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Serum Elastase Inhibitor Deficiency and α_1-Antitrypsin Deficiency in Patients with Obstructive Emphysema

Abstract. *A decreased inhibition of pancreatic elastase has been detected in the serums of six patients with α_1-antitrypsin deficiency. Five have severe clinical and physiological pulmonary emphysema. This observation extends the defect of inhibition by serum to a second, biologically active proteolytic enzyme in this form of familial emphysema.*

In one type of dysproteinemia of human serum, α_1-antitrypsin, a main component of the α_1-globulin fraction of serum, is deficient (*1*). This dysproteinemia is associated with severe pulmonary emphysema beginning at an early age (*2*). In one family reported by Eriksson (*2*), the amounts of trypsin inhibitor in the serums of various members were either normal, moderately decreased (60 percent of normal), or greatly decreased (less than 10 percent of normal). Eriksson suggested that the concentration of α_1-antitrypsin was under genetic control. Cases have since been reported on the association between α_1-antitrypsin deficiency and familial, chronic, obstructive lung disease (*3–6*); these findings reinforce the concept of genetic transmission of the defect as an autosomal recessive with emphysema occurring only in the homozygotes. We now report that the serums of individuals with severe pulmonary emphysema which are deficient in α_1-globulin antitrypsin are also deficient in an inhibitor of pancreatic elastase.

The combined deficiencies were found in six patients [five white males (C.S., age 26; A.Sa., age 47; W.P., age 51; E.M., age 46; A.S., father of C.S., age 72) and one white female (M.A., age 35)]. With the exception of A.S., all patients have clinical and physiological evidence of pulmonary emphysema.

Inhibition of elastase was measured by exposure of a commercial elastin-orcein complex (Sigma) to bovine crystalline pancreatic elastase (Worthington) in the presence of serum, according to the method of Sachar *et al.* (*7*) as modified by Mandl *et al.* (*8*). Results are reported as units of elastase inhibited by 1 ml of undiluted serum, with 1 unit equivalent to the elastase activity releasing all the orcein from 1 mg of the elastin-orcein complex (*8*). In practice, values are converted from the inhibition observed at serum dilutions of 1 to 50.

We measured antitrypsin activity, by the method of Blackwood and Mandl (*9*), with crystalline trypsin (Tryptar, Armour) and a substrate of benzoyl-arginine naphthylamide hydrochloride (Mann Chemical) at *p*H 7.3. All tests were done in duplicate; serums were tested on more than one occasion, and a reference serum from a normal subject with normal inhibitory activity as well as uninhibited controls without human serum were tested each time.

Antibody to α_1-antitrypsin was made by immunizing rabbits with a normal human serum fraction containing the α_1-globulin obtained by elution from diethylaminoethyl-Sephadex columns. The amount of α_1-antitrypsin in the serums of the patients was measured by its reaction with prepared and commercial rabbit antibody to human α_1-antitrypsin (Hoechst Pharmaceutical) by Ouchterlony double diffusion on agar gel.

Three groups of patients were tested: (i) twenty (14 males and 6 females) normal, healthy adults (ages 20 to 65 years); (ii) nine subjects (three females, six males) with chronic obstructive lung disease (ages 44 to 67 years); and (iii) six subjects with α_1-antitrypsin deficiency.

The inhibition found at a dilution of 1:50 for serum tested against elastase and at a dilution of 1:60 against trypsin was plotted. Elastase inhibitor activity has been expressed as units of elastase inhibited per 1 ml of undiluted serum. Antitrypsin activity has been plotted as the number of milligrams of trypsin inhibited per milliliter of undiluted serum. There is no statistical difference in elastase inhibition or trypsin inhibition between the group of 20 normal subjects and the 9 subjects with chronic obstructive lung disease (Fig. 1). The average normal inhibitory activity per milliliter of serum is 55 ± 4 units of elastase and $1.27 \pm .08$ mg of trypsin. However, patients C.S, A.Sa., and W.P. all had 0 units of inhibition for elastase and inhibited 0.25 to 0.4 mg of trypsin. Patient M.A. inhibited 9 units of elastase and 0.3 mg of trypsin per milliliter of serum. The moderately deficient subjects E.M. and A.S. demonstrated 45 to 60 percent of the normal inhibitory activity for each enzyme.

The titer of α_1-antitrypsin against rabbit antiserum was less than 5 percent of normal for C.S., W.P., and A.Sa., and 25 percent of normal for A.S. and E.M.

All five subjects who demonstrated pulmonary emphysema had the following in common: (i) early age of onset, usually in the 3rd or 4th decade; (ii) absence of a history of cough and sputum either before or as a part of their current respiratory complaints; and (iii) radiographic evidence of hyperradiolucent lungs with bullae without cardiomegaly.

Pulmonary function tests (Table 1) indicate severe bronchial obstruction and a large total lung capacity, with a large functional residual capacity and residual volume. Diffusing capacity for carbon monoxide was reduced in A.S. and A.Sa. as measured by the steady-state method. With the exception of W.P., the arterial carbon dioxide tension was normal or low; arterial oxygen saturation was above 90 percent in each

Table 1. Measurements of pulmonary function in five patients with combined elastase and trypsin inhibitor deficiency.

Patient	Percent predicted for				FEV_1/FEV (%)*	Nitrogen % $\bar{p}$ 7 min of 100% O_2†	Arterial		DL_{co} (ml min^{-1} $mm\text{-}Hg^{-1}$)‡	Pulmonary artery pressure (mm-Hg)	Cardiac output (liter min^{-1} m^{-2} bsa)§
	Total lung capacity	Vital capacity	Residual volume	Maximum breathing capacity			Oxygen saturation (%)	Tension CO_2 (mm-Hg)			
C.S.	137	59	245	23	38	6.4	93	35	8	30/14	4.6
A.Sa.	123	57	257	25	31	12.3	93	30	8.5		
W.P.	120	41	135	27	34		71	52			
M.A.	168	66	355	24	31		90	32			
E.M.	142	58	370	32	33	7.2	91	40		29/14	2.4

* FEV_1/FEV, percentage of the forced expiratory volume expired in 1 second. † Concentration of alveolar nitrogen after breathing 100 percent oxygen for 7 minutes (normal, <2.5 percent). ‡ Pulmonary diffusing capacity for carbon monoxide measured by steady-state method with arterial tension CO_2 and Bohr dead space. § Body surface area in square meters.

instance, thus indicating a relatively minor degree of pulmonary ventilation-perfusion disturbance. In C.S. and E.M., the resting pulmonary artery pressure and cardiac index were normal despite the evidence of hyperinflation and severe bronchial obstruction.

The clinical and physiological characteristics of the pulmonary disease in these subjects adhere closely to cases of familial emphysema previously reported in association with α_1-antitrypsin deficiency.

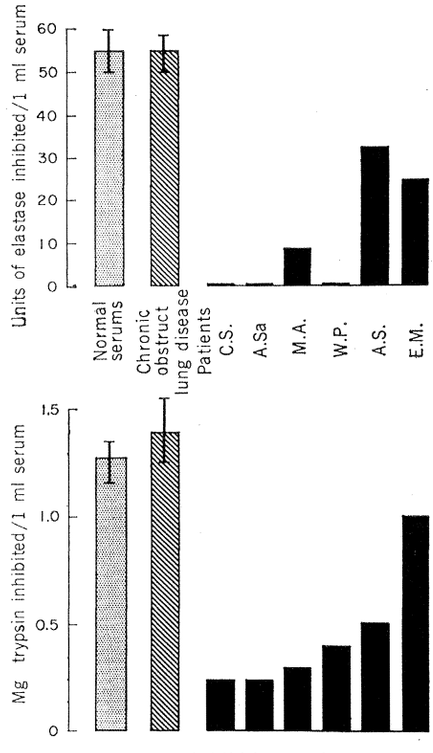

Fig. 1. Elastase inhibition (above) and trypsin inhibition (below) by serums of normal subjects, patients with chronic obstructive lung disease, and five patients and one normal relative (A.S.) with serum inhibitor deficiencies. The values for the serums of normal subjects and for patients with chronic obstructive lung disease are the average for 20 subjects and for 9 subjects, respectively; brackets indicate the range.

These subjects fall into the category of the "emphysematous" group identified by Burrows *et al.* (*10*) and Fletcher *et al.* (*11*) or the type A emphysema identified by Nash *et al.* (*12*) in contrast to those in whom chronic bronchitis without lung destruction predominates. Thus, our results reinforce the concept that a form of early onset emphysema exists where tissue destruction predominates and which may be secondary to an intrinsic biochemical defect which results in primary parenchymal destruction. The extraordinarily low values of elastase inhibition in the serums of four of these patients are the lowest values reported for this inhibitor in human subjects (*13, 14*).

A failure of inhibition of a second enzyme whose proteolytic activity is highly specific for elastin extends the potential for pathogenetic mechanisms in α_1-globulin deficiency. As suggested by Kueppers and Bearn (*15*), proteolytic substances in leukocytes or bacteria may become overoperative in vivo in the presence of a reduced serum inhibitory capacity. The specific components of α_1-globulin which inhibit elastase remain unknown and may possibly be the same component of α_1-globulin which inhibits trypsin. Normal human serum contains other trypsin inhibitors, and about 10 percent of the inhibitory capacity for trypsin in serum resides in the α_2-globulin fraction, which is not impaired in the patients with emphysema. However, the α_2-globulin does not inhibit elastase, an indication that part of the inhibitory capacity is not overlapping (*16*).

The possibility that more than one proteolytic inhibitor may be abnormal in pulmonary emphysema may be relevant to the observation that some subjects deficient in α_1-antitrypsin do not manifest pulmonary emphysema (*1, 4*).

The mechanisms by which deficiency of inhibition of elastase and trypsin may lead to destruction of lung parenchyma remain unknown. With regard to elastase, the administration of this enzyme parenterally or directly changes the mechanical characteristics of whole lung in vivo (*17*) and airways in vitro (*18*). Also, failure of inhibition of proteolytic enzymes may lead to an increased breakdown or disturbed resynthesis of alveolar connective tissue proteins. The constant physical stress of the lung during normal ventilation might precipitate gross structural defects in the connective tissue of this organ while such defects remain covert in other organs without similar mechanical functions.

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Atherosclerotic Plaque: X-ray Diffraction Investigation

Abstract. *Human atherosclerotic plaque material continuously maintained in an aqueous environment has been subjected to examination by x-ray diffraction. The first diffraction pattern from single crystals of human biological apatite was obtained from the plaque material of a freshly excised plaque when it was equilibrated with its aqueous environment. As the plaque material dried, the discrete spots characteristic of single crystal diffraction disappeared, leaving only the powder pattern of apatite.*

The atherosclerotic plaque is a lesion that represents the culminative trauma of atherosclerotic disease. The plaque, which predominantly occurs in man, can be found in the large and medium-sized arteries. Although no explanation completely accounts for all the aspects of atherogenesis, it is generally believed that atherosclerosis results from lipid accumulation in the intimal wall of the artery caused by a local breakdown of the normal process of lipid diffusion (*1*). This initial accumulation of lipid is followed by further deposits of organic cholesterol and other lipids and inorganic material (*2*).

X-ray studies (*3*) established that the major crystalline constituent of all mineralized tissue was the polycrystalline form of the calcium phosphate mineral apatite. Calcified intimal atheroma of the coronary arteries were examined by Carlstrom *et al.* (*4*), who used x-ray powder diffraction techniques. The diffraction patterns indicated a crystalline structure due to the presence of apatite. In all cases, the biological apatite structure consisted of relatively small crystallites, and x-ray diffraction investigation of calcified tissue (*5*) substantiated these findings. The powder patterns were consistent with the patterns obtained from apatite with small crystallites. Posner and Termine (*6*) recognized the presence of polycrystalline hydroxyapatite as well as amorphous calcium phosphate in bone; Perloff and Posner (*7*) prepared the first pure hydroxyapatite suitable for x-ray diffraction studies of single crystals.

Atherosclerotic material was examined by x-ray diffraction to determine to what extent structural order exists in the plaque and to what extent this structure is influenced by its environment. Aortas taken from recently deceased, aged, normal males were placed immediately into physiological saline solution. The moisture equilibrium between the plaque and its surrounding fluid was thus maintained. Plaques from different sections of the aorta were stripped from the arterial wall, and small test specimens were excised from the plaque so as not to include surface material.

Small test samples were placed in a 1-mm glass capillary tube that contained a reservoir of solution at each end. Care was taken not to have the solution in direct contact with the plaque specimen, so that no x-rays would pass through the solution before reaching the sample. The test specimen in the sealed capillary tube was exposed to nickel-filtered copper radiation in a Weissenberg camera for 2 hours. Specimens were examined from 1 to 7 days after autopsy. Tests were run as the plaque samples remained stationary, as the sample went through a 15° oscillation, or as the sample went through a 360° rotation. The capillary tube was then opened at one end and the solution was allowed to evaporate slowly for about a week. The same samples were tested on successive days as the plaque dried so that a change in the diffraction pattern with loss of moisture could be noted.

Five plaque samples from different areas in two aortas taken from recently deceased aged males were examined. Tests were also run, on successive days, on the glass capillary tube containing solution only. Plaque samples in physiological saline were stored in a sealed container at about 5°C for x-ray examination at a future date. Samples stored in this manner were examined as early as 1 day after autopsy and as long as 1 year later with no change in the diffraction pattern.

Since there is some evidence for the presence of hydroxyapatite in calcified tissue (*3*), a control pattern of hydroxyapatite was obtained. This control pattern was obtained by placing powdered calcium tribasic phosphate in a 0.5-mm glass capillary tube for exposure to x-rays for ½ hour to 2 hours.

Initially, as the plaque sample was equilibrated with the solution, two types of patterns were obtained. Continuous rings characteristic of polycrystalline material were obtained, as well as discrete spots characteristic of single crystals. In all cases the discrete spots fell on the continuous rings. Scattering maximums were obtained at scattering angles greater than 160°. As the sample dried the discrete spots disappeared, leaving only the rings. The rings from the plaque sample were compared with those obtained from the hydroxyapatite powder. They were identical.

Discrete diffraction spots falling on powder rings identified as hydroxyapatite were recorded only when the atherosclerotic plaque material was equilibrated with an aqueous environment. These discrete spots disappeared, leaving only rings as the plaque specimen dried. These findings indicate that in vivo plaques contain single crystals of apatite. Although biological apatites in the polycrystalline state have been observed in the human body this is the first observation of single crystals of biological apatite.

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