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GLYCOPROTEIN METABOLISM AND BASEMENT MEMBRANE ABNORMALITIES IN DIABETES MELLITUS

by

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ABSTRACT

Basement membrane is a glycoprotein structure resembling the collagens. Peptide chains are randomly layered over one another, and linked together by covalent bonds. Carbohydrate is present in both a glucosylgalactosyl disaccharide unit and in a complex heteropolysaccharide unit. In diabetic glomerular basement membrane the number of disaccharide but not of heteropolysaccharide units becomes increased. The cause of this increase is unknown. Basement membrane has an exceedingly slow formation rate, and in man is relatively inaccessible for study. Serum glycoproteins have a relatively rapid formation rate and are readily accessible. Thus, a study of their structure and metabolism in health and in diabetes might serve as a model applicable to the basement membrane disorder of diabetic micro-angiopathy. Orosomucoid, a circulating hepatic-synthesized protein of unknown function, was chosen for this purpose, because it is present in serum at a relatively high concentration (65 mg%) and with a high carbohydrate content (40%). Methods were developed for the isolation and measurement of stable and radioactively-labelled serum orosomucoid, and for measurement of orosomucoid hexose content. Then two studies were carried out.

In the first study, the distribution of serum orosomucoid levels in 10 diabetic subjects proved to be significantly lower than that in 10 healthy subjects ($p<0.025$), while that of the orosomucoid hexose content tended to be higher in the diabetic subjects than in the healthy subjects ($p<0.1$). In health, the serum

orosomucoid level showed a strong negative correlation with the plasma triglyceride level ($r = -0.88$; $p < 0.001$), but no tendency toward a correlation with either the blood glucose level or the plasma cholesterol level. In diabetes, the serum orosomucoid level likewise showed a strong negative correlation with the plasma triglyceride level ($r = -0.71$; $p < 0.05$), but also a significant inverse relationship with both the fasting blood glucose level ($r = -0.66$; $p < 0.05$) and with the average quality of long-term diabetes control by treatment ($p < 0.05$). It also showed a tendency to correlate inversely with the plasma cholesterol level ($r = -0.58$; $p \geq 0.05$). The orosomucoid hexose content did not correlate significantly with any other subject characteristic, including the orosomucoid level. The various observations suggest that the relationship between orosomucoid and glucose levels in diabetes is not a direct one, but rather a reflection of the higher triglyceride levels prevailing in subjects with less well-controlled diabetes. The cause of this unexpected relationship of orosomucoid to triglyceride levels is unknown. Structural abnormalities of the carbohydrate component of circulating orosomucoid appear to occur in the diabetic state; their cause also is unknown. The findings, in general, encourage the view that an in-depth study of serum glycoproteins may be relevant to an understanding of long-term diabetes pathology.

The second study was directed toward measuring the entry rate of circulating glucose into serum orosomucoid. It was necessary to design the methodology and kinetics analysis

in preliminary studies; the entry rate of circulating alanine into serum albumin was selected as a prototype. On the basis of the experience with alanine-albumin incorporation, the measurement of the incorporation of circulating glucose into serum orosomucoid was successfully carried out in a healthy subject. 60% of orosomucoid carbohydrate was derived from circulating glucose. A comparative study of the rates of incorporation of circulating glucose into orosomucoid in health and in diabetes now appears feasible. This may provide insight into the etiology of the abnormalities in serum orosomucoid hexose content in diabetes, and thus relate to an understanding of the alterations in amount and composition of basement membrane which occur in diabetic microangiopathy.

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INTRODUCTION

The existence of macromolecular compounds containing both peptide and carbohydrate has been known for decades (1-4). The distinctive components of these complex substances are the amino sugars. The term "muco-substances" designates this entire group of macromolecules, the term "muco" referring to the presence of amino sugars and not to the viscous properties possessed by only some of these compounds. The "muco-substances" are subdivided into two distinct groups, the mucopolysaccharides and the glycoproteins (3-5). In the older literature, investigators referred to both glycoproteins and mucopolysaccharides as mucoproteins (4,5). This terminology was a descriptive form used to designate proteins with a relatively high carbohydrate content and is no longer used.

Mammalian mucopolysaccharides, which are found mainly in the connective tissue, are acidic substances containing uronic acids, sulphate esters, or both, in addition to the amino sugars and are exemplified by such substances as hyaluronic acid, chondroitin sulphate, heparin, and keratosulphate. In the native state, the carbohydrate component of these compounds is linked to the peptide by

an ionic or very labile covalent bond (3-5). The two components can therefore readily be dissociated without drastically altering the rest of the molecule by physical procedures such as changes in pH or in ionic strength (2,4,5).

Glycoproteins differ from mucopolysaccharides in that they contain no uronic acids (3-5) and few (3) or no (5) sulphate esters. In these compounds, the carbohydrate and peptide components are firmly linked by covalent bonds (1, 3-6) and can only be separated by drastic chemical treatment (2,4,5).

Although glycoproteins are not characterized by a unique amino acid content or sequence (1.6), they do contain a characteristic group of sugar components (1,5,6). These include the amino sugars, glucosamine and galactosamine, usually present in their N-acetyl form; the neutral hexoses, D-galactose, D-mannose, and D-glucose; the pentose D-xylose; the methyl pentose L-fucose; and the N- and O-acetyl and N-glycolyl derivatives of neuraminic acid, collectively known as the sialic acids (1,2, 4-6).

The carbohydrate moiety of a glycoprotein may contain as few as two or as many as seven of these sugar types and may constitute from less than 1% to more than 80% of the weight of the molecule. In contrast to the

peptide chain, the composition of the carbohydrate component of a given glycoprotein is somewhat inconstant. This phenomenon, termed "microheterogeneity", has been observed in almost all glycoproteins. Despite these intermolecular and intramolecular variations, glycoproteins may still be considered as a group due to the presence of the covalent carbohydrate-peptide linkage and the characteristic group of sugars (1,6).

Glycoproteins are widely distributed in animal tissues and account for a major portion of the carbohydrate present in polymer form. More recently, glycoproteins have also been found in plants and microorganisms (6). Table 1 exemplifies the diveristy and ubiquitous nature of these carbohydrate-containing proteins. Included are all of the plasma proteins with the exception of albumin, proteins of mucous secretions, hormones, enzymes, soluble and insoluble components of connective tissue, and both cellular and extracellular membranes. Of these groups, only the extracellular basement membrane will be discussed emphasizing both its structure and its metabolism in health and diabetes.

STRUCTURE AND METABOLISM OF BASEMENT MEMBRANES IN HEALTH

Basement membranes are extracellular membranes which react intensely with the periodic acid-Schiff stain,

indicating their glycoprotein nature. Histochemically, use of this stain has demonstrated the wide distribution of basement membranes throughout various tissues of the body. For example, they are important components of the capillaries of the vascular tree, the alveoli of the lungs, the renal glomeruli and tubules, and the eye, being represented by the lens capsule, Descemet's membrane of the cornea, and Bruch's membrane of the retina (6,7).

The two most apparent physiologic roles of basement membrane are those of selective filtration and of support (6,7). The role of basement membrane material as a filter is best illustrated by the basement membrane of the renal glomerulus. In the glomerulus the basement membrane constitutes the only complete barrier between plasma and filtrate since the endothelial and epithelial cells, the other two components of the glomerular capillary walls, are discontinuous. The glomerular basement membrane serves as a passive filter whose porosity is probably determined by the structural arrangement of its peptide chains and carbohydrate units (6,7). The basement membrane of less specialized capillaries, such as those found in muscle, are also believed to play a role in filtration. However, in this type of capillary the layer of endothelium overlying the membrane is continuous and therefore assists the filtrative function (7).

The lens capsule of the eye, which completely envelopes the lens, is composed of basement membrane material and illustrates the important role that basement membrane may assume as a supportive structure (6,7).

Another important structural protein in the body is collagen since it is the principal component of connective tissue. Analysis of basement membrane and of collagen has indicated striking similarities between these two compounds.

Composition Of The Normal Glomerular Basement Membrane and Its Relationship to Collagen

Collagen, the insoluble fiber of connective tissue, is the most abundant protein in the body and accounts for 25-33% of the total protein (6% of body weight). Under the electron microscope, collagen fibers are shown to be composed of smaller fibrils having characteristic cross-striations. X-ray diffraction studies of the collagen fibrils indicate that they are composed of long, narrow, assymetric molecules having a helical structure resembling a triple-stranded cable (8).

The amino acid composition of the collagens, illustrated in Table 2, is highly characteristic of this family of proteins. Glycine, the simplest amino acid, is the most abundant in collagen and accounts fo 33% of the total amino acid residues. Alanine, the next simplest

amino acid, accounts for 11% of the total amino acid residues while the imino acids, proline and hydroxyproline, together account for a further 22%. These four amino acids therefore account for two-thirds of the entire collagen molecule (9). It is important to note that the collagens are among the few proteins known to contain hydroxylysine or a substantial amount of hydroxyproline. Cysteine, cystine, and tryptophan are absent (8).

Under the electron microscope, the glomerular basement membrane appears amorphous and does not show the fibrillar structure characteristic of collagens. X-ray diffraction studies, however, give a molecular pattern similar to that obtained with the collagens (10).

Detailed chemical analyses of canine (10), bovine (11), and human (12) glomerular basement membrane indicate that it belongs to the collagen family of proteins. The occurrence of hydroxylysine, and a significant amount of hydroxyproline and proline, as well as the presence of one-fifth of the total amino acids as glycine is indicative of this fact (10). The glomerular basement membrane does differ from the collagens, however, in a few respects. Quantitatively, there is less glycine, proline, and hydroxyproline and more hydroxylysine than in the collagens. Half-cystine, an amino acid not present in collagen, does occur in the basement membrane.

The presence of a relatively large amount of carbohydrate compared to less than 1% in collagen further differentiates this material from vertebrate collagens. As is indicated in Table 3, the total carbohydrate content comprises about 7% of the dry weight of the glomerular basement membrane and is present in the form of glucose, galactose, mannose, fucose, glucosamine, galactosamine, and N-acetylneuraminic acid. The occurrence of glucose is of particular importance since this sugar is not a common component of glycoproteins and so far has been detected only in collagen and basement membranes (11). The occurrence of hydroxylysine-linked carbohydrate also appears to be a characteristic structural feature of both basement membrane and collagen since a disaccharide containing glucose and galactose linked to hydroxylysine has been found in both these substances. The hydroxylysine-linked carbohydrate of basement membrane, however, differs from that of collagen since, in addition to the presence of glycosylgalactosyl disaccharide units, a substantial proportion of the carbohydrate of the collagen molecule occurs in the form of single galactose residues linked to hydroxylysine.

The absence of hexuronic acids and sulphate esters in the glomerular basement membrane indicates there are no mucopolysaccharides present (11, 12).

Structure of the Carbohydrate Component of Normal Glomerular Basement Membrane

Investigation of the glycopeptides resulting from the combined collagenase-pronase digestion of human (12) and bovine (13) glomerular basement membrane has indicated that the carbohydrate is distributed in two distinct types of units.

One of these units is a heteropolysaccharide accounting for 45-50 percent of the carbohydrate in the membrane. It has a molecular weight of 3200 and, in its completed form, is composed of 4 galactose, 3 mannose, 5 N-acetylglucosamine, 3 sialic acid, and 1 fucose residues. No glucose is present (12, 13). The heteropolysaccharide unit is believed to be a branched structure in which oligosaccharide chains consisting of the sequence sialic acid (or fucose) ---------> galactose ---------> N-acetylglucosamine are linked to an inner core consisting of mannose and N-acetylglucosamine residues (7). In this type of unit, carbon 1 of the most internal glucosamine is attached to asparagine in the peptide chain. The heteropolysaccharide unit of the basement membrane is very similar, both in composition and molecular weight, to the carbohydrate units of certain other glycoproteins such as fetuin, thyroglobulin, and orosomucoid and, like these glycoproteins, exhibits microheterogeneity with the oligosaccharide chains existing in various stages

of completion (13).

The other carbohydrate unit is a disaccharide containing glucose and galactose linked to the hydroxyl group of hydroxylysine. Detailed investigation has indicated it to have the structure illustrated in Figure 1. Glucose is linked by an α- glycosidic bond to carbon 2 of galactose, the galatose being linked by a β-glycosidic bond to the hydroxyl group of hydroxylysine in the peptide chain (14). This disaccharide unit accounts for the remaining 50-55 percent of the carbohydrate of the normal basement membrane (12). Since the carbohydrate is approximately evenly divided by weight between the disaccharide and heteropolysaccharide units, it has been calculated that there are approximately ten disaccharide units for every heteropolysaccharide (13).

Structure of the Peptide Component of Normal Glomerular Basement Membrane

Figure 2 illustrates schematically the location of the carbohydrate on the peptide chains and the relationship of these chains to each other on a section of glomerular basement membrane. The peptide chains are randomly layered over each other with the disaccharide and heteropolysaccharide units being attached to hydroxylysine and asparagine residues respectively. The entire structure is held together by covalent disulphide bonds as well as another type of bond of undetermined nature (7).

Biosynthesis of the Normal Basement Membrane

The assembly of such a complicated structure as the basement membrane requires many steps for its completion. Included are the genetically coded synthesis of the peptide chain, the subsequent hydroxylation of proline and lysine, the enzyme-determined attachment of monosaccharide residues to form both types of carbohydrate unit, and the establishment of cross-links to form the insoluble end product. The various stages involved in the assembly of the basement membrane are schematically depicted in Figure 3. The synthesis of the peptide takes place prior to the attachment of the carbohydrate by the well established mechanisms of protein synthesis involving ribosomes, soluble RNA, messenger RNA, cofactors, and soluble enzymes. This is followed by the hydroxylase mediated hydroxylation of proline and lysine. The stepwise attachment of sugar residues then occurs through the action of specific glycosyltransferases which mediate the transfer of one sugar at a time from its nucleotide form to its appropriate location on the basement membrane precursor (7).

The assembly of the disaccharide unit of the basement membrane is mediated by a galactosyltransferase and a glucosyltransferase. These enzymes are highly specific and their level of activity is believed to reflect the rate of basement membrane synthesis (15-17). The

assembly of the disaccharide unit is illustrated in Figure 4. The galactosyltransferase transfers galactose from UDP-galactose to the hydroxyl group of hydroxylysine of the nascent basement membrane to form the glycopeptide bond (16). The glucosyltransferase then transfers glucose to the hydroxylysine-linked galactose residue. While UDP-glucose is the most active nucleotide for this reaction, CDP- and TDP-glucose can also serve as the glycosyl donor (15,18).

The synthesis of the heteropolysaccharide unit of the basement membrane probably proceeds through the action of several glycosyltransferases (18). Studies on thyroglobulin have indicated that the heteropolysaccharide unit of this glycoprotein is assembled after completion of the peptide chain by the sequential enzymatic attachment of monosaccharide units to the growing carbohydrate chain (19). The sequence of events involved in the synthesis of the heteropolysaccharide unit of the basement membrane has not as yet been determined, but is probably similar to that of thyroglobulin.

STRUCTURE AND METABOLISM OF BASEMENT MEMBRANES IN DIABETES MELLITUS

Chronic diabetes is characterized by a general hypertrophy of basement membranes. It is this process which contributes largely to blindness, uremia, and the

eventual demise of the diabetic patient. Biochemical studies of this process have been limited to the renal glomerulus. The glomerular basement membrane in diabetes undergoes marked thickening and becomes defective in its function as a selective filtration barrier. As a result, patients with diabetic nephropathy lose increasing quantities of protein in the urine. This suggests that diabetes leads not only to an increased amount of basement membrane material but also to alterations in its structure (12).

Composition of the Diabetic Glomerular Basement Membrane

Chemical analysis of human diabetic glomeruli has, in fact, indicated not only an increased amount of basement membrane material, but also a distinctive alteration in its composition (12). The results are summarized in Table 4. The diabetic basement membrane was found to have a composition similar to normal except for an increased amount of hydroxylysine and a corresponding elevation in the number of hydroxylysine-attached glucosylgalactose units, the percentage of the hydroxylysine residues substituted with the disaccharide remaining the same in the normal and the diabetic membrane. The increased hydroxylysine content was accompanied by a proportional decrease in lysine content. This indicates that an abnormal number of lysine residues are hydroxylated, but that the underlying structure of the peptide chains

is probably not changed. Comparison of the other amino acids of normal and diabetic glomerular basement membrane showed no significant differences except for a slight increase in the glycine and hydroxyproline content of the diabetic membranes.

The amount of glucose, which occurs uniquely in the hydroxylysine-linked disaccharide unit, was significantly elevated in the diabetic membrane. However, the amounts of mannose and hexosamine, which occur only in the heteropolysaccharide unit, were similar in the diabetic and normal basement membrane, indicating that the asparagine-linked heteropolysaccharide unit does not change in the diabetic membrane. The ratio of disaccharide: heteropolysaccharide units increased from 11.3:1 in the normal basement membrane to 14.6:1 in the diabetic basement membrane (12).

The diabetic glomerular basement membrane, although thickened, paradoxically exhibits increased porosity. It is interesting to speculate whether it is the extra disaccharide units which alter the porosity by affecting cross-linking originating from lysine or hydroxylysine, or by sterically interferring with the layering of the peptide chains.

The Role of Altered Glucose Metabolism in Basement Membrane Biosynthesis

As was previously illustrated, after the genetically coded synthesis of the peptide chain is completed, there are a series of noncoded steps not directly under genetic control. These are the hydroxylation of lysine and proline, the stepwise attachment of sugar residues to form both types of carbohydrate units, and the formation of cross-links between the peptide chains. These various noncoded steps are primarily controlled by enzyme specificity, substrate availability, and cofactor requirements. Environmental factors influencing any of these steps could therefore be reflected in the final basement membrane product (7). Thus, it is important to evaluate to what extent the altered carbohydrate metabolism of diabetes could affect the membrane. In this regard, it is necessary to appreciate the central role of glucose in the biosynthesis of the monosaccharide constituents of glycoproteins.

Figure 5 illustrates the metabolic pathways involved in the biosynthesis of these monosaccharide derivatives. It should be noted that the original glucose skeleton is modified through series of activating reactions to form the various sugar nucleotides which ultimately become incorporated into glycoproteins. The monosaccharide

constituents of glycoproteins, with the exception of galactose, are not available in significant amounts from the diet. Glucose, being the main sugar available in the diet and the sole sugar formed by the liver for transport to other tissue (4,5), must be the principal precursor available for synthesis of the sugar components of glycoproteins by both the liver and peripheral tissue.

Because of the central role of glucose in the biosynthesis of the sugar components of glycoproteins, it is pertinent to assess the effect of insulin deficiency on the synthesis of these components. A comparative study of the rates of biosynthesis from glucose of liver glycogen and protein-bound glucosamine in the alloxan diabetic rat has indicated that insulin deficiency causes no impairment in the rate of synthesis of protein-bound glucosamine, while glycogen synthesis is reduced to negligible levels. The synthesis of the protein-bound glucosamine of serum, kidney, lung, testes, and spleen is also undiminished in the insulin-deficient state (20). Elevations in serum protein-bound sialic acid, glucosamine, fucose, and particularly galactose, in diabetes suggest that the biosynthetic pathways of these sugars also are not dependent upon insulin (21). These findings indicate that not all pathways of glucose utilization are under the control of insulin and that, in particular, the biosynthesis of glycoproteins by various cells is probably insulin-independent.

Basic to an understanding of this difference in the effect of insulin deficiency on various pathways of glucose metabolism is a consideration of the three distinctive patterns of glucose utilization observed in mammalian cells:

1. e.g. muscle. Insulin facilitates glucose penetration into the cell but has no effect on the phosphorylating enzyme (hexokinase).
2. e.g. liver. Glucose freely enters the cell and insulin acts by inducing synthesis of the specific hexokinase, glucokinase, which makes it possible to use glucose for metabolic purposes and for glycogen storage.
3. e.g. brain, kidney, liver. Glucose freely enters the cell and an insulin-independent hexokinase is present. Control of glucose utilization is dependent on the level of glucose in the extracellular fluid.

The liver utilizes glucose by both the second and third mechanisms since it has both an insulin-sensitive glucokinase important for glycogen synthesis and an insulin-independent hexokinase probably involved in glycoprotein synthesis (7).

In the diabetic subject, the first two pathways are impeded due to insulin-deficiency, and an elevation of the blood glucose level results which is primarily due to

underutilization, and secondarily due to increased gluconeogenesis. Under conditions such as this, it is likely that the third pattern of glucose utilization would be favored and that glucose would be shunted from the routes dependent on insulin to those that do not require this hormone. The resultant increase in the availability of activated sugar nucleotides could lead to an increased synthesis of glycoproteins, including basement membrane, particularly if carbohydrate attachment is the rate-limiting step in the formation of these molecules (7). Such an overproduction of basement membrane in diabetes, along with the slow rate of membrane degradation (7), could account for the accumulation of excess basement membrane material. A sequence of events such as this would directly relate the altered glucose metabolism observed in diabetes to the development of basement membrane lesions observed in diabetic microangiopathy. Such an hypothesis is attractive, but at present is speculative, because the rate-controlling factors in basement membrane synthesis are unknown.

Nevertheless, there is recent direct evidence that the metabolism of basement membrane is influenced in some way by insulin deficiency. The levels of activity of the various enzymes involved in basement membrane synthesis are believed to reflect the overall rate of synthesis of the membrane (15, 16, 17). Activity of the glucosyltransferase of rat kidney was recently measured in alloxan-diabetic rats and was found to be significantly

increased over the activity found in normal littermates (22). Administration of insulin to the diabetic rats resulted in the return of these values toward normal levels. However, whether these changes in enzyme activity were mediated directly by insulin lack, indirectly by substrate availability, or by some other factor, remains to be determined.

It should be mentioned that some investigators contend that the microangiopathy of diabetes does not result directly or indirectly from insulin lack, but rather is a separate genetically-determined component of the disease. This view is based mainly upon an apparent lack of correlation between the severity and control of angiopathy and of the metabolic aspects of diabetes, and upon the finding by electron microscopy of basement membrane abnormalities in genetically predisposed individuals before the metabolic disorder is detectable by conventional means (23). These observations are disputed (7), and the subject is one of great controversy at the present time. In my opinion, the occurrence of typical diabetic microangiopathy in experimentally induced, non-genetic diabetes in animals, and in patients with secondary diabetes due to chronic pancreatitis or hemochromatosis (1,7), is strong evidence that the angiopathy is in some way an acquired disorder dependent upon the metabolic disturbances of the disease.

SERUM GLYCOPROTEINS IN HEALTH AND DIABETES MELLITUS

Study of the circulating glycoproteins in health and in diabetes is not only of primary interest, but may also be relevant to an understanding of the alterations in the amount and composition of basement membrane which occur in diabetic microangiopathy.

It has been seen that basement membrane is a glycoprotein structure resembling the collagens. The structure of the carbohydrate subunits has been ascertained, and the alteration in this structure which occurs in diabetes has probably been identified. However, it is clear from the preceding discussion that the cause of this change in structure, and the means that might be used to prevent it, are unknown. It could result from the general abnormalities of substrate control in diabetes; it could be a direct effect of insulin at some metabolic control point in the basement membrane itself; it could be a result of an abnormality of some other hormone, such as somatotropin; it could reflect some concomitant inherited change in the genetic control of basement membrane synthesis; it could reflect alterations in genetic control by the presence of a virus; or it could be due to none of these things but to some as yet unimagined factor. Even in animals the study of the cause of the structural changes may be difficult because of the slow synthesis rate and prolonged turnover time of basement membrane. In man, because of these properties, together with the inaccessibility of the

involved organs, the study of basement membrane kinetics in relation to diabetic microangiopathy may prove to be impossible.

It is for these reasons that changes in circulating glycoproteins in diabetes may be relevant to the problem of microangiopathy. The serum glycoproteins are readily accessible, and they have relatively short turnover times. Although their metabolism may not directly reflect the metabolic sequence of events occurring in the basement membrane, it may serve as a model to provide some general insight into control factors in health and the influence of diabetes upon them. Incidentally, the serum glycoproteins are also of primary interest in that they represent a very large, and as yet unstudied, carbohydrate pool.

Previous studies have implicated a relationship between serum glycoprotein levels and diabetes mellitus. This relationship, however, is obscure and controversial. Considerable data in the literature indicate the level of protein-bound carbohydrate to be elevated in all diabetic individuals (24-31). However, other data relate the response only to diabetes with vascular complications (32-34). These findings are further obscured by the fact that the level of protein-bound carbohydrate has been found to be markedly increased in a wide variety of pathological conditions including neoplasia (31, 33-37), myocardial infarction (33, 34), tuberculosis (2,4,5,36), rheumatoid arthritis (2,4,5,36), pneumonia (2,5), renal disease (2,31),

lupus erythematosus (2), rheumatic fever (2), fractures (2), gout (2), scurvy (2) and pregnancy (2). As well, there is a gradual increase in serum glycoprotein levels with age (2,38). It may be noted that there are many circulating glycoproteins, that they have usually been studied in groups, that methods for separating them have been imprecise, and that, for most of them, nothing is known of their function in health nor of the significance of changes in disease.

My experimental study has been concerned with an assessment of two aspects of the metabolism of a representative serum glycoprotein. Orosomucoid has been chosen for this purpose. A method has been developed for the specific isolation and measurement of stable and of radioactively-labelled serum orosomucoid. This isolation has allowed the hexose content of the molecule to be measured. A study of serum orosomucoid levels and hexose content in health and in diabetes will be presented first. This will be followed by presentation of a probing study designed to illustrate a technique for the quantitative measurement of the rate of incorporation of circulating glucose into serum orosomucoid.

CHARACTERISTICS OF SERUM OROSOMUCOID

One of the most widely investigated and best known of the serum glycoproteins is the α_1-acid glycoprotein or orosomucoid (3). The former name refers to the acidic nature of the substance (due to the terminal

sialic acid residues and the high content of aspartic and glutamic acids 3,37) and to the fact that it is found in the α_1 electrophoretic band; the latter name refers to its source and nature ("oros" being the Greek word for "serum", "mucoid" implying the presence of covalently-linked carbohydrate, 2). Older descriptive terms for orosomucoid such as α-1-glycoprotein (2), small acid glycoprotein (2), low molecular weight glycoprotein (39), α_1-seromucoid (39), α_1-orosomucoid (39), M-1 (5), and MP-1 (2,39) have now been discarded.

Like other glycoproteins, orosomucoid gives typical color reactions for protein and for carbohydrate, and contains a peptide skeleton with a number of polysaccharide side branches attached (3). Structural studies indicate the presence of a single polypeptide chain of approximately 210 amino acids (40) forming the core of the molecule, with 5-8 polysaccharide units (1,3,40,41), each containing 8-15 monosaccharide residues (1,41), covalently linked to the core by a β-glycosidic bond between N-acetylglucosamine of the polysaccharide and the β-carboxamide nitrogen of asparagine in the peptide chain (41). The amino acid (42-45) and carbohydrate (3,5,37, 43-46) content have been elucidated by various workers and are presented in Tables 5 and 6 respectively. The molecular weight has been estimated to be from 41,000 to 44,100 (3,37,43-46).

Orosomucoid is the most carbohydrate-rich glycoprotein isolated so far from human serum (3,5,44,47), approximately 40% of the molecule being carbohydrate and the remaining 60% of the molecule being protein, and it constitutes from 5-10% of the total protein-bound carbohydrate of the serum (2,3,5,28). Estimates of the concentration of orosomucoid in normal human serum (Table 7) vary, and range from as low as 20-40 mg/100 ml to as high as 125 mg/100 ml. Most studies, however, fix the concentration at from 70-75 mg/100 ml.

Studies on orosomucoid have added to an understanding of how the presence of carbohydrate may contribute to the polymorphism seen in many plasma proteins. Five to nine polymorphic forms of this protein are seen upon starch-gel electrophoresis having identical amino acid and carbohydrate compositions. Removal of the sialic acid, however, reduces the number of these forms to two, suggesting that the multiple forms observed in the native protein are due to the sialic acid residues. Since the polymorphism of the native protein is observed only at acid pH values, in the titration range of the sialic acid, it has been proposed that the multiple forms are due to the positional isomerism in the attachment of sialic acid to galactose, which could give rise to different pK values for the carboxyl groups of this sugar (1,6).

Radioisotopic studies have indicated that the isolated rat liver is capable of incorporating C^{14}-glucose into the galactose, mannose, and glucosamine of orosomucoid, and C^{14}-leucine into its protein components (54), providing direct evidence that the liver is a site of synthesis of both the carbohydrate and protein moieties of orosomucoid. Whether or not circulating glucose and amino acids are the principal precursors in vivo has not been determined. The possibility of other sites of synthesis also has not been investigated. An in vivo study employing C^{14}-glucosamine as the radioactive precursor has shown that this sugar can also be incorporated directly by the liver into the carbohydrate component of orosomucoid (55): eighty percent of the recovered protein-bound radioactivity was present as glucosamine, while the remaining twenty percent of the label was mainly in the sialic acids, and none appeared in hexose or amino acid. It is not likely, however, that extrahepatic glucosamine is a significant precursor in vivo.

Only one study of the turnover rate of plasma orosomucoid in man has been attempted (37). It showed I^{131}-orosomucoid to have an average half-life of 5 to 6 days.

Only one comparative study of serum orosomucoid levels in health and in diabetes has been conducted (25). This study, using an immunodiffusion technique for

orosomucoid estimation, has shown the serum orosomucoid level to be slightly increased in diabetes.

The biological function of orosomucoid remains unknown (37,47,55). Recent studies have shown that orosomucoid is capable of binding the steroids corticosterone, testosterone, and progesterone (42,56). Although the association constant of the progesterone-orosomucoid complex is greater than that of the progesterone-albumin complex, only a minor portion of circulating progesterone is bound to orosomucoid because of the low concentration of this serum glycoprotein (42). In this regard, it is interesting to note that serum orosomucoid levels have been observed to be decreased in pregnant women and women on oral contraceptives (57). The physiological significance of these observations has not been determined.

EXPERIMENTAL:

SECTION 1: SERUM OROSOMUCOID LEVEL AND HEXOSE CONTENT IN HEALTH AND DIABETES

SUBJECTS:

The characteristics of all subjects are shown in Table 8.

The healthy subjects were medical students and hospital staff from the Winnipeg General Hospital. None had a previous history of any metabolic disorder or a close family history of diabetes mellitus.

The diabetic subjects were from the diabetes clinic at the Winnipeg General Hospital. All had insulin-treated diabetes of the juvenile-onset type. None had a history of any metabolic disorder other than diabetes mellitus.

All subjects were male. No subject had any evidence of recent infection as determined by history, physical examination and serum protein electrophoresis.

EXPERIMENTAL PROCEDURE:

The subjects continued their usual diet and activities, and the diabetic subjects their usual treatment. They were fasted overnight and had a 30 ml blood sample obtained between 0800 and 1000 hours the next morning. The blood sample from the diabetic subjects

was obtained before they received their morning insulin. The samples were used for determination of fasting blood glucose level, serum orosomucoid level and hexose content, serum electrophoresis, and plasma triglyceride, cholesterol, and creatinine levels.

A two-hour timed urine sample was obtained from the diabetic subjects the same morning for determination of albumin content and creatinine clearance. The presence or absence of retinopathy and peripheral neuropathy, as well as the status of the peripheral vessels (dorsalis pedis, posterior tibial), was determined by physical examination of the diabetic subjects at the time of the study.

ANALYTICAL METHODS:

1. Serum Orosomucoid:

None of the methods described in the literature for orosomucoid isolation were suitable for my purposes. These methods involve large-scale fractional precipitation, large-scale ion-exchange chromatography, or small-scale immunodiffusion techniques. Too much blood was required for the large-scale techniques. Although a technique employing quantitative immunodiffusion might have solved this problem, one commercially available anti-orosomucoid serum (Schwarz/Mann) proved to be very non-specific, and this possibility was not pursued further. Furthermore, my second series of experiments (to be presented in the

next section) required specific isolation of orosomucoid from serial blood samples for radioisotope estimation. It was therefore necessary that a new method for orosomucoid isolation be developed which was capable of yielding sufficient pure orosomucoid for specific-activity determination from a small amount of blood. Thus, considerable time was spent developing my own technique for serum orosomucoid isolation.

Pure orosomucoid was isolated from 5.0 ml serum by means of an adaptation of the DEAE cellulose method of Whitehead and Sammons (58) and of Whitehead (51). Orosomucoid is adsorbed by DEAE cellulose at a controlled pH and is selectively eluted by changing ionic concentration and pH. Further purification by half-saturation with ammonium sulphate removes contaminating globulins. Desalting and concentration to a final volume of 5.0 ml leaves a protein solution suitable for both qualitative and quantitative analysis.

The complete method is described below.

(a) Reagents:

All water used is glass distilled.

(1) Sodium Acetate, pH 4.9, 0.02 M Na^+ (Buffer A):

3.281 grams sodium acetate (granular) were dissolved in about 1800 ml water, the pH adjusted to 4.9 with acetic acid, and the solution diluted to 2000 ml. pH was re-checked before each use.

(2) Sodium Acetate, pH 4.3, 0.05 M Na^+ (Buffer B):

8.203 grams sodium acetate (granular) were dissolved in about 1800 ml water, the pH adjusted to 4.3 with acetic acid and the solution diluted to 2000 ml. pH was rechecked before each use.

(3) Sodium Acetate, pH 4.0, 0.1 M Na^+ (Buffer C):

16.406 grams sodium acetate (anhydrous) were dissolved in about 1800 ml water, the pH adjusted to 4.0 with acetic acid, and the solution diluted to 2000 ml. pH was re-checked before each use.

(4) 0.1 M Sodium Acetate in 0.5 M Sodium Chloride, pH 4.0 (Buffer D):

16.406 grams sodium acetate (anhydrous) and 58.4 grams sodium chloride were dissolved in about 1800 ml water, the pH adjusted to 4.0 with acetic acid, and the solution diluted to 2000 ml. pH was re-checked before each use.

(5) 1 M Acetic Acid:

28.5 ml glacial acetic acid were diluted to 500 ml with water.

(6) Saturated Ammonium Sulphate:

375 grams ammonium sulphate were dissolved in 500 ml water by heating to about 40°C. The solution was cooled to room temperature and filtered through a double thickness of

Whatman No. 1 filter paper. The solution was stored at 4°C in a polyethylene bottle.

(b) Column Preparation:

Whatman microgranular DE 32 anion-exchange cellulose (Reeve Angel Co., New Jersey) was used for all column chromatographic procedures and was prepared according to the manufacturer's recommendation.

A clean 20 ml Multifit glass syringe was siliconized by coating the inside of the barrel thoroughly with Dricote (Fisher Scientific Co.). A 1.95 cm (dia.) nylon support screen and support net (Pharmacia Fine Chemicals) were placed perfectly flatly across the inside base of the syringe barrel and held in place with a rubber "O" ring (13/16" O.D.). The syringe was clamped vertically to a suitable stand and attached via an 18 gauge needle and polyethylene tubing to a Technicon Proportioning Roller Pump (Model-1) having a manifold prepared to pump buffer to and from the column at a rate of 0.8 ml/min. (0.045 mm I.D. standard tubing). A DEAE cellulose suspension was poured into the syringe to give a packed volume of 13 ml. In preparation for sample application, the column was equilibrated by pumping excess Buffer A through it until the effluent was pH 4.9.

(c) Separation and Purification:

5.0 ml serum were diluted with 25 ml water and pH adjusted to 4.9 with 1 M acetic acid. The solution

was allowed to stand at room temperature for 10 minutes and the pH was re-checked and adjusted if necessary. Any precipitate was removed by centrifuging for 10 minutes at 2500 rpm in a 50 ml conical centrifuge tube and the supernatant was transferred to the prepared cellulose column. After the sample had run into the cellulose, the column was washed with 50 ml Buffer B and the effluent discarded. A protein mixture containing the orosomucoid was then eluted from the column with Buffer C and collected in 2.5 ml fractions (approximately 12-15 required) until O.D. at 280 nm (1 cm light path) was less than 0.05. A typical elution pattern is presented in Figure 6. The column could be re-used after washing with Buffer D to remove any remaining protein followed by equilibration with Buffer A. Fractions with O.D. at 280 nm greater than 0.075 were pooled and an equal volume of cold saturated ammonium sulphate was added. The solution was mixed well and stored at $4^{o}C$ for 48 hours before filtration through a double thickness of Whatman No. 1 filter paper. The paper was rinsed with 5.0 ml of a 1:1 solution of Buffer C and saturated ammonium sulphate and the washing was added to the filtrate. The filtrate was dialyzed for 48 hours to remove salts. This was followed by concentration *in vacuo* at $37^{o}C$ to a final volume of 5.0 ml.

(d) Qualitative and Quantitative Analysis:

The protein in the final aqueous solution was not precipitated by the following reagents: 0.6 M perchloric acid, 5% trichloroacetic acid, 0.2 M sulfosalicylic acid, or half-saturated ammonium sulphate. It was precipitated readily with 5% phosphotungstic acid in 2 N HCl. Conventional electrophoresis of the isolated protein in Beckman B-2 (barbital) buffer, pH 8.6, revealed the mobility of an α_1-globulin. The specificity of the method of isolation was investigated by immunoelectrophoresis and Ouchterlony agar diffusion. Immunoelectrophoresis, in which antisera to normal human serum and to orosomucoid (Hoechst Pharmaceutical Co.) were used, demonstrated the presence of only one precipitin line in the α_1-globulin region. Ouchterlony agar diffusion indicated the formation of only one precipitin line against antiserum to whole human serum.

The protein-bound hexose (galactose and mannose) content of the isolated orosomucoid was determined on 0.2 ml of the final aqueous solution by the orcinol method of Winzler (2) with a galactose standard. Methods of assay for its other carbohydrate components were not developed during this study.

The protein content of the isolated orosomucoid was determined on 1.0 ml of the final aqueous solution by the tyrosine method of Winzler (2). Calculations were

based on the assumption of the presence of 11 moles tyrosine per mole of orosomucoid, and a molecular weight of 44,100 (45).

2. Blood Glucose:

The blood glucose level was determined on 1 ml whole blood by the Technicon N-2a automated ferricyanide method.

3. Plasma Triglyceride:

The plasma triglyceride level was determined on 1 ml plasma by the automated fluorometric method of Kessler (59).

4. Plasma Cholesterol:

The plasma cholesterol level was determined on 1 ml plasma by the Technicon N-24a automated colorimetric method.

5. Plasma and Urine Creatinine:

The plasma and urine creatinine levels were determined on 1 ml each of plasma and urine by the automated Technicon N-11a method. The levels were used for calculation of creatinine clearance according to the following formula:

$$\frac{\text{urine concentration (mg\%)} \times \text{urine volume (ml)} \times 1.73}{\text{plasma concentration (mg\%)} \times \text{time (min)} \times \text{body surface area}(m^2)} = \text{ml/min/1.73m}^2$$

6. Serum Electrophoresis:

Paper electrophoresis of 6.0 µl of serum was carried out on a Beckman Model R paper electrophoresis cell according to the manufacturer's recommendation.

7. Urine Albumin:

The presence or absence of urine protein (albumin) was determined on 1 ml urine by the turbidometric method of King and Haslewood (60).

RESULTS:

The results of all biochemical determinations in the healthy and the diabetic subjects are presented in Table 8.

Serum orosomucoid levels and hexose content in the two groups are compared in Figure 7. The distribution of serum orosomucoid levels in the diabetic subjects was significantly lower than that in the healthy subjects ($p<0.025$). The serum orosomucoid hexose content, however, tended to be greater in the diabetic subjects than in the healthy subjects ($p<0.1$), with five out of the ten diabetic subjects having values higher than the range seen in health.

The presence or absence of relationships of the serum orosomucoid level and hexose content to the other subject characteristics within the healthy and diabetic groups are summarized in Table 9. Those relationships which are statistically significant are illustrated in Figures 8, 9 and 10. The pattern of these relationships was somewhat unexpected.

In health, the serum orosomucoid level showed a strong negative correlation with the plasma triglyceride level (Figure 8), but no tendency toward a correlation with either the blood glucose level or the plasma cholesterol level. The orosomucoid hexose content did not correlate significantly with any other subject characteristic, including the orosomucoid level.

In diabetes, the serum orosomucoid level again varied inversely with the plasma triglyceride level (Figure 8). In this group, however, it also showed a significant inverse relationship with both the fasting blood glucose level (Figure 9), and with the average quality of long-term diabetes control (Figure 10). Furthermore, it showed a tendency to correlate inversely with theplasma cholesterol level (Table 9). Again, the orosomucoid hexose content did not correlate significantly with either the orosomucoid level or with any other subject characteristic. That it may be influenced in at least some subjects by the quality of long-term diabetes control is suggested by the distribution of the data in Figure 10.

DISCUSSION:

Serum orosomucoid level in health:

The serum orosomucoid levels in the healthy subjects were comparable to those observed by most previous workers (Table 7), and ranged from 48 to 83 mg/100 ml. The determinants

of this intersubject variability, like the biologic function of orosomucoid, are unknown. Unexpectedly, the orosomucoid level showed a strong inverse relationship to that of serum triglyceride, while showing none to that of blood glucose. It is difficult even to speculate about the reason for this relationship, in part because the determinants of intersubject variability in the serum triglyceride level likewise are unknown. It could be that in those individuals with higher triglyceride levels there is diversion of hepatic substrate from protein to lipid synthesis; somewhat against this possibility is the absence of a relationship of the orosomucoid level to the semi-quantitative estimates of the other serum protein levels. It could be that higher orosomucoid levels reflect, in some way, the lesser hepatic capacity or disposition to synthesize the apoprotein moiety of the very-low-density serum lipoprotein complex (61). It could be that the removal rates of the lipoprotein complex and of orosomucoid from the serum are inversely related in some way. It could be that the levels of these two moieties are not directly influenced by each other at all, but rather by some more fundamental underlying characteristic of hepatic metabolism. There is no information at present with which to consider a choice between these and other possibilities. It does, however, seem possible to suggest the overall conclusion that, whatever the function of serum orosomucoid, this substance is related in some way to the circulating-lipid control system, and that

further examination of this relationship in the lipoprotein disorders including diabetes, and in varying nutritional states, may direct some light upon this problem.

Serum orosomucoid level in diabetes:

The distribution of the serum orosomucoid levels in the diabetic subjects was lower than in the healthy subjects (Figure 7). This observation is different from that of Cleve et al (25), who by an immunodiffusion assay giving an unusually high level for serum orosomucoid in health (Table 7), found the level to be still higher in diabetes; the reason for this difference in findings is presumably methodologic. The serum orosomucoid levels further proved to be inversely related to the fasting blood glucose concentration, and to the average quality of long-term blood-glucose control by treatment. This finding is in contrast to the absence in the healthy subjects of a relationship between orosomucoid and glucose concentration. In the diabetic, as in the healthy subjects, the orosomucoid level related inversely to that of triglyceride. These several observations seem to suggest that the orosomucoid-glucose relationship in diabetes is not a direct one, but rather is more probably a reflection of the higher triglyceride levels which are present in subjects with less well-controlled diabetes. Inasmuch as there is no quantitative information as to the relative contributions of increased production and of decreased removal to the hyperlipoproteinemia present in diabetes, these observations do not clarify the nature of the

relationship between orosomucoid and triglyceride levels. Studies of orosomucoid in maturity-onset diabetes and in coexisting diabetes and primary hyperlipoproteinemia, and of changes in orosomucoid levels in response to alterations in various kinds of treatment, may cast some light upon this question.

Serum orosomucoid hexose content in health:

The serum orosomucoid hexose content in health was comparable to that found in previous studies (Table 6). Unlike that of the level of serum orosomucoid, the variability of its hexose content was low, and probably not outside the range of variability of the method. It showed no relationship to the serum orosomucoid level. These findings imply that in health there was little intersubject heterogeneity of orosomucoid structure, at least with respect to hexose.

Serum orosomucoid hexose content in diabetes:

In distinct contrast, the intersubject variability of orosomucoid hexose content in diabetes was much greater, with the values in half the subjects ranging up to 60 percent above the highest value in health. This implies that there was heterogeneity of orosomucoid structure in the diabetic individuals. A number of suggestions can be made about this finding:

(1) It may explain the observations of earlier workers (24-34) that serum glycoprotein levels are increased in diabetes; the index of glycoprotein concentration employed in their assays was protein-bound carbohydrate.

(2) It indicates that structural abnormalities of circulating glycoproteins do occur in the diabetic state, the study of which therefore may further understanding of the pathogenesis of the structural abnormalities of glycoproteins in basement membrane.

(3) The occurrence of an abnormality in a circulating glycoprotein selected for study more or less at random, suggests that diabetes may be found to include a general disorder of glycoprotein synthesis, rather than one specific for vascular tissue. Such a general disorder would be somewhat inconsistent with the concept that the basement membrane abnormality reflects a separately inherited component of diabetes (23), and would be more consistent with the view that it is a result of the chronic metabolic disturbance.

(4) Spiro originally proposed the hypothesis that, in the diabetic state, increased amounts of sugar nucleotides may become available to cells involved in basement membrane synthesis due to shunting of glucose from insulin-dependent to insulin-independent pathways, and that, if the attachment of carbohydrate is rate-limiting, an accelerated formation of basement membrane could result (7). More recently, he has shown that the activity of at least one of the key enzymes involved in the synthesis of the carbohydrate units of the basement membrane, glucosyltransferase, becomes elevated in the insulin-deficient state, and that this increased activity can be suppressed by insulin administration (22). These recent observations indicate

that the basement membrane alterations of diabetes are probably a sequela of the hormonal deficiency of this disease, but they leave open the question of whether the basement membrane lesions are the direct result of hyperglycemia and substrate shunting, as he originally proposed, or whether they are due to some other, less apparent, action of insulin on basement membrane metabolism. The present demonstration of an abnormality in carbohydrate content of serum orosomucoid in juvenile-onset diabetes tends to favor the former hypothesis.

The purpose of the experiments described in the next Section was to initiate a quantitative study of the contribution of circulating glucose to the carbohydrate component of serum orosomucoid.

EXPERIMENTAL:

SECTION 2: ENTRY RATE OF CIRCULATING GLUCOSE INTO SERUM OROSOMUCOID

Studies designed to measure the entry rate of circulating radioisotope-labelled precursors into serum proteins in man are lacking, and only one such *in vivo* study in animals was found (62). Thus, the feasibility of induction in a serum protein pool of a labelling pattern suitable for conversion-ratio analysis by an isotope infusion of practical duration and dose, was unknown. Accordingly, before the attempt to measure the entry rate of circulating glucose into serum orosomucoid, the methodology and kinetics analysis were developed in preliminary studies of the entry rate of circulating alanine into serum albumin, these being a precursor and a serum protein present in high concentration and with well-known kinetic properties (63, 64).

SUBJECTS:

The characteristics of all subjects are shown in Table 10. The healthy subjects were from the minor-surgery or ophthalmology clinics, and the diabetic subjects from the diabetes clinic, at the Winnipeg General Hospital. The diabetic subjects discontinued their treatment. Three had relatively mild maturity diabetes (FBS $<$ 200 mg/100 ml), and three had more severe maturity diabetes (FBS $>$ 200 mg/100 ml). No subject had a history of any systemic disorder other than diabetes. All were over

40 years of age as directed by the Atomic Energy Commission of Canada. The experimental procedure was explained to each subject before consent was granted.

EXPERIMENTAL PROCEDURES:

1. Entry Rate of Circulating Alanine into Serum Albumin:

The subjects came to the laboratory at 0630 hours, having fasted overnight, and rested for one-half hour. During this period an indwelling catheter was inserted into a forearm vein. At 0700 hours a constant infusion of 80 μC of U-C^{14} alanine (99.7% purity; New England Nuclear), diluted in about 190 ml normal saline, was begun through this catheter and administered over 8 hours by means of a Technicon Proportioning Roller Pump (Model-1). Blood samples for estimation of stable and radioactive alanine and albumin levels were obtained through an indwelling catheter inserted into a vein of the opposite forearm at 120, 180, 240, 300, 345, 390, 435 and 462 minutes after the start of the infusion. One month after this experiment the vascular pool size was measured by means of an intravenous injection of radioiodinated serum albumin (64).

2. Entry Rate of Circulating Glucose into Serum Orosomucoid:

Techniques were the same as for the U-C^{14} alanine infusion. The infusate was 100 μC of U-C^{14} glucose (98% purity; New England Nuclear). Blood samples were drawn at 240, 300, 360, 420, and 480 minutes for analysis of stable and radioactive

plasma glucose and serum orosomucoid levels. Vascular pool size was determined later.

ANALYTICAL METHODS:

1. Stable and Isotopic Plasma Alanine:

Simultaneous analysis of stable and radioactive plasma alanine was performed on a Technicon Amino-Acid Analyzer. Method NC-1 was modified (65) by substitution of a short column and by addition of a stream-splitter which diverted a portion of the effluent to a fraction collector to permit liquid scintillation counting (Nuclear Chicago, Unilux II) of the radioactive alanine peak. The remainder of the effluent was directed to the colorimeter in the conventional manner.

2. Stable and Isotopic Serum Albumin:

Albumin for specific-activity determination was isolated from 1 ml serum by means of a Sephadex G-200 column (1.5 x 84 cm) according to the method of Killander (66) and Flodin and Killander (67). The albumin fractions were dialyzed to remove salts and concentrated to a final volume of 0.8 ml. A 0.5 ml portion was counted for 20 minutes in the liquid scintillation counter. A 0.2 ml portion was used for determination of protein content according to a standard Biuret method (68). A 6 µl portion was analyzed on a Beckman Model R paper electrophoresis cell; the isolated protein solution was 92 $\pm$ 2 S.D.% albumin (n = 61 determinations).

The serum albumin level was determined by conventional paper electrophoresis.

3. Stable and Isotopic Serum Orosomucoid:

The level of stable serum orosomucoid was determined as outlined in Section 1.

Radioactive orosomucoid was isolated from two 5-ml portions of serum as in Section 1, pooled, and concentrated in vacuo at 37°C to a volume of 5.0 ml. A 4.0 ml portion was counted for 100 minutes in the liquid scintillation counter. A 0.5 ml portion was used for determination of protein content by a standard tyrosine method as outlined in Section 1.

4. Stable and Isotopic Plasma Glucose:

The stable plasma glucose level was determined by the Technicon N-2a automated ferricyanide method.

Plasma radioactive glucose was isolated by column chromatography on the Technicon Amino-Acid Analyzer during the same procedure used to isolate alanine (65). Comparison of results obtained by this new method with those obtained by the standard gluconate method indicates close agreement (65).

RESULTS:

1. Entry Rate of Circulating Alanine into Serum Albumin:

Figure 11 represents the changes in specific activity of plasma alanine and of serum albumin during the U-C^{14} alanine

infusion in subject B.K. Whereas the specific-activity curve of plasma alanine had equilibrated after two hours, the albumin pool, being large and of slow turnover rate, showed a constant rate of rise in specific activity. This linear rise represents the initial portion of a prolonged exponential curve, and indicates that during the infusion the rate of loss of counts from the albumin pool was negligible. Therefore, its slope reflects the entry rate of counts from the equilibrated alanine pool into the albumin pool. The slope was calculated by the least squares method. The following calculations were then performed:

(1) Circulating-alanine turnover rate

$$= \frac{\text{U-C}^{14}\text{ alanine infusion rate (dpm/min)}}{\text{alanine equilibrium specific activity (dpm/}\mu\text{M)}}$$

$$= \frac{339,430}{746}$$

$$= 455\ \mu\text{M/min.}$$

(2) Entry rate of circulating alanine into the serum albumin pool

$$= \text{slope of albumin specific activity (dpm/}\mu\text{M/min)} \times \frac{\text{intravascular albumin pool size (}\mu\text{M)}}{\text{alanine equilibrium specific activity (dpm/}\mu\text{M)}}$$

$$= \frac{2.471 \times 2093}{746}$$

$$= 6.93\ \mu\text{M/min.}$$

(3) Percent of circulating-alanine turnover converted to serum albumin

$$= \frac{\text{rate of alanine to albumin (}\mu\text{M/min)} \times 100}{\text{alanine turnover rate (}\mu\text{M/min)}}$$

$$= \frac{6.93 \times 100}{455}$$

$$= 1.5\%$$

The results for all subjects are shown in Table 11. The fraction of labelled alanine appearing in serum albumin was higher in severe maturity diabetes (1.7 ± S.E. 0.3%) than in milder diabetes (1.0 ± 0.02%) and in health (1.3 ± 0.1%).

2. Entry Rate of Circulating Glucose into Serum Orosomucoid:

Figure 12 represents the changes in specific activity of plasma glucose and of serum orosomucoid during the U-C^{14} glucose infusion in subject Y.K. As in the alanine-albumin experiments, the specific-activity curve of the precursor plasma glucose pool had equilibrated after two hours, whereas the recipient serum orosomucoid pool showed a constant rate of rise in specific activity. Therefore the same calculations could be performed:

(1) Circulating-glucose turnover rate =

$$\frac{\text{U-}C^{14}\text{ glucose infusion rate (dpm/min)}}{\text{glucose equilibrium specific activity (dpm/}\mu\text{M)}}$$

$$= \frac{334,590}{720}$$

$$= 465\ \mu\text{M/min.}$$

(2) Entry rate of circulating glucose into the serum orosomucoid pool

= slope of orosomucoid specific activity (dpm/μM/min) x

$$\frac{\text{intravascular orosomucoid pool size (}\mu\text{M)}}{\text{glucose equilibrium specific activity (dpm/}\mu\text{M)}}$$

$$= \frac{2.865 \times 11}{720}$$

$$= 0.044\ \mu\text{M/min.}$$

(3) Percent of circulating-glucose turnover converted to serum orosomucoid

$$= \frac{\text{amount of glucose to orosomucoid } (\mu M/min) \times 100}{\text{glucose turnover rate } (\mu M/min)}$$

$$= \frac{0.044 \times 100}{465}$$

$$= 0.01\%$$

DISCUSSION:

1. Entry Rate of Circulating Alanine into Serum Albumin:

No precedent was found in man for the measurement of the rate of entry of circulating precursors into a serum protein. The test of the feasibility of such a procedure based upon alanine incorporation into albumin was successful: the precursor pool equilibrated rapidly; radioisotope levels in the recipient pool were sufficient at the allowable dose level to be measured readily; entry was a stable, linear function of time.

The fraction of labelled alanine appearing in serum albumin represents only the contribution of circulating alanine to this end product. It seems likely that this alanine is incorporated as the intact molecule, without previous deamination and admixture into a common precursor pool; hydrolysis and amino-acid radioanalysis of the albumin would be needed to confirm that the radioactivity is confined to the alanine residues. The approximate total rate of incorporation of alanine into albumin can be estimated from the average serum albumin replacement rate of 200 mg/kg/day (64, 69), and from the specific alanine

content of albumin (70). Thus, an estimate of the fraction of this total provided by circulating alanine can be derived. In the healthy, overnight fasted subjects this estimate was 40 ± SE 4%. The validity of this value is supported by its similarity to those obtained in overnight fasted rats by Gan and Jeffay (71) and by Loftfield and Harris (72) for the contribution of circulating amino acids to the intrahepatic free amino acid pool. The balance of the pool is thought to be derived from endogenous hepatic amino acids which have not circulated, although in the case of alanine the potential *in vivo* contribution of pyruvate by transamination seems not to have been measured. In severe maturity diabetes the estimated fractional contribution of circulating alanine to serum albumin was 55 ± SE 3%, somewhat higher than in health. This could reflect depletion of hepatic precursors by the excess gluconeogenesis which occurs in maturity diabetes (73).

An attempt to measure the entry rate of circulating alanine into orosomucoid was unsuccessful, presumably because of its low alanine content (Table 5).

2. Entry Rate of Circulating Glucose into Serum Orosomucoid:

This measurement was attempted on the basis of the experience with alanine-albumin incorporation. The kinetics observed in the precursor and recipient pools were similar to those in the prototype experiments. The specific activities of orosomucoid were similar to those of albumin at corres-

ponding times during the infusion; the levels of counts per sample volume, were very much lower because the concentration of orosomucoid is only about 1/100 that of albumin. They reached 2½ times background at the end of the infusion.

The fraction of labelled glucose appearing in serum orosomucoid was only 0.01%. Again, this measurement represents only the contribution of circulating glucose to this end product. If one assumes that glucose is incorporated equally into all carbohydrate components of serum orosomucoid (Figure 5), it follows that the approximate total rate of incorporation of glucose into orosomucoid can be estimated from the average serum orosomucoid turnover time of 8 days (37, 74), and from the total number of carbohydrate residues per mole (Table 6). Thus, the approximate fraction of this total provided by circulating glucose can be derived. This fraction was 60%, and thus similar to the corresponding fractions in the alanine infusion studies. A comparative study of the rates of incorporation of circulating glucose into orosomucoid in health and diabetes now appears feasible. It may provide some insight into the etiology of the abnormalities in serum orosomucoid hexose content in diabetes demonstrated in Section 1, and thus may relate to an understanding of the alterations in amount and composition of basement membrane which occur in diabetic microangiopathy.

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TABLE 1 BIOLOGICAL CLASSIFICATION OF GLYCOPROTEINS

GROUP	EXAMPLES
Plasma	Fetuin, α_1-acid glycoprotein, transferrin, ceruloplasmin, haptoglobin, α_2-macroglobulin, Barium α_2-glycoprotein, fibrinogen, β-lipoprotein, thyroxine-binding globulin, corticosteroid-binding globulin
Immunoglobulins	IgG, IgA, IgM
Urine	Tam and Horsfall glycoprotein
Hormones	Chorionic gonadotrophin, follicle-stimulating hormone, interstitial cell-stimulating hormone, thyroid-stimulating hormone, thyroglobulin
Enzymes	Pancreatic ribonuclease and deoxyribonuclease, Takaamylase, fungal glucose oxidase, stem bromelain, kidney γ-glutamyl transpeptidase, liver β-glucuronidase, N-acetylglucosaminidase, serum cholinesterase and atropinesterase, horseradish peroxidase, fungal glucoamylase and chloroperoxidase, pepsin and pepsinogen, yeast invertase
Egg white	Ovalbumin, ovomucoid, avidin, ovotransferrin
Mucins	Submaxillary glycoproteins, ovarian cyst glycoproteins, sulfated gastric, colonic and submaxillary glycoproteins
Connective tissue	Proteoglycans, collagens, aorta glycoproteins, acid glycoproteins of bone, keratan sulfate-proteins
Extracellular membranes	Glomerular basement membrane, lens capsule, Descemet's corneal membrane, Reichert's yolk sac membrane, annelid cuticle, plant cell wall, yeast cell wall
Cellular membranes	Plasma membranes, intracellular membranes, platelet membranes, visual pigment, E. coli cell membrane
Phytohemagglutinins	Soy bean, wax bean, potato, black locust, meadow mushroom

Spiro, R.G. (6)

TABLE 2 COMPOSITION OF HUMAN SKIN COLLAGEN

COMPONENT	RESIDUES PER 1000 TOTAL AMINO ACID RESIDUES
Glycine	330
Proline	128
3-Hydroxyproline	1
4-Hydroxyproline	93
Alanine	110
Aspartic Acid	45
Glutamic Acid	73
Serine	36
Threonine	18
Valine	24
Methionine	6
Isoleucine	10
Leucine	24
Lysine	27
Hydroxylysine	6
Histidine	5
Arginine	51
Phenylalanine	12
Tyrosine	3
Half-cystine	0

White, A., Handler, P.,
Smith, E.L. (8)

TABLE 3 COMPOSITION OF NORMAL HUMAN GLOMERULAR BASEMENT MEMBRANE

COMPONENT	RESIDUE WEIGHT (gm/100 gm dry wt. ± S.D.M.)	RESIDUES PER 1000 TOTAL AMINO ACID RESIDUES
Hydroxyproline	7.51 ± 0.35	84.1
Aspartic acid	5.87 ± 0.37	64.6
Threonine	2.72 ± 0.23	34.1
Serine	3.49 ± 0.23	50.8
Glutamic acid	8.83 ± 0.87	86.7
Proline	6.09 ± 0.69	79.4
Glycine	9.95 ± 0.37	220.9
Alanine	3.87 ± 0.18	68.9
Valine	2.93 ± 0.24	37.4
Methionine	1.27 ± 0.08	12.7
Isoleucine	2.53 ± 0.13	28.3
Leucine	4.88 ± 0.23	54.8
Tyrosine	1.97 ± 0.21	15.4
Phenylalanine	2.90 ± 0.34	26.3
Hydroxylysine	2.39 ± 0.19	21.3
Lysine	2.91 ± 0.21	28.8
Histidine	1.66 ± 0.08	15.4
Arginine	5.79 ± 0.36	47.1
Half-cystine	1.71 ± 0.09	21.0
Tryptophan	0.25	2.0
Amide nitrogen	0.87	(64.9)
Glucose	2.03 ± 0.07	15.8
Galactose	2.34 ± 0.13	18.3
Mannose	0.55 ± 0.02	4.32
Fucose	0.14 ± 0.007	0.98
N-Acetylglucosamine	1.21 ± 0.02	7.40
N-Acetylgalactosamine	0.16 ± 0.004	0.99
N-Acetylneuraminic acid	0.62 ± 0.025	2.67
Glucosylgalactosyl-hydroxylysine		16.13 ± 0.76

Beisswenger, P.J.,
Spiro, R.G. (12)

TABLE 4 COMPARISON OF SEVERAL COMPONENTS OF THE NORMAL AND DIABETIC HUMAN GLOMERULAR BASEMENT MEMBRANE

COMPONENT	RESIDUES PER 1000 TOTAL AMINO ACID RESIDUES ± S.D.M.	
	NORMAL	DIABETIC
Hydroxylysine	24.7 ± 1.06	30.21 ± 0.96
Lysine	25.4 ± 0.94	19.70 ± 0.34
Lysine plus hydroxylysine	50.1 ± 1.49	49.9 ± 0.98
(Lysine/hydroxylysine)	(1.03 ± 0.09)	(0.65 ± 0.03)
Glucose	17.8 ± 1.01	22.8 ± 1.15
Mannose	4.73 ± 0.25	4.70 ± 0.17
Hexosamine	10.26± 0.46	10.47 ± 0.82
(Disaccharide/heteropolysaccharide)	(11.3)	(14.6)
(Percentage of glycosylation of hydroxylysine)	(72.5 ± 4.3)	(75.7 ± 4.2)
Glucosylgalactosylhydroxylysine	15.9 ± 1.08	20.8 ± 1.31

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TABLE 5 AMINO ACID COMPOSITION OF OROSOMUCOID

AMINO ACID	NUMBER OF AMINO ACID RESIDUES PER MOLE[a]				PERCENT BY WEIGHT OF AMINO ACID RESIDUES PER MOLE
	Ganguly and Westphal (42)	Walborg and Ward (43)	Weimer, Mehl, and Winzler (44)	Schultze and Heremans (45)	Schultze and Heremans (45)
	[b]		[c]		
Alanine	11	13	-	9	1.5
Arginine	10	9	9	10	3.4
Aspartic acid	26	24	25	22	5.7
Cystine	-	3	} 2	-	-
Cysteine	5	-	}	3	0.7
Glutamic acid	36	36-37	32	36	10.5
Glycine	8	10	5	8	1.0
Histidine	4	4	4	4	1.1
Isoleucine	11	10	11	10	2.5
Leucine	17	18-19	18	15	3.9
Lysine	16	17	15	15	4.5
Methionine	2	1-2	2	1	0.4
Phenylalanine	11	11	11	10	3.3
Proline	9	11	9	8	1.8
Serine	8	10	11	7	1.4
Threonine	20	18	18	18	4.0
Tryptophan	-	4	5	3	1.4
Tyrosine	13	12	5	11	4.1
Valine	-	12-13	11	10	2.1
Amide NH_3	-	-	-	4	1.5
Total	207	223	193	204	54.8%

TABLE 5 (continued)

a. Based on an orosomucoid molecular weight of 44,100 except for the data of Ganguly and Westphal (42) who assumed a molecular weight of 41,000.

b. These results were expressed as residues per 100,000 grams. To convert them to residues per mole I have multiplied by the factor 0.41.

e.g. alanine = 26 residues / 100,000 g.

$$\underline{\text{OR}} \quad 26 \times \frac{41{,}000}{100{,}000} = 11 \text{ residues/mole}$$

c. These results were expressed as grams per 100 grams protein. I have converted them to residues per mole as follows:

$$\frac{\text{Weight of a.a.}}{\text{mol. wt. of a.a.}} \div \frac{\text{Weight of orosomucoid (100 g.)}}{\text{mol. wt. of orosomucoid (44,100)}}$$

e.g. arginine = 3.65 g./100 g. protein

$$\underline{\text{OR}} \quad \frac{3.65}{174.20} \div 0.00226 = 9 \text{ residues/mole.}$$

TABLE 6 CARBOHYDRATE COMPOSITION OF OROSOMUCOID

COMPONENT	PERCENT BY WEIGHT OF CARBOHYDRATE RESIDUES PER MOLE					NUMBER OF CARBOHYDRATE RESIDUES PER MOLE	
	Kent (3)	Spiro (5)	Eylar and Jeanloz (46)	Weisman, Goldsmith, Winzler and Lepper (37)	Weimer, Mehl, and Winzler (44)	(45)	(43)
Hexose	13.6-16.8	11.3	13.2	15	16.4	33	27
Acetyl Hexosamine	12.4-14.3	15.2	15.3	-	-	26	21
Hexosamine	-	-	-	14	11.9	-	-
Sialic Acid	11.0-14.7	10.8	10.9	16	-	16	16
Fucose	0.7- 1.1	1.0	0.7	Small amount	-	2	3-4
Total	37.7-46.9	38.3	40.1	45	41.8	77	67

TABLE 7 OROSOMUCOID CONCENTRATION IN NORMAL HUMAN SERUM

REFERENCE	SEROM OROSOMUCOID CONCENTRATION (mg/100 ml)
Kent (3)	20-40
Tokita & Schmid (48)	45
Sundblad & Wallin-Nilsson (49)	68
Snyder & Ashwell (35)	69
Johnson, Schmid & Alper (50)	70
Whitehead (51)	73 (28 - 124)
West & Hong (30)	75
Winzler (56)	75
Schultze & Heremans (45)	75-100
Hardwicke (52)	75-125
Kornel, Schrohenloher & Caldwell (53)	78
Cleve, Alexander, Mitzkat, Nissen & Salzmann (25)	106

TABLE 8: PHYSICAL CHARACTERISTICS OF SUBJECTS AND BIOCHEMICAL DETERMINATIONS

HEALTHY SUBJECTS	PHYSICAL CHARACTERISTICS				SERUM PROTEIN ELECTROPHORESIS (g/100 ml)					DIABETES CHARACTERISTICS								
	Age (yr)	Ht. (ft)	Wt. (lb)	% Ideal Wt.	Albumin	Globulins α_1	α_2	β	γ	Age of Onset (yr)	Duration (yr)	Insulin Requirement (U/day)	Usual Quality of Control	FBS (mg/100ml)	Plasma Triglyceride (mg/100 ml)	Plasma Cholesterol (mg/100 ml)	Creatinine Clearance (ml/min/1.73 m²)	Urine Albumin (mg/2
E.S.	25	5'11"	170	108	4.6	0.2	0.5	1.0	1.0	-	-	-	-	73	126	166	-	-
D.M.	25	6' 1"	150	90	5.4	0.2	0.4	0.6	0.8	-	-	-	-	76	60	152	-	-
B.P.	26	5'11"	132	84	5.3	0.2	0.4	0.7	0.9	-	-	-	-	78	100	130	-	-
G.R.	24	6' 0"	205	127	4.6	0.3	0.8	1.1	1.1	-	-	-	-	78	168	198	-	-
K.T.	28	6' 2"	195	114	5.1	0.2	0.4	0.5	0.6	-	-	-	-	79	67	162	-	-
T.H.	26	5'10"	170	111	5.4	0.1	0.3	0.6	0.7	-	-	-	-	83	76	142	-	-
R.B.	29	5'11"	230	146	5.3	0.2	0.5	0.7	1.1	-	-	-	-	87	96	184	-	-
H.S.	29	6' 0"	205	127	5.5	0.2	0.4	0.7	1.0	-	-	-	-	87	67	146	-	-
R.C.	25	6' 0"	175	108	5.1	0.2	0.5	0.7	0.8	-	-	-	-	88	120	134	-	-
E.B.	23	5'10"	150	98	5.3	0.2	0.4	0.7	0.8	-	-	-	-	90	130	154	-	-
DIABETIC SUBJECTS																		
R.W.	24	5'10"	172	111	5.2	0.2	0.4	0.7	0.9	18	6	45	Good	131	106	194	125	0
R.V.	32	5' 8"	119	82	5.1	0.2	0.5	0.7	0.9	15	17	68	Fair	150	102	182	121	0
R.S.	34	5'11"	150	96	4.8	0.3	0.5	0.6	0.7	29	5	24	Good	191	92	244	109	7
G.G.	24	5'10"	155	102	5.3	0.3	0.5	0.7	0.8	15	9	70	Good	231	106	154	136	0
R.L.	30	5' 4"	136	106	4.1	0.3	0.7	0.8	0.9	12	18	28	Good	249	106	158	174	0
S.S.	22	5' 9"	184	124	4.5	0.3	0.6	0.8	0.8	19	3	75	Fair	268	306	218	-	-
G.M.	21	5'10"	148	97	5.0	0.3	0.7	0.9	1.2	20	1	30	Good	276	160	208	107	0
J.T.	20	6' 0"	170	105	3.7	0.3	0.9	0.9	1.0	3	17	54	Poor	328	104	212	123	1445
R.P.	20	5' 6"	123	91	5.4	0.3	0.7	0.9	1.5	13	7	80	Poor	356	222	214	102	99
S.M.	19	5' 6"	129	95	5.4	0.2	0.8	0.7	0.5	8	11	65	Fair	384	306	240	166	4

INATIONS

e	Plasma Cholesterol (mg/100 ml)	Creatinir Clearance (ml/min/ 1.73 m²	Urine Albumin (mg/2hr)	B.P. (mmHg)	Retinopathy	Peripheral Neuropathy	Peripheral Vessels	SERUM OROSOMUCOID Level (mg/100 ml)	Hexose Content (mg/100 ml)
	166	-	-	-	-	-	-	49	16
	152	-	-	-	-	-	-	83	14
	130	-	-	-	-	-	-	62	15
	198	-	-	-	-	-	-	52	15
	162	-	-	-	-	-	-	82	18
	142	-	-	-	-	-	-	71	17
	184	-	-	-	-	-	-	72	17
	146	-	-	-	-	-	-	71	17
	134	-	-	-	-	-	-	50	15
	154	-	-	-	-	-	-	48	14
	194	125	0	125/85	Absent	Absent	Normal	58	29
	182	121	0	110/80	Absent	Absent	Normal	51	25
	244	109	7	110/75	Absent	Absent	Normal	51	17
	154	136	0	108/78	Absent	Slight	Abnormal	55	16
	158	174	0	112/88	Slight	Absent	Normal	53	13
	218	-	-	140/90	Absent	Absent	Normal	49	19
	208	107	0	120/78	Absent	Absent	Normal	54	17
	212	123	1445	160/90	Slight	Slight	Normal	51	27
	214	102	99	110/80	Absent	Slight	Normal	51	13
	240	166	4	110/70	Absent	Slight	Normal	39	21

TABLE 9 PRESENCE OR ABSENCE OF RELATIONSHIPS OF SERUM OROSOMUCOID LEVEL AND HEXOSE CONTENT TO OTHER SUBJECT CHARACTERISTICS

	HEALTHY SUBJECTS		DIABETIC SUBJECTS	
	Serum Orosomucoid Level	Serum Orosomucoid Hexose Content	Serum Orosomucoid Level	Serum Orosomucoid Hexose Content
	r(p)	r(p)	r(p)	r(p)
Serum orosomucoid level	-	0.47 (>0.1)	-	0.06 (>0.8)
Serum orosomucoid hexose content	0.47 (>0.1)	-	0.06(>0.8)	-
Fasting blood glucose level	-0.15 (>0.6)	0.04 (>0.9)	-0.66(<0.05)	-0.34(>0.3)
Plasma triglyceride level	-0.88(<0.001)	-0.44(>0.2)	-0.71(<0.05)	-0.18(>0.6)
Plasma cholesterol level	-0.07(>0.8)	0.13(>0.7)	-0.58(>0.05)	0.12(>0.7)
Duration of diabetes	-	-	-0.14(>0.6)	0.21(>0.5)
Daily insulin requirement	-	-	-0.32(>0.3)	0.07(>0.8)
Quality of control (good vs fair & poor)	-	-	p*<0.05	p*>0.4
Presence or absence of complications	-	-	p*>0.4	p*>0.3

* Student's t test

TABLE 10: CHARACTERISTICS OF SUBJECTS

	Subject	Sex	Age	Ht. (ft)	Wt. (lb)	% Ideal Wt.*	Fasting Plasma Glucose Level (mg/100 ml)	Fasting Plasma Alanine Level (μM/l)
1. Entry rate of circulating alanine into serum albumin.								
(a) Healthy subjects	M.T.	F	45	4'11"	163	157	92	267
	C.H.	M	54	5' 4"	135	102	100	359
	H.S.	F	60	5' 3"	120	103	102	277
(b) Mild maturity-onset diabetic subjects	A.B.	F	54	5' 0"	125	111	136	323
	W.V.	M	78	6' 2"	170	100	182	261
	H.W.	M	73	5' 3"	152	117	197	343
(c) Severe maturity-onset diabetic subjects	B.K.	F	49	5' 4"	197	150	237	336
	V.M.	F	47	5' 5"	193	147	316	174
	L.M.	F	43	5' 1"	200	182	328	329
2. Entry rate of circulating glucose into serum orosomucoid.								
(a) Healthy subject	Y.K.	F	43	4'10"	99	100	87	-

* Metropolitan Life Insurance Tables

TABLE 11 ALANINE-ALBUMIN INTERCONVERSIONS

	CIRCULATING-ALANINE TURNOVER RATE (μM/min)	CIRCULATING-ALANINE TO ALBUMIN (μM/min)	(percent of alanine turnover)
(a) Healthy subjects			
M.T.	209	3.19	1.5
C.H.	320	3.45	1.1
H.S.	232	3.32	1.4
(b) Mild maturity-onset diabetic subjects			
A.B.	271	2.75	1.0
W.V.	245	2.50	1.0
H.W.	272	2.62	1.0
(c) Severe maturity-onset diabetic subjects			
B.K.	455	6.93	1.5
V.M.	270	5.74	2.1
L.M.	510	6.89	1.4

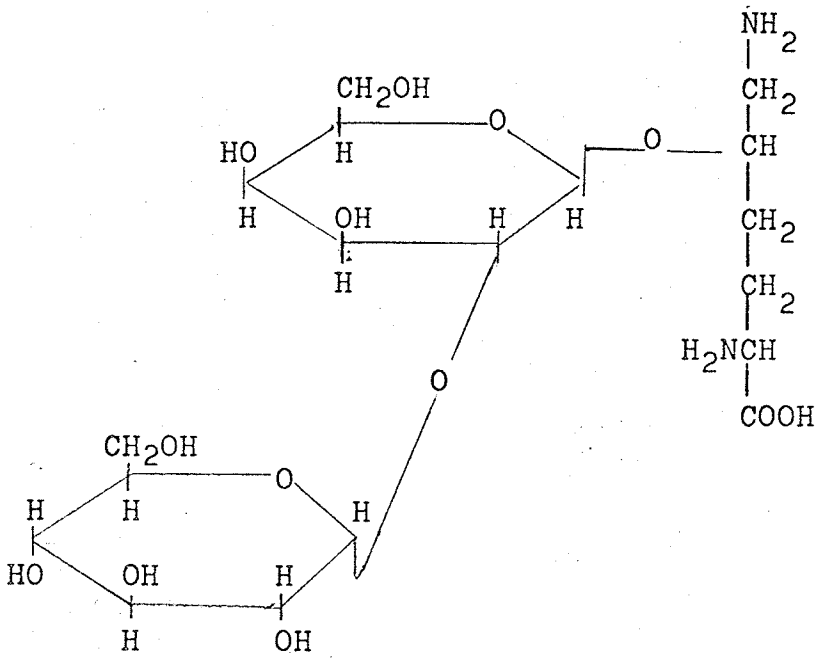

FIG. 1 STRUCTURE AND PEPTIDE ATTACHMENT OF THE DISACCHARIDE UNIT OF THE GLOMERULAR BASEMENT MEMBRANE:

2-O-α-D-glucopyranosyl-O-β-D-galactopyranosylhydroxylysine

Spiro, R.G. (14)

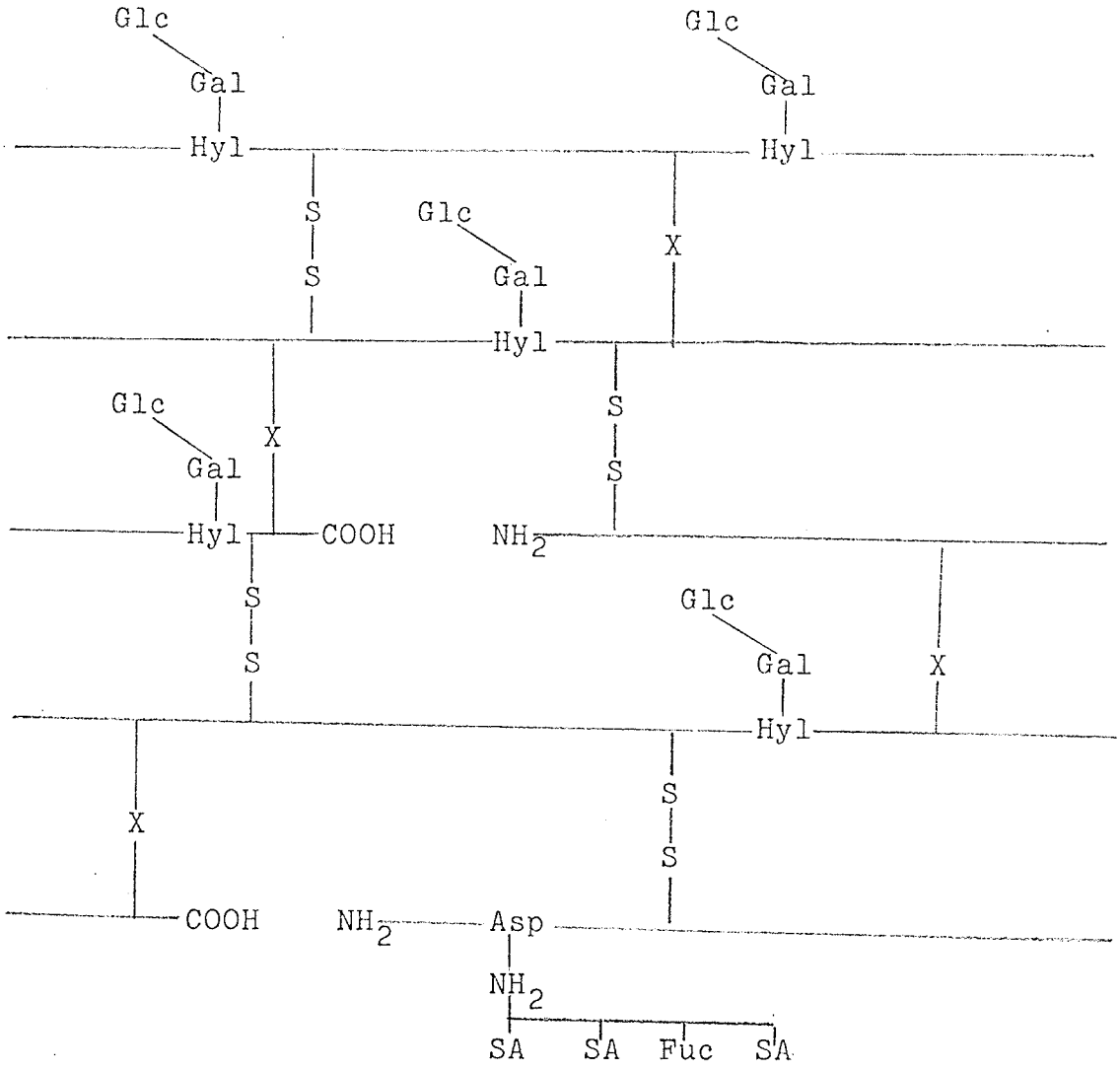

FIG. 2 SCHEMATIC REPRESENTATION OF GLOMERULAR BASEMENT MEMBRANE

Glc = glucose, Gal = galactose, SA = sialic acid, Fuc = fucose, Hyl = hydroxylysine, $AspNH_2$ = asparagine, -S-S- = disulphide bonds, -X- = undetermined covalent bonds

Spiro, R.G. (7)

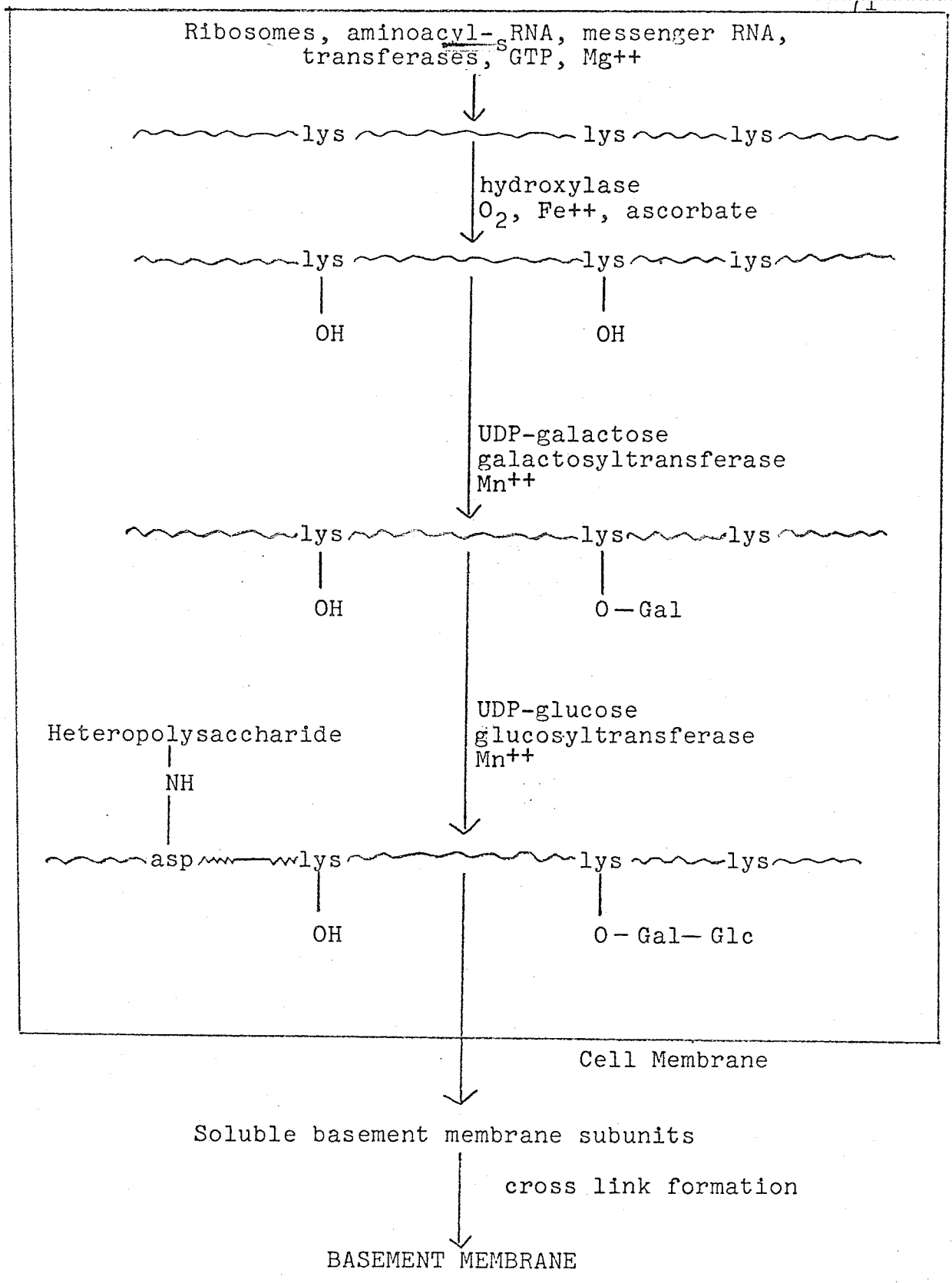

FIG. 3 SCHEMATIC REPRESENTATION OF STEPS INVOLVED IN THE SYNTHESIS OF BASEMENT MEMBRANE

Spiro, R.G. (7)

(1) UDP-Gal + Hyl - (basement membrane) $\xrightarrow{Mn^{++}}$

O-β-D-Gal-(1⟶5)-Hyl- (basement membrane) + (UDP)

(2) UDP-Glc + O-β-D-Gal-(1⟶5)-Hyl- (basement membrane) $\xrightarrow{Mn^{++}}$

O-α-D-Glc-(1⟶2)-O-β-D-Gal-(1⟶5)-Hyl-(basement membrane)

+ (UDP)

FIG. 4 REACTION SEQUENCE FOR THE BIOSYNTHESIS OF THE HYDROXYLYSINE-LINKED DISACCHARIDE UNIT OF BASEMENT MEMBRANES AND COLLAGENS

(a) Enzyme responsible for sequence (1) is UDP-galactose: hydroxylysine-(basement membrane) galactosyltransferase.

(b) Enzyme responsible for sequence (2) is UDP-glucose: galactosylhydroxylysine-(basement membrane) glucosyltransferase.

Spiro, R.G., Spiro, M.J. (17)

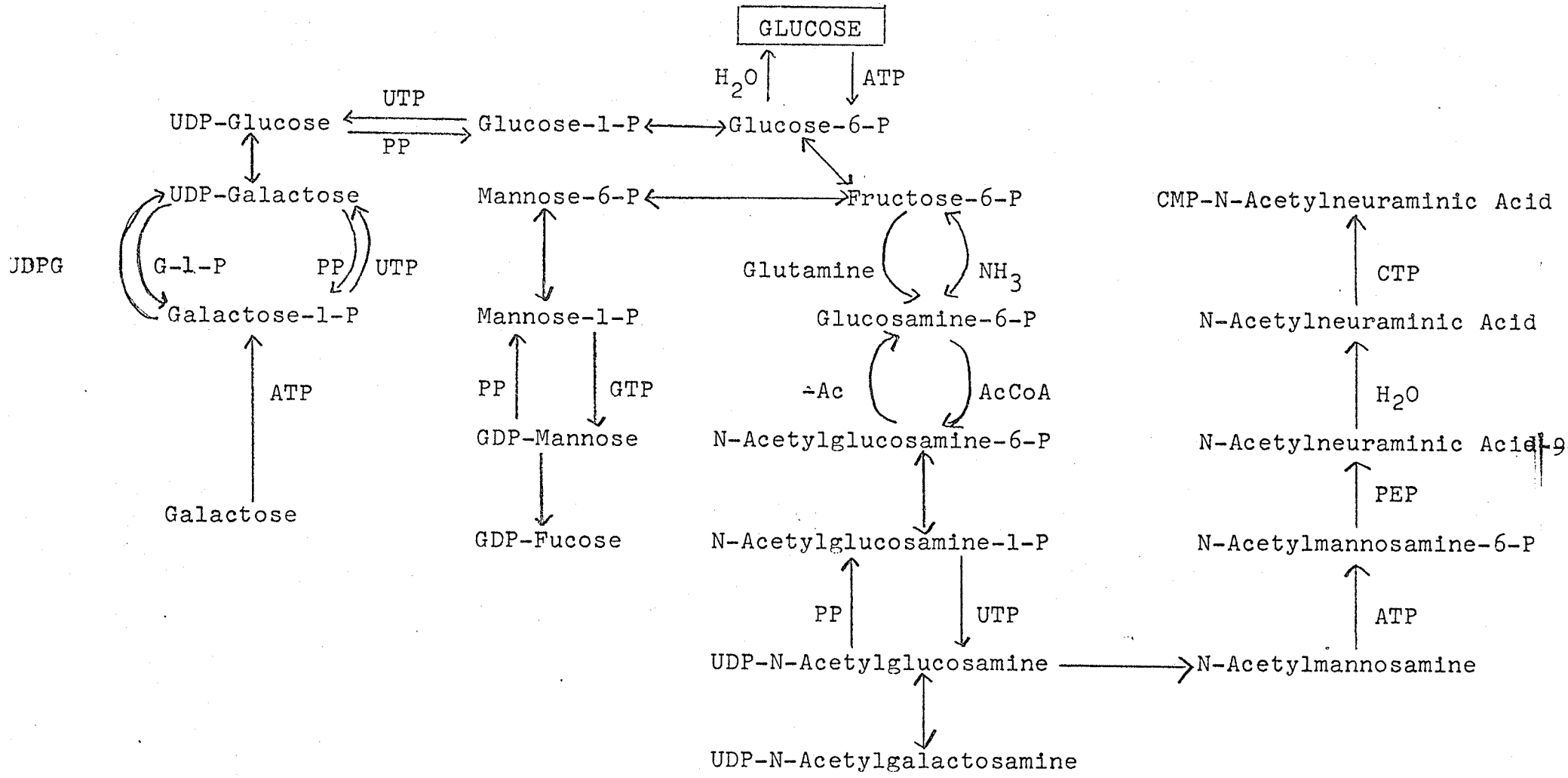

FIG. 5 PATHWAYS FOR THE BIOSYNTHESIS OF THE MONOSACCHARIDE CONSTITUENTS OF GLYCOPROTEINS

UTP = Uridine triphosphate; UDP = Uridine diphosphate; GTP = Guanosine triphosphate; GDP = Guanosine diphosphate; CTP = cytidine triphosphate; CMP = Cytidine monophosphate; ATP = Adenosine triphosphate; UDPG = Uridine diphosphate glucose; G-1-P = Glucose-1-Phosphate; AcCoA = Acetylcoenzyme A; PEP = Phosphoenol pyruvate; PP = inorganic pyrophosphate.

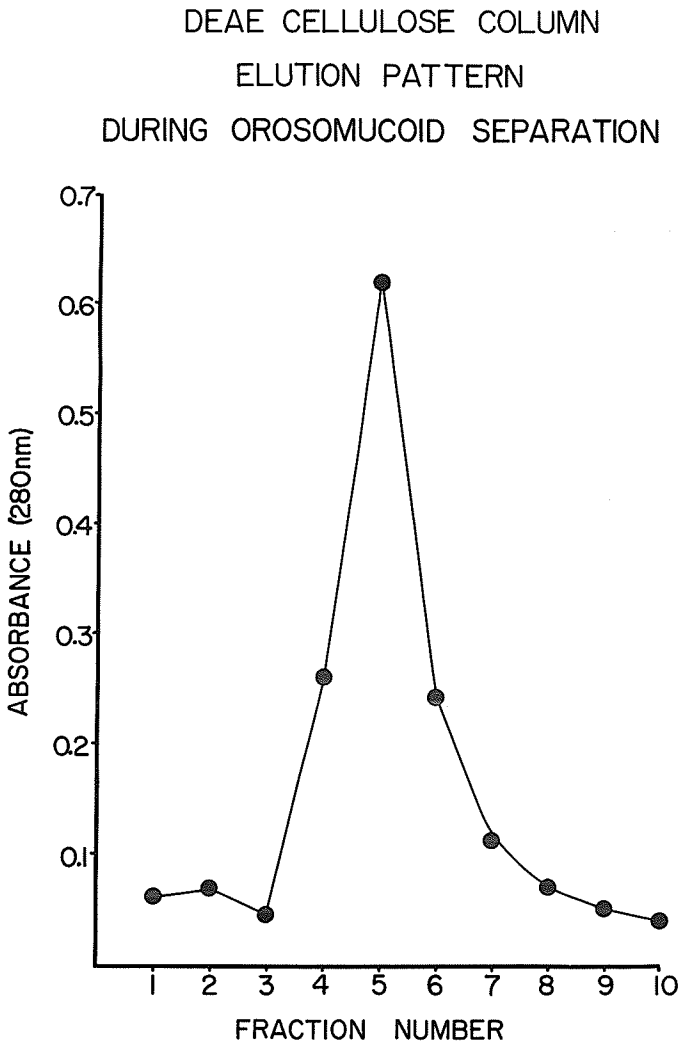

FIG. 6 DEAE CELLULOSE COLUMN ELUTION PATTERN DURING OROSOMUCOID SEPARATION

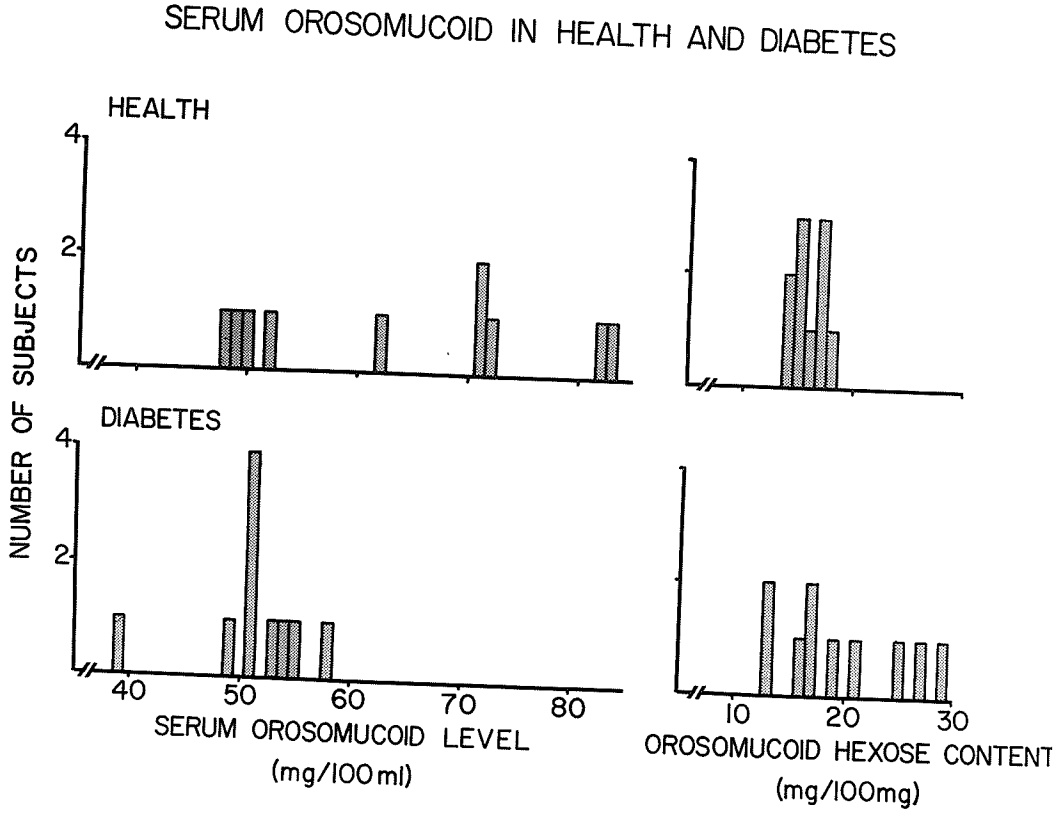

FIG. 7 SERUM OROSOMUCOID LEVELS AND HEXOSE CONTENT IN HEALTH AND DIABETES

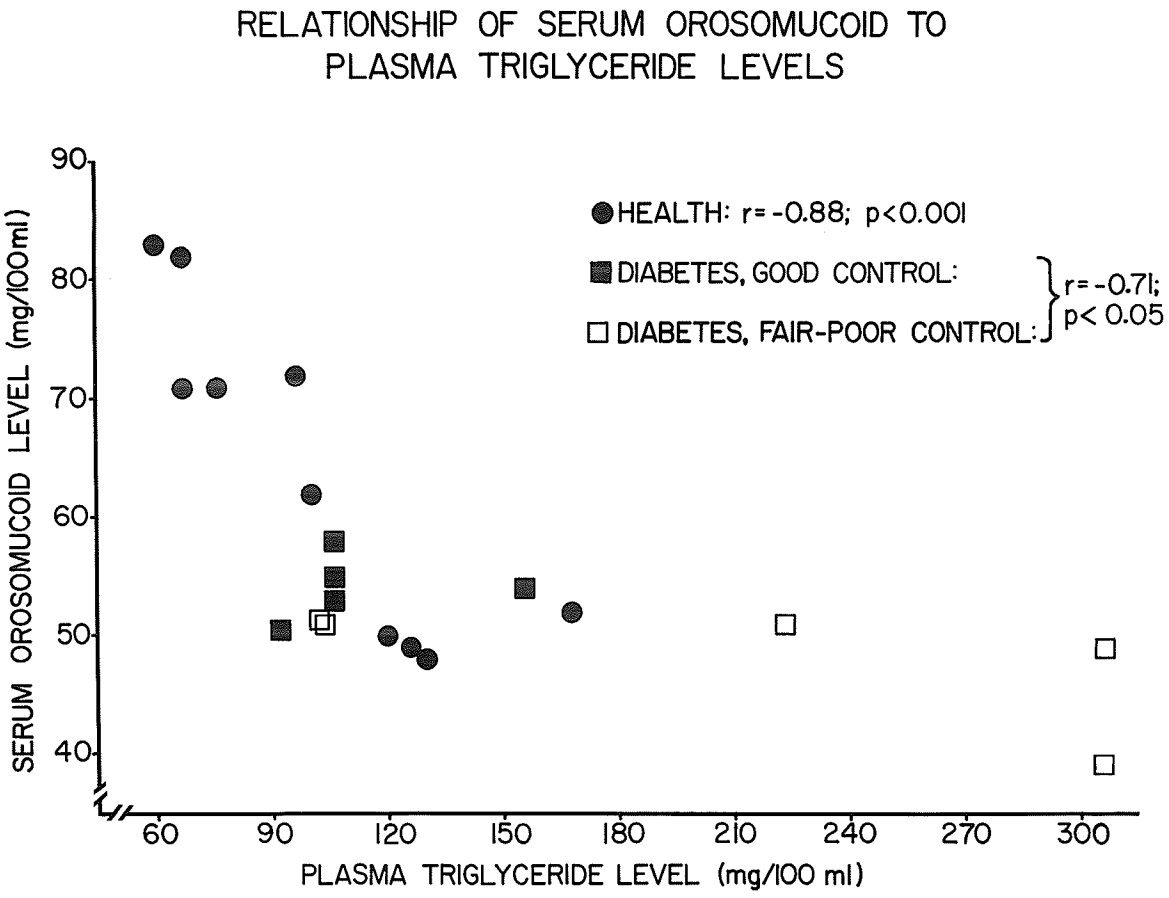

FIG. 8 RELATIONSHIP OF SERUM OROSOMUCOID TO PLASMA TRIGLYCERIDE LEVELS

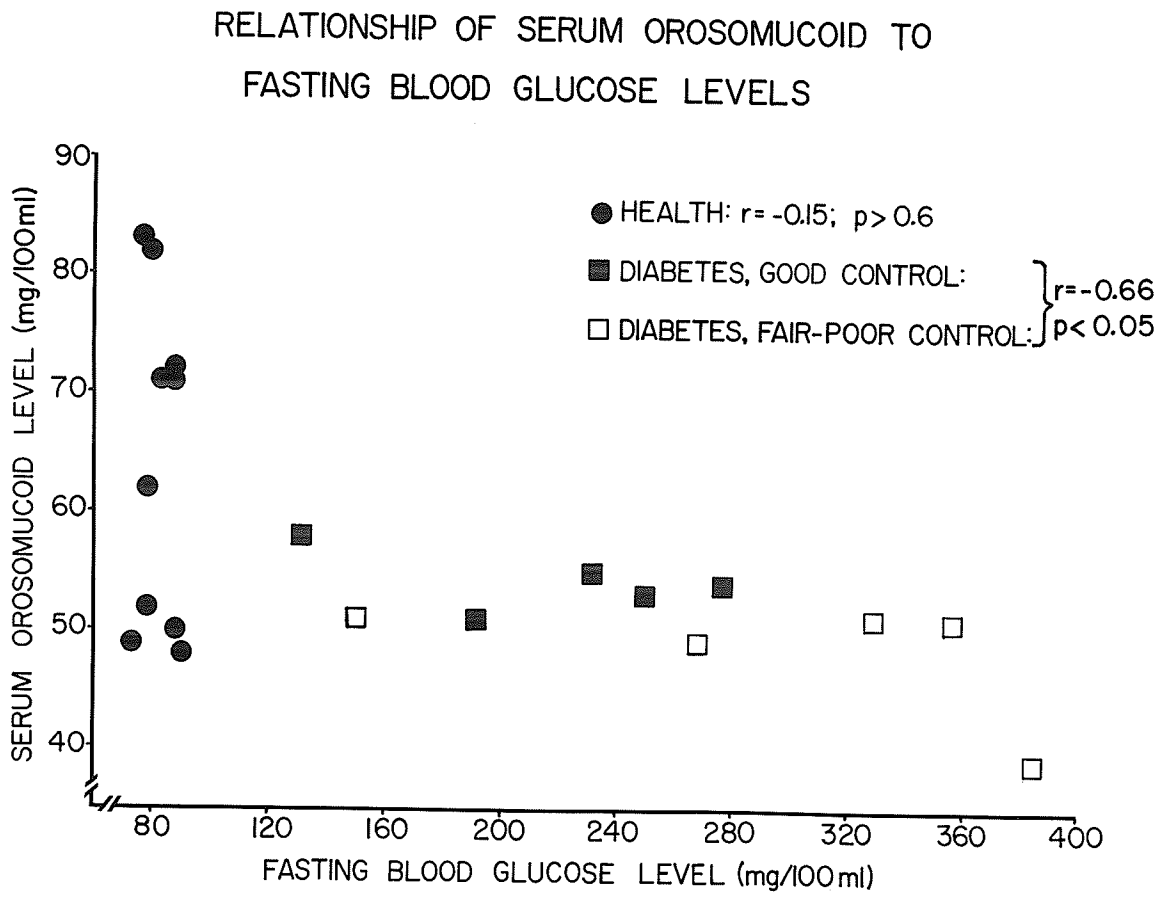

FIG. 9 RELATIONSHIP OF SERUM OROSOMUCOID TO FASTING BLOOD GLUCOSE LEVELS

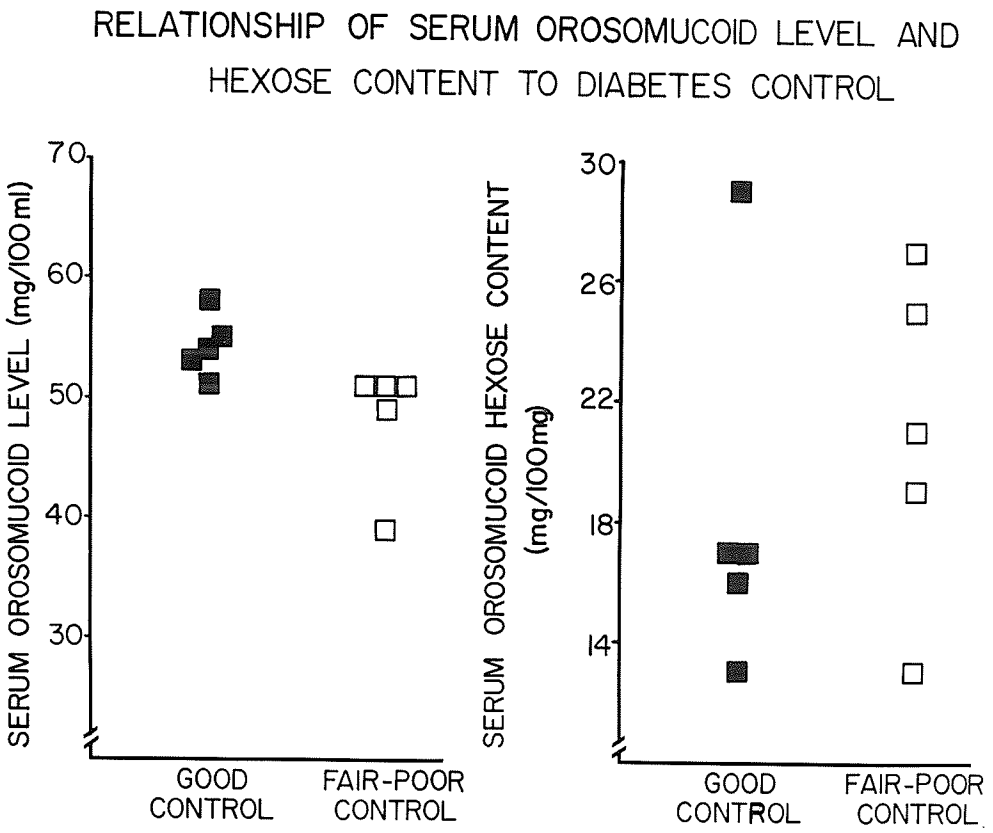

FIG. 10 RELATIONSHIP OF SERUM OROSOMUCOID LEVEL AND HEXOSE CONTENT TO DIABETES CONTROL

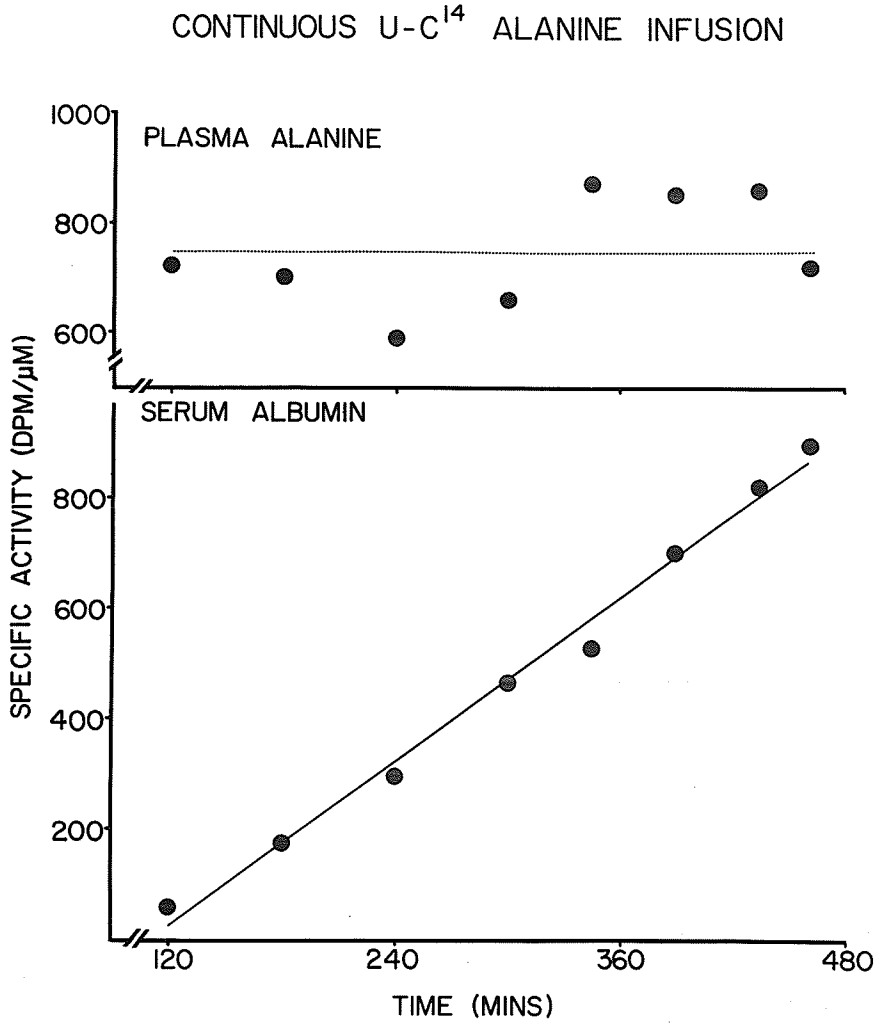

FIG. 11 THE SPECIFIC ACTIVITY-TIME CURVES OF PLASMA ALANINE AND SERUM ALBUMIN DURING THE U-C^{14} ALANINE INFUSION IN SUBJECT B.K.

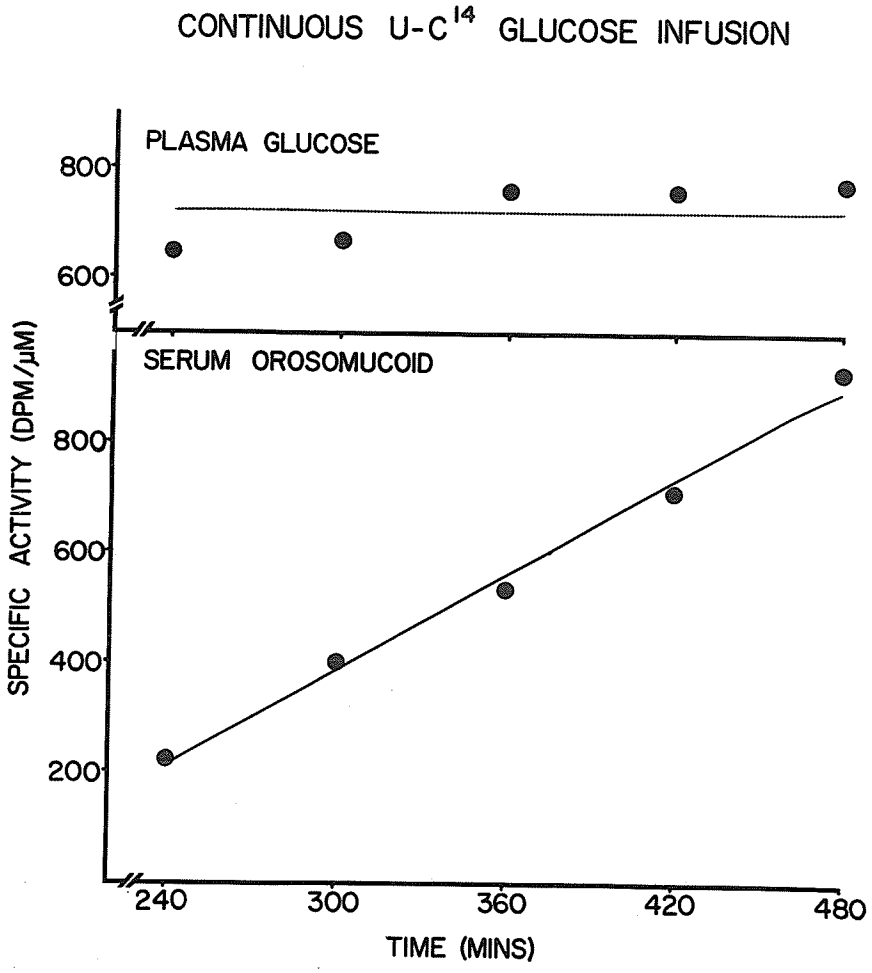

FIG. 12 THE SPECIFIC ACTIVITY-TIME CURVES OF PLASMA GLUCOSE AND SERUM OROSOMUCOID DURING THE U-C^{14} GLUCOSE INFUSION IN SUBJECT Y.K.