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Protein Abnormalities in Adult Respiratory Distress Syndrome, Tuberculosis, and Cystic Fibrosis Sera[1] (42448)

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Abstract. Crossed immunoelectrophoresis (X-IEP) revealed several abnormalities in serum proteins from patients with adult respiratory distress syndrome (ARDS), tuberculosis (TB), and cystic fibrosis (CF). The two quite different kinds of pulmonary disease, one acute (ARDS) and the other chronic (TB and CF) exhibited serum changes specific for each disease and abnormalities associated with inflammation and pathogenesis, in general. In ARDS sera, most proteins were extremely low, presumably due to leakage into the lungs through damaged tissue, while the acute-phase proteins, orosomucoid, α_1-antitrypsin, α_1-antichymotrypsin, and haptoglobin, were markedly high when compared to the overall protein pattern. The extremely high α_1-antichymotrypsin values were not seen in corresponding TB and CF sera. Numerous TB patients had elevated α_1-antitrypsin, α_1-antichymotrypsin, and haptoglobin, but only the α_1-antitrypsin population mean was significantly different from normal. Gc-globulin, ceruloplasmin, and β-lipoprotein were higher and α_1-lipoprotein and inter-α-trypsin inhibitor lower than normal. All other quantitative serum changes were not statistically significant. Surprisingly, all TB patients belonged to the Gc-1-1 genotype in contrast to the Gc-1-1, Gc-1-2, Gc-2-2 polymorphisms of the other populations. CF homozygote sera revealed statistically significant increases in the acute-phase proteins, α_1-antitrypsin, α_1-antichymotrypsin, and haptoglobin, while orosomucoid, transferrin, IgA, and IgG tended to be higher than normal. The tendency for higher levels of transferrin indicated possible iron deficiency in some patients. In contrast, prealbumin, α_1-lipoprotein, and inter-α-trypsin inhibitor were significantly depressed in CF patients. CF heterozygotes shared the decrease of α_1-lipoprotein with the patients while exhibiting small but significant depressions of α_2-macroglobulin and IgG. Though not statistically significant, lowered concentrations of α_1-antitrypsin were evident for the heterozygotes.

Crossed immunoelectrophoresis (X-IEP)[3] is a method for identifying and quantitating many serum proteins simultaneously (1–3). As a result of its multifactorial, analytic capabilities, X-IEP can definitively identify normality and abnormality in profiles of serum proteins obtained from small populations of subjects or experimental animals (3–7). This makes it useful for rapidly and economically screening patients with various classes of illnesses in a remarkably comprehensive way for pathognomonic changes in their serum antigens.

In this report, we compare and contrast serum protein analyses of three pulmonary diseases which are distinctly different in nature and causes. Adult respiratory distress syndrome (ARDS) is an acute, often fatal illness induced by several diverse predisposing conditions (8–11). It is a disease that is still being characterized and its causes defined (9, 12). Tuberculosis (TB) and cystic fibrosis (CF) are chronic illnesses. While TB is an infectious disease, whose pathogenesis and treatment have been well studied (13), CF is a genetic disease, frequently accompanied by pulmonary complications, whose fundamental cause(s) remains unknown (14, 15). A number of studies have suggested the presence of various CF "factors" or modifications of other serum constituents (14–19), but, as yet, no

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[3] Abbreviations used: Ct, carbamylated transferrin; PA, prealbumin; Alb, albumin; Or, orosomucoid; α_1Lp, α_1-lipoprotein; α_1At, α_1-antitrypsin; α_1B, α_1-B-glycoprotein; α_1X, α_1-antichymotrypsin; IαI, inter-α-trypsin inhibitor; Gc, Gc-globulin; α_2HS, α_2HS-glycoprotein; Cr, ceruloplasmin, Hpt, haptoglobin; α_2M, α_2-macroglobulin; Hpx, hemopexin; Tr, transferrin; IgA, immunoglobulin A; IgG, immunoglobulin G; βLp, β-lipoprotein; C3, C3 complement; IgM, immunoglobulin M; ARDS, adult respiratory distress syndrome; TB, tuberculosis; CF, cystic fibrosis; X-IEP, crossed immunoelectrophoresis.

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consistent CF-specific serum antigen abnormalities have been found.

Apparently, X-IEP has never been used to study ARDS, and few investigations of TB and CF have utilized its analytic power (20–22). Our data will show that X-IEP does detect distinctly abnormal profiles of serum proteins for each of these diseases while offering comprehensive comparisons.

Materials and Methods. *Human sera.* Serum from two ARDS patients were provided by Dr. Thomas Petty, Pulmonary Division, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado, after more than 60 hr of treatment. Dr. Thomas Hyers, Veterans Administration Hospital, Denver, provided specimens from eight ARDS patients with various associated complications, specifically burns, cirrhosis, diabetic ketoacidosis, ethanol doxepin overdose, hypotension, and sepsis. Sera had been obtained upon hospitalization (0 hr), and after 36 and 60 hr of treatment.

Serum samples from 10 TB patients were obtained from Dr. Paul Davidson, National Jewish Hospital and Research Center, Denver, Colorado. These patients, still with active disease, had already received extensive treatment.

Sera from 16 CF patients (age range: 2–32 yr; average 20 yr) and 12 adult CF heterozygotes (age range: 30–74 yr; average age 49 yr) were obtained from Drs. Thomas Petty and Richard Simon, Respiratory Care Unit, Department of Medicine and Surgery, University of Colorado Health Sciences Center, and from Dr. Warren Warwick, CF Center and Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota.

All samples were stored at −20°C before use. Most sera were frozen and thawed more than once during this study, affecting the C3 globulin (23) and preventing us from obtaining a consistent value for it. In contrast, the other serum components remain immunoelectrophoretically stable under these conditions (23).

Crossed immunoelectrophoresis. X-IEP was performed as previously described (24, 25) using polyspecific rabbit antiserum to normal human serum prepared in this laboratory (4, 24).

Identification and measurement of serum proteins. The serum proteins (antigens) had been identified previously in X-IEP (4) (Fig. 1). The concentrations of each antigen were measured by considering the areas under the precipitation loops as representative of the amounts of protein in the serum (2, 26, 27). After the calculation of areas, the ratio between these and that of the external control, carbamylated transferrin (Ct; 28) were obtained. A known amount of carbamylated transferrin was added to every sample and, therefore, could be used for comparing different sera (3, 28). The resulting ratios were used for all quantitative analyses and comparisons of serum proteins with sera of 15 individuals of the normal population (age range: 23–61 yr; mean age: 37 yr).

Qualitative electrophoretic deviations were identified by X-IEP pattern of test samples with that of a representative sample pooled from sera of the normal population. With the exception of Gc-globulin, little electrophoretic variation was detected among normal patterns.

Results. *Quantitative alterations: ARDS (an acute pulmonary disease).* The serial collection of blood from a number of ARDS patients with different clinical complications, as either a cause or a result of ARDS, enabled us not only to monitor protein changes over several days, but also to detect the effects of various complications on the serum alterations. In general, most patients showed decreased concentrations of serum proteins during the 60 hr. Many of the protein levels for patients with sepsis were extremely low, with either α_1Lp, βLp, IαI, or IgM being undetected. These low concentrations increased substantially after 60 hr, but still did not approach normal concentrations. The Or, α_1At, and α_1X values for one sepsis patient were exceptionally high (3, 3.5, or 8.5 times normal, respectively). In some patients, α_1At and α_1X concentrations were highest and Hpx levels were lowest at 36 hr. One patient (etiology: cirrhosis, sepsis) was exceptional in having both IgA and IgG markedly elevated even when all of the other proteins were depressed. The means for 0-, 36-, and 60-hr samples, generally, did not reflect individual trends. Nevertheless, comparisons of the overall ARDS protein means with the normal population (Fig. 2) revealed that virtually all serum changes were statistically significant, with the exceptions of haptoglobin and IgA.

Tuberculosis (a chronic disease). As depicted in Fig. 3, there was a trend in the TB population toward decreased prealbumin, α_1-

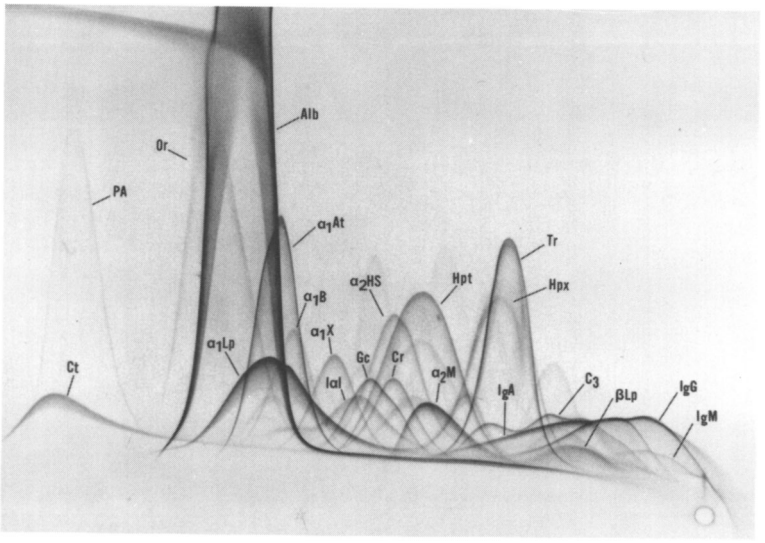

FIG. 1. X-IEP pattern of pooled, human serum using rabbit polyspecific antiserum to normal, whole human serum. Ct, carbamylated human transferrin, an internal control reference antigen of known concentration; PA, prealbumin; Alb, albumin; Or, orosomucoid (α_1-acid glycoprotein, α_1S); α_1LP, α_1-lipoprotein (high density lipoprotein); α_1At, α_1-antitrypsin; α_1B, α_1-B-glycoprotein; α_1X, α_1-antichymotrypsin; IαI, inter-α-trypsin inhibitor; Gc, Gc-globulin; α_2HS, α_2HS-glycoprotein; Cr, ceruloplasmin; Hpt, haptoglobin; α_2M, α_2-macroglobulin; Hpx, hemopexin; Tr, transferrin; IgA, immunoglobulin A; IgG, immunoglobulin G; βLp, β-lipoprotein (low density lipoprotein); C3, C3 complement; IgM, immunoglobulin M. (From Emmett and Crowle, 1981, with permission.)

lipoprotein, inter-α-trypsin inhibitor, hemopexin, and transferrin levels and increased concentrations of orosomucoid, albumin; α_1-antitrypsin, α_1-antichymotrypsin, Gc-globulin, ceruloplasmin, and IgA. But, only the changes in α_1Lp, α_1At, IαI, Gc, and Cr were

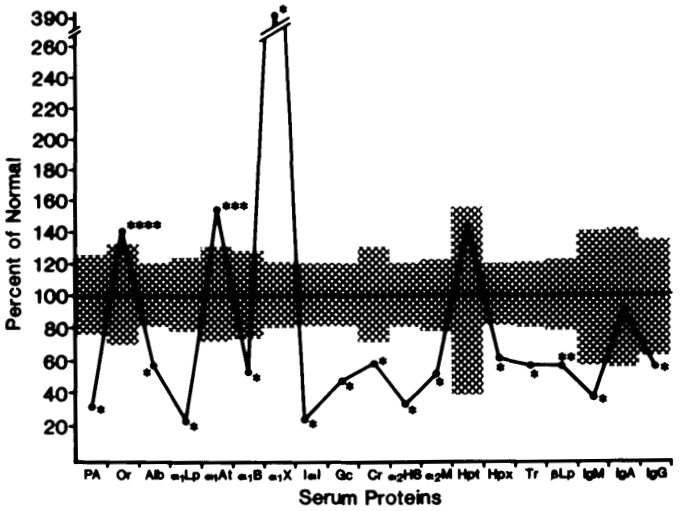

FIG. 2. Comparison of ARDS and normal protein population means. Each normal mean is represented as 100% normal. The enclosed areas depict the normal standard deviation for each particular protein. Abbreviations as in Fig. 1. Levels of significant differences: *$P < 0.01$; **$P < 0.02$; ***$P < 0.025$; ****$P < 0.05$.

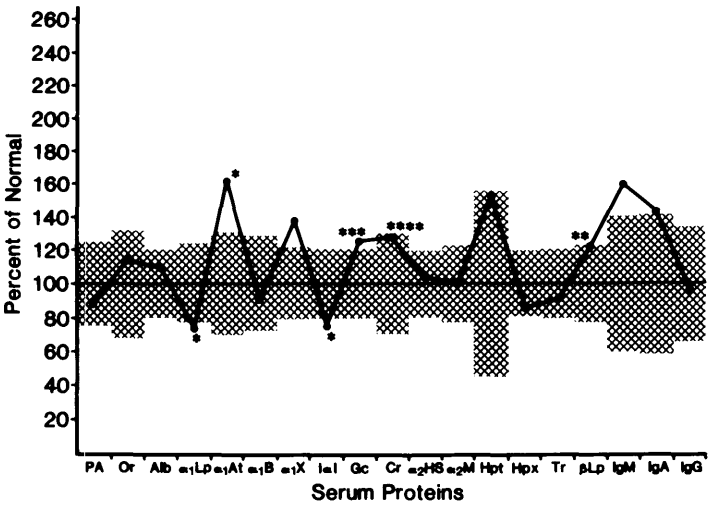

FIG. 3. Comparison of TB and normal population means. Abbreviations as in Fig. 1. Levels of significant differences: $*P < 0.01$; $**P < 0.02$; $***P < 0.025$; $****P < 0.05$.

statistically significant. The increase in βLp levels was found to be small but also significant.

Cystic fibrosis (a chronic, genetic disease). In general, the number of protein abnormalities was greater in adolescents and adults than in the young children. Figure 4 shows that decreases of PA, α_1Lp, and IαI were common to CF patients of all ages. Increases of the acute-phase proteins, α_1At, α_1X, and Hpt, were also significantly different from normal. A tendency toward increased Or, Tr, IgA, and IgG concentrations were evident, but these changes were not statistically significant.

Cystic fibrosis heterozygotes. X-IEP profiles of heterozygote sera were more normal than those of CF patients. However, these individuals usually showed increases of two or more of the acute-phase proteins. Comparisons with normal sera demonstrated noticeably decreased levels of PA, Alb, α_1Lp, α_2M, IgA, and IgG. Interestingly, most of these subjects

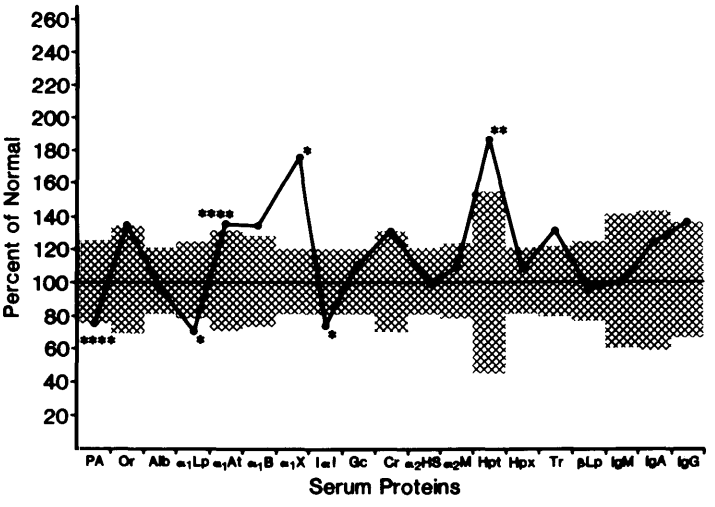

FIG. 4. Comparison of CF homozygote and normal population means. Abbreviations as in Fig. 1. Levels of significant differences: $*P < 0.01$; $**P < 0.02$; $***P < 0.025$; $****P < 0.05$.

showed *decreased* concentration of α_1-antitrypsin. As shown in Fig. 5, only the changes in α_1Lp, α_2M, and IgG were statistically significant.

Changes in IgA and IgG. Use of X-IEP profiles for internal quantitative comparisons between different serum proteins revealed differential changes of the major immunoglobulins (Table I). Even though the IgA/IgG ratios of ARDS and TB patients overlapped with normal values, the ratio means in ARDS and TB populations were significantly higher than normal ($P < 0.05$). IgA/IgG ratios of the CF homozygotes were virtually identical in range and mean values to the normal values, while CF heterozygote values tended to be slightly higher but not significantly different.

Qualitative alterations. As illustrated in Fig. 6, the prealbumin precipitation loop was anodically shifted in all ARDS sera. The haptoglobin loop for patients with sepsis contained a noticeable *cathodic* tail possibly due to binding with one of the immunoglobulins. Evidence of α_1At binding to proteases was also common. TB and CF homozygote sera also exhibited α_1At-protease binding.

Our X-IEP analyses were able to differentiate the allelic genotypes of Gc-globulin as revealed by their electrophoretic variance (Gc-1-1, loop anodal to ceruloplasmin; Gc-2-2, loop above ceruloplasmin; Gc-1-2, double loop) (29, 30). Each population demonstrated differential genetic polymorphism of this protein, as shown in Table II. ARDS and CF homozygote and heterozygote populations exhibited slight shifts from normal. Surprisingly, Gc analyses revealed that *all* TB patients in this study were homozygous for the Gc-1 allele.

Discussion. In this study, we have been able to identify multiple, consistent serum protein abnormalities in three representative human pulmonary diseases, ARDS, TB, and CF. This was possible despite our using small populations of patients because of the multifactorial quantitations and internal ratio comparisons that X-IEP analyses provide (6, 31–33). The findings thus confirm the special usefulness of X-IEP for this purpose.

We detected trends of change for several serum antigens consistent for each disease, those in ARDS being particularly striking. Some protein changes were shared by all three diseases. Three or more of the acute-phase proteins (34) were usually increased, including orosomucoid, α_1-antitrypsin, α_1-antichymotrypsin, and haptoglobin. In addition, decreases in α_1-lipoprotein and inter-α-trypsin inhibitor were common.

Particularly valuable may be our finding, though expected, of a profound drop of most serum antigens in ARDS sera and the excellent proof it gives of the usefulness of internal comparisons that X-IEP uniquely provides to establish serum antigen quantitative abnormalities. Thus, the absolute concentrations of

FIG. 5. Comparison of CF heterozygote and normal population means. Abbreviations as in Fig. 1. Levels of significant differences: *$P < 0.01$; **$P < 0.02$; ***$P < 0.025$; ****$P < 0.05$.

TABLE I. COMPARISON OF IgA/IgG RATIOS IN ARDS, TB, AND CF SERA

Population	Range[a]	Mean	Significance
Normal (15)[b]	1.46 ± 0.67	1.09 ± 0.06	—
ARDS (10)	2.63 ± 0.97	1.53 ± 0.16	$P < 0.05$
TB (10)	3.65 ± 0.84	1.66 ± 0.21	$P < 0.05$
CF Homozygote (16)	1.48 ± 0.52	1.03 ± 0.06	N.S.[c]
CF Heterozygote (12)	2.10 ± 0.79	1.25 ± 0.08	N.S.

[a] Calculated from the X-IEP area determinations for IgA and IgG from each serum.
[b] Number in population.
[c] Not significant from normal.

some acute-phase proteins in ARDS sera were normal (e.g., haptoglobin). But, compared with the majority of other profoundly depleted antigens in individual sera, indicating general loss of serum proteins from the circulation (12), they more correctly would be interpreted as being high. In this situation, a "normal" value for some antigens would suggest that these were being overproduced enough to maintain their "normal" concentrations despite rapid losses from the circulation (6, 25). This is an important new observation for ARDS that obviously was easily made with X-IEP, where it would have been considerably more difficult with other monospecific techniques. For ARDS, then, we found sharp elevations of the acute-phase proteins, indicating acute disease, and extreme depression of other antigens representing severe tissue damage and loss of intravascular proteins, for example, in the lungs (12).

Two of the ARDS patients had very high concentrations of IgA and IgG. This was not due to sepsis, because this response was lacking in other patients with sepsis. We can speculate that it was due to polyclonal increase such as associated with cirrhosis and with the recovery stages in burns (35), or represent some kind of auto antibody production (36).

As reported earlier (20–22), TB and CF as chronic, albeit severe, diseases do not cause such marked quantitative serum abnormalities. Mostly, in sera of these diseases, the abnormalities we saw were acute-phase responses, which could be known consequences of inflammation (34). Concentrations of α_1-lipoprotein and inter-α-trypsin inhibitor usually were low, but not as much as in ARDS sera.

The extremely high α_1-antichymotrypsin in ARDS sera was unique among the diseases in our study. In TB, this protein tended to be above normal but not significantly. It was significantly above normal in CF, but only about half as high as in ARDS. This extreme rise seems to be a shared hallmark of severe acute reactions, for we have seen it also in patients with extensive burns (7) and with toxic shock syndrome (29).

Other aspects of X-IEP serum profiles, however, are different in other acute diseases. In toxic shock, for example, acute-phase pro-

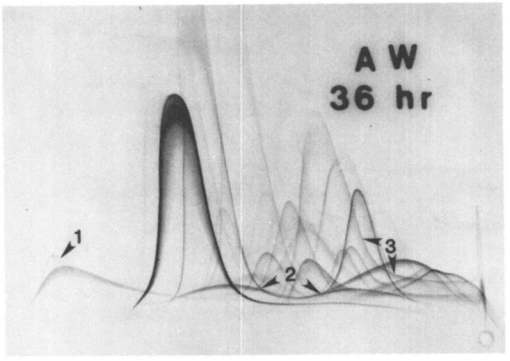

FIG. 6. Qualitative protein alterations in ARDS sera. (1) Anodic shift of prealbumin; (2) cathodic tail of α_1At as evidence of protease binding; (3) cathodic tail on Hpt merging with spike near origins.

TABLE II. Gc-GLOBULIN GENETIC POLYMORPHISM

Population	% Gc-1-1	% Gc-1-2	% Gc-2-2
Normal (15)[a]	53	40	7
ARDS (10)	40	50	10
TB (10)	100	0	0
CF Homozygote (16)	53	31	13
CF Heterozygote (12)	50	50	0

[a] Number of individuals in population in parentheses.

teins are elevated, and prealbumin, α_1-lipoprotein, and inter-α-trypsin inhibitor levels are depressed as in ARDS, but many serum proteins remain at normal concentrations (29). Thus, further studies probably will be able to define an X-IEP profile that is pathognomonic for ARDS.

Our X-IEP analyses also identified Gc-globulin genotypes. We noted the predominance of the Gc-1-2 heterozygote in ARDS, a shift toward this Gc heterozygosity in CF heterozygotes, and a shift toward the Gc-2-2 homozygote in CF homozygotes. Other investigations have noted population shifts toward the Gc-1-1 genotype associated with leprosy, uterine cancer, psoriasis, neurodermitis, diabetis mellitus, and rheumatoid arthritis, and toward Gc-2-2 for some cancers (37). Most surprising, however, was a complete absence of allelic polymorphism in our TB population; all were Gc-1-1. Calcification of the tubercle is associated with the containment of the bacilli (38). Allelic variability in the binding and transport of vitamin D, a regulator of calcium metabolism (30), by Gc-globulin may affect this process. Recently, a regulatory role of vitamin D metabolites on the phagocytic functions of macrophages has been demonstrated (39, 40). This could also be affected.

The changes in IgA/IgG ratios evident in ARDS, TB, and CF populations reflect preferential alterations in the relative amounts of these immunoglobulins, not the milligram per milliliter amount of IgA to IgG in normal serum. Rather, due to technical reasons, the normal mg ratios (IgA/IgG = 1/4) is represented in this immunological system by precipitation loops of relatively equal areas. Nevertheless, the increase in the area of the precipitation loop is an accurate measurement of the same change in actual mg levels.

Specific polyclonal IgA increases occur in a number of diseases including cirrhosis, alcholic hepatitis, and rheumatoid arthritis (41). In TB, it could have resulted from increased lymphatic transport of IgA into the blood as a result of pulmonary infection (42). In contrast, the ARDS ratio resulted from marked IgG deficiency possibly through preferential loss into the lung. The relatively high levels of IgA and IgG in CF sera could be due, in part, to production of antibodies against the opportunistic pathogen, *Pseudomonas aeruginosa,* as regularly occurs in this disease (43).

As might have been expected, because our analyses used antiserum to normal serum, we found no unusual specific markers for CF. Yet, we did find some interesting serum protein abnormalities similar to those previously described (21, 22). CF sera had below-normal prealbumin. This abnormality may have profound pleiotropic effects on development of CF. Prealbumin transports the retinol-binding protein–vitamin A complex in serum (44, 45). Since the plasma levels of retinol-binding proteins are already known to be decreased in cystic fibrosis patients (46), prealbumin deficiency further would inhibit vitamin A transport. Furthermore, recent reports have suggested that either prealbumin (47, 48) or a serum thymic factor carried by prealbumin (49) is important to the differentiation and maturation of T cells. Thus, the pathological alteration of this single protein could not only affect the delivery of vitamin A to tissues but also disturb the cellular immunity of these patients.

There was a tendency toward elevated transferrin in our samples of CF sera, indicating iron deficiency in some patients (50) and confirming previous findings (21, 22), but the differences between CF and normal values for transferrin were not statistically significant. The concentrations of a few serum proteins normally vary quite widely in the general population (e.g., haptoglobin), while other proteins including transferrin are very constant (e.g., inter-α-trypsin inhibitor and α_2-macroglobulin) (6). Our lack of statistical significance for differences between means of CF and normal transferrin probably reflects a pathological effect itself, hypervariability of serum proteins in this syndrome, which was also seen for other serum proteins.

Changes in the acute-phase protease inhibitors in CF were mixed. α_1-Antitrypsin and α_1-antichymotrypsin concentrations increased, perhaps as a protection against tissue damage (e.g., in the lungs), while the other two principal antiproteases of serum responded quite differently. Inter-α-trypsin inhibitor dropped, a finding seen in all the diseases we have studied. The importance of this drop is unknown. α_2-Macroglobulin remained quantitatively and qualitatively unchanged. The functioning of this major serum antiprotease in CF serum is controversial (15, 19).

In contrast to CF homozygotes, the het-

erozygotes exhibited few X-IEP serum abnormalities. Interestingly, these apparently healthy subjects did share the decrease of α_1-lipoprotein with the CF patients. This new finding together with the slight, but significant, deficiencies of α_2-macroglobulin and IgG may represent subtle hints of an underlying genetic predisposition toward CF. Since α_2-macroglobulin has been shown to normally decrease (51), increase (2), or remain relatively constant (52) during the 37–45 yr range (and vs the heterozygote population) (19), the effect of age on the small but significant heterozygote decrease is uncertain.

In addition, many heterozygotes had lower α_1-antitrypsin than normal, resulting in a slightly decreased mean value for this population as a whole. This deficiency has been noted before (18), but not pursued. Since this mean did not statistically differ from the normal, our analyses are, as yet, only suggestive.

With X-IEP in this study, we have detected a number of abnormalities in relatively small populations, some previously undetected. X-IEP has also shown its capability to efficiently distinguish pathologic changes among different diseases. Even though many of the regulatory mechanisms associated with serum protein alterations remain to be elucidated, documentation of these changes contributes to the understanding of more general clinical symptoms.

We greatly appreciate the efforts of many individuals for providing the serum samples used in this study. We also acknowledge the help of Drs. John Repine and Richard Fox and Ms. Ione Brown.

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