A Possible Experimental Approach to the Association of Hereditary α_1-Antitrypsin Deficiency and Pulmonary Emphysema.* (31006)

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Human serum has long been known to inhibit the proteolytic activity of trypsin. One ml of normal serum inhibits approximately 1.2 mg of trypsin(1,2) and approximately 90% of the total serum inhibitory capacity can be ascribed to the activity of the α_1-antitrypsin. The remaining 10% is contributed by at least 3 other inhibitors: the α_2-macroglobulin, less commonly known as the serum plasmin inhibitor(2), the inter-alpha trypsin inhibitor(3) and a third component, not yet well characterized, which migrates somewhat slower than the α_1-antitrypsin in starch gel using a Tris-lithium borate buffer(4).

It has recently become evident that a certain proportion of individuals homozygous for the α_1-antitrypsin deficiency gene suffer from pulmonary emphysema(5,6). However, since not all homozygous deficient individuals have pulmonary emphysema, and because the disease usually does not develop before the thirtieth year of life, it is clear that one or more additional factors may be necessary to produce the disease. Our studies, in addition to those of other authors, suggest that inflammatory processes in the lungs may also play a contributory, possibly decisive, role in the manifestation of the clinical disease. It is well known that proteolytic enzymes can destroy lung tissue(7). Some of the proteinases liberated at the site of inflammation may be of bacterial origin while others may be released from the cells of the host. If the proteolytic enzymes liberated were not inhibited, destruction of lung tissue could occur.

Materials and methods. Serum electrophoresis was performed in a horizontal starch gel system as described by Smithies(8) using a Tris-lithium borate buffer of pH 8.65, 0.2 M. Following electrophoresis a longitudinal strip of starch 2 mm wide and 2 mm thick was removed and placed on top of a fibrin containing agar gel of 1 mm thickness (1% agar or agarose; 0.1% bovine fibrin from Mann Research Laboratories, barbital lactate buffer pH 8.65, .051 M) and left in a moist chamber. After 10-12 hours 2 mm wide troughs were cut parallel to the starch strip at each side, 1 cm apart. The starch strip was removed and the troughs filled with a 0.001% trypsin solution or with the supernatant obtained from bacterial cultures which were grown overnight in Staphylococcus Medium #110 (without gelatin and agar at 37°C)(9).

After trypsin or the supernatant from bacterial cultures had been placed in the troughs the plates were kept at room temperature in a moist chamber. Digestion, as indicated by clearing of the fibrin gel, or its inhibition could be readily observed when the plates were examined after 12 to 15 hours. The bacteria employed in the present study were *Staphylococcus aureus* type Georgia (kindly supplied by Dr. R. Schaedler) and *Proteus vulgaris,* obtained from the sputum of a patient with α_1-antitrypsin deficiency and pulmonary emphysema.

Leucocytes were isolated from 500 ml of heparinized human blood(10) washed several times with 0.15 M sodium chloride solution and frozen and thawed 3 times. The remaining suspension was centrifuged and the supernate was concentrated. Free hemoglobin, released from contaminating erythrocytes, was removed by pevikon block electrophoresis (11). After this step, the electrophoretic fractions with proteolytic activity were concentrated to a total of 0.6 ml. Fibrin-agar electrophoresis was performed according to the technique of Heimburger *et al*(12). Porcine pancreatic elastase was obtained from

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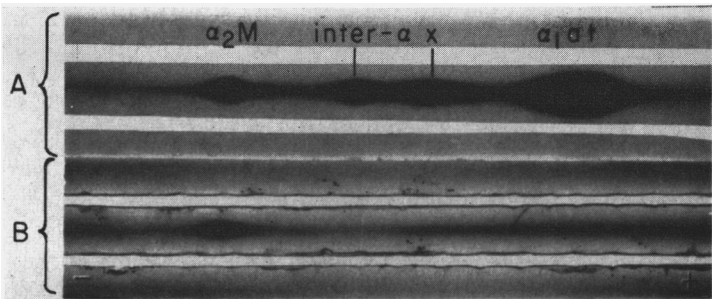

FIG. 1. Combined starch gel electrophoresis and fibrin-agar diffusion of human serum. Part A shows 4 inhibitors present in human serum. α_2 M, (α_2-macroglobulin); inter-α, (inter-alpha inhibitor); X, (an unknown inhibiting fraction); α_1 at, (α_1-antitrypsin). Trypsin diffusing from the 2 upper troughs is inhibited by all 4 serum proteinase inhibitors. Part B shows a starch gel electrophoresis and fibrin-agar diffusion of normal human serum. Both lower troughs contain supernatant from a *Staphylococcus aureus* culture. Inhibition of the enzymatic activity is restricted to the α_2-macroglobulin. A culture of *Proteus vulgaris* gave identical results.

Worthington Biochemicals Corp. A solution containing 5 mg/ml elastase in a barbital buffer of pH 8.6 was used for the fibrin-agar electrophoresis.

Results. Although no inhibition of proteolytic activity was observed in the α_1 region using the supernatant of bacterial cultures, a distinct zone of inhibition in the region of the α_2-macroglobulin was seen. The soluble proteolytic enzymes from *Staphylococcus aureus* and *Proteus vulgaris* were not inhibited by the α_1-antitrypsin. However, using the proteolytic enzymes from human leucocytes (Fig. 2A) a zone of inhibition in the α_1 and α_2 region was clearly visible. The inhibition zone in the α_1 region was shown to be due to the α_1-antitrypsin (Fig. 2B). α_1-antitrypsin can also be shown to inhibit elastase (Fig. 3).

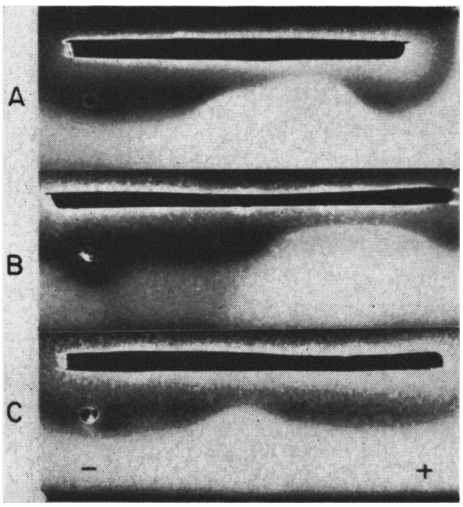

FIG. 2. Fibrin-agar electrophoresis of a normal serum (A), an isolated α_1-antitrypsin preparation (B), and a serum of a homozygous α_1-antitrypsin deficient individual (C). Human leucocyte extract was placed in all three troughs. Inhibition of these proteolytic enzymes in the α_1 and α_2 region can be seen (A). Inhibition by an isolated α_1-antitrypsin is apparent in B. No inhibition occurred in the α_1 region of serum from an α_1-antitrypsin deficient individual. Inhibition due to α_2-macroglobulin is still apparent.

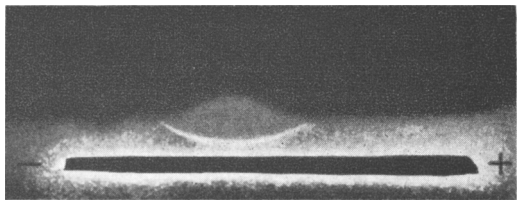

FIG. 3. Fibrin-agar electrophoresis of an isolated α_1-antitrypsin preparation. Porcine elastase is diffusing from above. Note the inhibition of elastase digestion by α_1-antitrypsin. A specific antiserum diffusing from below identifies the α_1-antitrypsin.

Discussion. The results obtained suggest that when leucocytes present in inflammatory exudates become necrotic and liberate their proteolytic enzymes, digestion of lung tissue might take place were it not for the presence of the serum α_1-antitrypsin. When this protein is absent, as in the case of a homozygous α_1-antitrypsin deficient individual, digestion of lung tissue may occur. It has been reported that human lung tissue, unlike bovine lung tissue, does not possess a special proteinase inhibitor(13). Thus the lack of the main serum proteinase inhibitor might be

expected to cause structural damage to the lung, particularly in alveolar tissue, during an inflammatory process. A recent report that the pathologic appearance of pulmonary emphysema may be produced in rats by intratracheal injection of papain(7) lends some support to this hypothesis.

Individuals heterozygous for the α_1-antitrypsin deficiency gene, who show intermediate levels (50-60% of normal) of α_1-antitrypsin, are apparently able to increase the serum antitrypsin activity in response to various pathologic conditions. Although there has not been an opportunity to follow the antitrypsin level of a known heterozygote during inflammation, it is probably relevant that in a large group of hospital patients with various conditions (chronic bronchitis, sarcoid, neoplasia) the observed heterozygote frequency was lower than in a general population(14).

Since the elastase used in our experiments was strongly inhibited by α_1-antitrypsin, the possibility that elastase may play a role in the development of emphysema should be considered. Thus far, however, there is no evidence that elastase is present in lung tissue under physiological conditions. It is, of course, quite conceivable that bacterial elastases may also prove to be of importance. Recent electron microscopic observations, however, suggest that the primary lesion in pulmonary emphysema is not in the elastic fibers but in the alveolo-capillary membrane (15).

The physiological significance of an increase in the level of antitryptic activity in response to inflammatory processes(16,17) and the possible harmful effects of an inherited deficiency of the principal serum inhibitor, the α_1-antitrypsin, are more readily understood in the light of these experiments.

Summary. Proteolytic enzymes from various cellular sources were tested against proteinase inhibitors of human serum. Serum α_1-antitrypsin was shown to inhibit the proteolytic enzymes of human leucocytes. This finding was interpreted in relation to the association between α_1-antitrypsin deficiency and pulmonary emphysema.

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