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13. We thank Drs. W. Guralnick and E. Rosenberg for their interest in this work and their cooperation in securing teeth suitable for study, and also A. Weinberg, B. Shapiro, and O. Sardinas for technical assistance. We thank Dr. M. S. Rodriguez for sending us a copy of his thesis. Supported by grants from the United States Public Health Service (AM-06375-02) and the John A. Hartford Foundation, Inc., to the Harvard Medical School and the Massachusetts General Hospital, and the Colgate-Palmolive Company and the American Chicle Company to the Forsyth Dental Center.
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Hereditary Deficiency of Serum α_1-Antitrypsin

Abstract. *Deficiency of the serum α_1-antitrypsin appears to be under genetic control. The level of this protein is reduced to less than 10 percent of the norm in individuals homozygous for the trait, who may suffer from pulmonary emphysema. Heterozygous individuals have a concentration of serum α_1-antitrypsin between 50 and 60 percent of normal, but appear to be in good health. The estimated heterozygous frequency of the trait in a small white population in Georgia is 2.1 percent.*

It has become clear since the work of Camus and Glay (*1*) that human serum inhibits the proteolytic activity of trypsin, but only recently was it established that this property resides in two different serum proteins (*2*). Most of the serum antitryptic activity (85 to 90 percent) is associated with an α_1 protein which is designated the α_1-antitrypsin; the remaining 10 to 15 percent resides in the α_2-macroglobulin fraction.

The α_1-antitrypsin, a glycoprotein isolated and characterized by Schultze (*3*), contains 12.5 percent carbohydrate. It has a molecular weight of 60,000 and a sedimentation constant of 3.5*S*. In starch-gel electrophoresis at *p*H 8.5 it migrates as a single band in the post-albumin region.

Laurell and Erikson (*4*) described several individuals in whose serums the concentrations of α_1-antitrypsin were greatly decreased; in all instances the detectable amount was less than 10 percent of the norm. Subsequently Erikson reported a family whose serum antitrypsin was either normal, moderately decreased (40 percent normal), or greatly decreased (less than 10 percent normal), according to the individual; he suggested that the concentration of serum α_1-antitrypsin was under genetic control (*5*). Moderate deficiency of α_1-antitrypsin was apparently compatible with normal health, but several individuals in whom only very small quantities were detectable suffered from pulmonary emphysema (*5*).

Since the association of deficiency of serum α_1-antitrypsin with pulmonary emphysema appeared more prevalent than could be expected by chance, a pilot study was undertaken on 99 random patients attending the emphysema clinic at Bellevue Hospital. Serum α_1-antitrypsin was examined by starch-gel electrophoresis in a barbital buffer at *p*H 8.5 (*6*) and by fibrin-agar electrophoresis (*7*); its activity was quantitatively determined by observing its inhibitory effect on tryptic digestion of the chromogenic substrate *N*, α-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (*8*). Inhibitory activity of the serum α_2-macroglobulin was estimated from the fibrin-agar electrophoresis.

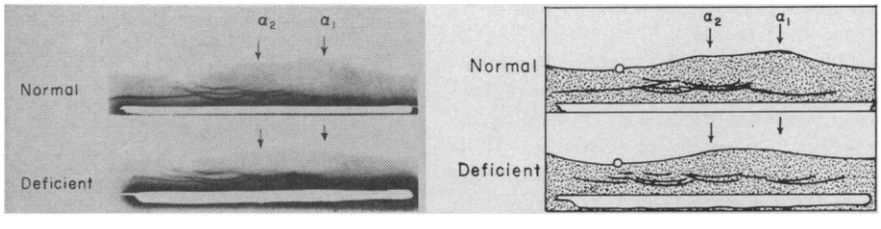

Fig. 1. Fibrin-agar electrophoresis of normal serum and serum deficient in α_1-antitrypsin. Horse antiserum against whole human serum is allowed to diffuse from the antibody trough to identify the serum proteins. A solution of trypsin diffuses from the upper trough, digesting the fibrin in the agar. Inhibition of fibrin digestion in the α_1 and α_2 regions in normal serum is demonstrated by the two broad peaks on the upper edge of the shaded area, whereas there is very little inhibition in the α_1 region of the serum deficient in α_1-antitrypsin. The important features of the photograph are shown in the drawing (right).

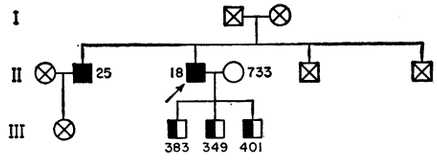

Fig. 2. Pedigree. The numbers are the numbers of micrograms of trypsin inhibited per milliliter of serum. Solid square, presumptive homozygote; half-solid square, presumptive heterozygote; open circle, normal; crossed circle, not examined.

In all but one of the patients (A.B.) the serum antitrypsin was approximately normal. Fibrin-agar electrophoresis of this one serum deficient in α_1-antitrypsin resulted in a markedly decreased peak of inhibition in the α_1 region; the α_2-macroglobulin peak of inhibition appeared normal (Fig. 1). Quantitative analysis confirmed that the serum antitrypsin activity was greatly decreased; the α_1-antitrypsin in 1 ml of normal serum inhibits approximately 745 μg trypsin, compared to 18 μg trypsin for A.B.'s serum. A.B. was a 53-year-old Caucasian male with clinical and x-ray findings consistent with the diagnosis of pulmonary emphysema. His arterial oxygen saturation was normal (94 percent) at rest but abnormally low (83 percent) during exercise. He was unusual among those whose emphysema is severe enough to disable them, in having a relatively well-preserved maximum breathing capacity (84 liters/min), a large vital capacity (5.6 liters), and an unusually large total lung capacity (12 liters). His older brother had similarly decreased serum α_1-antitrypsin activity (25 μg of trypsin inhibited by 1 ml of serum); although not examined, he was in apparent good health. A.B.'s three sons had α_1-antitrypsin activities approximately 50 percent of normal (383, 349, and 401 μg of trypsin inhibited by 1 ml of serum, respectively) (Fig. 2); they were healthy and showed no evidence of pulmonary emphysema. The wife of A.B. was healthy, and the α_1-antitrypsin activity of her serum was normal.

These findings, in conjunction with those of Erikson (*5*), suggest that the deficiency is inherited as a single-gene effect. Individuals with a serum α_1-antitrypsin concentration less than 10 percent of the norm would be presumed homozygotes, whereas those whose concentration was 50 to 60 per-

cent of the norm would be heterozygous for the abnormal gene. Whether the small amount of α_1-antitrypsin found in the affected homozygotes is structurally normal must await further study.

In view of the apparent innocuous nature of the gene in single dose, an attempt was made to estimate the frequency of the trait in a random population by surveying 193 whites from a community in Georgia (*9*); all serums were examined by starch-gel electrophoresis. Diminution in intensity of the α_1-antitrypsin band was further investigated by quantitative determination of the serum α_1-antitrypsin activity. All serums were examined by both methods, but it became apparent that the starch-gel-electrophoretic technique alone was a fairly reliable screening method for detecting deficiency of this protein (Fig. 3). Four of the 193 individuals studied had serum α_1-antitrypsin concentrations approximately 50 percent of the norm (342 ± 78.5 μg of trypsin inhibited by 1 ml of serum); they corresponded to a heterozygous frequency of the trait of 2.1 percent. The calculated homozygous frequency in this small sample of the population is approximately 1 per 10,000 (this estimate is necessarily very provisional). Further studies in several populations will be required to establish variations in frequency of this serum polymorphism and to elucidate the possible relation of serum α_1-antitrypsin deficiency to certain types of pulmonary emphysema.

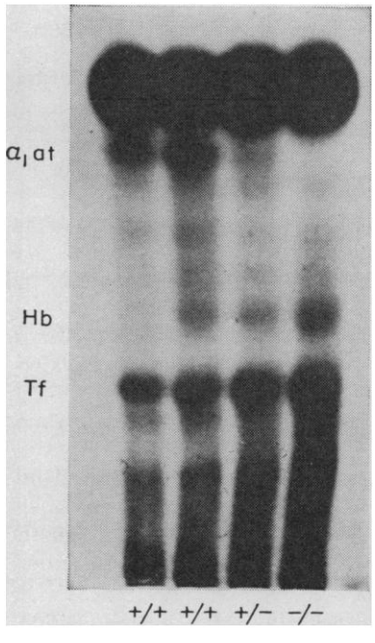

Fig. 3. Genetic variants of the serum α_1-antitrypsin. Starch-gel electrophoresis of two normal serums (+/+), one heterozygote (+/−), and one homozygous deficient individual (−/−). Note the decrease in intensity of the α_1-antitrypsin band ($\alpha_1$at) in +/— and —/— individuals. Hb, free hemoglobin; Tf, transferrin.

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9. The serum samples were supplied by Dr. C. Hames, Claxton, Georgia. Work supported in part by PHS grant 5T1GM 577 and H3341 from the National Institute of General Medical Sciences, and by grant U-1067 from the Health Research Council of the City of New York. We thank Drs. A. Cournand, J. H. McClement, J. B. Cromie, and A. Davis for the opportunity to study the Bellevue Hospital patients.

29 September 1964

Serum Albumin: Polymorphism in Man

Abstract. *Serums from 1015 individuals, mainly Norwegians, were studied by starch-gel electrophoresis. All but one showed the same albumin phenotype. The appearance of the exceptional sample on starch gel fits with that of a heterozygote. A genetic theory of two alleles* Al^F *and* Al^S *is proposed.*

Several examples of a genetically determined condition in man, usually called abnormal bisalbuminemia, have been reported (*1, 2, 3*). This condition is characterized by two serum albumin zones instead of the usual one. In animals such as horses (*4, 5*), cattle, sheep, pigs (*4*), and chickens (*6*) similar variations in the albumin have been observed. However, in most animal species three phenotypes are found showing distributions fitting with a genetic theory of two alleles which have been named Al^F and Al^S (*4, 6*). In connection with investigations of albumin polymorphism in domestic species we have carried out comparative studies of human albumin and now report results on serum albumin polymorphism in man.

A total of 1015 serums were investigated, 800 of them from pregnant women living in various parts of Norway. Two hundred samples came from seamen of whom 75 percent were Norwegians, and 15 came from Africans in a Congo hospital.

Albumin types were determined by starch-gel electrophoresis, using a method based upon Poulik's (*7*) horizontal discontinuous system. The serum samples were diluted 1:3 with distilled water, and the *p*H of the gel buffer was usually 7.8. No other details are given because separation of the major albumin fractions is easy with ordinary electrophoretic methods.

Two phenotypes were observed (Fig. 1). The common type designated FF appears as a major band with a very weak one in front. Separation of al-

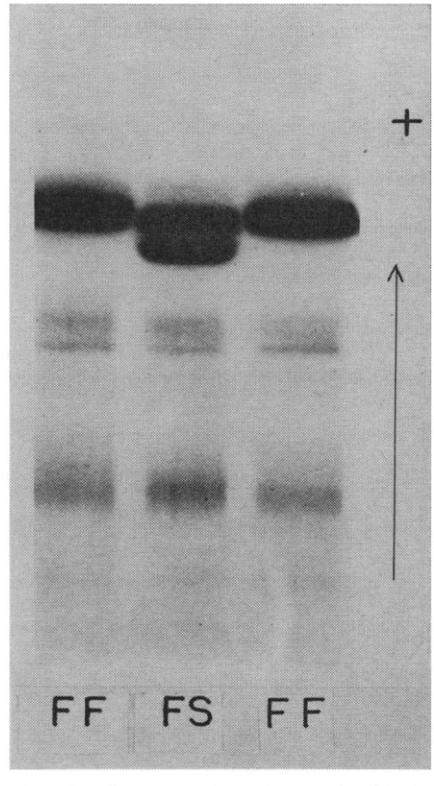

Fig. 1. Gel stained with amido black, showing three electrophoretic patterns of two albumin phenotypes. The origin is at the base.