Measurement of Circulating Desialylated Glycoproteins and Correlation with Hepatocellular Damage

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A B S T R A C T   Addition of increasing amounts of ^{125}I-labeled desialylated thyroxine-binding globulin (DTBG) to hepatic cell membranes resulted in a progressive increase in binding. Saturability of membrane sites was indicated by a concentration beyond which further increases in [^{125}I]DTBG resulted in no further binding. The binding curve for [^{125}I]DTBG was similar to binding curves of desialylated orosomucoid, fetuin, and ceruloplasmin.

An inhibition assay system using hepatic cell membranes showed that desialylated orosomucoid had a greater affinity for membrane binding sites than did DTBG but desialylated fetuin and ceruloplasmin bound less avidly than DTBG.

Serum from normal persons and patients with a variety of illnesses was tested for its ability to inhibit [^{125}I]DTBG binding. The inhibitory activity of 1 ml of normal serum was equivalent to that of 0.2–2 μg DTBG. Patients with Laënnec's cirrhosis, biliary cirrhosis, and hepatic metastases had greatly increased inhibitory activity in their serum. Patients with jaundice due to extrahepatic obstruction had inhibitory activity not significantly different from that found in normal serum.

Column chromatography of normal serum on Sephadex G-200 resulted in inhibitory activity throughout the range of protein molecular weight. Desialylation of normal serum with neuraminidase enhanced the inhibitory activity but did not change the distribution of the activity. Gel chromatography of cirrhotic serum showed markedly increased inhibitory activity associated with the macroglobulins and the 4.5S peak and a new peak of inhibitory activity in the low molecular weight area was also seen.

Inhibition of desialylated glycoprotein binding to liver cell membranes by serum from patients with hepatocellular disease raises the possibility that desialylated serum glycoproteins accumulate in the circulation and that patients with compromised hepatocellular function may no longer be able to clear them from the circulation. Alternatively, accumulation of desialylated glycoproteins in the circulation could result from defective protein synthesis by the diseased liver.

INTRODUCTION

The presence of carbohydrate chains attached to many of the serum proteins has long been recognized. Nevertheless, the biologic significance of these sugar moieties has remained obscure. Ashwell and his colleagues have postulated that sialic acid–a nine carbon acidic monosaccharide which occurs only in the terminal position of glycoprotein carbohydrate side chains–appears to play a key role in determining the circulating life of serum glycoproteins (1–3). They have presented evidence that exposure of the penultimate galactosyl moiety of glycoprotein carbohydrate side chains by removal of the terminal sialic acid groups results in rapid clearance of many serum glycoproteins from the circulation and that the glycoproteins thus cleared can be recovered in the liver (1–3). This phenomenon has been demonstrated for orosomucoid, fetuin, haptoglobin, α_2-macroglobulin, ceruloplasmin, human chorionic gonadotropin and follicular stimulating hormone (2). These observations have been extended by their demonstration that isolated hepatic cell plasma membranes contain specific saturable binding sites for the desialylated glycoproteins (4).

Pathologic processes in the liver are often accompanied by changes in serum glycoproteins. Diffuse parenchymal liver disease usually presents with decreased serum al-

bumin and polyclonal increase in serum gamma globulin (5). Increased α_1-antitrypsin levels may contribute to the apparent rise in gamma globulin seen with electrophoresis (6). Beta globulin is often increased in primary biliary cirrhosis (7) and α_2-macroglobulin may rise in inflammatory exacerbations (8).

Observations made in this laboratory have shown that serum of cirrhotic patients often contains an additional thyroxin (T_4)[1]-binding protein which has an electrophoretic mobility slower than that of the glycoprotein thyroxine-binding globulin (TBG) (9). Other evidence previously reported has suggested that this slowly moving T_4-binding protein represents a partially desialylated form of TBG. At that time we proposed that hepatocellular destruction with fibrotic replacement of liver tissue might impair the clearance of desialylated glycoproteins from the circulation (9).

We now present evidence that human TBG can be added to the list of serum glycoproteins which bind to hepatic cell plasma membranes upon desialylation. We also show that desialylated TBG can be used in a hepatic cell plasma membrane inhibition assay as developed by Van Lenten and Ashwell (4) to demonstrate levels of circulating desialylated glycoproteins in normal human serum and markedly increased levels in serums from patients with liver disease.

METHODS

TBG was purified from pooled serum by affinity chromatography as previously described (10). Samples of orosomucoid and ceruloplasmin were kindly furnished by Dr. K. Schmid, Boston University, and Drs. G. Jamieson and M. Jett, American National Red Cross. Fetuin was purchased from Grand Island Biological Co., Grand Island, N. Y. Desialylated glycoproteins were prepared either by incubation with *Vibrio cholerae* neuraminidase or by treatment with 0.1 N H_2SO_4. When *V. cholerae* neuraminidase (Behringwerke AG, Marburg-Lahn, W. Germany) was used, 100 μg of protein in acetate buffer at pH 5.4 and calcium chloride in a final molarity of 5 mM was incubated with 5 units of neuraminidase. A "unit" of *V. cholerae* neuraminidase is defined by the manufacturer as that amount of enzyme which will release 1 μg N-acetylneuraminic acid per 15 min. When H_2SO_4 was used for desialylation, 100 μg of glycoprotein was combined with H_2SO_4, final concentration 0.1 N, and incubated for 1 h at 80°C.

Iodination was performed by using the chloramine-T method of Greenwood and Hunter (11) as modified by Levy, Marshall, and Velayo (12). About 0.5 mCi ^{125}I was used per 10 μg protein yielding specific activities of about 50 μCi/μg. The iodinated protein was usually diluted with unlabeled protein to obtain a specific activity of about 10 μCi/μg for use in the liver cell membrane binding system. The preparations of labeled glycoproteins were used for 2 wk and then discarded.

Rat liver cell plasma membranes were prepared by Ray's modification (13) of a method reported by Neville (14)

[1] *Abbreviations used in this paper:* DTBG, desialylated thyroxine-binding globulin; TBG, thyroxine-binding globulin; T_4, thyroxine.

using differential centrifugation in a discontinuous sucrose gradient. Protein concentrations were determined by the method of Lowry, Rosebrough, Farr, and Randall (15). Each membrane preparation was assayed by measurement of the marker enzyme, alkaline phosphatase. In our hands membrane preparations contained about four times the activity of this enzyme as those reported by Van Lenten and Ashwell (4) and about 1/3 the activity reported by Ray (13). The membranes were stored at − 60°C until used. Different preparations of membranes demonstrated little variation either in the binding curves or maximum binding capacity for desialylated TBG. The frozen membranes could be used successfully for at least 6 wk.

Binding of desialylated glycoproteins to rat liver cell plasma membranes was carried out as described by Van Lenten and Ashwell (4). Plasma membranes containing 50 μg protein were incubated with radioiodinated desialylated glycoproteins in an N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES), sodium chloride, calcium chloride buffer, pH 7.3, containing 1% bovine serum albumin as described by Van Lenten and Ashwell (4). Incubation was carried out in a shaking water bath at 37°C for 1 h. After incubation the unbound radioactivity was separated from the membrane-bound radioactivity by filtration through a Whatman GF/C glass fibre filter which was then dried and counted for radioactivity in a Nuclear-Chicago well type scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) whose efficiency was about 50%. Blanks to determine nonspecific binding of radioactivity to the filters were included in each assay. These consisted of adding labeled desialylated glycoprotein to an ice-cold mixture of buffer and membranes and immediate filtration. Blanks were in all cases less than 3% of the total radioactivity. The counts obtained from the blanks were subtracted from those obtained from the incubated samples.

Two control experiments were carried out to exclude the possibility that the presence of neuraminidase used to desialylate the glycoproteins had any effect on binding of the desialylated glycoproteins to liver cell plasma membranes, since Van Lenten and Ashwell have shown membrane sialic acid to be essential for binding of desialylated glycoproteins (4). In the first experiment desialylated glycoprotein binding to the membranes was measured in the presence of increasing amounts of neuraminidase. A decrease in binding occurred only at neuraminidase concentrations greater than 5 units/50 μg membrane protein—an amount 2,000 times greater than the concentration of neuraminidase present in our standard assay. The second experiment demonstrated no change in binding after the neuraminidase used to desialylated glycoproteins had been removed by passing the glycoprotein-neuraminidase mixture through an affinity column of Sepharose to which a specific neuraminidase inhibitor, p-aminophenyloxamic acid, had been coupled via a tripeptide "arm" (16).

The inhibition assay was carried out in the same manner, except that the membranes were incubated with unlabeled glycoprotein or serum aliquot to be tested for 1 h at 37°C. Then an amount of ^{125}I-labeled desialylated glycoprotein previously determined to be sufficient to saturate the membrane binding sites was added. Incubation was continued for an additional hour and the mixture filtered. The inhibition of binding of labeled desialylated glycoprotein caused by varying amounts of unlabeled inhibitor was quantitatively related to the concentration of the latter, as shown previously (4). In most cases the ^{125}I-labeled glycoprotein used in this assay system was desialylated TBG (DTBG). In addition to the blanks described above controls consisting
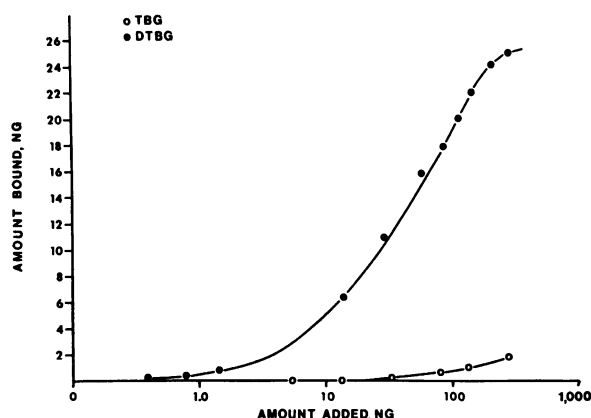
FIGURE 1 Binding of [^{125}I]desialylated TBG (DTBG) and [^{125}I]TBG (TBG) to rat liver cell plasma membranes. Specific activity of DTBG, 54 μCi/μg. Specific activity of TBG, 62 μCi/μg.

of increasing known concentrations of unlabeled DTBG were run with the inhibition assay. Inhibition was expressed in terms of nanograms DTBG equivalents, that is, the nanograms of DTBG required to cause equivalent inhibition.

Serum samples, kindly furnished by Dr. Clive Hamlin of the Institute of Pathology, Case Western Reserve University, were collected in red-top Vacutainer tubes (Becton, Dickinson & Co., Rutherford, N. J.) and were separated from the red cells within 2 h. All specimens were stored at 4°C and were stable for up to 2 wk when assayed in the inhibition binding assay. Normal serum stored at − 20°C for 3 yr showed a twofold increase in the expected inhibitory level. Inhibitory activity in serum was stable after heating to 65°C for 30 min.

Column chromatography of normal serum and serum from patients with hepatic cirrhosis was done on Sephadex G-200 using a 1.5 × 100-cm Glenco column (Glenco Scientific, Inc., Houston, Tex.). The gel was equilibrated with 0.06 M Tris-HCl, pH 8.6, and elution of the serum sample was done with the same buffer. A flow rate of 6 ml/h was used and 1.2-ml aliquots were collected. The optical density at 280 nm was used for determining protein concentration.
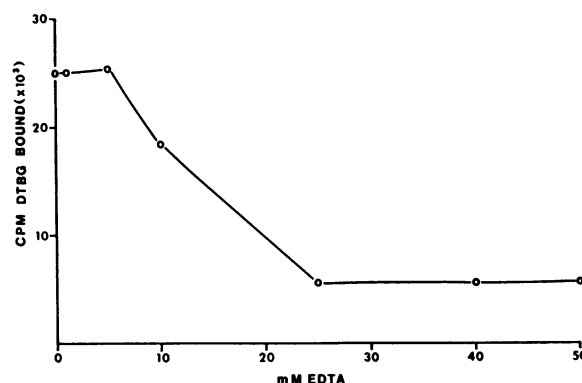

FIGURE 2 The effect of EDTA on binding of DTBG to liver cell membranes. Conditions for the assay were as given in Methods with the exception that the indicated concentration of EDTA was present in solution with 10 mM Ca^{++}.

RESULTS

A typical curve describing [^{125}I]DTBG binding to rat liver cell plasma membranes is shown in Fig. 1. Addition of increasing amounts of the desialylated glycoprotein resulted in a progressive increase in the amount bound; further increases in [^{125}I]DTBG concentration result in no further binding, indicating the saturability of the binding sites. At the lowest level of [^{125}I]DTBG added, approximately 95% of the counts were bound; when saturation was reached approximately 3% of the added radioactivity was bound. Native (fully sialylated) [^{125}I]TBG bound to the membranes only negligibly under these conditions (Fig. 1). As shown by Van Lenten and Ashwell (4), the presence of Ca^{++} was required for binding in this system (Fig. 2).

The results of inhibition assays in which purified unlabeled desialylated glycoprotein inhibitors were preincubated with the membranes are shown in Fig. 3. Unlabeled DTBG prevented the binding of approximately
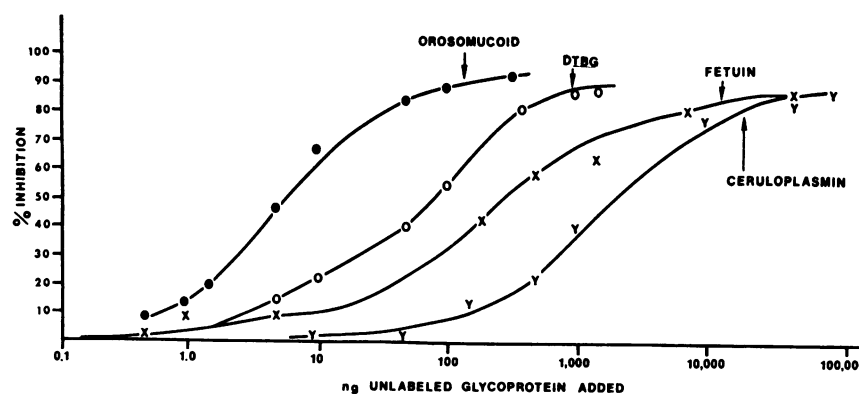

FIGURE 3 Inhibition of [^{125}I]desialylated orosomucoid binding to liver cell plasma membranes by nonradioactive desialylated glycoproteins. For conditions of the inhibition assay see Methods.
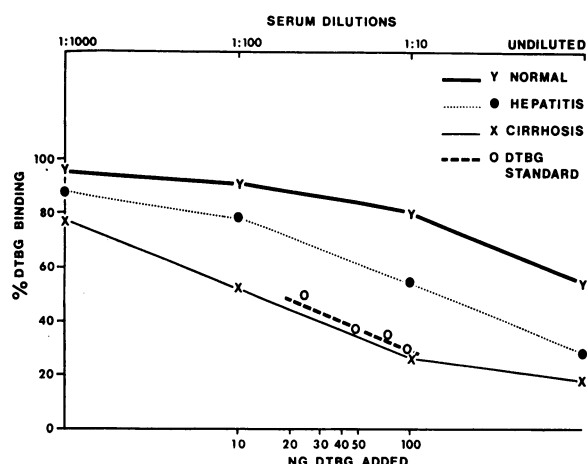
FIGURE 4 Effect of serum dilutions on the inhibition of binding of [^{125}I]DTBG to rat liver cell plasma membranes, top abscissa. A standard curve of [^{125}I]DTBG binding is also depicted, bottom abscissa. 10-μl serum samples were added to standard incubation mixture. 125 ng labeled DTBG was used in the competitive binding assay of normal, hepatitis, and cirrhotic serums.

90% of [^{125}I]desialylated orosomucoid at a concentration of 2 μg/ml. Desialylated orosomucoid appeared to have a greater affinity than DTBG for the plasma membrane binding sites as shown by a shift of the inhibition curve to the left by nearly a full order of magnitude of concen-
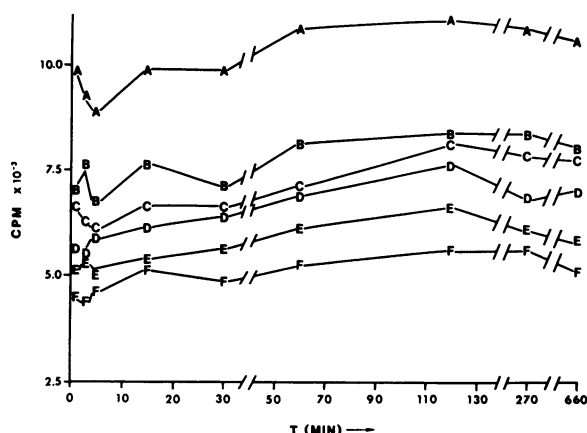

FIGURE 5 Inhibition of liver cell plasma membrane binding of ^{125}I-labeled DTBG by various dilutions of serum from a patient with hepatic cirrhosis as a function of the time of incubation. The serum sample was incubated with membranes for the indicated length of time and then an amount of [^{125}I]DTBG known to saturate the binding sites was added and the incubation continued for an additional hour. The ordinate represents cpm [^{125}I]DTBG bound to membrane; the fewer counts bound, the greater the inhibition by the serum. A—A represents inhibition of [^{125}I]DTBG by unlabeled DTBG. B—B, C—C, D—D, E—E, and F—F represent 1:16, 1:8. 1:4, 1:2, and undiluted cirrhotic serum, respectively. 10-μl serum samples were added to standard incubation mixtures.

tration. Similarly, desialylated fetuin and desialylated ceruloplasmin appeared to bind less avidly than DTBG. The standard deviation of duplicate samples within a single assay was ±2%. The standard deviation of 12 identical inhibition assays run over a period of 3 wk was ±8%; greater deviation of values appeared to occur with aging of the radiolabeled protein.

Untreated serum from normal individuals and from patients with a variety of illnesses preincubated with plasma membranes inhibited the binding of [^{125}I]-DTBG in a manner analogous to the desialylated purified proteins (Fig. 4). The similarity of the inhibition curves of purified desialylated glycoproteins and whole serum suggested an interaction at the same binding sites on the liver cell membranes. Experiments were performed to measure binding inhibition of mixtures of normal and abnormal serums of known individual inhibitory activity. The total inhibitory activity of the combined serums was always recoverable to within 10% of the values expected from determinations using the individual serums.

Varying the time of membrane preincubation with serum from 60 min to 12 h did not affect the level of inhibition of [^{125}I]DTBG binding.

In order to examine the possibility that the inhibition of membrane binding of [^{125}I]DTBG by whole serum might be due to a circulatory neuraminidase which might destroy the membrane binding sites or to a circulating galactosidase which might cleave the exposed penultimate galactose from the desialylated [^{125}I]DTBG, thereby preventing membrane binding of the labeled glycoprotein, parallel incubations of membranes in the presence of increasing amounts of serum were performed and the time-course of binding observed. It was expected that if the serum contained factors other than desialylated glycoproteins that interfered with binding, the binding curves might not demonstrate parallelism once maximal inhibition was reached. Fig. 5 shows that the inhibition curves are parallel once maximal binding was reached.
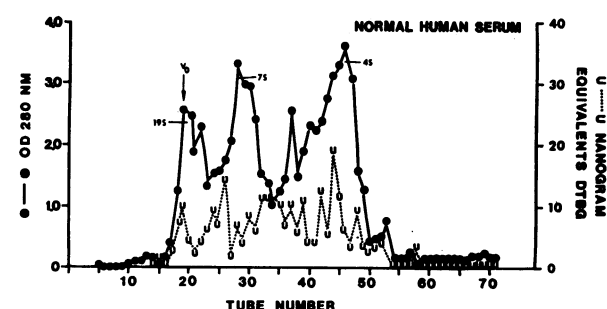

FIGURE 6 Column chromatography of normal human serum on Sephadex G-200 and the inhibition of liver cell plasma membrane binding of [^{125}I]DTBG, expressed as nanogram equivalents DTBG, by each fraction. 1 ml serum applied to a 1.5 × 100-cm column. Flow rate, 6 ml/h. 1.2-ml fractions. Vo, void volume as determined by blue dextran.
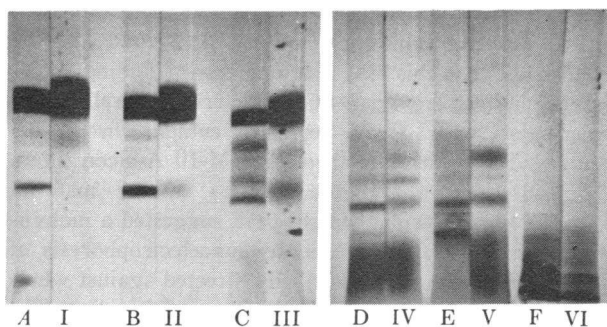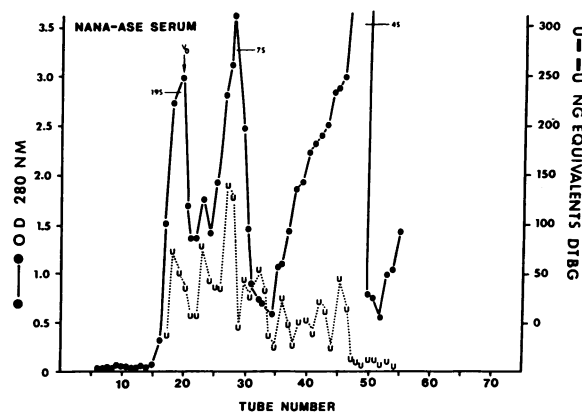
FIGURE 7 Disk gel electrophoretograms of fractions from Sephadex G-200 column chromatography of ml of normal and cirrhotic serum. Gels I through VI (normal serum) represent tube numbers 24, 31, 34, 40, 46, and 50, respectively, of Fig. 6 and gels A through F (cirrhotic serum) represent tube numbers 20, 26, 30, 35, 42, and 46, respectively, of Fig. 10. Amido-black stain. Electrophoresis in Tris-glycine buffer, pH 8.6, for 40 min at 3 mA constant current per tube. Anode at bottom.

FIGURE 8 Column chromatography of normal human serum treated with *V. cholerae* neuraminidase on Sephadex G-200. The inhibition of [^{125}I]DTBG binding, expressed as nanogram equivalents DTBG, to liver cell plasma membranes by each fraction is shown by the dotted line. 1 ml serum incubated overnight at room temperature with 5 units *V. cholerae* neuraminidase was applied to the column. 1.2-ml fractions. Flow rate, 6 ml/h. V_0, void volume as determined by blue dextran. NANA-ase, *N*-acetylneuraminic acidase.

Normal serum was fractionated by column chromatography on Sephadex G-200 (see Methods) and the aliquots were tested for their ability to inhibit [^{125}I]DTBG binding (Fig. 6). Peaks of inhibitory activity were present throughout the range of serum protein molecular weights. There was relatively little inhibition shown in the aliquots containing carbohydrate-poor 7S immunoglobulin and in those containing carbohydrate-free albumin. Peaks of inhibitory activity roughly coincided with aliquots from segments of the chromatographic pattern known to correspond to carbohydrate-rich proteins (17).

Disk gel electrophoretograms of several aliquots from the gel chromatographic separation (Fig. 7) demonstrate the presence of numerous proteins in each fraction. It is therefore probable that inhibitory activity is not confined to species of a single molecular weight or a single electrophoretic mobility.

Exposure of normal human serum to *V. cholerae* neuraminidase (5 units/ml) followed by fractionation on Sephadex G-200 produced fractions with markedly elevated levels of inhibition (Fig. 8). Except for a peak correspondng to 7S protein, the pattern of inhibition in a qualitative sense was not significantly different from that of untreated serum.

A number of normal serums and serums from patients with a variety of illnesses were tested for their ability to inhibit [^{125}I]DTBG binding (Fig. 9). The range of inhibitory activity of 10 μl of normal serums was equivalent to 2–10 ng DTBG. Serum from patients with Laënnec's cirrhosis showed inhibition equivalent to 30–80 ng DTBG and two cases with biliary cirrhosis showed inhibition equivalent to 22–50 ng DTBG. Two patients with hepatic metastases showed elevated inhibi-

tory levels equivalent to 42 and 70 ng DTBG and one patient with a history of alcoholism and a primary hepatoma demonstrated inhibition equivalent to 55 ng DTBG.

On the other hand, four patients with jaundice secondary to common bile duct obstruction by tumor had levels of inhibition less than 20 ng DTBG equivalents. Similarly, one patient with ascites secondary to pancreatitis and another patient with abnormal liver function tests due to congestive heart failure had inhibitory activity equivalent to 6 and 17 ng DTBG. Two patients with markedly abnormal rheumatoid factor latex fixation

FIGURE 9 Inhibitory activity of serums from normal individuals and from patients with a variety of illnesses. CHF, congestive heart failure; LFT, liver function tests.
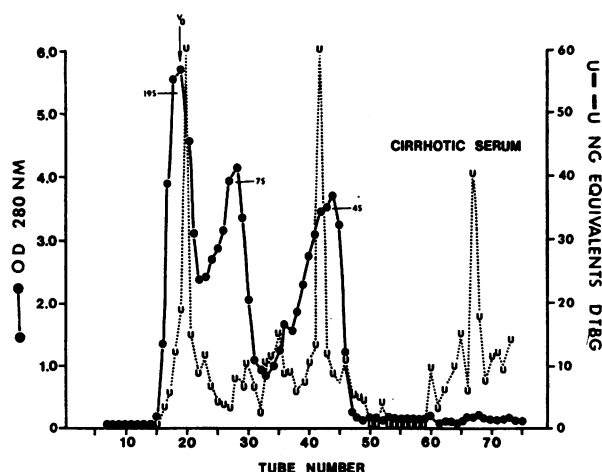
FIGURE 10 Column chromatography on Sephadex G-200 of serum from a patient with hepatic cirrhosis. Inhibition of liver cell plasma membrane binding of [^{125}I]DTBG, expressed as nanogram equivalents DTBG, is shown for each fraction. 1 ml serum was applied to the column. 1.2-ml fractions. Flow rate, 6 ml/h. V_0 = void volume as determined by blue dextran.

tests also had serums with inhibitory activity within the normal range of values. In our small series of ten patients with no demonstrable hepatic disease there were two who had abnormal levels of serum inhibitory activity. One was a 17-yr-old boy 1 yr post-pulmonary lobectomy for staphylococcus abscess who had an upper respiratory infection that was probably of viral etiology. The second was a 27-yr-old alcoholic with normal liver function tests and a lobar pneumococcal pneumonia.

When the results of inhibition of [^{125}I]DTBG binding by serum from patients with a variety of hepatic disease were compared with standard liver function test results in these patients, no simple correlation could be found between serum inhibitory activity and levels of alkaline phosphatase, serum glutamic oxalacetic transaminase, total bilirubin, serum albumin, or serum globulin.

Column chromatography on Sephadex G-200 of a sample of serum from a patient with cirrhosis (Fig. 10) showed the expected reversal of albumin to globulin ratio compared to normal serum. The amount of inhibitory activity was greater than that of normal serum fractions and the pattern of inhibition was unique in showing three spikes of activity corresponding to the macroglobulins, to globulins with a molecular weight roughly equal to that of albumin, and a small peak of protein more retarded and therefore presumably of lower molecular weight than albumin. This same pattern of optical density and inhibitory activity was observed in three additional patients with end-stage cirrhosis whose serum was fractionated on Sephadex G-200. There did not ap-

pear to be any simple relationship between optical density at 280 nm and inhibitory activity. It should also be pointed out that this was not a universal finding in all serums obtained from patients with cirrhosis. Preliminary experiments with G 25 Sephadex column chromatography and ultrafiltration through UM-10 Amicon filters (Amicon Corp., Lexington, Mass.) of the material from the low molecular weight peak suggested a molecular weight of 10,000 or less. Immunoelectrophoresis of this material with rabbit antibody directed against whole human serum failed to produce any precipitin line. The significance of this last peak of inhibitory material is not known and is currently under investigation.

DISCUSSION

When present, sialic acid occurs as the terminal nonreducing sugar of carbohydrate chains of glycoproteins (18). The penultimate sugar is typically galactose (19). Morell, Gregoriadis, Scheinberg, Hickman, and Ashwell (2) showed that desialylation and injection of purified ceruloplasmin resulted in the rapid hepatic uptake of the glycoprotein from the circulation and that the integrity of the exposed galactose moiety was an absolute requirement for clearance. Similar results for other serum glycoproteins were shown including orosomucoid, fetuin, thyroglobulin, haptoglobin, α-macroglobulin, human chorionic gonadotropin, and follicle-stimulating hormone (2). In addition, Van Lenten and Ashwell (4) have demonstrated specific saturable binding sites for a number of desialylated glycoproteins on a plasma membrane fraction of rat liver cells. However, the binding process in both the in vivo system, observed by injecting desialylated glycoproteins into the circulation, and the in vitro system, using the membrane binding assay, was followed by using exogenously prepared desialylated glycoproteins. There was no evidence that serum glycoproteins exist in a desialylated form in vivo or that hepatic plasma membrane binding normally plays a role in their turnover. Our finding of a partially desialylated form of TBG in serum from patients with hepatic cirrhosis (9) led us to investigate the possible physiologic significance of hepatocellular desialylated glycoprotein binding.

We have presented evidence that the hormone transport protein, TBG, may now be added to the group of serum glycoproteins which, after desialylation, bind to liver cell membranes. The inhibition curves shown here indicate that desialylated TBG binds to liver cell plasma membranes more avidly than desialylated fetuin or ceruloplasmin, but less strongly than desialylated orosomucoid. Quantitative determination of affinity constants employing Scatchard plots has not been possible because of the failure to demonstrate reversibility of binding under the conditions of the assay. These findings are

consistent with our observations that desialylated TBG is rapidly cleared from the circulation by the liver in rats, rabbits, and humans (20).

We have attempted to demonstrate the presence of desialylated glycoproteins in serum by its ability to compete with the binding of a purified desialylated glycoprotein by the liver plasma membrane preparation. For convenience, we chose TBG. In these studies competitive activity in both normal and cirrhotic serums has been demonstrated. The inhibition of membrane binding of desialylated TBG by serum suggests that the circulation normally has detectable levels of partially desialylated glycoproteins. Evidence for this includes (a) activity in an inhibition assay previously shown to be quite specific for desialylated glycoproteins and glycopeptides (4), (b) the occurrence of peaks of inhibition throughout the molecular weight range of serum proteins, (c) a similar but enhanced pattern of inhibition after column chromatography of serum pretreated with neuraminidase, and (d) the similarity of the inhibition curves of serum and of desialylated purified glycoproteins.

The physiologic significance of hepatocyte plasma membrane binding is suggested by the glycoprotein alterations observed in serum from cirrhotic patients. These serums showed a marked elevation of inhibitory activity which we believe reflects an increase in levels of desialylated glycoproteins. This interpretation is supported by our earlier studies of TBG from patients with cirrhosis (9) which showed the appearance of a partially desialylated form of this protein in some of their serums. An increase in desialylated glycoproteins in serum from patients with hepatic cellular damage could result from several abnormal conditions which may accompany cirrhosis such as: (a) pathologic alteration of hepatocyte function resulting in the synthesis and release of glycoproteins into the circulation with incomplete carbohydrate chains, (b) pathologic decreases in the number of hepatocyte membrane binding sites, (c) desialylation of serum glycoproteins at a rate in excess of uptake by a damaged liver, (d) presence in the serum of substances which may compromise the ability of desialylated glycoproteins to bind to the hepatocyte membranes. These abnormalities, either separately or in combination, could result in an increase in the steady-state level of serum desialylated glycoproteins.

If one assumes that the in vitro inhibition binding assay is a good reflection on the in vivo binding process, then the fourth possibility is considered unlikely because of the following observations. Increasing serum concentrations yield parallel inhibition curves (Figs. 4 and 5) which are essentially stable with time, suggesting the absence of a serum neuraminidase activity which inactivates membrane binding sites or of galactosidase

activity which might destroy the ability of radiolabeled desialylated TBG to bind. Furthermore, the results of gel filtration chromatography, of both normal and cirrhotic serums, demonstrating substances with inhibitory activity throughout the molecular weight range of serum glycoproteins rule out the possibility that a single species could be responsible for the inhibition.

Cirrhotic serums demonstrated the most significant increases in inhibitory activity compared to normal serums in the macroglobulin and 7S globulin fractions, as well as substance(s) with a lower molecular weight than albumin. Further work will be required to correlate these findings with the increases in α_2-macroglobulin, haptoglobin, 7S globulins, and α_1-antitrypsin known to occur in cirrhosis (5).

The gamma globulin fraction from either normal or cirrhotic patients was a poor inhibitor of the membrane binding system, but treatment of serum with neuraminidase caused a significant increase in inhibitory activity. Experiments currently in progress indicate that desialylated immune globulins bind to liver cell membranes competitively with other desialylated glycoproteins. This binding might be expected and is consistent with the carbohydrate structures of IgG, IgM, and IgE, which have been elucidated by Kornfeld and co-workers (21–23). Although gamma globulin may be less susceptible to the in vivo mechanism for desialylation than the majority of glycoproteins, a case report has appeared describing a monoclonal gammopathy in association with cirrhosis in which the IgG was deficient in sialic acid (5). A specific interaction between the desialylated IgG and IgM was found, suggesting an antibody-antigen complex. Thus, the removal of sialic acid from circulating IgG may "uncover" an antigenic site to which autoantibodies might be produced. Accordingly, the hepatic removal of desialylated glycoproteins from the circulation may not be just a "passive" catabolic process, as suggested by Van den Hamer, Morell, Scheinberg, Hickman, and Ashwell (1) but may protect the organism against production of autoantibodies to "damaged" proteins.

Serum from patients with cirrhosis has also been reported to contain increased levels of a soluble galactosyltransferase (24). A preliminary report (25), as yet not confirmed, suggests that the binding site for desialylated glycoproteins on the liver cell is a membrane-bound galactosyltransferase. A soluble receptor site in the serum for desialylated glycoproteins could effectively compete with the hepatic membrane binding sites and result in impaired hepatic uptake. In addition, the increased galactosyltransferase activity in serum of patients with cirrhosis could reflect degradation of liver membrane binding sites. As yet, the mechanism(s) of desialylation of serum glycoproteins is not understood.

The clinical importance of the inhibition assay must await the study of a larger number of patients. However, Laënnec's and biliary cirrhosis, primary and metastatic liver carcinoma, and hepatitis appear to be associated with elevated levels of circulating desialylated glycoproteins. Patients with abnormal liver function tests due to congestive heart failure, pancreatitis, or extrinsic biliary tract obstruction had normal inhibitory activity. We are currently engaged in following the time-course of desialylated serum glycoprotein levels of a larger number of patients with a variety of pathologic entities in order to evaluate the clinical value of this test in determining diagnosis and prognosis of hepatic disease.

ACKNOWLEDGMENTS

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