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19. Tests of alcohol preference repeated 2 weeks to 6 months after the infusion sequence, again with the same 3 to 30 percent sequence, revealed a similar range of high intakes of alcohol in terms of grams per kilogram of body weight. In still other experiments in which a palatable solution of saccharin was offered in the third drinking tube, other rats being infused with THP nevertheless drank significantly more alcohol than under control conditions (C. L. Melchior and R. D. Myers, in preparation). This shows that THP simply does not cause a loss of taste discrimination.
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Influence of Cadmium on Human Alpha-1-Antitrypsin: A Reexamination

An inherited deficiency in the major proteinase inhibitor in human plasma, alpha-1-antitrypsin (AAT), is associated with chronic obstructive lung disease (*1*). This may result from the unregulated action of proteolytic enzymes released in the lung by leukocytes and alveolar macrophage cells (*2*). An increased incidence of emphysema is found in industrial workers exposed to cadmium over long periods of time (*3*). In addition, the cadmium concentrations in emphysematous lungs are increased as compared to that of normal lungs (*4*); also cadmium accumulates in the human body as a consequence of cigarette smoking (*5*).

Chowdhury and Louria found a progressive decrease in both the trypsin inhibitory capacity (TIC) and the AAT levels as assayed by radial immunodiffusion (RID) when increasing levels of cadmium were added to either plasma or partially purified AAT (*6*). Other heavy metals (Pb, Hg, Ni, Fe, and Zn) had no effect. Chowdhury and Louria used a cadmium reference solution in dilute nitric acid, as provided by Fisher Scientific Company (*7*). They suggested that the toxic effects of cadmium on the human lung might be a consequence of its specific interaction with AAT. However, from our own studies, we conclude that the

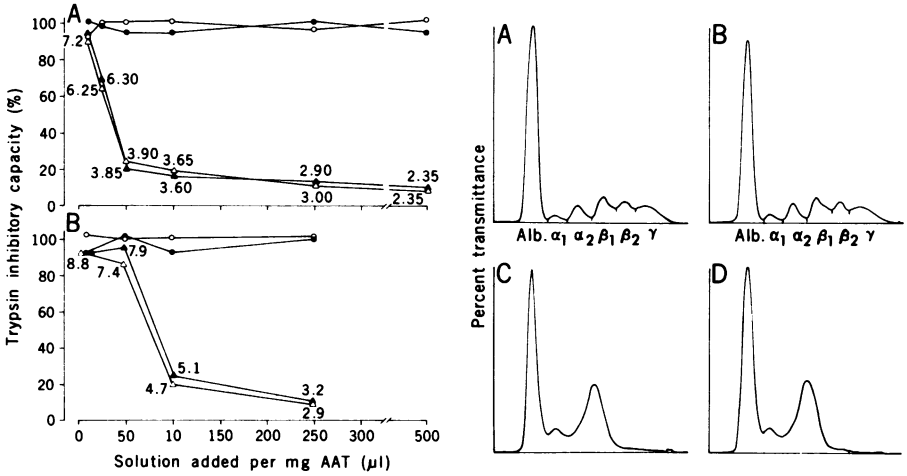

Fig. 1 (left). Trypsin inhibitory capacity (TIC) after incubation of (a) alpha-1-antitrypsin in 0.2*M* tris-HCl buffer, *p*H 8.0, and (b) normal human plasma (diluted 1:1 with saline) with different media at 37°C for 1 hour; o——o cadmium nitrate or ●——● cadmium acetate in the tris buffer, ▲——▲ cadmium reference solution (Fisher), ▲——▲ 0.29*N* nitric acid. The AAT concentration was 1 mg/ml in (a) and (b). The cadmium concentration of all solutions was 1 mg/ml. The TIC values are relative to either a control solution of AAT or normal human plasma; *p*H values are indicated on the graph for those experiments where acidic media were used. The AAT concentrations as measured by RID decreased in parallel with the TIC data reported. Fig. 2 (right). Cellulose acetate electrophoretic patterns of (A) normal human plasma; the same after incubation of 1 ml with 250 μl of (B) cadmium nitrate in 0.02*M* tris-HCl buffer, *p*H 8.0; (C) 0.29*N* nitric acid; (D) cadmium reference solution (Fisher). In experiments (C) and (D), more than 80 percent of immunologically active AAT was lost, as indicated by RID.

reported in vitro effects of cadmium on AAT can be attributed solely to the protein denaturing properties of the nitric acid.

The concentration of cadmium in the reference solution was 1 mg/ml in 0.29*N* nitric acid. We added from 0.01 to 0.50 ml of this solution to purified AAT (1 mg/ml) (*8*) in 0.02*M* tris buffer, *p*H 8.0. After the mixture was incubated for 1 hour at 37°C, TIC (*9*) and RID (*10*) assays showed a parallel decrease in the biological and immunological activity of AAT, respectively. These changes, however, were always accompanied by an increased acidity of the solution. To examine the contribution of *p*H to the observed decrease in AAT, portions of the cadmium solution were lyophilized and reconstituted with tris buffer, *p*H 8.0. Using either this media or cadmium acetate in tris, we could not demonstrate any effect on TIC (or AAT concentration) even when we used as much as ten times the concentration of metal reported to give almost complete inhibition. However, 0.29*N* HNO_3 containing no cadmium produced a decrease of AAT equivalent to that found with the cadmium reference solution (Fig. 1a).

When heparinized human plasma (diluted 1 : 1 with saline) was substituted for pure AAT solutions and the experiment was repeated, AAT was again found to vary with *p*H and not with the metal concentration (Fig. 1b). In order to determine the independent effects of cadmium and nitric acid on other plasma proteins, we developed cellulose acetate electrophoretic patterns from the treated plasma. At 250 μg of cadmium (reconstituted in tris buffer) per milliliter of diluted plasma, no change was detectable. However, an equivalent amount of cadmium introduced from the Fisher reference media resulted in major electrophoretic changes, which were closely paralleled by 0.29 HNO_3 alone (Fig. 2).

Therefore, the hypothesis that cadmium neutralizes the immunological and biological activity of AAT cannot be confirmed at dosages of 500 to 1000 percent of those reported. Previous results can be accounted for on the basis that the reference solution of cadmium employed resulted in acidification and denaturation of AAT. The adverse effects of low *p*H on the biological properties of AAT have been described (*11*).

Cadmium has complex effects on the biochemistry of mammalian organisms in general, and on the lung in particular. A large number of enzyme activities are either elevated or depressed in individuals with cadmium poisoning (*12*). Alveolar cellular proliferation results, although the precise biochemical mechanism that leads to this cellular response is uncertain (*13*). The result is a progressive remodeling of the normal lung structure into an emphysematous form. This sequence of events is not the result of any specific chemical interaction between cadmium and AAT.

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We have shown that cadmium standard solution in a dose-related fashion (Fisher Scientific Company, Springfield, N.J.) produces a marked decrease in both antitrypsin concentration (AAT) and trypsin inhibitory capacity (TIC) in normal human plasma (*1*). We concluded from our investigation that the said effect of cadmium might be a factor in the etiology of emphysema in industrial workers exposed to cadmium. Fisher Scientific did not specify the concentration of the nitric acid in the bottle. We have taken the Pb, Zn, and Ni reference solutions, which are also in dilute nitric acid and found that the Cd reference solution did produce antitrypsin and TIC changes, whereas the other reference solutions did not. We did not focus on the effects of *p*H alone in these simultaneous determinations because of the differences among the metals. We agree with Glaser *et al.* (*2*) that part of the reduction in TIC and AAT concentration could be due to *p*H effect. However, the *p*H we have subsequently determined (Beckman research *p*H meter) was found to be different from estimations of Glaser *et al.* For example, using 0.1 ml of Fisher Cd reference solution in human plasma in saline (1 : 1 dilution), we found the *p*H to be 5.99 as opposed to the 4.7 determined by Glaser *et al.* The difference in *p*H between normal and treated plasma in our case was *p*H 1.48, whereas with Glaser *et al.*, the *p*H difference was 4.7; the reason for the discrepancy is not clear. Furthermore, with the addition of 0.1 ml of 0.25*N* HNO_3 (which the Fisher Scientific Company indicates is at present in Cd reference solution) we found the change in *p*H to be 0.96 compared to the 3.3 found by Glaser *et al.* At the low *p*H used by Glaser *et al.*, one would expect a bigger TIC drop because of protein denaturing effect. In our study, we did not carry out the experiment beyond the opalescent point. The mixture starts to become cloudy with addition of more than 0.1 ml of the Cd reference solution (equivalent to 5000 μg of Cd per 100 ml of plasma). We did not proceed further, but Glaser *et al.* did proceed much beyond this point.

Finally, we have given $CdCl_2$ to mice and reduced plasma antitryptic activity in vivo; no changes in plasma *p*H were observed in these mice. We agree that in vitro the effect on antitryptic activity may be a combination of *p*H and cadmium, but we believe that if protein denaturation is avoided, we can show an effect of cadmium not shared by other trace metals studied. The in vivo studies in mice also suggest that cadmium exerts this effect independently of *p*H.

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Influence of cadmium on human alpha-1-antitrypsin: a reexamination

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