Identification of a Major Heparin-Precipitable Protein in Human Serum and Its Relationship to Cystic Fibrosis

RITA MARGOLIES, BRUCE GRAY, AND THOMAS F. BOAT⁽²⁵⁾

Department of Pediatrics, Case Western Reserve University, Cleveland, Ohio, USA

Summary

Heparin binding to serum proteins and their subsequent precipitation is reportedly increased in cystic fibrosis (CF). We have confirmed this finding for CF patients over the age of 12 [11.34 ± 2.42 mg/ml precipitated protein for normals ($n = 19$) versus 17.46 ± 4.60 mg/ml for CF patients ($n = 37$), $P < 0.001$; 0.629 ± 0.098 mg/ml precipitated heparin for normals versus 0.789 ± 0.206 for CF patients, $P < 0.01$]. We have also shown that patients with a variety of pulmonary diseases unrelated to CF do not show this effect. When the amounts of protein and heparin precipitated are compared with the amount of IgG found in the whole serum sample, the correlation coefficients (protein $r = 0.77$; heparin, 0.74 ; $n = 81$) are significant at a level of $P < 0.001$. In addition, the report that young CF patients exhibit hypogammaglobulinemia prompted us to examine serum samples from CF patients and age-matched controls under the age of 12. No differences were found.

To investigate the molecular basis for this effect, sera from patients with CF and from age-matched controls were precipitated with 50 mg% heparin at pH 5.57. Pellets resolubilized in 8 M urea were fractionated on DEAE-Sephadex and analyzed by double-immunodiffusion, SDS-PAGE, immunoelectrophoresis, and radial immunodiffusion. IgG constituted 55-56% of the eluted protein. When serum from all donors was fractionated by Staph A-Sepharose into IgG and non-IgG fractions, 85-89% of heparin precipitable protein was in the IgG fraction.

Speculation

We conclude from this study that the major heparin-precipitable protein in human serum at pH 5.57 is IgG. The increased amounts of protein precipitated from serum of older patients with cystic fibrosis merely reflects the elevated levels of IgG in their serum. These findings do not support the hypothesis of Pearson and Lubin (16) that a generalized defect in protein glycosylation is the basis of the heparin effect, although a specific alteration of IgG oligosaccharides is not ruled out. In addition, this study of the heparin effect does not provide any evidence that there is an alteration in glycoprotein:glycosaminoglycan interaction in cystic fibrosis.

Cystic fibrosis (CF) is the most common genetic disorder among Caucasians in the United States, with an estimated incidence of one in every 2000 live births. It is widely accepted that CF is inherited as an autosomal recessive trait, and thus a defect in a single protein is to be expected.

Pearson and Lubin (15) have reported that precipitates formed by addition of heparin at pH 5.57 to CF sera have higher protein and heparin contents than precipitates formed in control sera. In addition, they reported that serum proteins from patients with cystic fibrosis had increased heparin-precipitating capacity. In a later paper (16), the same authors confirmed these findings and reported altered carbohydrate composition for glycoproteins precipitated from CF serum.

These findings prompted us to identify the proteins precipitated from CF and control sera by heparin at pH 5.57. The literature provides little information, inasmuch as most serum protein-heparin interactions have been studied at neutral or basic pHs.

Because of its polyanionic character, we anticipated that heparin would interact most strongly with positively charged serum proteins, i.e., the gamma globulins. IgG, which represents the bulk of this class of proteins, is elevated in serum samples from many patients with cystic fibrosis (12, 13, 18, 21). Matthews *et al.* (12) and Weeke *et al.* (21) both report that this elevation of serum IgG is correlated with severity of disease.

The results of this study show that IgG is indeed the major protein precipitated by heparin at pH 5.57 and suggest that precipitation of increased amounts of IgG is largely responsible for previous observations of heparin interactions with CF serum components.

MATERIALS AND METHODS

Specimens. Nonfasting venous blood samples were allowed to clot at 4°C. Following centrifugation, the serum was removed, aliquoted into plastic tubes (Falcon 2054), and stored for varying periods at -70°C. Samples were obtained with informed consent. The protocol was approved by the Human Experimentation Committee of University Hospitals.

Subjects. The patients in this study were diagnosed as having CF by clinical criteria and elevated sweat chloride concentrations. Severity of disease was graded using the clinical scoring method outlined by Doershuk, *et al.* (3). For this study, patients with scores over 75 are considered to have mild disease; 75-50, moderate disease; and less than 50, severe disease. The control population consisted of normal healthy volunteers and of patients with non-CF related pulmonary diseases. A detailed description of the subjects used in each study is provided in the appropriate sections.

Materials. Porcine heparin (Grade I) and Staph A-Sepharose were obtained from Sigma Chemicals. IgG Quantiplate kits were obtained from Kallestad and DEAE-Sephadex from Pharmacia. Prepoured immunoelectrophoresis plates were obtained from Mayloy. Spectropor dialysis tubing (molecular weight cutoff: 6000-8000) was used throughout. Reagents for polyacrylamide electrophoresis were supplied by Eastman.

Quantitation of protein and heparin. The amount of heparin precipitated was quantitated indirectly by measuring the amount of uronic acid in the pellet. The carbazole method, as modified by Bitter and Muir (1) and using glucuronic acid as standard, was employed. The % of uronic acid (weight for weight) in the heparin used was also determined (31-33%). Since heparin is an alternating copolymer of glucosamine and glucuronic acid, the amount of heparin in the precipitate could be calculated.

Protein was determined by the method of Lowry *et al.* (10) except when assaying the DEAE-Sephadex column fractions. In this case, the fluorescamine assay of Udenfriend *et al.* (20) was used because of its greater sensitivity. Bovine serum albumin was

used as standard, except for the Staph A experiment where bovine IgG was used because this was the class of protein being studied.

Heparin precipitation of serum protein. The method of Pearson and Lubin (15) was employed for the precipitation of serum proteins with heparin at pH 5.57. Briefly, 1 ml of serum was added to 30 ml of 50 mM ammonium acetate buffer, pH 5.5 containing 0.5 mg of porcine heparin per ml (the final pH of this solution was pH 5.57). The resulting precipitate was collected by centrifugation, washed with ammonium acetate buffer, and lyophilized. The pellet was resuspended in 1 ml of 0.5 M sodium bicarbonate in 10% ethanol and after 1 h incubation, 0.2 ml of 3N NaOH was added and the incubation was continued for 30 min., until the pellet was completely solubilized.

Analysis of unprecipitated serum proteins. In order to analyze the unprecipitated serum components, the supernatants were decanted from the protein-heparin precipitates and dialyzed against 5% BaCl₂ to remove the unbound heparin (2). The heparin-barium complex was removed by centrifugation (8000 rpm, 4°C, 30 min.) and the supernatant was then dialyzed extensively against glass distilled water, lyophilized, and resuspended in 50 mM barbital buffer, pH 8.0. This preparation is referred to as the supernatant.

DEAE-Sephadex chromatography. Heparin was separated from the precipitated proteins by DEAE-Sephadex chromatography. This procedure also results in the fractionation of these proteins. The protein-heparin precipitate from 1.5 ml of serum was dissolved in 3 ml of 50 mM Tris-HCl, (pH 8.0) containing 8 M urea and applied to a 1.0 by 13 cm DEAE-Sephadex (A-50) column that had been equilibrated with the sample buffer. After the column had been washed with 15 ml of buffer, it was eluted with a 100 ml NaCl gradient (0–1.0 M). Fractions of 1.15 ml were collected at a flow rate of 0.47 ml/min.

The protein content of the column fractions was monitored by following the absorbance at 280 nm. The proteins eluted as two peaks, which were dialyzed extensively against glass distilled water, lyophilized, and then resuspended in a volume of barbital buffer equivalent to that of the original serum sample. The ionic strength of the fractions was monitored by a Radiometer-Copenhagen conductivity meter, type CDM3.

Immunoanalytical methods. Immunoelectrophoresis of column fractions was performed with 50 mM barbital buffer, pH 8.0. A constant current of 25 mA per plate was applied and the voltage never exceeded 300 mV. "Hoat" antihuman serum antiserum (Kallestad) was used in this system. IgG was quantitated by radial immunodiffusion. Double immunodiffusion analysis was carried out by the method of Ouchterlony and Nilson (14).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS, 10% polyacrylamide slab gels were used according to the method of Laemmli and Favre (7). Samples in 5% 2-mercaptoethanol and 0.5% SDS were electrophoresed for approximately 3 h at 4°C at a current of 35 mA per gel. Proteins were visualized using Coomassie Blue R-250 stain.

Preparation of IgG and non-IgG serum fractions. Staph Protein A covalently coupled to Sepharose 4B was equilibrated in 0.1 M phosphate buffer, pH 7.0 at 4°C. Columns containing 1 ml of settled gel were poured and washed extensively with the starting buffer. Serum samples were centrifuged at 3000 rpm for 10 min to remove particulate matter and a 0.5 ml aliquot applied to each column. The columns were then washed with 30 ml of phosphate buffer, at which point the eluant no longer absorbed at 280 nm. The IgG fraction was then eluted with 6 ml of 1 M acetic acid. Both fractions were immediately dialyzed against glass distilled water and lyophilized. The fractions were resuspended in a volume of phosphate buffer equivalent to the original serum sample volume, heparin precipitated, and analyzed for protein and heparin.

Data analysis. Students *t* test for unpaired samples was used to determine the significance of difference between two means; 0.05 (two-tailed analysis) was used as the level of significance.

RESULTS

Analysis of serum protein-heparin precipitates. Table 1 data show that significantly more protein and heparin were precipitated from

serum samples of CF subjects over the age of 12 than from age-matched normal serum samples. Additionally, the protein/heparin combining ratio *i.e.*, mg of protein precipitated per mg of heparin precipitated, was significantly higher ($P < 0.01$) in patients with CF (22.71 ± 5.87) when compared with normal controls (18.3 ± 3.46). The concentration of IgG in CF sera was higher than in control sera.

Data from the two control groups were analyzed separately. No significant differences were found (Table 2). Thus both groups were designated as the control population for this study.

Table 3 presents an analysis of the data from older patients with CF according to the severity of their disease. All parameters showed significant differences when compared with the control group except the amount of heparin precipitated by serum and the IgG levels in serum of patients with mild disease.

When serum samples were studied from CF donors who were younger than age 12 and who had mild disease, no significant differences from age-matched control values were found (Table 4).

In no case was the concentration of total protein in serum significantly different between CF and control donors.

Characterization of precipitated serum proteins. Precipitated proteins from serum samples from ten normal individuals and nine individuals with cystic fibrosis were solubilized as described and qualitatively analyzed by immunoelectrophoresis, immunodiffusion, and SDS-PAGE.

Table 1. Heparin-protein precipitate data for donors age 12 and over¹

	Non-CF <i>n</i> = 19 (mg/ml of serum)	Cystic fibrosis <i>n</i> = 37 (mg/ml of serum)	<i>P</i>
Protein precipitated	11.34 ± 2.42	17.47 ± 4.64	<0.001
Heparin precipitated	0.629 ± 0.098	0.789 ± 0.206	<0.01
IgG	10.73 ± 2.49	13.93 ± 3.85	<0.001

¹ Control population, 10 healthy volunteers (six males and four females) and nine patients with non-cystic fibrosis related pulmonary disease who ranged in age from 13–30 years. Cystic fibrosis population, 37 patients (21 males and 16 females) ranging in age from 14–32 years.

Table 2. Heparin-protein precipitate data for control group according to disease state

	Normal <i>n</i> = 10 (mg/ml of serum)	Lung disease ¹ <i>n</i> = 9 (mg/ml of serum)	<i>P</i>
Protein precipitated	11.29 ± 1.86	11.42 ± 3.03	n.s. ²
Heparin precipitated	0.615 ± 0.155	0.646 ± 0.077	n.s.
IgG	9.54 ± 2.11	10.8 ± 3.09	n.s.

¹ Infectious disease, four donors; bronchitis and asthma, five donors.

² Not significant, n.s.

Table 3. Heparin-protein precipitate data for CF donors (> 12 years old) according to severity of disease¹

	Mild <i>n</i> = 13 (mg/ml of serum)	Moderate <i>n</i> = 14 (mg/ml of serum)	Severe <i>n</i> = 10 (mg/ml of serum)
Protein precipitated	15.34 ± 3.35 ²	18.29 ± 4.68 ²	19.11 ± 5.39 ²
Heparin precipitated	0.688 ± 0.164	0.781 ± 0.151 ³	0.932 ± 0.254 ²
IgG	11.77 ± 3.80	15.14 ± 3.17 ²	15.28 ± 3.75 ²

¹ Levels of significance are determined by comparison with control group. Similar results are obtained if these data are compared to patients with non-cystic fibrosis related lung disease.

² $P < 0.001$.

³ $P < 0.01$.

Table 4. Heparin-protein precipitate data for donors age 11 and under

	Non-CF <i>n</i> = 16 (mg/ml of serum)	Cystic fibrosis <i>n</i> = 15 (mg/ml of serum)
Protein precipitated	8.19 ± 1.91	8.51 ± 2.95
Heparin precipitated	0.489 ± 0.092	0.397 ± 0.154
IgG	8.22 ± 1.83	7.37 ± 2.95

Precipitated proteins from all 19 individuals consistently eluted as two peaks. The first peak contained proteins that did not bind to the ion exchanger and was designated Peak 1; the bound proteins, which were released between 5 and 25 mS, were designated Peak 2. No carbazole reacting material was found in either peak.

Immunoelectrophoresis of whole serum, Peak 1 (DEAE-Sephadex) proteins solubilized from heparin precipitate, and supernatants resulted in the migration patterns shown in Figure 1. The most electronegative gamma globulins were strikingly absent in the supernatant fraction, but were recovered in the precipitate fraction, Peak 1. When the Peak 2 precipitate fractions were electrophoresed, most of the precipitin bands were found in the gamma globulin region, with occasional weak bands in the beta region (results not shown). This pattern was consistent for the 5 CF and 5 control serum samples examined, although the bands seen with normal sera were usually weaker than those from patients with cystic fibrosis.

Double diffusion analysis of Peaks 1 and 2 from serum samples of six patients and six controls revealed the presence of IgG in both peaks. No IgM, IgA, or lysozyme were detected, although these proteins were found in the supernatant fraction. The C3 component of complement was found in the supernatant and in both DEAE-Sephadex column fractions.

Column fractions from six normal individuals and six patients with CF were examined by SDS-PAGE and no qualitative differences between the two groups were found. Two protein bands that comigrated with IgG heavy and light chains were consistently found in both DEAE-Sephadex peaks of urea-solubilized precipitates. Other less prominent, but higher molecular weight protein bands appeared in Peak 2 fractions from control and CF sera. These have not been identified. Figure 2 shows a typical gel with samples from DEAE-Sephadex Peaks 1 and 2, supernatants, and whole serum from a normal donor and a CF donor.

Protein and immunoglobulin concentrations in DEAE-Sephadex fractions of solubilized precipitates were measured to determine if there were qualitative differences with respect to total protein and/or IgG content (Table 5). The total amount of protein eluting from the DEAE-Sephadex column was significantly higher for serum samples from patients with cystic fibrosis. The amount of IgG in Peak 2 was three times higher for donors with cystic fibrosis; however, because of the wide variation among individ-

Fig. 1. Immunoelectrophoresis of (A) whole serum, (B) supernatant, (C) Peak 1 from DEAE fractionation of normal serum precipitate, and (D) Peak 1 from cystic fibrosis serum.

Fig. 2. Typical SDS-PAGE of DEAE-Sephadex column fractions. Lanes 1, 9, and 18 contain the standards, phosphorylase b, bovine serum albumin, carbonic anhydrase, and α -lactalbumin. Lanes 2, 10, and 17 contain partially purified IgG as standard. Lane 3 contains Peak 1a from a normal individual, Lane 4 contains the same material from a patient with cystic fibrosis; Lane 5—Peak 1b, normal; Lane 6—Peak 1b, cystic fibrosis; Lane 7—Peak 2a, normal; Lane 8—Peak 2a, cystic fibrosis; Lane 11—Peak 2b, normal; Lane 12—2b, cystic fibrosis; Lane 13—supernatant, normal; Lane 14—supernatant, cystic fibrosis; Lane 15—serum, normal; Lane 16—serum, cystic fibrosis. All lanes contain approximately the same amount of protein. Note the faintness of the IgG bands in the supernatants.

Table 5. Analysis of DEAE-Sephadex fractions

	Peak 1		Peak 2		Total	
	N	CF	N	CF	N	CF
Protein eluted (mg per 1.5 ml sample)	3.27 ± 1.61	4.02 ± 1.53	1.53 ± 1.8	3.25 ± 1.60 ¹	4.80 ± 1.80	7.25 ± 2.55 ²
IgG eluted (mg)	2.28 ± 1.18	2.58 ± 1.38	0.32 ± 0.42	1.00 ± 1.00	2.64 ± 1.15	3.58 ± 2.19

¹ $P < 0.005$.² $P < 0.01$.Table 6. Results of heparin precipitation of protein fractions from Staph A-Sepharose chromatography¹

	Fraction A		Fraction B	
	Normal	Cystic fibrosis	Normal	Cystic fibrosis
Staph A fractions (mg of protein)	45.64 ± 9.45	51.69 ± 13.3	12.36 ± 5.58	16.15 ± 5.97
Protein precipitated ² (mg)	0.86 ± 0.67	1.37 ± 1.06	6.44 ± 2.40	8.02 ± 3.07
Heparin precipitated (mg)	0.216 ± 0.124	0.287 ± 0.196	0.897 ± 0.417	0.998 ± 0.344

¹ Per ml of serum.² Protein was determined by the method of Lowry, *et al.* (10) using bovine IgG as standard. Fig. 3. A comparison between the amount of IgG in the serum sample and the amount of protein precipitated. The correlation coefficient is 0.77 ($n = 81$, $P < 0.001$).

uals, these data are not statistically significant. It should also be noted that the ratio of IgG to total protein in each fraction was identical for the two groups.

Serum fractionation. In order to confirm that IgG is the major heparin-precipitable protein, serum samples from 10 normal and 12 CF individuals were fractionated into non-IgG (Fraction A) and IgG (Fraction B) fractions by using Staph Protein A-Sepharose 4B to bind immunoglobulins of all subclasses except IgG3, which represents only about 5% of the total (15). The two resulting fractions were heparin precipitated and the pellets were analyzed for protein and heparin.

As can be seen from Table 6, approximately 80–85% of the precipitable protein in serum is contributed by Fraction B, which contains predominately IgG. Indeed, in some cases, virtually no precipitated protein could be detected in Fraction A.

Direct correlation of serum IgG concentrations with protein precipitation. Figure 3 shows the relationship between the amount of precipitated protein and the levels of IgG in whole serum from all donors. The correlation coefficient for the amount of precipitated protein and the levels of IgG in the serum samples is 0.77, $n = 81$,

$P < 0.001$, and for precipitated heparin and IgG the correlation coefficient is 0.74, $n = 81$, $P < 0.001$. When the correlation coefficient is computed only for older cystic fibrosis patients, the results are very similar and are still significant at a level of $P < 0.001$.

DISCUSSION

This study was initiated to re-examine the work of Pearson and Lubin (15, 16) who reported that heparin-protein precipitates from sera of cystic fibrosis patients contain both more heparin and more protein than precipitates from control sera. Our findings confirm these results with several exceptions. The amount of protein precipitated was significantly higher for serum samples from older cystic fibrosis patients regardless of the severity of disease. In addition, the protein/heparin combining ratio in our study is higher for CF serum samples, when compared to controls, while Pearson and Lubin (15, 16) reported it to be lower. The reason(s) for discrepancies between the two studies cannot be explained by procedural differences since the methods described by Pearson and Lubin were followed exactly.

We have extended these observations by demonstrating that at pH 5.57 the major heparin precipitable protein in human serum is IgG. The evidence is as follows: (1) a prominent precipitin band appears only in the gamma globulin region after immunoelectrophoresis of DEAE-Sephadex fractions from urea-solubilized heparin-protein precipitates. This band is missing in the supernate. Double immunodiffusion of the precipitate fractions shows reactivity with anti-IgG antiserum but not with anti-IgM, IgA, or lysozyme antisera; (2) SDS-PAGE of the DEAE-Sephadex fractions from solubilized precipitates (especially Peak 1) reveals major bands that comigrated with heavy and light chains of IgG; (3) over 50% of the serum protein precipitated by heparin and then eluted from DEAE-Sephadex columns was found by radial immunodiffusion to be IgG; (4) when whole serum is fractionated on Staph A-Sepharose into non-IgG and IgG fractions, 85–89% of the total protein precipitated by heparin resides in the IgG fraction; and (5) furthermore, we have shown that increased amounts of precipitate formed in CF sera are closely correlated with elevated IgG levels.

Different values for the amount of protein in NaOH-solubilized precipitates (Table 1), the amount of protein recovered from DEAE-Sephadex chromatography of urea-solubilized precipitates (Table 5), and the amount of protein precipitated after Staph

Protein A fractionation (Table 6), can be attributed to the different methods of protein estimation and standards used in each of these experiments. First, when the Fluram method of protein estimation is used with bovine serum albumin as standard, the protein content of a sample containing predominantly IgG is underestimated by about 60% (unpublished data). Thus, if one corrects the data shown in Table 5 for the 60% underestimate and compares it with the data shown in Table 1, a 66% recovery is calculated. This figure is roughly comparable with the usual recovery of IgG from DEAE (4). In addition, the measured levels of IgG in urea-treated samples may not be as high as expected because there may have been a loss of antigenically reactive IgG. Recovery data from the DEAE column are not reported because the amounts of urea and Tris required to solubilize the pellets interferes with both the Fluram and Lowry methods of protein estimation. Finally, heparin precipitation of serum fractions may not mimic precipitation from whole serum. However, this experiment did show that approximately one-half of the serum IgGs are capable of interacting with heparin and that other serum proteins are considerably less so.

There are several reports in the literature that the concentrations of IgG are elevated in many patients with cystic fibrosis, particularly in those over the age of 10. Matthews *et al.* (12) examined 419 patients with cystic fibrosis. They found that, compared with normals, of 178 patients 10–20 years old, 24% had elevated serum levels while of 87 patients over the age of 20, 47% had elevated levels. On the other hand, in patients under the age of 10 only 6.5% had elevated levels, while 22% had low levels. In our study, in patients over 12, over 50% had elevated IgG levels, and only 10% had low levels compared with age-matched controls. In younger patients, this was reversed: 50% had low levels while only 13% had high levels compared with age-matched controls. In our study, protein-heparin precipitates from patients under the age of 12 also showed no differences from normals in the amount of protein or heparin precipitated. This supports our contention that increased levels of precipitable proteins are related to elevated concentrations of serum IgG.

Can the increase in heparin-precipitable protein be largely accounted for by the increase in IgG levels seen in patients with cystic fibrosis? While our studies show a mean increase in IgG levels of 3.2 mg/ml, Weeke *et al.* (21) report that on average, patients with cystic fibrosis have an increase of 5 mg of IgG per ml of serum and Schwartz (18) reports that the serum levels in older patients are increased by 6.86 mg IgG per ml of serum, which compares favorably with the 6.13 mg of extra protein in precipitates of cystic fibrosis serum (see Table 1). None of the other 17 proteins examined by Weeke *et al.* showed an increase in CF serum samples that even approached this magnitude, except for α_2 -macroglobulin, which showed an average increase of 1.3 mg. The absence of any precipitin bands in the alpha globulin region on immunoelectrophoresis of the precipitated proteins is a strong indication that this protein was not precipitated to any significant degree. It is unlikely that other serum proteins, alone or in combination, contribute substantially to the heparin precipitation effect in CF serum.

One possible reason for the scatter in the data presented in Table 5 may be differences in the individual immune responses. It is well documented that the humoral response to specific types of antigens (*i.e.*, polysaccharide, protein, etc.) is subclass restricted (17, 23) and that the isoelectric points of the IgG subclasses vary; IgG1, pI 6.8 to < 9, IgG2, pI 6.3–8.4; IgG3, pI 8.0–9.0; IgG4, pI < 6.2 (22). Thus the concentrations of the various subclasses can vary depending on which antigen has elicited a response. In addition, Shakib *et al.* (19) have reported that CF patients have altered ratios of serum IgG subclasses. Because of the electrostatic nature of the heparin-protein complex (8), changes in the relative amounts of the four IgG subclasses would be expected to affect the amount of protein and heparin precipitated.

An alternate hypothesis is suggested by Levy, *et al.* (9) who have demonstrated that all eight heparin-binding myeloma proteins studied had cross-reacting idiotypes not found in 92 nonhe-

parin binding monoclonal IgGs. No other common feature was found, *i.e.*, light chain type, isoelectric point, or subclass. Perhaps serum from CF patients has larger amounts of IgGs with idiotypes similar to those found by Levy, *et al.*

Pearson and Lubin (16) have put forth the hypothesis that "the glycoprotein biosynthetic mechanism in cystic fibrosis is genetically altered and produces glycoproteins of abnormal carbohydrate composition which combine with normal, acid glycosaminoglycans to form insoluble, viscous complexes that hinder normal cellular and subcellular transport mechanisms and metabolic processes." This hypothesis is based on the analysis of the carbohydrate composition of the protein-heparin precipitates, the results of which are as follows: a 27% decrease in sialic acid, a 27% increase in fucose, and a 31% decrease in hexose in glycoproteins precipitated from cystic fibrosis serum as compared with controls, although no differences in the carbohydrate composition of whole serum were found (16). Accordingly we are currently investigating whether the carbohydrate content of IgGs from CF serum is different from that of IgGs from normal serum.

Since we have shown that only one major class of serum proteins is precipitated by heparin, a generalized defect in glycosylation is not suggested by the altered CF serum protein-heparin interactions, although a specific alteration in IgG glycosylation is not ruled out. This conclusion is also supported by the work of Hunt and Summers (6), who showed that G protein of vesicular stomatitis virus is normally glycosylated when grown in CF fibroblasts, and of Margolies (11), who examined the levels of fucosyl- and sialyltransferases in CF fibroblasts and lymphoid lines and found no alterations in these enzyme activities. We suggest that the previously reported alterations of the carbohydrate content may reflect differences in the IgG subclass distribution in CF (19).

Our observations also make a generalized alteration in glycoprotein: glycosaminoglycan interaction an unlikely explanation for the heparin effect first described by Pearson and Lubin (16). However, serum-type glycoproteins are synthesized by a mechanism entirely different from that of mucous-type glycoproteins (5, 20). Therefore failure to uncover abnormalities of serum glycoproteins does not preclude alterations of mucous glycoprotein synthesis or metabolism in CF.

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24. The authors would like to thank Dr. P.-W. Cheng, Dr. L. Poncz, and Dr. R. N. Smith for their helpful suggestions. We are grateful to Dr. S. Polmar for providing us with partially purified IgG, to Dr. J. Scillian for putting antisera and equipment at our disposal, and to Dr. R. Stern for procuring blood samples.
25. Requests for reprints should be addressed to: Thomas F. Boat, Rainbow Babies and Childrens Hospital, 2101 Adelbert Road, Cleveland, OH, 44106.
26. This research was supported by NIH Grant AM 08305, a Parker B. Frances Foundation Fellowship, and the Rainbow Chapter of the Cystic Fibrosis Foundation.
27. Presented in part at the Annual Meeting, Society for Pediatric Research, San Francisco, CA, 1981.
28. Received for publication April 16, 1981.
29. Accepted for publication September 23, 1981.