port from animal data (*19*)] is that changes in serotonin turnover are part of a general stress reaction in certain individuals, and the depressed mood may be another facet thereof.

A most interesting question is how our findings are related to the well-known catecholamine hypothesis of affective disorder (*20*). According to this hypothesis, noradrenergic functions are disturbed in depression. Do disturbances of serotonergic and noradrenergic functions coexist, or is there another subgroup of disturbed noradrenaline turnover within the depressive spectrum? Further investigation may help to clarify this issue. Our preliminary findings indicate, however, that a negative correlation may indeed exist between concentrations of the noradrenaline metabolite, 4-hydroxy-3-methoxyphenyl glycol (HMPG) in CSF and severity of illness in endogenously depressed patients, but only in those who belong to the upper 5-HIAA mode.

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Influence of Cadmium and Other Trace Metals on Human α_1-Antitrypsin: An in vitro Study

Abstract. *The effect of trace metals on plasma α_1-antitrypsin was studied in vitro by adding known concentrations of trace metals, either alone or in combination, to plasma. Cadmium was the only trace metal that reduced the concentration of α_1-antitrypsin and depressed the trypsin inhibitory capacity. No such effects were found with divalent lead, mercury, nickel, iron, and zinc ions. The present study appears to offer a plausible explanation for the emphysema that occurs in industrial workers exposed to cadmium.*

Considerable attention has been paid to the association between familial α_1-antitrypsin deficiency and pulmonary emphysema (*1, 2*). Although the role of intermediate α_1-antitrypsin deficiency in predisposing to pulmonary emphysema is still controversial (*2, 3*), the association with severe deficiency appears well established. Emphysema can also be caused by prolonged exposure to cadmium (*4–6*).

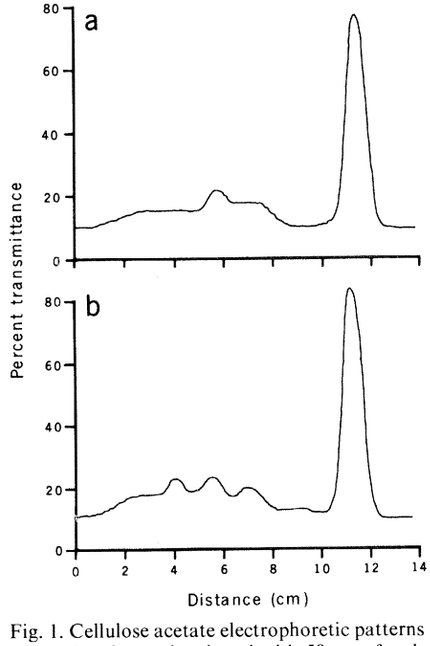

Fig. 1. Cellulose acetate electrophoretic patterns of (a) the plasma incubated with 50 μg of cadmium per milliliter and (b) normal human plasma (control). Percent transmittance (ordinate) and distance (abscissa) are in arbitrary units.

Since exposure to cadmium and severe α_1-antitrypsin deficiency are associated with emphysema, we wondered whether cadmium or other trace metals would alter human α_1-antitrypsin.

Experiments were carried out by adding a graded amount of trace metal in concentrations of 5 to 50 μg/ml to plasma and incubating the mixture at 37°C for 1 hour. Further experiments were conducted by combining two metals together, in each case one of the two being cadmium. Plasma blanks were incubated in triplicate for each set of experiments. After incubation the samples were centrifuged at 5000 rev/min and the following assays were performed in triplicate. α_1-Antitrypsin content was determined by a radial immunodiffusion method (*7*); trypsin inhibitory capacity (TIC) was measured according to the method of Dietz *et al.* (*8*); serum protein concentration was determined by cellulose acetate membrane electrophoresis with a Beckman Microzone electrophoresis apparatus (*9*); and total protein was determined by biuret reaction (*10*).

Figure 1 shows the electrophoretic pattern of normal human plasma and of plasma treated with cadmium solution, the concentration of cadmium being 50 μg/ml. The α_1-globulin peak is virtually absent in the plasma to which cadmium had been added. A decrease in the peak corresponding to the β-globulin fraction was also observed. Treatment of plasma with equivalent concentrations of Pb^{2+}, Hg^{2+}, Ni^{2+}, Fe^{2+}, and Zn^{2+} produced no such effects. The effects of various concentrations of

cadmium on α_1-antitrypsin content and its respective TIC are shown in Fig. 2. A commercial preparation (Worthington Biochemicals) of purified α_1-antitrypsin was studied simultaneously. The presence of even 10 μg of cadmium per milliliter produced a sharp drop in α_1-antitrypsin content; the decrease was far greater at higher concentrations. At a concentration of 50 μg of cadmium per milliliter, the antitrypsin content in plasma dropped to 6 mg/100 ml. Compared to the diminished plasma antitrypsin content, the decrease in plasma TIC was less pronounced. Purified α_1-antitrypsin showed a marked drop in TIC at lower cadmium concentrations than were required for equivalent reductions in plasma; the TIC continued to drop at a gradual rate with increasing cadmium concentrations.

In contrast to the above studies, other trace metals (Pb^{2+}, Hg^{2+}, Fe^{2+}, Zn^{2+}, and Ni^{2+}) at concentrations equimolar to those of cadmium produced virtually no effect on either the antitrypsin content or plasma TIC. These results indicate that interaction of cadmium with α_1-antitrypsin is specific. In order to demonstrate further the specificity of the reaction or the binding characteristics, the cadmium-containing plasma was extensively dialyzed in 0.1 percent buffer (*p*H 7.4) treated with ethylenediaminetetraacetate. No change was noticed in either the antitrypsin content or the TIC values observed in the plasma before dialysis.

In an attempt to determine whether other trace metals would diminish or increase the effects of cadmium on the antitryptic activity of the plasma, cadmium and another metal were added to plasma. The combinations of cadmium-zinc, cadmium-iron, cadmium-nickel, and cadmium-lead did not produce greater decreases of antitryptic activity than did cadmium alone at the same concentration. However, the combination of cadmium and mercury seemed to be more effective in decreasing the antitryptic activity than cadmium alone.

Our studies indicate that, among several trace metals studied, cadmium was the only one that reduced the plasma antitrypsin content and TIC in a dose-related fashion. Decrease of the α_1-antitrypsin content and its respective TIC by cadmium suggests an interacting system of substantial specificity, since most other serum components remained unchanged. The decrease of β-globulin as compared to α_1-globulin is, however, interesting and merits further investigation. As reported by Lieberman and Gawad (*11*), chloroform-shaken plasma also produced a decrease in the α_1-globulin content and this also requires further investigation. Other trace

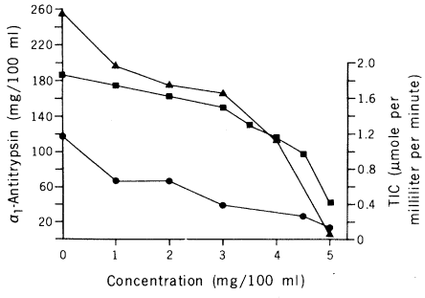

Fig. 2. Effect of cadmium on α_1-antitrypsin and trypsin inhibitory capacity (TIC). The abscissa represents concentrations of cadmium in milligrams per 100 ml. ▲—▲, α_1-Antitrypsin (plasma); ■—■, TIC (plasma); and ●—●, TIC (α_1-antitrypsin from commercial source).

metals, under similar circumstances, showed no specificity or interaction at all. Decrease of either antitrypsin content or TIC seemed to be directly related to the cadmium concentration in blood.

Studies carried out on 243 individual blood samples in 19 cities in the United States showed that the concentration of cadmium in blood ranged between 0.005 and 0.14 μg/ml (*12*). However, among cadmium-poisoned industrial workers, a thousandfold increase has been reported in both blood and tissue concentrations (*4, 5, 13*). A cadmium-binding protein has been found in the liver (*14*), but thus far no such substance has been detected in the lung that could account for such a massive deposition of this trace metal in that tissue. In the absence of a tissue mechanism to handle cadmium, exposure to industrial aerosols would be expected to produce a significant increase of cadmium in the lung and a secondary increase in concentrations in blood and in other tissues.

The concentrations of cadmium we used in vitro are far greater than concentrations of cadmium found in blood of normal adults, but they are comparable to blood concentrations in industrially poisoned workers. Furthermore, in those workers dying with cadmium-induced emphysema, concentrations of 50 to 600 mg per gram of lung tissue have been noted (*4, 5*); these are far above the concentrations we used (1 to 5 mg/100 ml).

Evidence of abnormal storage of α_1-antitrypsin in liver parenchymal cells has been demonstrated by Sharp (*15*) and by Aagenaes *et al.* (*16*). Cohen (*17*) has found α_1-antitrypsin in human alveolar macrophages. Since it appears that α_1-antitrypsin does reach pulmonary parenchymal cells, it is expected that the effects of cadmium on α_1-antitrypsin would be of greater significance at a local lung level than in plasma. There are many α_1-antitrypsin phenotypes and certain of these appear more predisposed to complicating emphysema, and it is possible that individuals with intermediate α_1-antitrypsin deficiency, such as that found with the MZ phenotype (*18*), might be more susceptible to cadmium-induced emphysema. We have not yet determined whether cadmium destroys α_1-antitrypsin, inactivates it without destroying it, or alters the phenotype.

In summary, we believe these investigations show a specific effect of cadmium on α_1-antitrypsin and TIC that is not shared by other trace substances studied. If decreased α_1-antitrypsin is an important factor in the etiology of emphysema, then the present study appears to offer a potential explanation for the emphysema reported in some industrial workers exposed to cadmium.

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