SERUM GLYCOPROTEINS
(PREALBUMIN, HAPTOGLOBIN AND ALPHA$_2$-MACROGLOBULIN) IN DIABETIC KETOACIDOSIS AS AN INDEX FOR HEPATIC FUNCTION

THESIS

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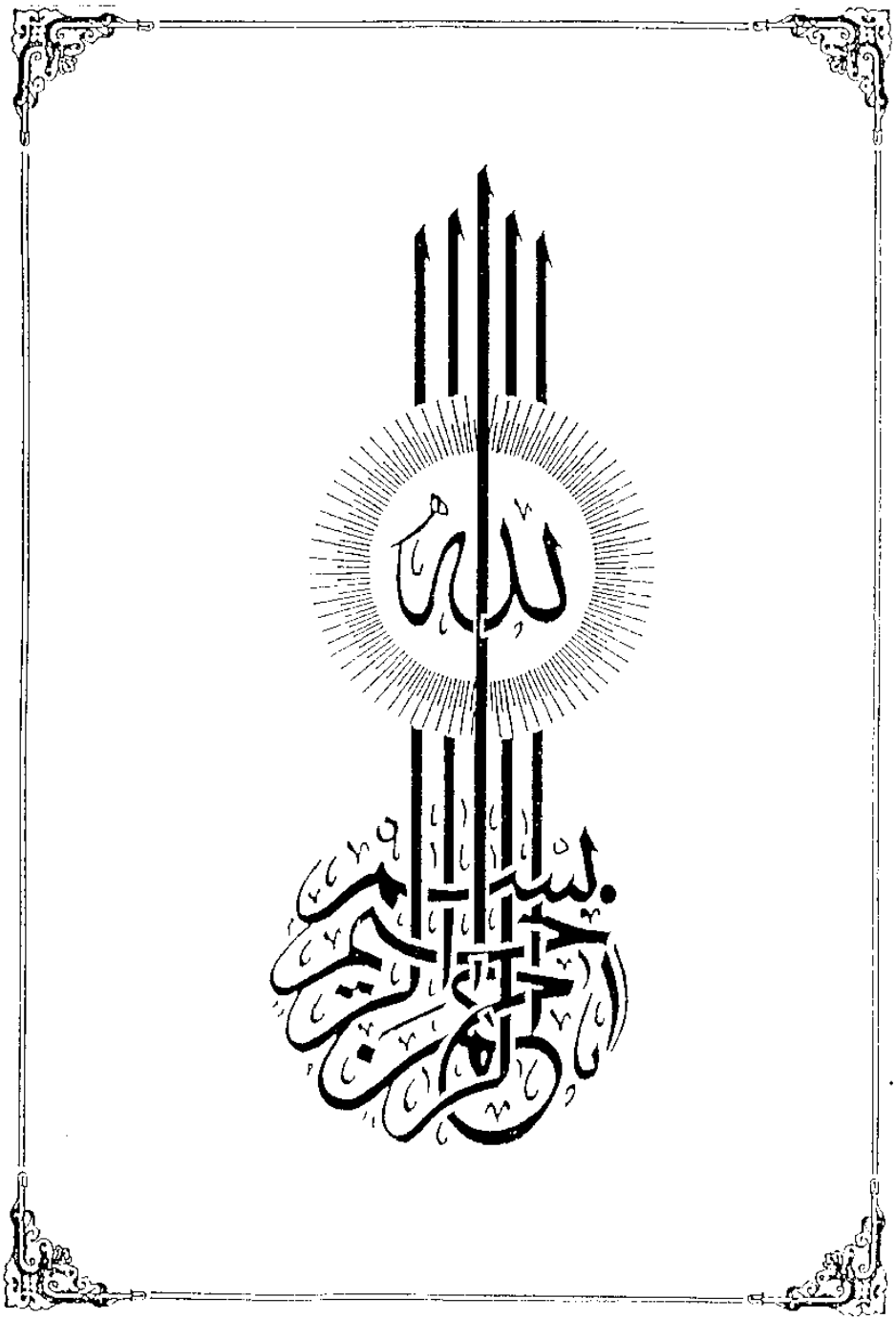

CONTENTS

AIM OF THE WORK

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Hepatic changes in diabetes may mimick alcoholic hepatitis and cirrhosis (Miller et al., 1979). They may range from fatty change (or glycogen deposition) to pericentral fibrosis, though the progression to actual cirrhosis is still controversial (Falchuk et al., 1980).

In uncomplicated diabetes, the liver may be enlarged and the convential enzymatic liver functions may be mildly affected and these changes may be accentuated by occurence of diabetic ketoacidosis (Sherlock, 1981).

Serum glycoproteins are manufactured by hepatocytes and changes in their levels reflect changes in liver cell function earlier than other tests. In alcoholic liver, it was found that giving a dose of ethanol alters the levels of serum glycoproteins in patients having pericentral necrosis and fibrosis (Kaku et al., 1982).

In analogy, the aim of this work is to study the levels of serum glycoproteins in diabetics with hepatomegaly and to assess the effect of ketoacidosis on their levels.

REVIEW OF LITERATURE

GLYCOPROTEINS

A great number of the proteins that are found in nature have carbohydrate covalently linked to the peptide portion and are accordingly termed glycoproteins. These conjugated proteins are represented by many substances of biologic importance, including enzymes, hormones, antibodies and membranes. The collagens, major structural proteins of the body, have now been clearly shown to belong to the glycoprotein family, and the presence of a large number of carbohydrate-containing proteins in plasma and mucous secretions has been appreciated for a long time .(Spiro, 1969).

Increasing attention has been called to the medical importance of the glycoproteins, for they have been implicated, either directly or indirectly, in pathologic processes ranging from neoplasia to diabetes mellitus .

A major portion of the carbohydrate in animal tissues is found in conjugated form attached by covalent bonds to either proteins (glycoproteins) or lipids (glycolipids). Glycoproteins are a most diverse group of compounds, since the covalent association of carbohydrate with protein occurs

in a wide range of protein types. They have no unique amino acid composition, but do contain a characteristic group of sugars that include D-galactose, D-mannose, D-glucose, D-fucose, D-xylose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and the various derivatives of neuraminic acid (the sialic acids).

The carbohydrate content of glycoproteins may vary from less than 1 per cent to more than 80 per cent of the weight of the molecule, and as few as two to as many as seven sugar types may be present in a given protein. Since glycoproteins span the whole spectrum of protein types, from the soluble globular plasma proteins to the insoluble collagens, and membranes, it is apparent that the occurrence of carbohydrate in these molecules does not impart any unique properties. What characterizes glycoproteins as a group is the occurrence of covalently attached carbohydrate units that have structural features in common and require similar enzymatic mechanisms for their assembly and degradation. (Spiro, 1969).

STRUCTURE OF GLYCOPROTEINS

A structural investigation of a glycoprotein requires the solution of a number of problems encountered around the carbohydrate portion of the molecule : -

1. It is necessary to determine the nature of the carbohydrate units among which the sugar components of the glycoproteins are distributed in regard to a.size .
   b.number .
   c.composition .

2. The structure of these carbohydrate units has to be specified in terms of :
   a. the sequence of the monosaccharide residue .
   b. the type of linkage of the monosaccharides to each other .
   c. the length and number of the chains present .

3. The carbohydrate-peptide linkage is the unique structural characteristic of glycoproteins and has to be described in regard to the sugar and amino acid involved, as well as to the chemical nature of the bond .

4. Finally, the location of the carbohydrate unit (or units) along the peptide chain(or chains) needs to be determined. The amino acid sequence in the immediate vicinity of the linkage site is a structural feature of particular interest.

Fortunately, a sufficient number of purified glycoproteins from various sources have been studied with these problems in mind to permit generalizations about patterns of structure.

I. Nature of the carbohydrate units:

The carbohydrate units of glycoproteins can usually be isolated as glycopeptides with a minimum number of amino acids attached after extensive proteolytic digestion. From a study of these glycopeptides:

a. The size.
b. The number and
c. The composition of these units can be determined.

a. The carbohydrate units range in size from a molecular weight of approximately 3500 (as fetuin) to single monosaccharide residues of molecular-weight 162 (as in the collagens).
b. As regards the number of carbohydrate units, there may be only a single carbohydrate unit per molecule, as in ribonuclease B, ovalbumin, or deoxyribonuclease, or there may be as many as 800, as in the ovine submaxillary glycoprotein.(Spiro, 1970).

c. the composition: since glycoproteins vary so much in molecular weight from 14,500 for ribonuclease to 1 x 10^6 for the ovine submaxillary glycoprotein the average spacing of the carbohytrate units along the peptide chain (that is the number of amino acids per carbohydrate unit) is a better index of the extent of carbohydration of a protein than the number of units per molecule. This spacing of carbohydrate units may be seen to vary considerably with as many as 779 amino acid residues per carbohydrate unit in the IgG immunoglobulin, and as few as six amino acid residues per unit in the ovine submaxillary glycoprotein .

II. Structure of glycoprotein :

The structure of glycoproteins is studied in terms of variation in :

a . sequence .

b . linkages

A very common structural pattern, one of the first studied in detail is represented by the heteropolysaccharide unit of such glycoproteins as fetuin (Spiro , 1962) the $alpha_1$ acid glycoprotein (Jeanloz ,1966), the $alpha_2$ -macroglobulin (Dunn and Spiro ,1967) , thyroglobulin (Spiro ,1965) , chorionic gonadotrophin (Bahl ,1969) IgG (Rothfus , and Smith, 1963) and IgA immunoglobulin (Dawson and Clamp, 1968), and the glomerular basement membrane (Spiro,1967).

These carbohydrate units are extensively branched and consist of oligosaccharide chains with the sequence sialic acid (or fucose) ----- galactose ------ N.acetylglucosamine linked to a core of mannose and additional N-acetyl glucosamine .

To illustrate the structure as regards the sequence the type of linkage and the number of chains , fetuin will be discussed in detail .

In each of the carbohydrate units of fetuin there are four such chains in which sialic acid(N-acetylneuraminic acid) is linked by an alpha - ketosidic bond to carbon 3 of galactose, which in turn is linked by a beta glycosidic bond to carbon 4 of N-acetyl glucosamine.
However, the linkage of galactose to the N-acetyl glucosamine in these types of chains is B (1-4) in a large number of glycoproteins studied, including fetuin (Spiro ,1962) , $alpha_1$ acid glycoprotein (Eylar and Jeanloz, 1962) thyroglobulin (Spiro and Spiro, 1968), $alpha_2$ - macroglobulin (Dunn and Spiro, 1967) and an aorta glycoprotein (Radhakrishnamurthy , and Berenson, 1966).

The fine structure of the internal portion of this type of unit is less well known, although it has been shown in fetuin to consist of three mannose and two N-acetylglucosamine residues. The three mannose residues are linked to each other, with the two N-acetyl glucosamines appearing more internally, adjacent to the glycopeptide bond (Spiro, 1964) . In the human IgG immunoglobulin, it has also been indicated that two N-acetyl glucosamines are linked together to form the most internal part of the carbohydrate unit, with one of these taking part in the attachment to the peptide chain (Rothfus & Smith ,1963) . Studies on fetuin (Spiro, 1962) have indicated that at least some of the mannose residues are the branch points to which the oligosaccharide chains are attached .

A somewhat simpler type of unit, consisting only of mannose and N-acetylglucosamine residues, is to be found in ovalbumin (Johansen et al., 1961) , thyroglobulin (Spiro ,1965) , ribonuclease B (Plummer and Hirs, 1964) and deoxyribonuclease (Catley et al., 1969).

The most detailed studies on the structure of these units have been carried out on ovalbumin (Montgomery et al., 1965) and a structural formulation has been proposed,

It appeared that two of the three N-acetyl glucosamine residues in the carbohydrate unit are again located in the most internal positions, with one taking part in the linkage to the peptide chain. Similarly the two N-acetyl glucosamine residues of ribonuclease B are believed to be in similar internal positions (Tarentino et al., 1969).

The carbohydrate units of glycoproteins from mucous secretion and those with blood group activity contain galactose, N-acetyl glucosamine, N-acetyl galactosamine, sialic acid and fucose, but do not have mannose residues. In such glycoproteins as those from porcine submaxillary gland, fucose and sialic acid residues are linked to a core of galactose and hexosamine residues.(Spiro, 1969).

Very simple carbohydrate units occur in some glycoproteins, such as the N-acetyl neuraminic acid-N-acetyl-galactosamine disaccharide found in ovine submaxillary mucin (Graham, and Gottschalk, 1960), and the glucose-galactose disaccharide unit found in basement membranes (Spiro, 1967) and collagens (Spiro, 1969) and N-acetyl-galactosamine in glycoproteins from mucous secretions. (Spiro, 1970).