W. D. Studies on blood changes in pneumococcus infections. J. Exp. Med. 33, 627, (1921).

11. Barnard, R. D. Pharmaceutic utilization of materials derived from erythrocytes. J. Am. Pharm. Assoc. 34, 177, (1945).

12. Paul, W. D., Kemp, C. R. Methemoglobin a normal constituent of blood. Proc. Soc. Exp. Biol. Med. 56, 55, (1944).

13. Zeynek, R. Von. Uber krystallisirtes cyanhamoglobin. Z. Physiol. Chem. 33, 426, (1931).

14. Keilin, D. Combination fo methemoglobin with hydrogen sulfide. Proc. Roy. Soc. (London) B113, 393, (1933).

15. Keilin, D., Hartree, E.F. Combination of methemoglobin with peroxides. Proc. Roy. Soc. (London) B 117, 1, (1935).

16. Ville, Derrien. Modification du spectre de la methemoglobine sous l'action du fluorure de sodium. Compt. Rend. 140, 743, (1905).

17. Barnard, R. D. The reactions of nitrite with hemoglobin derivitives. J. Biol. Chem. 120, 177, (1937).

18. Anson, M. L., Mirsky, A. E. The equilibria between native and denatured hemoglobin in salicylate solutions and the theoretical consequences of the equilibrium between native and denatured protein. J. of Gen. Physiol. 17, 399, (1933).

19. Keilin, D. Comparitive study of turicin and hematin and its bearing on cytochrome. Proc. Roy. Soc. (London). B100, 129, (1926).

20. Levy, M. The preparation and some properties of the crystalline methemoglobin of the horse. J. Biol. Chem. 89, 173, (1930).

21. Conant, J. B. The Oxidation of Hemoglobin and Other Respiratory Pigments. The Harvey Lectures. Series 28, p. 159, (1933).

22. Brooks, M. Treatment of methemoglobin in blood by glucose. Proc. Soc. Exp. Biol. Med. 32, 63, (1934).

23. Warburg, O., Christain, W. Uber Aktivierung der Robisonchen Hexose Monophosphoraure in roten Blutzellen und die Gewinnung aktivierender Ferment Losungen. Biochem. Z. 242, 206, (1931).

24. Halliburton, W. D. The Essentials of Chemical Physiology. Longmans Green and Co. London. (1896).

25. Drabkin, D. K., Austin, J. H. Spectrophotometric studies of methemoglobin. J. Biol. Chem. 112, 67, (1935).

26. Michel, H. O., Harris, J. S. The blood pigments. J. Lab. Clin. Med. 25, 445, (1940).