

Serum Concentration of Human Alpha₂ HS Glycoprotein during the Inflammatory Process

EVIDENCE THAT ALPHA₂ HS GLYCOPROTEIN IS A NEGATIVE ACUTE-PHASE REACTANT

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ABSTRACT A nonspecific opsonin function has been ascribed to human alpha₂ HS glycoprotein. Its serum level has been shown to be decreased in trauma patients. Recent studies from this laboratory revealed a heterogeneity among the final products obtained in the course of the preparation of the protein. To date, no definitive agreement existed with regard to a molecular homogeneous entity of alpha₂ HS glycoprotein (Ba-alpha₂ glycoproteins). The purpose of the current work was to study the variations in serum level of alpha₂ HS in patients suffering from an acute inflammatory process of bacterial etiology and to determine whether a decrease in alpha₂ HS was accompanied by the appearance of fragments of this protein in the serum. A method of preparing alpha₂ HS was thus developed, using an immune adsorbent as a final purification step. In an intermediary step of the preparation, alpha₂ HS was found to bind zinc ions when metal chelate affinity chromatography was employed. Immunologically and physico-chemically pure alpha₂ HS was obtained. The protein consists of a unique polypeptide chain of about 50,000 daltons and has a unique amino-terminal residue, alanine. However, the protein maintained its molecular integrity with difficulty, and spontaneous fragments ranging from 30,000 to <10,000 daltons were produced in some of the preparations. No major modification in the molecular structure of the protein was

noted in the sera of subjects suffering from an acute inflammatory process. Serum level of alpha₂ HS and alpha₁ antitrypsin (AT) was determined in 23 patients. When the acute-phase (AP-)reactant alpha₁ AT was increased (difference with normal mean >+2 or +3 SD), the sera showed a large decrease in alpha₂ HS (difference with normal mean <-2 or -3 SD). The serum level of alpha₂ HS, albumin, alpha₂ macroglobulin, and of positive AP-reactants, orosomucoid, alpha₁ AT, haptoglobin, and ceruloplasmin was evaluated in a longitudinal study of seven patients. The results were submitted to a principal components analysis. Alpha₂ HS showed a negative correlation with the AP-reactants alpha₁ AT, orosomucoid, and haptoglobin ($P < 0.05$) and a positive correlation with albumin ($P < 0.05$); these findings indicate that alpha₂ HS is a negative AP-reactant. In addition, analysis of the principal components confirms the strong analogy between alpha₂ HS and albumin and indicates that serum level behavior of the AP-reactants during the course of the disease closely depends on the protein studied.

INTRODUCTION

Acute-phase (AP-)¹ reactants consist of the protein components of plasma. Their synthesis increases (positive

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¹ *Abbreviations used in this paper:* alpha₁ AT, alpha₁ antitrypsin; alpha₂ M, alpha₂ macroglobulin; AP-reactant, acute-phase reactant; Hp, haptoglobin; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; ZCAC, Zinc-chelate affinity chromatography.

AP-reactants) or decreases (negative AP-reactants) during diseases accompanied by an inflammatory process. These proteins are synthesized essentially by the liver, the mechanism of their inflammation-induced synthesis is not elucidated (1).

Alpha₂ HS is a normal human plasma globulin first described in 1960 (2) and partially characterized in terms of its chemical composition and physico-chemical properties (3, 4). In normal subjects, immunoelectrophoretic alpha₂ HS variants, both slow migrating and fast migrating, were described; the variations depended on the length of the serum storage period (5). The same variants could be induced by proteases and neuraminidase, indicating a peculiar fragility of the protein. To date, the primary structure of the protein is unknown; recently, however, the purification of alpha₂ HS has been facilitated by zinc-chelate affinity chromatography (6). Alpha₂ HS was found to be 0.40–0.85 g/liter in normal serum (7) but no precise information concerning its modifications in disease was available until Van Oss et al. (8) discovered that the plasma level of alpha₂ HS was decreased in trauma patients.

In an attempt to elucidate the structure of alpha₂ HS and its metabolism in the human, we reexamined the process of obtaining this protein and studied the variations in its plasma level and immunochemical structure under pathological conditions. In this work, we report the kinetics of serum alpha₂ HS concentration during the course of an acute inflammatory process of bacterial etiology in parallel with that of some well-known AP-reactants. In addition, we report some precise details concerning the structure of alpha₂ HS and its physico-chemical modifications during the process of purification.

METHODS

Isolation of the alpha₂ HS glycoprotein

Alpha₂ HS was prepared from normal human serum by a two-step procedure.

STEP 1

Ammonium sulphate precipitation, ion-exchange chromatography, zinc-chelate affinity chromatography, and preparation of a monospecific anti-alpha₂ HS immune serum. Whole blood was drawn from healthy human donors (Blood Transfusion Regional Center, Bois Guillaume, France), was allowed to clot for 1 h at room temperature and to retract for 1 h at 4°C. A solution of saturated ammonium sulphate adjusted at pH 7.0 was added, drop by drop, during magnetic stirring to reach 1.5 M in the serum containing 0.2 M ϵ -aminocaproic acid (Sigma Chemical Co., St. Louis, Mo.). The precipitate, allowed to settle overnight at 4°C, was washed with 1.5 M ammonium sulphate and allowed to settle a second time at 4°C overnight. After centrifugation, the precipitate was dissolved in phosphate-buffered saline (PBS) and then dialyzed against the PBS until sulphate ions were eliminated. The solution was dia-

lyzed against a 0.0250 M acetate buffer pH 5.0 containing 0.05 M ϵ -amino-caproic acid and applied to a column (2.5 × 50 cm) of CM-Sepharose CL-6B (Pharmacia Fine Chemicals Co., Uppsala, Sweden), previously equilibrated with the same buffer. The proteins were eluted at room temperature with the 0.025 M acetate buffer pH 5.0 and then step-wise with increasing concentrations of 0.02–0.2 M NaCl in the 0.025 M acetate buffer. Alpha₂ HS was eluted between 0 and 0.06 M NaCl. The fractions containing this glycoprotein were pooled, concentrated, dialyzed against a 0.05 M Tris-HCl buffer pH 8.0 containing 0.15 M NaCl and 0.05 M ϵ -aminocaproic acid, and the solution was applied to a column of zinc-chelate affinity chromatography (ZCAC), as reported earlier (6). The protein solution applied to the column was eluted with a 0.05 M Tris-HCl buffer pH 8.0 containing 0.15 M NaCl and then step-wise with a 0.1 M Na phosphate buffer pH 6.5 containing 0.8 M NaCl at room temperature followed by a 0.1 M acetate buffer pH 4.5 containing 0.8 M NaCl. The column was finally eluted with 0.05 M EDTA, pH 7.0, in 0.5 M NaCl. Two peaks were obtained: 85% of the alpha₂ HS glycoprotein was absorbed by the zinc chelate at pH 8.0 and was eluted at pH 6.5 (6) in the second peak. Serum alpha₂ Zn, a glycoprotein that has a isoelectric point and molecular weight near that of alpha₂ HS (9) was eluted in the first peak with other alpha glycoproteins. Alpha₂ HS contained in the second peak of ZCAC was used for the preparation of a monospecific antiserum against this protein.

Preparation of antiserum against alpha₂ HS glycoprotein.

Antiserum against the isolated protein was raised in rabbits given 0.5 mg of protein emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.), into the hind footpads. Booster doses were given subcutaneously after 3 wk and at weekly intervals thereafter. Satisfactory antibody titers were obtained 5 wk after the first injection. Immunoelectrophoresis of this antiserum displayed a major precipitin line in the alpha₂ region and a minor gamma line when reacted against human whole serum. The major alpha₂ precipitin line was identified by immunodiffusion studies. This line showed a reaction of identity with the precipitin line obtained with an anti-alpha₂ HS antiserum (Behringwerke A.G., Marburg, Federal Republic of Germany). The second minor precipitin line was identified by immunoelectrophoresis as an immunoglobulin (Ig)G line against a solution of purified human IgG globulins (10 mg/ml).

Partially purified IgG was prepared from the rabbit anti-human alpha₂ HS antiserum by precipitating that fraction with an equal volume of a 70% ammonium sulphate solution twice at 4°C. The globulin solution was absorbed with human IgG cross-linked with glutaraldehyde (10) to render it monospecific: 1 g of an human IgG solution was prepared by ion-exchange chromatography of human serum on a DEAE-cellulose column (Eastman Kodak Co., Rochester, N. Y.) equilibrated with a 0.005 M sodium phosphate buffer pH 6.5 and eluted with the same buffer. 50 ml of the solution containing 20 mg IgG/ml in a 0.2 M acetate buffer, pH 5.0, and 400 mg of bovine serum albumin (Poviet, Amsterdam, Netherlands) was added. The solution was adjusted at 110 ml with 0.2 M acetate buffer pH 5.0 and 22 ml of a 2.5% aqueous solution of glutaraldehyde (Taab Laboratories, Emmer, Green-Readings, England) was added drop by drop. The gel formed was allowed to stand for 3 h at room temperature and was homogenized. The suspension was dispersed in 0.1 M phosphate buffer, pH 7.4 and was centrifuged for 15 min at 3,500 g and 4°C. The operation was repeated three times until the supernate had an absorbance of 0 at 280 nm. The insoluble protein was suspended in 0.2 M glycine buffer, pH 2.8 and stirred 15 min at room temperature. The gel was centrifuged and neutralized with potassium phosphate 1 M. After

centrifugation for 15 min at 3,500 g, the gel was kept in PBS. 150 ml of partially purified immunoglobulins anti-human α_2 HS glycoprotein adjusted at 10 mg/ml were mixed with the insoluble protein and stirred gently for 60 min at room temperature, then kept overnight at 4°C. After centrifugation at 4,000 g for 15 min, the supernate was kept and tested by immunoelectrophoresis against whole human normal serum. A precipitation line was seen only in the α_2 region. By immunodiffusion, this line showed a reaction of identity with the line obtained with a commercial anti- α_2 HS-immune serum (Behringwerke A.G.). The immunoglobulins anti-human α_2 HS glycoprotein so obtained were used in the next step.

STEP 2

Preparation of an immune adsorbent anti- α_2 HS glycoprotein. The anti- α_2 HS immune adsorbent was prepared by coupling the IgG fractions (35% ammonium sulphate precipitate) of rabbit antiserum, obtained in step 1, to cyanogen bromide-Sepharose. 150 ml of Sepharose-4B (Pharmacia Fine Chemicals) was washed with distilled water at 25°C and the pH was adjusted at pH 7.5. 15 g of cyanogen bromide (Fluka A.G., Switzerland) were dissolved in 150 ml of distilled water at 25°C and slowly added to ice-cold Sepharose-4B. 12 min later the pH was stabilized at 11.5 with NaOH 10 N. The gel was then washed with cold distilled water and with CO_2HNa 0.1 M pH 9.0. The gel was immediately transferred to a beaker containing 110 ml of a solution of 1,500 mg of the anti- α_2 HS rabbit immunoglobulins obtained in step 1, dialyzed against a 0.1-M CO_2HNa buffer, pH 8.3, containing 0.5 M NaCl. The coupling reaction was allowed to proceed for 1 h at room temperature and overnight at 4°C. The gel was washed with 0.1 M CO_2HNa , pH 8.3, containing 0.5 M NaCl, and suspended in an ethanolamine 1 M solution during 2 h. The gel was washed with an 0.1-M acetate Na buffer, pH 4.0, followed by 0.01 M Na phosphate buffer containing 0.5 M NaCl and 1% NaN_3 . The gel (150 ml) was finally transferred in a column (32 \times 2.6 cm).

To human fresh serum from healthy human donors, obtained as indicated in step 1, a solution of saturated ammonium sulphate adjusted at pH 7.0 was added drop by drop during magnetic stirring to reach 1.5 M. The precipitate, allowed to settle overnight at 4°C, was washed with 1.5 M ammonium sulphate, pH 7.0 and allowed to settle a second time at 4°C overnight. After centrifugation, the precipitate was dissolved and dialyzed against PBS. 10 ml of the ammonium sulphate precipitate containing 560 mg protein was dialyzed against a 0.01-M Na phosphate buffer, pH 7.0, containing 0.5 M NaCl and 0.05 M ϵ -aminocaproic acid and applied to the anti- α_2 HS cyanogen bromide-Sepharose column. The column was eluted step-wise by a 0.01-M Na phosphate buffer, pH 7.0, containing 0.5 M NaCl followed by a 0.01-M Na phosphate buffer containing 0.5 M NaCl and 2 M guanidine HCl, and finally by a 0.01 M Na acetate buffer, pH 6.0, containing 3 M SCNNa and 0.05 M ϵ -aminocaproic acid. The protein fractions were pooled, concentrated, and subjected to immunoelectrophoresis and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Immunochemical analysis

Immunoelectrophoresis was performed according to Scheidegger (11). Double immunodiffusion in gel was carried out by the method of Ouchterlony (12). Quantitative determination of serum α_2 HS, albumin, and α_1 antitrypsin, was achieved by single radial immunodiffusion (13), using monospecific immune sera obtained from our laboratory and by

commercial immunoplates for haptoglobin, orosomucoid, ceruloplasmin, and α_2 macroglobulin (Partigen plates, Behringwerke A.G.). The reference serum was obtained from the same manufacturer. When no further extension of precipitin rings was observed, the diameter was measured with a magnifying comparator (Behringwerke A.G.). The haptoglobin phenotypes were ascertained by starch gel electrophoresis of whole serum in discontinuous buffers of pH 8.6 and were visualized by staining of the haptoglobin-hemoglobin complex with the benzidine reagent (14). Serum haptoglobin levels determined by Partigen plates were corrected in terms of the observed phenotype using the conversion factor indicated by the manufacturer.

Other methods

SDS-PAGE was carried out as described by Weber and Osborn (15) with a 10% cross-linked gel in 1% SDS. A protein concentration of 150 μg was applied onto the gel. In some experiments the sample to be studied was incubated overnight at 37°C in a final volume containing 5 M urea and 10% SDS. Electrophoresis was conducted in a 10% acrylamide gel containing 10% SDS. Proteins standards employed were human serum albumin (mol wt 68,000), ovalbumin (mol wt 43,000), trypsin (mol wt 23,000), and ribonuclease (mol wt 13,700). For the determination of polypeptides in the range of 3,000–22,000 daltons, insulin chain B (mol wt 3,400), aprotinin (mol wt 6,500), and cytochrom C (mol wt 12,500) (Boehringer Mannheim GmbH Biochemica, Federal Republic of Germany) were used for markers and electrophoresis was run with a 7.5% polyacrylamide gel. The behavior of α_2 HS in SDS-PAGE was studied with increasing concentrations of the acrylamide gel (4, 6, 10, and 12%) as described (16), and the mobility of the protein was compared with that of ribonuclease, trypsin, and human albumin. To detect any anomaly in protein migration the relative mobility of the protein was plotted vs. gel concentration (T) using the Ferguson equation: $\log(R_f) = \log(Y_0) - KrT$, where Y_0 is the extrapolated relative mobility at zero gel concentration and Kr is the retardation coefficient (16). A modified immunoelectrophoresis bidimensional method (17) was used to obtain an identification of the protein mixtures containing fragments related to α_2 HS. The first stage was carried out by SDS-PAGE in glass tubes (15). After the first stage was completed, the gel was removed and immersed in 800 ml of 0.05 M barbital buffer for 30 min. The rod gel was then longitudinally sliced into two equal parts with a scalpel. One-half was then placed in a groove (80 \times 7 mm) cut in an agarose glass plate (260 \times 125 mm; 1% agarose in 0.05 M barbital buffer pH 8.2). Electrophoresis was carried out at 4–5 V/cm until the bromophenol blue used in the first stage had fully migrated from the gel rod into the agarose plate. A channel (2 \times 8 mm) was then cut into the agarose gel plate, parallel to the acrylamide gel and filled with anti- α_2 HS. Preliminary studies showed that 8 mm was the appropriate distance between the channel and the acrylamide gel for obtaining optimal antigen:antibody ratio and permitting a distinct development of precipitin arcs in our experimental conditions. After diffusion, the agarose plate was rinsed in saline for 3 d and then stained with Coomassie Blue. NH_2 -terminal group was determined by the dansyl chloride method described by Gray (18). Dansyl amino groups were identified by thin-layer silica gel chromatography using the system of Gros and Labouesse (19).

Clinical data

Among 40 patients hospitalized in the Department of Infectious Diseases, 23 (15 males, 8 females) were selected for

study on the basis of: (a) an infection of proven bacterial etiology, and (b) laboratory evidence of acute inflammation. This evidence included: increased leukocyte count at admission and/or increased erythrocyte sedimentation rate; a rise in the α_2 region observed after classical zone agar gel electrophoresis of serum samples; an increase of the α_1 and α_2 regions of the immunoelectrophoretic patterns; and a decrease of albumin and β_2 regions as demonstrated by modifications of the albumin, α_1 , antitrypsin (AT), haptoglobin, and transferrin precipitin arcs and a measured increase of α_1 antitrypsin level (Table I). Samples of patient blood were obtained at regular intervals throughout the disease. When possible, control blood samples were obtained at recovery. Sera were stored at -20°C until used. The serum level of α_2 HS was determined in a serial study of 110 sera samples from the 23 patients. 70 sera were analyzed for α_1 , AT because α_1 , AT was measured only at the outset, the middle, and the end of the disease in some patients. The serum levels of albumin, α_2 HS, orosomucoid, α_1 , AT, haptoglobin (Hp), α_2 macroglobulin (α_2 M), and ceruloplasmin (322 analyses, Table II) were evaluated in a longitudinal study of the serum of 7 among the 23 patients studied.

The three reasons for choosing these seven patients were: the degree of laboratory evidence of acute inflammation and of initial modifications of α_1 , AT serum level, the length of their hospitalization, and the possibility of obtaining blood samples until the end of the disease. Identical serial protein determinations were made in a matched control population of five normal human subjects (four males, one female). The serum concentrations of α_2 HS, α_2 M, orosomucoid, albumin, and ceruloplasmin were determined in 38 healthy registered blood donors, but no records of the state of health of this group were available.

Statistical analysis

The results of quantitative determinations obtained from 45 blood samples (Table II) were submitted to a principal component analysis (20). Correlation matrix and principal components were calculated separately for the following two groups of proteins: α_2 HS, Hp, α_1 , AT, albumin, and orosomucoid (group I); and α_2 HS, α_2 M, and ceruloplasmin (group II). Results were computed on a HP 9825 calculator (Hewlett-Packard Co., Palo Alto, Calif.). So that each of the seven patients be equally represented in the analysis, each sample was assigned a weight inversely proportional to the total number of samples obtained from a single patient. Effects caused by the wide range of the different protein concentrations were corrected by standardizing the variables. Quantitative determinations in serum protein concentration could not be considered as reflecting an independent random variable, because concentrations for each patient depended on the phase of the disease. Nevertheless, the significance of the difference between zero and the linear correlations is roughly evaluated by using the distribution tables of this coefficient at level 0.05. The principal components were ranked according to their decreasing variance. For each of them were given: variance, percentage of total variance, and the components of its unitary vector in the initial axis system. Coordinates of the initial unitary vectors in the factorial axis system and projection of samples on each factorial axis were also algebraically and graphically obtained from the computer but have not been included in this paper.

RESULTS

Purification of α_2 HS glycoprotein. The chromatographic elution pattern of a 1.5-M ammonium sul-

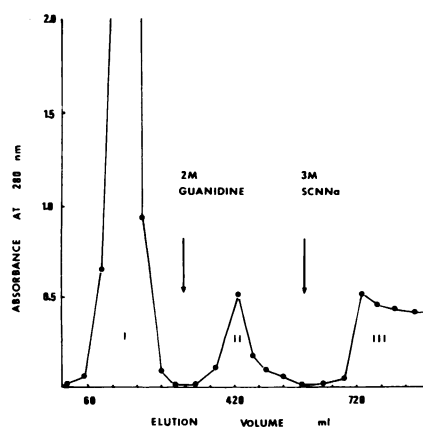


FIGURE 1 Affinity chromatography of a 1.5 M ammonium sulphate precipitate from normal human sera on anti- α_2 HS Sepharose. The sample (560 mg) was applied to a 2.6×32 -cm column of anti- α_2 HS Sepharose equilibrated with 0.01 M sodium phosphate, 0.5 M NaCl, and 0.05 M ϵ -aminocaproic acid at pH 7.0. The column was then washed with 300 ml of equilibrating buffer, followed by 300 ml of 2 M guanidine-HCl in the equilibrating buffer and by 300 ml of 0.1 M sodium acetate buffer at pH 6.0 containing 3 M SCNNa and 0.05 M ϵ -aminocaproic acid. Protein fractions were pooled as indicated by the arrows. The plateau in III was caused by the absorbance of sodium sulphocyanate at 280 nm.

phate precipitate obtained from normal human sera and absorbed onto an immune affinity column prepared with an anti- α_2 HS immune serum is shown in Fig. 1. An isolated and pure form of α_2 HS was obtained in peak III of the column (Fig. 2). SDS-PAGE of this fraction (Fig. 2B) revealed an intense band contiguous to a very faint band, when electrophoresis was carried out with a 10% cross-linked acrylamide gel in the presence of mercaptoethanol (15). Measurements at the level of the upper band gave a mol wt of $50,100 \pm 1,700$ (\pm SD; 28 determinations) and a mol wt of $47,000 \pm 1,900$ at the level of the lower band. However, when electrophoresis was carried out in 6% acrylamide and in 1% SDS, a single band of 50,000 mol wt was seen (Fig. 2A). Moreover when electrophoresis was conducted in 10% SDS, 10% acrylamide gel after incubating samples in 5 M urea, and 10% SDS, a single band of 50,000 daltons was also obtained (Fig. 2C). Consequently, the minor band of 47,000 daltons seen in Fig. 2B was considered an artefact. NH_2 -terminal analysis of α_2 HS showed it to have a single residue, alanine.

When α_2 HS was studied in SDS-PAGE (15) with different acrylamide concentrations (16) its behavior in gel was comparable with that of albumin and human globulins of known molecular weights (see Other Methods), because the extrapolated value of the mobility of the protein, e.g. the R_f vs. gel concentration (16), indicated that it did not differ from that of the former globular proteins.

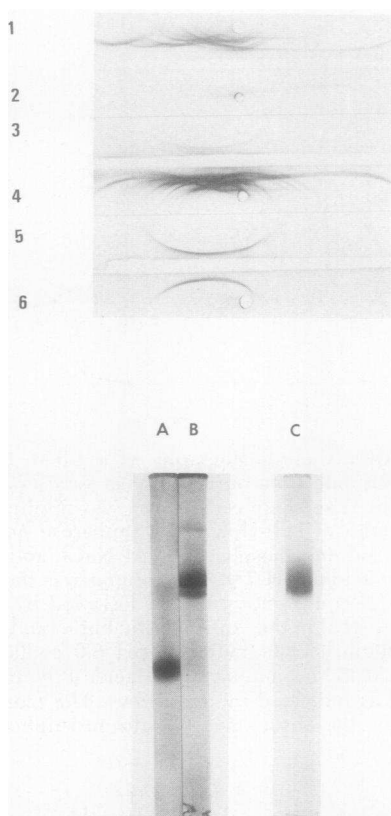


FIGURE 2 Immuno-electrophoretic patterns of the three peaks of the affinity-chromatography anti- α_2 HS column (Fig. 1). (1) The concentrated peak I. (2) The concentrated peak II. (3) The concentrated peak III. (4) Normal human serum; developed with an antitotal normal human serum. (5) The concentrated peak III. (6) Normal human serum; developed with a monospecific anti- α_2 HS immune serum. Below: SDS-PAGE with mercaptoethanol of the concentrated peak III of the affinity-chromatography column. (A) In 6% acrylamide. (B) In 10% acrylamide. (C) SDS-PAGE, without mercaptoethanol of the concentrated peak III diluted with an equal volume of 2% SDS-10 M urea solution, in a 10% acrylamide gel containing 10% SDS.

Molecular instability of α_2 HS. In other experiments, it was established that α_2 HS did not always remain in a homogeneous and intact molecular form. Immuno-electrophoresis of Fig. 3 revealed a bifid arc of the protein, and SDS-PAGE of the reduced proteins produced three bands. The bands were characterized by a two-dimensional SDS-PAGE immuno-electrophoresis overloading the gel (350 μ g) in the first dimension. Four bands were obtained. The upper band, with $\approx 75,000$ mol wt, did not react against anti- α_2 HS immune serum. Because, in preliminary experiments not shown, the last unidentified protein had been separated by Sephadex G-100 and did not react against anti- α_2 HS, the upper band (Fig. 3B) should be considered an impurity. The three bands, which devel-

oped a precipitin line, had a 50,000, 30,000, and $<10,000$ mol wt, respectively. Polypeptides of small molecular weight ($<10,000$), when measured in SDS-PAGE with polypeptide markers of small molecular weight, gave two poorly resolved bands of 6,000 and 4,700 daltons (not shown).

When α_2 HS was prepared after passing the ammonium sulphate precipitate through a CM-Sephadex column followed by a ZCAC (6) α_2 HS was obtained at both pH 8.0 and 6.5. α_2 HS eluted at pH 6.5 showed a modified precipitin arc (Fig. 2A). It displayed three bands in SDS-PAGE under reducing conditions after separation onto the anti- α_2 HS column (Fig. 4C). Immunodiffusion experiments indicated that an α_2 HS fragment had been eluted from the ZCAC column at pH 8.0 (Fig. 4B).

Serum level of α_2 HS, albumin, α_2 M, and positive AP-reactants during the course of acute inflammatory conditions. Mean serum level of α_2 HS in the 38 controls samples was 0.595 ± 0.120 g/liter (SD); α_2 M was 2.42 ± 0.68 g/liter (SD); orosomucoid, 0.86 ± 0.16 g/liter (SD); ceruloplasmin, 0.37 ± 0.07 g/liter (SD); albumin, 39 ± 8 g/liter (SD). Table I indicates a significant decrease of α_2 HS in 23 studied patients. Difference with normal mean was mean -3 SD (6 patients), mean -2 SD (16 patients), mean -1 SD (1 patient). In comparison, an inverse increase of α_1 AT was noted. Serial determinations

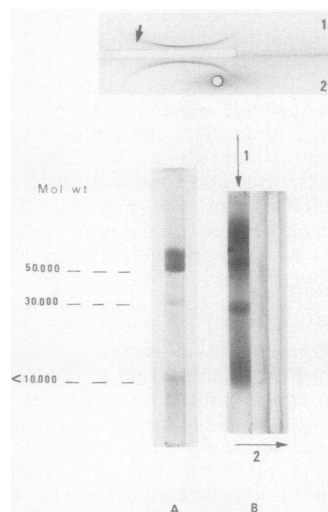


FIGURE 3 Immuno-electrophoretic pattern of an α_2 HS preparation (obtained as in peak III of Fig. 1) showing a modification of the precipitin arc, as indicated by the arrow 1. In 2: normal human serum; developed with an anti- α_2 HS immune serum. Below: (A) SDS-PAGE of the same α_2 HS preparation in 10% acrylamide with mercaptoethanol. (B) Bi-dimensional method of identification of the polypeptides obtained in A. Arrow 1 indicates the anodal migration in the first step of SDS-PAGE and arrow 2, the direction of the diffusion of the α_2 HS immune serum in the second step. The colored gel indicates the position of the protein bands.

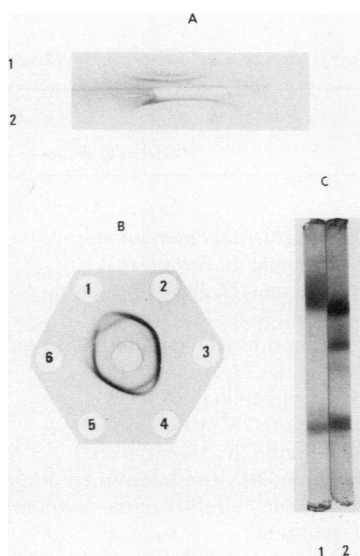


FIGURE 4 (A) Immuno-electrophoretic pattern of the concentrated peak I (1) and peak II (2) of a ZCAC-column, developed with an anti- α_2 HS immune serum. (B) Ouchterlony analysis (anti- α_2 HS immune serum) of (1, 4) concentrated peak I of the ZCAC-column (2) normal human serum (3, 5) concentrated peak II of the ZCAC-column (6) a polypeptide fragment of α_2 HS obtained in another preparation (not shown) by using a ZCAC column. (C) Peak II was passed through the anti- α_2 HS Sepharose column: the fraction III (eluted as in Fig. 1) was analyzed by SDS-PAGE. 10% acrylamide, (1) without mercaptoethanol, (2) with mercaptoethanol.

of the above serum proteins were made in seven patients (Table II) and in five normal subjects (Table III).

Values obtained for α_2 HS in normal subjects showed no level lower than 1 SD. Moreover, subject (H.M.), who used oral contraceptives, showed a significant α_2 HS increase (>2 SD), parallel to that seen for α_1 AT and ceruloplasmin.

Table II shows a gradual increase of α_2 HS throughout the disease in the seven patients examined with initial values below 2 or 3 SD; normal values were reached at days 250, 202, 50, and 46, respectively, in patients R.E., F.A., Th.A.M., and B.J.M. A parallel evolution was seen for albumin level. On the other hand, the high Hp, α_1 AT, and orosomucoid levels at the outset of the disease had returned to normal at the days cited above. In patients V.R. and R.R., the last samples still showed a decreased α_2 HS level and increased Hp and α_1 AT. Patient G.M. had a normal initial α_2 HS level. However, evolution of α_2 HS showed a distinct increase in the last sample, suggesting a high, normal-range α_2 HS value. In parallel, the last values for albumin, α_1 AT, Hp, and orosomucoid had returned to normal. Values for α_2 M and ceruloplasmin fell within normal range (7). However, it was noted that ceruloplasmin levels decreased in patients Th.A.M., B.J.M., and G.M. They

increased slightly in V.R. and R.R., and remained nearly stable in R.E. and F.A.

Immunochemical structure of α_2 HS in serum from patients suffering an acute inflammatory process. Sera from patients (S.E. and F.A.) with decreased α_2 HS levels of 0.17 and 0.13 g/liter, respectively (Table I), were analyzed by immune diffusion (Fig. 5) in comparison with normal human fresh serum and against an α_2 HS fragment as produced in Fig. 4. Precipitin lines indicated that α_2 HS from these patients was antigenically identical to normal α_2 HS and with that from patients S.E. and F.A. These results indicate that no gross modification occurred in the molecular structure of α_2 HS during the inflammatory process.

Factorial analysis of the serum levels of α_2 HS, Hp, α_1 AT, albumin, and orosomucoid. Significant positive correlations ($P < 0.05$) were obtained for the following pairs: α_2 HS and albumin, Hp and α_1 AT, α_1 AT and orosomucoid. Significant negative correlations ($P < 0.05$) were obtained for the following pairs: α_2 HS and Hp, α_2 HS and α_1 AT, α_2 HS and orosomucoid, Hp and albumin, α_1 AT and albumin (Table IV). The closest correlation was found between α_2 HS and albumin (0.74). Table V shows that the trihedral (1–3) encompasses nearly the whole percentage of total variance (0.906), with a sharp prevalence for the first principal component (0.586). The position of each of the five variables with regard to the first component is coherent with the algebraic signs of the paired linear correlation coefficients. In addition, the value of the first component is strikingly dependent on the day of blood sampling. For each patient (except R.R., in whom the disease was quasi nonevolutive), the outset of the inflammatory process was characterized by samples having highly positive values for the first component, and the end of the disease by samples presenting highly negative values for the same component. Thus, the first principal component appears, in a unidimensional pattern, to evaluate the degree of inflammation through the measurement of serum concentration of AP-reactants. The coefficient of orosomucoid for component 2 was very high (0.807) with regard to those of the four other proteins (≤ 0.364). This particular variation tendency could be responsible for the poor linear correlation between orosomucoid on the one hand, and Hp and albumin on the other hand. For each patient, the values of the third component retained the same algebraic sign throughout the disease and showed little variation in absolute value. The only exception is four samples in three different patients. This comportment, which was not dependent on the day of blood sampling, was probably related to individual particularities. Components 4 and 5 did not furnish sufficient information to deserve special interpretation.

TABLE I
Serum Level of Alpha₂ HS and Alpha₁ AT in 23 Patients Suffering from an Acute Infectious Disease

Patient	Age	Sex	Day*	α_2 -HS†	Difference from normal mean	α_1 -AT‡	Difference from normal mean	Underlying disease
	yr			g/liter	SD	g/liter	SD	
V.R.	65	M	3	0.26	<-2	4.78	>+3	Meningitis (<i>D. Pneumoniae</i>)
R.E.	72	F	10	0.14	<-3	4.66	>+3	Septicemia (<i>S. Aureus</i>)
L.A.	55	F	13	0.29	<-2	3.36	>+1	Septicemia (<i>D. Pneumoniae</i>)
L.D.	19	M	7	0.37	\approx -2	4.05	>+2	Typhoid fever (<i>S. Typhi</i> .)
Th.A.M.	49	F	11	0.24	\approx -3	4.44	>+2	Urinary infection with initial bacteriemy (<i>E. Coli</i>)
S.E.	76	M	4	0.17	<-3	5.31	>+3	Erysipelas (<i>S. Aureus</i>)
C.G.	48	M	8	0.27	<-2	4.50	>+2	Septicopyemia (<i>S. Aureus</i>)
L.M.	57	F	11	0.29	<-2	ND	—	Meningitis (<i>N. Meningitidis</i>)
L.H.	17	M	7	0.26	<-2	4.00	+2	Meningitis (<i>N. Meningitidis</i>)
P.A.	68	F	3	0.36	\approx -2	ND	—	Pneumopathy (undetermined etiology)
F.A.	56	M	6	0.13	<-3	4.53	>+2	Anaerobic cellulitis of the scrotum (Fournier's gangrene)
L.G.	50	F	11	0.27	<-2	5.25	>+3	Pulmonary superinfection during tetanus
L.C.	21	M	5	0.32	<-2	4.29	>+2	Bacterial meningitis
A.J.	49	M	5	0.20	<-3	5.25	>+3	Meningitis (<i>S. Pyrogenes</i>)
B.G.	75	F	12	0.33	<-2	2.95	none	Facial cutaneous staphylococci
B.B.	51	M	6	0.35	\approx -2	4.90	>+3	Meningitis (<i>N. Meningitidis</i>)
Q.M.	55	F	10	0.30	<-2	4.83	>+3	Septicemia (<i>K. Pneumoniae</i>)
B.J.M.	18	M	8	0.28	<-2	4.83	>+3	Septicemia (<i>C. Perfringens</i>)
J.G.	47	M	10	0.23	<-3	5.70	>+3	Pneumonia
G.M.	27	M	7	0.46	<-1	5.25	>+3	Staphylocodermatitis
M.F.	30	F	5	0.31	<-2	4.42	>+2	Meningitis (<i>N. Meningitidis</i>)
R.R.	51	M	22	0.27	<-2	4.40	>+2	Septicemia (<i>S. Aureus</i>)
C.T.	48	F	24	0.25	<-2	4.92	>+3	Septicemia

* The day indicated corresponds to the day when serum level of alpha₂ HS is the lowest after onset of the disease.

† Mean serum level of alpha₂ HS in 38 normal subjects: 0.595±0.120 g/liter (SD).

‡ Mean serum level of alpha₁ AT in normal subjects: 2.70±0.650 g/liter (SD).

Correlations between protein levels of alpha₂ HS, ceruloplasmin, and alpha₂ M. None of the paired linear correlations between protein serum levels is significant at level 0.05, (data not shown).

DISCUSSION

A human plasma alpha₂ glycoprotein was described in 1960 by Heremans (2) and studied by Schmid and Burgi (3) under the name Ba-alpha₂ glycoproteins. It was finally termed alpha₂ HS by Schultze et al. (4) in honor of these first workers (2, 3). Until now, it was thought that this glycoprotein could consist of two proteins, as judged by starch electrophoresis. These two proteins had different terminal amino acids (3, 7). The present isolation procedure yielded an homogeneous preparation and SDS-PAGE and NH₂-terminal analysis demonstrated that alpha₂ HS consists of a single polypeptide chain of 50,000 daltons. In our opinion, the reason this protein was earlier thought to consist of two molecular entities can be ascribed to a peculiar fragility of the protein. In our work, the demonstration of spon-

taneous alpha₂ HS fragments indicated that the protein could not always remain intact in vitro. Similar fragments have apparently been pointed out previously but were not physico-chemically analyzed. In this study, small peptides and a major polypeptide of 30,000 daltons were spontaneously obtained. In our current work, these spontaneous fragments could be obtained by serum proteases, because trypsin in vitro produced alpha₂ HS fragments of a size similar to those spontaneously obtained. Alpha₂ HS fragility could be caused by the fact that it is the serum protein that contains the greatest amount of hydrophobic residues (27). The high amount of proline amino acids in alpha₂ HS, which prevents formation of alpha helix, could cause an increased sensitivity to proteolytic enzymes. Moreover, because hydrophobic bondings are unstable at low temperature, nonpolar groups could represent an additional factor of fragility. Hamberg et al. (22) indicated that kininogens and alpha₂ HS display similar physico-chemical properties and postulated that some relationship might exist between these two proteins. It would be interesting to compare the sensitivity of

TABLE II
Pertinent Data on Seven Patients with an Acute Infectious Disease Taken during the Course of the Disease

Patient	Age	Sex	Underlying disease	Day*	Leukocyte count	Erythrocyte sedimentation rate	Serum concentration						Ceruloplasmin
							Albu- min	Alpha ₂ HS	Oroso- mucoid	Alpha ₁ - AT	Hp	Alpha ₂ M	
	yr				cells/mm ³						g/liter		
V.R.	65	M	Meningitis (<i>D. Pneumoniae</i>) following an otitis.	3	24,600 PMN = 92%	ND	32	0.26	1.35	4.78	7.86 HP 2.1	2.10	0.36
				5	26,000 PMN = 85%	ND	37	0.30	1.20	4.29	7.47	2.22	0.34
				7	9,600 PMN = 73%	57/92	33	0.30	1.50	4.17	5.65	1.88	0.30
				9	8,600 PMN = 62%	54/88	33	0.29	1.30	3.00	5.33	1.76	0.27
				11	9,700 PMN = 62%	54/95	34	0.30	1.45	3.58	6.04	1.76	0.32
				21	ND	ND	38	0.35	1.45	2.98	5.65	2.10	0.41
				77	ND	ND	41	0.40	1.00	2.44	5.00	2.98	0.54
				260	ND	ND	36	0.40	0.85	3.26	3.82	2.72	0.51
R.E.	72	F	Septicemia (<i>S. Aureus</i>) with empyema from day 13 to day 20. Clinical recovery at day 70.	10	ND	ND	23	0.14	1.50	4.66	5.22 Hp 2.2	2.10	0.41
				13	13,000 PMN = 91%	ND	21	0.17	2.00	4.17	5.22	2.34	0.41
				17	ND	ND	28	0.18	2.30	4.66	5.58	2.22	0.41
				21	ND	ND	29	0.22	2.20	3.93	4.14	2.12	0.38
				25	ND	ND	29	0.24	2.20	3.69	3.84	2.50	0.48
				39	13,000 PMN = 90%	83/120	28	0.24	2.10	3.00	4.74	2.50	0.44
				70	ND	40/80	35	0.36	0.88	2.44	2.22	2.40	0.34
				100	ND	ND	44	0.40	1.20	2.44	3.15	3.00	0.46
				250	ND	25/35	49	0.51	0.80	2.52	1.23	3.32	0.40
Th.A.M.	49	F	Urinary infection (<i>E. Coli</i>) with initial bacteriemy.	11	19,600 PMN = 88%	34/77	37	0.24	1.98	4.44	4.77 Hp 1.1	2.10	0.50
				14	ND	45/79	33	0.32	1.44	3.36	3.69	2.12	0.46
				17	6,500 PMN = 80%	ND	46	0.34	1.34	2.98	2.83	2.50	0.49
				200	ND	ND	50	0.48	0.58	2.35	1.22	2.50	0.36
F.A.	56	M	Anaerobic cellulitis of the scrotum (Fournier's gangrene).	6	13,200 PMN = 85%	114/122	19	0.13	2.52	4.53	8.13 Hp 1.1	1.30	0.44
				10	17,600 PMN = 86%	ND	17	0.13	2.52	4.65	5.28	0.80	0.35
				13	ND	ND	15	0.14	1.98	3.81	4.99	0.90	0.32
				17	4,900 PMN = 79%	ND	15	0.15	1.80	3.25	4.46	1.00	0.38
				20	ND	ND	15	0.17	1.83	4.41	3.70	0.80	0.38
				23	6,900 PMN = 74%	ND	16	0.21	1.98	2.98	3.79	1.00	0.32
				27	ND	ND	26	0.23	1.80	2.60	3.93	1.20	0.32
				30	ND	ND	24	0.28	2.10	2.98	3.93	1.30	0.35
				33	ND	ND	32	0.29	2.00	4.29	3.93	1.50	0.38
				37	10,300 PMN = 65%	ND	39	0.32	1.98	2.88	4.46	1.40	0.38
				41	ND	ND	31	0.32	2.30	3.58	4.34	1.30	0.36
				44	15,800 PMN = 72%	ND	37	0.32	2.15	3.81	6.91	1.50	0.50
				90	9,800 PMN = 85%	72/115	39	0.35	1.62	2.77	4.58	1.20	0.38
R.R.	51	M	Pleuropulmonary and septicemic staphylococci.	22	12,100 PMN = 70%	ND	14	0.27	0.80	4.42	5.65 Hp 2.1	2.20	0.36
				25§	ND	ND	16	0.22	0.80	ND	ND	ND	ND
				28	12,000 PMN = 71%	ND	17	0.21	1.00	4.42	6.20	2.44	0.83
				32§	10,900 PMN = 50%	ND	18	0.25	0.90	ND	ND	ND	ND
				43	ND	ND	23	0.37	0.80	4.17	6.95	3.00	0.44
B.J.M.	18	M	Septicemia (<i>C. Perfringens</i>) following a cranial trauma. Clinical recovery at day 54.	8	25,700 PMN = 75%	ND	23	0.28	2.50	4.83	3.67 Hp 2.1	2.46	0.48
				13	21,700 PMN = 74%	ND	32	0.30	1.95	3.30	4.13	2.72	0.40
				18	14,200 PMN = 74%	ND	34	0.41	1.55	3.18	2.86	2.72	0.27
				54	8,900 PMN = 69%	ND	46	0.73	0.60	2.22	1.93	3.12	0.27
G.M.	27	M	Staphylococcal dermatitis of the shoulder. Clinical recovery at day 26.	3	12,200 PMN = 79%	66/105	37	0.50	2.07	4.42	4.92 Hp 1.1	1.73	0.51
				7	ND	ND	34	0.46	2.50	5.25	5.73	1.73	0.52
				13§	7,600 PMN = 67%	69/112	37	0.55	1.80	ND	ND	ND	ND
				26	ND	ND	48	0.73	1.24	3.66	2.46	1.84	0.44

* In the column: Day, the first date indicated corresponds to the first sample obtained after the clinical onset of the disease.

† Sample not included in the principal component analysis (group II).

§ Sample not included in the principal component analysis.

alpha₂ HS to enzymic attack with that of plasma kininogens that produce kinins after a proteolytic and estero-lytic cleavage of kininogen substrates. As is well-known, kinins play a pharmacological role in the inflammatory process (23).

Recent studies (24) indicate that plasma alpha₂ HS is concentrated extravascularly in the matrix of both adult and fetal bone, and is located in areas of mineraliza-tion. Triffitt et al. (25, 26) have shown that a rabbit alpha glycoprotein, which was a component of calcified

TABLE III
Pertinent Data on Five Normal Subjects

Subject	Age	Sex	Day of sampling	Serum concentration						
				Alpha ₂ HS	Hp	Alpha ₁ AT	Albumin	Orosomucoid	Ceruloplasmin	Alpha ₂ M
	<i>yr</i>						<i>g/liter</i>			
R.J.P. (Hp 2-2)	30	M	1	0.73	1.38	1.71	46	0.86	0.28	1.4
			6	0.53	1.20	1.99	45	0.62	0.24	1.3
			13	0.56	1.95	2.10	35	0.94	0.26	1.2
			24	0.58	1.56	2.41	38	0.80	0.28	1.4
			29	0.48	1.33	2.20	41	0.80	0.27	1.4
			36	0.56	1.23	2.34	39	0.76	0.29	1.4
L.J.P. (Hp 1-1)	41	M	1	0.76	ND	2.43	44	0.68	ND	ND
			6	0.65	1.17	1.87	37	0.46	0.28	1.3
			13	0.56	1.50	1.87	34	0.78	0.32	1.3
			20	0.76	1.50	1.96	43	0.60	0.34	1.4
			31	0.72	1.10	2.23	39	0.66	0.30	1.4
H.M. (Hp 2-1)	23	F	1	0.89	ND	3.03	ND	ND	ND	ND
			6	0.83	1.35	3.93	41	0.52	0.46	2
			13	0.94	1.85	3.58	46	0.44	0.52	2.10
			20	0.98	1.82	4.05	59	0.52	0.52	2.10
			29	0.84	1.56	3.69	37	0.56	0.45	2.2
J.F. (Hp 2-2)	26	M	1	0.60	ND	2.05	ND	ND	0.29	1.94
			7	0.62	0.68	1.96	48	0.66	0.28	1.84
			15	0.80	0.65	2.23	55	0.74	0.25	1.60
			25	0.72	0.59	2.13	32	0.74	0.28	1.96
			28	0.62	0.78	1.59	45	0.70	0.29	1.96
			41	0.69	0.60	2.20	54	0.74	0.29	1.48
			55	0.78	1.14	2.55	43	0.84	0.30	1.48
			72	0.90	2.40	2.96	52	0.94	0.34	2
			78	0.84	1.00	3.25	42	0.76	0.32	1.8
			93	0.74	1.23	2.60	48	0.66	0.31	2
			99	0.74	0.80	2.75	50	0.62	0.31	1.9
			119	0.74	0.62	2.35	42	0.72	0.28	1.8
			127	0.69	1.29	2.86	45	1.00	0.31	1.6
			136	0.74	0.38	2.65	51	0.80	0.28	1.7
			192	0.69	1.29	1.62	51	0.68	0.29	2
			220	0.63	0.75	2.15	34	0.56	0.24	1.6
			233	0.66	0.80	2.35	36	0.64	0.24	1.6
			333	0.61	0.99	3.34	46	1.00	0.26	1.6
			334	0.61	1.62	2.45	45	1.20	0.28	1.4
			335	0.66	2.40	2.75	53	1.52	0.40	1.7
			336	0.71	2.07	2.45	43	1.16	0.34	1.7
M.J.P. (Hp 2-1)	37	M	1	0.56	1.48	1.99	45	0.48	0.27	1.73
			7	0.51	1.35	1.90	52	0.46	0.22	1.38
			15	0.61	1.58	1.90	52	0.56	0.26	1.84
			25	0.52	1.46	1.90	52	0.56	0.26	1.50
			38	0.62	1.58	2.23	59	0.62	0.29	1.72

cortical bone matrix, was synthesized by the liver but not by bone. Similarly, a alpha₂ glycoprotein from bovine cortical bone (27) was shown to be present in the collagenase digest of decalcified bone matrix at a much

higher concentration than in the plasma. Because alpha₂ HS and these alpha₂ glycoproteins disclosed similar physico-chemical properties, it was thought that they fulfilled identical physiological functions (27, 28).

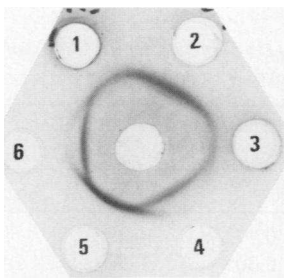


FIGURE 5 Ouchterlony analysis with an anti- α_2 HS immune serum of (1) patient S.E. (2, 5) normal human serum (3) patient F.A. (4, 6) an α_2 HS polypeptide fragment (peak I of Fig. 4).

These results, added to those indicating that α_2 HS was precipitated by calcium phosphate complexes (28) and those obtained in the present work demonstrating that α_2 HS could be purified after metal affinity, suggested that α_2 HS was able to bind metal ions. Van Oss et al. (29) reported that α_2 HS increased hydrophobicity of *Escherichia coli* and *staphylococcus aureus* and enhanced their phagocytosis. These authors postulated that α_2 HS could be analogous to a rat opsonic α_2 globulin (30), which is primarily involved in phagocytosis of nonbacterial particular matter. However, it has not been reported that α_2 HS is the analogue of α_2 opsonic protein from rat serum that exhibits completely different physico-chemical parameters and is devoid of cross-reactivity with α_2 HS. (31, 32). Therefore, the role of α_2 HS in the opsonic process of microorganisms merit further attention.

Our present study demonstrates that α_2 HS serum level is greatly decreased in patients who developed an acute inflammatory process after a severe bacterial infection. Because no fragments of the protein could be demonstrated in the serum of patients with low levels of α_2 HS, it was assumed that decreased levels were caused by impaired hepatic synthesis. An alternative possibility would be an increased degradation, possibly by lysosomal enzymes or other serum

proteolytic enzymes, with rapid removal of the degraded fragments from the serum making them undetectable. Correlations found between α_2 HS, on the one hand, and Hp, α_1 AT, albumin, and orosomucoid, which are all known to be AP-reactants (1, 33) on the other hand, indicate that α_2 HS is a negative AP-reactant. A very close similarity in the behavior of α_2 HS and albumin is proved by their very close positive paired correlation and by the near identity of their correlation with the two first principal components (group I). It would be of interest to assess serum levels of these two proteins in other types of illnesses. However, although inconclusive, it is noteworthy that in our parallel study, α_2 HS and α_1 AT serum levels of a woman taking oral contraceptives (34) were greatly increased in parallel. These facts suggest that, because serum levels appear to be opposite, different stimuli could also produce different hepatic responses.

The second principal component obtained in the analysis of group I clearly indicates a variation tendency involving orosomucoid to a large degree, but the other proteins not at all or only slightly. This phenomenon has not yet been explained. The nonsignificant correlation coefficient found between α_2 HS and ceruloplasmin could be explained by the tendency for ceruloplasmin level to remain high, while α_2 HS was already increasing in most of the patients. It should be noted that, principally for patients V.R. and R.R., biological recovery was incomplete, whereas clinical recovery was obtained. For these two proteins there may be a delay in the return to normal, which is responsible for the lack of linear correlation but does not exclude another type of correlation.

It has been shown that AP-reactants can be roughly divided into two groups with respect to their response time: the first group contains C-reactive protein. Kushner et al. (35) have recently shown that the rise in concentration of this protein began 1–2 h after injury, with maximum concentration reached at 50 h. Another group comprises orosomucoid, Hp, α_1 AT, and ceruloplasmin, the maximum serum levels of

TABLE IV
Paired Linear Correlations between the Serum Concentration of Five Proteins: α_2 HS Glycoprotein, Hp, α_1 AT, Albumin, and Orosomucoid, during the Acute Inflammatory Process (Group I)

	α_2 HS		Hp		α_1 AT		Albumin		Orosomucoid	
	r^*	P^\dagger	r	P	r	P	r	P	r	P
α_2 HS										
Hp	-0.52	<0.05	-0.52	<0.05	-0.36	<0.05	0.74	<0.05	-0.35	<0.05
α_1 AT	-0.36	<0.05	0.66	<0.05	0.66	<0.05	-0.59	<0.05	0.21	NS
Albumin	0.74	<0.05	-0.59	<0.05	-0.55	<0.05	-0.55	<0.05	0.49	<0.05
Orosomucoid	-0.35	<0.05	0.21	NS	0.49	<0.05	-0.23	NS	-0.23	NS

* r is calculated on 45 blood samples obtained in seven patients (Table II).

$^\dagger P$ indicates the probability of nonsignificant difference between r and zero. NS; not significant at level 0.05.

TABLE V
Principal Component Analysis Results for Five Serum Proteins: Alpha₂-HS Glycoprotein, Hp, Alpha₁ AT, Albumin, and Orosomucoid, during the Acute Inflammatory Process (Group I)

No. of factorial axis	Component variance	Percentage of total variance	Characteristic vector coordinates in initial axis system with regard to axis of				
			Alpha ₂ HS	Hp	Alpha ₁ AT	Albumin	Orosomucoid
1	2.931	0.586	-0.460	0.470	0.472	-0.493	0.319
2	0.909	0.182	0.289	-0.193	0.308	0.364	0.807
3	0.692	0.138	-0.574	0.516	0.490	0.193	-0.357
4	0.320	0.064	-0.267	0.637	-0.445	0.543	0.173
5	0.148	0.030	0.551	0.266	-0.494	-0.541	0.299

Protein serum concentration was studied on 45 blood samples obtained in seven patients (Table II). Total variance is five.

which are reached between 72 and 96 h after time injury (36). Therefore, it could be postulated that liver synthesis of these proteins is regulated by a different hypothetical mediator. Another possible explanation is that there may be different hepatic response times to an identical circulating mediator. So far, the nature and origin of liver cell stimulators are still unknown and experiments such as those using mediators obtained from leukocytes, or those in which histamine was used, did not provide convincing evidence that these substances are stimulating agents of the hepatocyte (1). Hormones should be considered humoral factors influencing the synthesis of these proteins (1) and recent studies (37) have indicated that the hepatic synthesis of alpha₁ glycoproteins and albumin from rat hepatocytes depends on the composition of the hormone mixture employed.

In conclusion, this study demonstrates that human alpha₂ HS protein is a negative AP-reactant, which may be used in *in vitro* and *in vivo* studies of human hepatic protein synthesis in physiological and pathological conditions.

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