Seromucoid in Hepatobiliary Disease

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With the technical assistance of Florence L. Jones

An increase in circulating carbohydrate-rich proteins has been known for some time to occur in diseases associated with neoplastic or inflammatory processes. These substances have been variously termed polypeptides (1-4), proteoses (5), mucoid-like material (6), seromucoid (7-10), and mucoprotein (11-15). It was only recently that the occurrence of a pathologic depression of the seromucoid (SM) level* was discovered, observed principally in hepatic disease (16-19). Thus, the incidence of a low SM level among 180 patients with either viral hepatitis or portal cirrhosis was about 80 per cent and compared favorably in diagnostic efficiency with the usual flocculation and other hepatic tests. On the other hand, only 2 per cent of 125 cases of obstructive jaundice showed a subnormal level, while 50 per cent had an increase (18, 19).

Since the test, in addition to its reputed diagnostic utility in such conditions as rheumatic fever and renal disease (20, 21), promised to be a useful means of distinguishing between “medical” and “surgical” jaundice (22)—an ever-present clinical problem—it was being considered by the authors for adoption in the routine hospital laboratory. As a necessary preliminary step, an attempt was made to reappraise the clinical significance of the test. The results of this study, which, in part, were in discord with the cited published findings, are reported here.

MATERIAL AND METHODS

Patients afflicted with various types of jaundice or hepatomegaly (other than that usually observed in congestive heart failure), or both,
were studied. Only those patients were included in this report whose diagnosis appeared established by autopsy, biopsy, surgical operation, or prolonged clinical observation. "Normal" controls were recruited from the hospital* and Public Health Service staffs; "hospital" controls included patients with such localized lesions as were unlikely to exert a significant systemic effect and to influence the SM level, such as healing fracture, cataract, or peptic ulcer.

Blood specimens for SM analysis were usually secured in the morning from subjects in the preprandial state, except for experiments designed to determine the effect of food intake on the SM concentration (see below). Serum was separated promptly upon adequate clot retraction and stored at \(-10^\circ\) to \(-15^\circ\), if not analyzed the same day. Results of the usual hepatic tests, including serum bilirubin (23), cephalin cholesterol flocculation (24), thymol turbidity (24), and alkaline phosphatase (25), were used for correlation with SM values only if the interval between respective blood collections was no greater than three days.

For the purpose of adequate comparison with Greenspan's data (18), his method of analysis—originally described by Simkin and coworkers (16)—was adopted, although some of its shortcomings were recognized early in the study (26). Serum (usually 4 ml., never less than 2 ml.) was treated with twice the volume of 0.6M perchloric acid. After exactly 10 minutes of standing and subsequent filtering through Whatman #5 paper, filtrate and 5% phosphotungstic acid (in 2N HCl) were, in a volume ratio of 3:1, placed in a 15 ml. centrifuge tube, gently mixed, and spun 15 minutes later at about 500 G, for 10 minutes. The seromucoid precipitate was washed once with the phosphotungstic acid solution and, after thorough drainage, dissolved in 3 ml. of 0.2N NaOH and 0.6 ml. of biuret reagent. After one hour's standing in a dark cabinet, the samples were read in a Beckman Model DU spectrophotometer at 540 μ. Calculation of the SM content was made by reference to a standard curve obtained by means of solutions of 50 to 200 mg. of casein per 100 ml. of 0.2N NaOH, treated with biuret as indicated above.

RESULTS

Analysis of SM values determined among the normal controls revealed no striking difference between the fasting and nonfasting groups, if one discounts the low upper extreme in the nonfasting female group because of the small number of observations (Table 1). In individual cases, however, food intake did seem to influence the SM level, contrary to Green-

* Grady Memorial Hospital, Veterans Administration Hospital, and Emory University Hospital, Atlanta, Ga.
span's experience (18), though not in a consistent manner (Fig. 1). Consequently, emphasis was placed on collecting blood for SM analysis from fasting subjects only. The range of values in the group of hospital controls was somewhat higher than among the "normals." In accordance with the findings of Greenspan and his associates (18), the range for females was distinctly lower than that for males in each control group. For each sex, however, the span of extreme values was considerably wider in this study than in the former authors' series, even though the latter included nearly four times as many observations. The lowest values in

* Range employed in diagnosis.

† Hospital controls.

Fig. 1. Influence of food intake upon seromucoid level.
the present control group were found in young and slender but obviously healthy nurses and medical technologists.

To a limited extent, these discrepancies can be explained by the difficulties encountered in analysis. The coefficient of variation (27) of duplicate determinations on 65 unselected consecutive sera was as high as 6.1 per cent.* In other words, the SM values that may be expected from a single serum range ±12% from the "ideal" or "true" value. If the latter were 50 mg./100 ml., 95% of the replicate determinations would fall between 44 and 56 mg. per 100 ml. The experimental error among values obtained from aliquots of a single serum analyzed several days apart was even greater, the coefficient of variation among aliquots of 10 such sera approaching 10 per cent. Among the factors (subsequently discovered) that may be responsible for this variation are differences in temperatures prevailing on different days of analysis (Fig. 2), as well as inadvertent changes in the procedure of pipetting and mixing serum and perchloric acid (26). The chief problem of precision is apparently inherent in this initial step in analysis, designed to separate the seromucoid from the other serum proteins. Analysis (in triplicates) upon 10 different perchloric acid filtrates yielded a coefficient of variation of 1%, and upon 30 different casein solutions, of 0.8% only.

In view of these limitations and the relatively small number of "nor-

* Winzler's method (11) yielded a coefficient of variation of 5 per cent.
mal” subjects studied, the 65 per cent confidence limits (27) were chosen to designate the normal range, which therefore constituted 45–80 mg./100 ml. for males and 30–65 mg./100 ml. for females. These extremes are in fairly close agreement with the 95 per cent confidence limits which represented the normal range of SM in Greenspan’s series.

Among 44 cases of viral hepatitis (including 13 instances of presumed virus B infection), 17, or 39 per cent, showed a subnormal SM upon initial examination (Table 2). This percentage increased to 49 in the 29 cases in whom subsequent SM determinations could be secured. Nine of the above 44 patients were found to have complicating conditions (recent

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<tr>
<th>Table 2. SEROMUCOID LEVELS IN 165 PATIENTS WITH HEPATOBILIARY DISEASE</th>
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<td><strong>Diagnosis</strong></td>
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<tr>
<td>Acute hepatitis</td>
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<tr>
<td>A or B virus</td>
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<td>Leptospirai</td>
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<tr>
<td>Amoebic</td>
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<tr>
<td>Septic</td>
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<td>Chronic liver disease</td>
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<td>Alcoholic cirrhosis</td>
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<td>Postnecrotic cirrhosis</td>
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<td>Hemochromatosis</td>
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<td>Sarcoïdosis</td>
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<td>Biliary obstruction</td>
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<td>Acute cholecystitis</td>
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<td>Common duct stone</td>
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<td>Congenital duct stenosis</td>
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<tr>
<td>Malignant</td>
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<td>Hepatoma</td>
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<td>Lymphoma</td>
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<td>Hemolytic jaundice</td>
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<td>Sickle cell anemia</td>
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<td>Transfusion reaction</td>
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<td>Malaria</td>
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* Parentheses denote complicating illness (in hepatitis and hemolytic jaundice groups).

* Carcinoma of gastrointestinal tract (4 cases), pancreas (12), biliary tract (2), kidney (1), and of unknown origin (7), causing jaundice and/or metastatic hepatomegaly.
Fig. 3. Relation of seromucoid to severity of viral hepatitis as denoted by serum bilirubin. In all but 9 patients, the serum bilirubin was above 4 mg./100 ml. on initial examination. Most of the corresponding seromucoid values were either normal or low. \( \chi^2 \) test (27) failed to suggest correlation between seromucoid and bilirubin.

Fig. 4. Relation of seromucoid to thymol turbidity in uncomplicated hepatic disease. Less than one third of the cases showed, upon initial testing, a normal thymol turbidity (below 5 units) while more than one half had a normal or elevated (negative) seromucoid. In 6 instances, seromucoid was positive and thymol negative; in 9, both tests failed to indicate hepatic disease. \( \chi^2 \) test (27) failed to suggest correlation between seromucoid and thymol turbidity.
surgical operations, pneumonia, pyelonephritis, mastitis, pregnancy), to which the failure of SM depression could be ascribed. Even if these cases were eliminated, reducing the group to 35 cases, there was still about one half of these which yielded a normal or, in 2 instances, elevated SM without evidence of an extrahepatic "SM-raising" process. While some of these patients had suffered a mild attack or were already in the recovery stage, as indicated by the low serum bilirubin level (Fig. 3), about one half of them showed considerable jaundice, malaise, and associated laboratory evidence of hepatic damage (Fig. 4) at the time of the SM test.

Absence of SM depression appeared to characterize a group of 6 patients presenting an acute hepatitis syndrome secondary to certain systemic disorders: infectious mononucleosis, leptospirosis (two), amoebiasis, acute bacterial endocarditis, and intoxication with bootleg whiskey.

Since complications likely to augment the SM level (chronic pyelonephritis, pneumonia, subphrenic abscess, lymphopathia venereum, urti-
caria, and delirium tremens) occurred in the majority of the 41 patients with alcoholic cirrhosis, it was not surprising that only 6 (15 per cent) showed a subnormal SM level. In 2 instances, a high initial SM was associated with prolonged fever and leukocytosis, attributed to enterогenous bacte-
eremia; subsidence of these signs of inflammation weeks later was accompanied by SM depression. Of the 14 patients in this group who showed no evidence of complications, there were still 2 with an increased and only 6 (43 per cent) with a low SM. The latter finding was also encountered in the 3 cases of postnecrotic cirrhosis, in 1 of the 2 cases of cardiac cirrhosis, and in 1 of 3 cases of sarcoidosis. SM was elevated in a patient with hemochromatosis but apparently not affected by fatty infiltration of the liver.

Thus, the incidence of significantly reduced SM levels in the group of

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<th>Table 3. Efficiency of Seromucoid and of Three Other Serum Tests in the Diagnosis of Hepatobiliary Disease</th>
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<td><strong>Diagnosis</strong></td>
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<tr>
<td>Viral hepatitis</td>
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<td>Cirrhosis</td>
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<td>Benign obstruction</td>
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<td>Malignant obstruction</td>
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* Parentheses denote number of patients in whom test was performed.
“primary” hepatic diseases was only 28 per cent, far below expectations based on Greenspan’s reports (17, 18). This disappointment was enhanced upon comparison with some of the usual hepatic tests, performed concomitantly with the SM test. The cephalin flocculation and thymol turbidity tests were positive approximately twice as often in viral hepatitis and nearly three times as often in cirrhosis (Table 3). Even after exclusion of cases with extrahepatic complications, the incidence of diagnostic SM levels was still below 50 per cent. These findings are strikingly different from Greenspan’s, who reported the incidence of a subnormal SM in hepatitis and cirrhosis as being 88 per cent and 70 per cent, respectively, while the cephalin flocculation and thymol turbidity tests gave less frequent positive results: 48 and 68 per cent, respectively, in hepatitis, and 41 and 39 per cent in cirrhosis.

With regard to the serum alkaline phosphatase, experiences of the authors correspond more closely with those of Greenspan’s in that this test yielded a positive result less often than the SM test (results of the two series are not directly comparable because of the differences in critical enzyme levels used).

On the other hand, in 55 cases of biliary obstruction (Table 2; Fig. 3) not a single instance of a subnormal SM was encountered while the two routine flocculation tests gave 20 per cent of “false” positive reactions (Table 3); the alkaline phosphatase failed in 27 to 43 per cent of the cases to show a marked elevation. In nearly three times as many cases of obstructive jaundice, Greenspan and his associates found the incidence of a subnormal SM level to be less than 3 per cent (19).

Hence, a subnormal SM level in the presence of jaundice is almost specific for a primary parenchymal disease, a statement that cannot be made for any of the commonly used hepatic tests. The SM test may therefore be most valuable in problems involving the differentiation of “medical” from “surgical” jaundice when the usual clinical and laboratory technics give equivocal results: a subnormal level weighs heavily in favor of “medical” jaundice. The utility of the test was clearly enhanced by the use of follow-up determinations (17, 21); not infrequently, when the first test gave a normal or borderline SM value, examination a few days later revealed a distinct tendency of the SM level to rise or fall, suggesting obstructive or parenchymal disease, respectively. A single normal or increased SM concentration per se was of little help in ruling out parenchymal disease, even in the absence of extrahepatic complication. However, when such a diagnosis appeared to be well established, the finding of an elevated SM suggested the possibility—and, in several cases, initiated the discovery—of either an extrahepatic (inflammatory
or neoplastic) complicating illness or an icterogenic agent other than the hepatitis virus (mononucleosis, leptospirosis, etc.). With about 80 per cent of both benign and malignant forms of biliary obstruction yielding high SM levels, the test could not be utilized to render a preoperative differentiation of the nature of the obstructive lesion.

DISCUSSION

Analysis of the data presented permits one to attribute a definite diagnostic value only to the finding of a subnormal SM level. Such a finding, in association with jaundice or hepatomegaly, or both, and in the absence of a nephrotic syndrome (20), of excessive hyperglobulinemia, and, perhaps, of certain endocrinopathies (19), is almost pathognomonic of hepatocellular disease. Normal or elevated levels are of very limited value and only occasionally make a decisive diagnostic contribution. In particular, high levels do not spell neoplastic disease, an observation which has been made in the past by Henry and coworkers (13) and, most recently, by Rhees and his associates (15). Although these investigators disregarded the existing sex difference in the normal SM concentration (Table 1) (17), their conclusions conform to Greenspan's (19) and the authors' experiences.

Recognition of abnormal (high or low) levels is obviously dependent

![Figure 5](image-url)  
**Fig. 5.** Relation of seromucoid to thymol turbidity in biliary obstruction. The seromucoid test gave no false-positive (subnormal) result but the thymol turbidity was increased in 9 cases.
upon the definition of the "normal range," which in the present study was quite arbitrarily established as the 65 per cent confidence limits of all normal values encountered. Had the 95 per cent limits been used (Table 1), which would be more acceptable from a statistical point of view, the incidence of subnormal values would have been far lower than above recorded, whereas the number of high levels would have decreased but slightly (Figs. 3-5). The absence of any apparent false-positive results furnished by the values herein designated as subnormal lends some justification to the choice of the normal range as presented; additional support stems from the close similarity to the normal extremes employed by Greenspan and coworkers (17).

However, these, as well as other problems of a more technical nature mentioned initially, throw a suspicious light on the reliability of the analytic method and on the biologic significance of the results. Sero-mucoid obviously does not represent a single substance but rather a serum fraction whose separation from the other serum proteins by means of perchloric acid or other reagents (sulfosalicylic acid, trichloracetic acid [5, 11]) is quite arbitrary. This fact is evident from the marked changes in SM values that can be produced by relatively small alterations in the concentration of either acid or serum proteins or in the proportion of acid to serum (11, 26, 28). Increases in serum globulin such as are observed in sarcoidosis or multiple myeloma apparently tend to reduce the SM level (18), presumably through physicochemical action.

Electrophoretic analysis of perchloric-acid-serum filtrates revealed the presence of at least 3 peaks (MP-1, MP-2, and MP-3) (11, 29). Chemically, carbohydrate (galactose-mannose), hexosamine and protein (measured as tyrosine) constituted, aside from lipids, the chief components, in a ratio of 15.1:11.9:4.2 (by weight, on a moisture-free basis). Since the carbohydrate:protein (tyrosine) ratio was apparently maintained when the SM (tyrosine) level was markedly elevated in patients with cancer or pneumonia, it was concluded that the material responsible for the increase was chemically similar (but not necessarily identical!) to that isolated from normal serum (12). However, subnormal SM values (expressed as casein) encountered in liver disease were associated with increased carbohydrate:protein ratios (17).

With the use of ammonium sulfate fractionation, an electrophoretically and ultracentrifugally homogeneous plasma mucoid (M-1, corresponding to MP-1) was prepared by Winzler and his associates (30, 31). This had a molecular weight of 44,100, the mobility of $\alpha_1$-globulin, and an isoelectric point of pH 1.8 at a buffer pH of 8.6; it contained approximately 26 per cent of carbohydrate (including hexosamine) and 10 per
cent of "sialic acid" (32). The substance was finally isolated in crystallized form by Schmid from Cohn's fraction VI (33). Variations of this "acid glycoprotein" due to disease have, in the absence of a practical clinical method, not been reported.

It is thus obvious that seromucoid, as determined in perchloric acid filtrates, does not represent a chemical or biologic entity. There is much question as to whether increases or decreases of certain of its constituents—protein, hexosamine, hexose polysaccharide, or others—necessarily reflect equivalent fluctuations of the whole complex. Consequently, any speculation about the mechanism responsible for changes in SM concentration found in various disease states are bound to be superficial and fraught with error.

It has been suggested that serum glycoproteins originate from the depolymerization of ground substance of connective tissue (34, 35). This process, normally under enzymatic and hormonal control, may be augmented by certain alterations in tissue metabolism, particularly those associated with destruction or proliferation, causing an excess of soluble, lower-molecular substances to diffuse into the circulation (34-38) and be excreted in the urine (39). These substances are only in part represented by seromucoid which is devoid of hexuronic acids (10, 11, 30, 32, 33), the regular constituents of tissue glycoprotein (40). Hexuronic acids (41, 42), as well as polysaccharides other than those contained in seromucoid, are normally present in serum (37) and may be pathologically increased. The decrease in the concentration of seromucoid (but not of those carbohydrate derivatives) occurring in liver disease suggests that the liver may act either as another source of SM or as an organ capable of removing it from the blood stream. The former possibility, implying inadequate synthesis by the diseased liver (18), is suggested by Werner's observations on the serum hexosamine response of rabbits subjected to blood loss; the usual increase failed to occur when liver damage was produced by means of phosphorus or chlorobenzene (43). On the other hand, total protein-bound hexose polysaccharides have been found in normal or increased serum concentrations in viral hepatitis (17). The role of the liver as a source of SM is also suggested by the finding of reduced $\alpha$-globulin in viral hepatitis (44, 45) and by the observation, based on C$^{14}$ isotope studies, that most of the major serum protein fractions stem from the liver, except for $\gamma$-globulin and minute amounts of $\beta$- and $\alpha$-globulins (46, 47).

As a working hypothesis, connective tissue and the hepatic parenchyma may be regarded as two independent sources of seromucoid, each source having a different type of response to physiologic stimulation or injury:
the former chiefly in the form of an increase, the latter by way of a decrease. The SM level, as analyzed at any given time, constitutes the sum of these responses. Thus, only marked preponderance of the response of one source over that of the other will result in a distinctly abnormal and diagnostically useful level. The combination of two severe responses, i.e., pneumonia and viral hepatitis, is liable to yield a normal level.

This theory leaves open the question of endocrine factors influencing the SM level (48–51) and, indeed, the distinct possibility that there may be many different sites of origin of SM and of different glycoproteins and their individual constituents, with different degrees or types of response to certain pathologic processes. Further clinical or physiologic studies of seromucoid would seem to be of little avail without the attainment of far greater precision in chemical identification and in analytic measurement of the substances involved. Any attempt at classification of diseases by seromucoid levels (19) must therefore be regarded as preliminary and subject to future revision.

SUMMARY AND CONCLUSIONS

Seromucoid was determined in 165 patients with jaundice or hepatomegaly, or both. Subnormal seromucoid concentrations were found in 39 per cent of 44 cases of viral hepatitis and in 15 per cent of 41 cases of alcoholic cirrhosis. This incidence increased to 50 and 43 per cent, respectively, when patients with complicating extrahepatic disease processes were excluded. No subnormal values were encountered in 55 cases of benign and malignant biliary obstruction which, however, yielded about 80 per cent of elevated values.

The empirical data point to the lower limit of the normal seromucoid range as providing a useful dividing line between obstructive and parenchymal jaundice: the finding of a subnormal level is strongly indicative of the latter. Normal or elevated levels may only occasionally be expected to make a decisive diagnostic contribution.

A more definitive evaluation of the clinical utility of this type of test must await improvements in analytic standardization, based on a more detailed knowledge of the chemical and physiologic identity of the compound than is available at present.

REFERENCES

41. Stary, Z., and Yuvanidis, M., Biochem. Z. 324, 206 (1953).