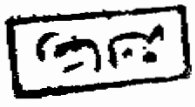
A STUDY ON KING AND ASATOOR METHOD FOR BLOOD GLUCOSE DETERMINATION

A THESIS

Submitted in Partial Fulfillment of
Requirement for the Degree of
M.S. CLINICAL PATHOLOGY



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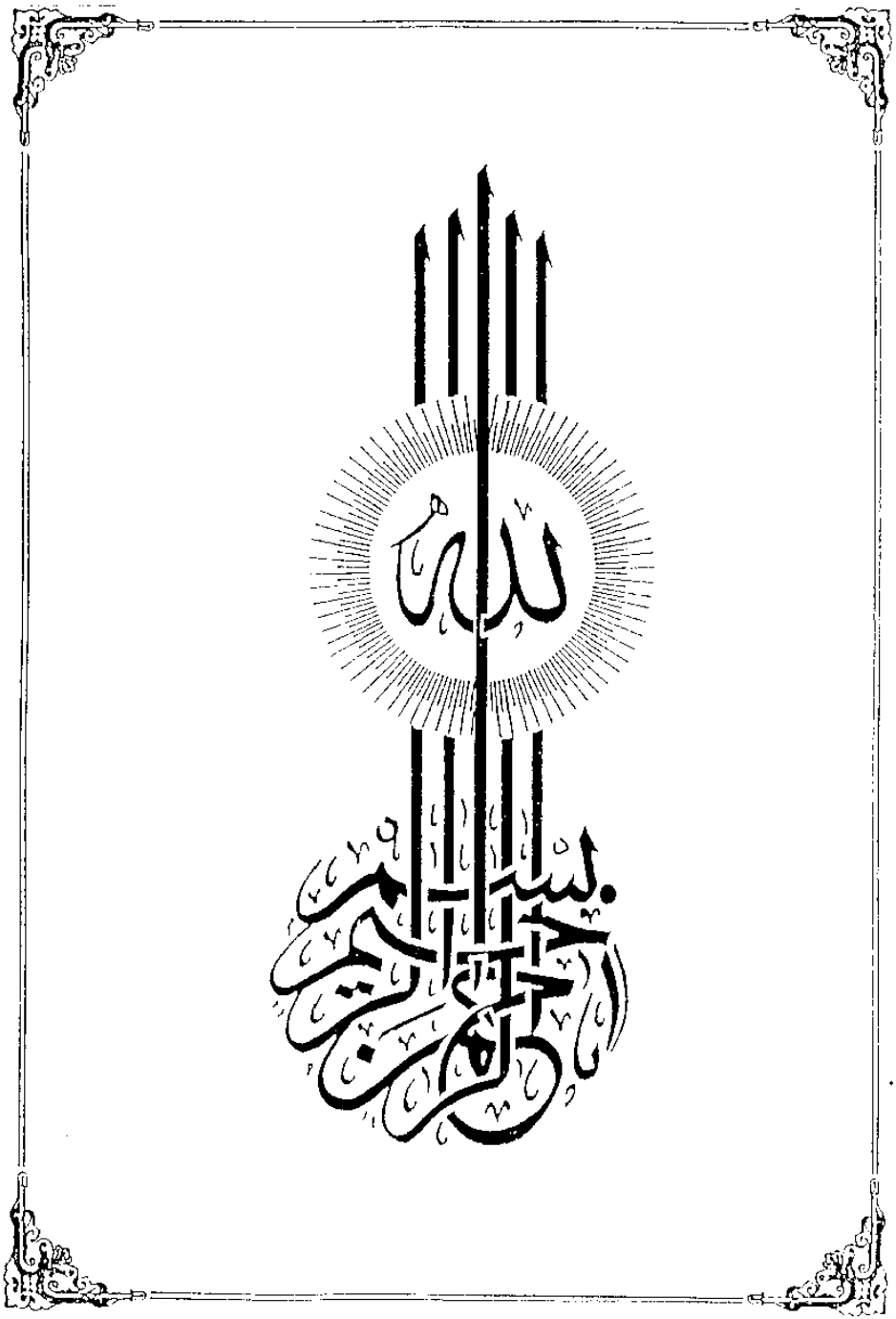
D E D I C A T I O N

THIS STUDY IS TO BE DEDICATED

TO MY FATHER PROF. DR. SALEM YASSIN

AND MY MOTHER DR. RAFAHIA KHALAF,

FOR THEIR GUIDANCE AND BLESSINGS.



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C O N T E N T S

INTRODUCTION AND AIN OF THE WORK

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In spite of the development of specific enzymatic methods for determination of blood glucose, most laboratories are still using the reduction methods. The best of the reduction methods is that of King and Asatoor. It measures true sugar and is very easy and cheap, but it suffers from one defect; the values below 100 mg percent are markedly lowered.

In this work we tried to lessen this lowering by studying the effect of potassium oxalate in Harding solution "B" half strength and in the anti-coagulant used. Precision and accuracy were studied by comparing the results of King and Asatoor method with those of glucose oxidase method.

REVIEW OF LITERATURE

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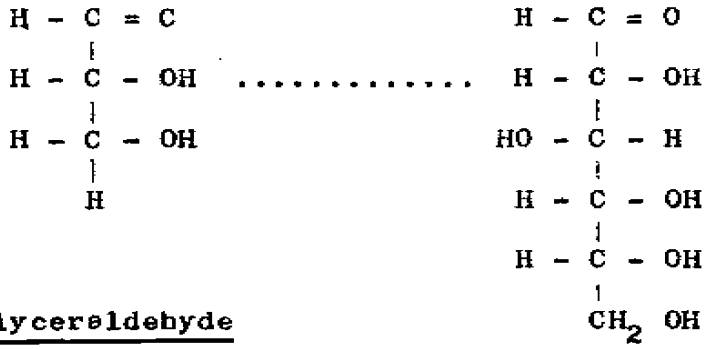
Determination of blood glucose is the procedure most frequently performed in hospital laboratories. It may be performed in the fasting and post prandial as well as other states.

I- Chemistry of Glucose:

Glucose is formed of six carbon atoms. It is an aldose because the first carbon atom is attached to an aldehydic group. Each of the five carbon atoms is attached to a hydroxyl group at one side and a hydrogen atom at the other side.

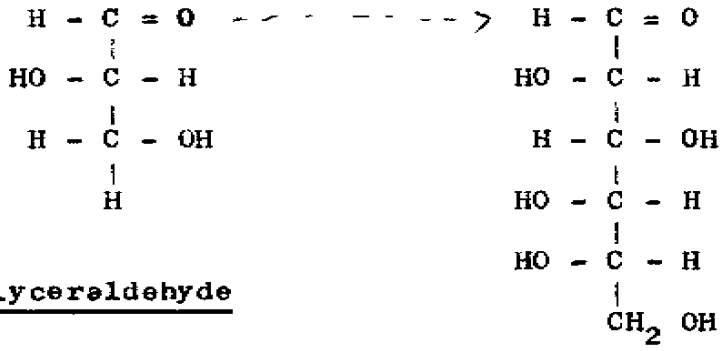
All simple sugars including glucose are regarded as derivatives of either D-glyceraldehyde which rotates the plane of polarized light to the right or L-glyceraldehyde which rotates the plane of polarized light to the left. For this reason glyceraldehyde, which is a triose has been called the reference sugar. This means that glucose with the same configuration as D-glyceraldehyde for the asymmetric carbon atom farthest from the aldehyde group is given the designation D and that which is like L-glyceraldehyde is given the prefix L, irrespective of their rotation of the plane of polarized light.

The D-glucose is written with the hydroxyl group on the carbon atom next to the last on the right and L-glucose with the hydroxyl group on the left.



D-Glyceraldehyde

D-Glucose

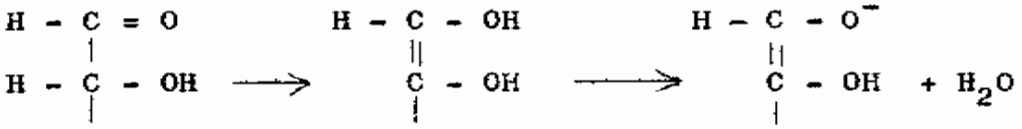


L-Glyceraldehyde

L-Glucose

The majority of glucose occuring in the body is of the D-configuration.

The formula of glucose can be written in either aldehyde or enol form. Shift to the enol form is favoured in alkaline solutions:



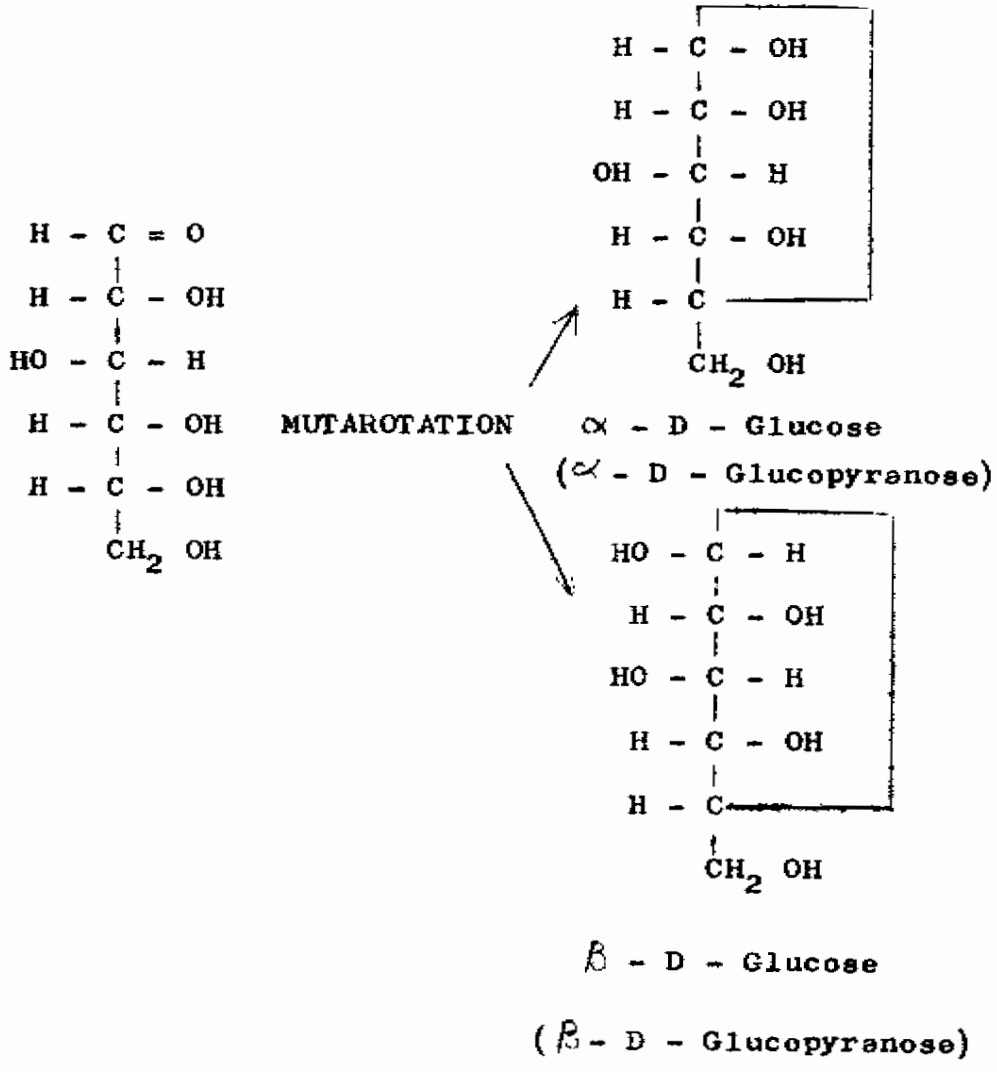
Aldehyde Enol Enol anion

The presence of a double bond and a negative charge in the enol ion form, makes glucose an active reducing substance.

Glucose in a hot alkaline solution, reduces metallic ions such as cuprous ions and the change of the colour can be used as an indication for the presence of glucose and for its quantitation.

The aldehyde and the alcohol groups of one molecule can react together to form a hemiacetal. In glucose the aldehyde group reacts with the hydroxyl group on carbon number 5. With this ring structure the hydroxyl group on the first carbon can be written

to the right and disignated alfa or to the left and disignated beta (Orten and Neubaus, 1970).



The common anhydrous crystaline glucose is in α - D form. The β - D form is obtained by crystalization from acetic acid. The two forms differ with respect to optical rotation of polarized light. The specific rotation for the α - D form is + 113° and for the β - D form is + 19.7°. Either form in aqueous solution gives rise to an equilibrium mixture that has a specific rotation of + 52.5°. The equilibrium established at room temperature is such that 36 percent of glucose exists in the α - form and 64 percent in the β form. The enzyme glucose oxidase reacts only with β - D - glucose. For this reason standard solutions prepared from pure anhydrous crystalline glucose, that is to say pure α - D form, used in glucose oxidase method, should be permitted to stand at least 2 hours in order to obtain equilibrium comparable to that in the test sample to be analysed. (Norbert W. Tietz, 1976).

II- Methods of Blood Sugar Determination:

(1) Chemical Methods:

In nearly all the chemical methods the blood sample must be deproteinized first. Claud Bernard (1849) used acetic acid and sodium sulfate to remove the proteins. Seegen (1892 - 93) employed ferric acetate. Abeles (1891) and Bang (1906) removed the proteins by the addition of an alcoholic zinc acetate solution. Rona and Michealis (1908) recommended the use of kaolin and colloidal iron. Benedict (1915) used picric acid for deproteinization. Folin (1919) discovered the use of tungestic acid as a deproteinizing agent. Benedict (1931) and Somogyi (1937) employed zinc sulfate and sodium hydroxide for deproteinization. Nelson (1944) used zinc sulfate and barium hydroxide instead of sodium hydroxide.

The amount of glucose in protein free filtrate is measured by either titrimetric or colourimetric methods.

a. Titrimetric Methods:

In the method of Bertrand (1906). Glucose was allowed to reduce cupric ions to cuprous ions. The precipitated cuprous oxide was filtered off, dissolved in a solution of ferric sulfate and sulfuric acid and titrated with potassium permenganate.

Shaffer and Hartman (1920 - 21) used iodometric titration. They prepared a combined alkaline - citrate - oxalate - copper sulfate reagent. This reagent contained potassium iodate and excess potassium iodide. It was heated with the solution to be analysed for its glucose content.

In one case the residual cupric salt is completely converted into cuprous iodide, with the liberation of an equivalent amount of iodine:

2 cupric ions + iodide $\longrightarrow$ cupric iodide + iodine

$$Cu^{++} + I^{-} \longrightarrow CuI + I_2 \quad \ldots (1)$$

In the other case cuprous salt is completely oxidized to cupric in the presence of known excess of iodine, with the conversion of the corresponding amount of iodine into iodide: