Cadmium-Induced Potentiation of Hexobarbital Sleep Time in Rats

In recent years cadmium has become recognized as a toxicologically important environmental contaminant. In addition, this metal has been implicated as an etiological factor in various pathological processes including testicular tumors, renal dysfunction, hypertension, atherosclerosis, growth inhibition, chronic diseases of old age, and cancer[1,2]. There is also an increasing awareness that some toxic effects of metals may be manifested in very subtle ways. Investigations in this laboratory have shown that cadmium pretreatment alters the pharmacological response to drugs. Three days after a single dose of cadmium acetate (2 mg/kg, i.p.) the duration of hexobarbital-induced sleep time and zoxazolamine paralysis times were significantly prolonged[3]. This study was undertaken to specifically determine the minimum effective dose of cadmium required to potentiate drug response and the peak time for this phenomenon.

Methods. Male, Sprague-Dawley derived rats (250 to 300 g) were obtained from Laboratory Supply Co., Indianapolis, Indiana. The animals were housed in community cages, allowed free access to food and water, and maintained in our animal quarters for 7 days prior to use.

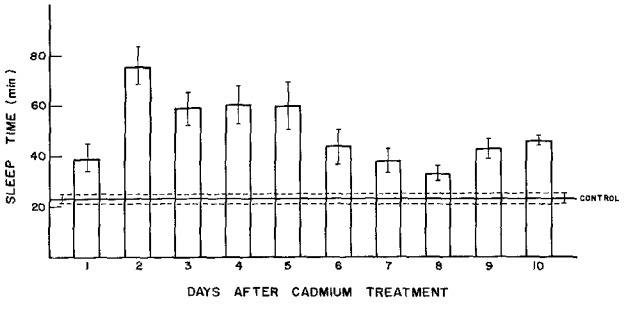
Cadmium was administered i.p. as the acetate salt. Control rats received an equivolume injection of saline. Sleep time was measured as the time interval between losing and regaining the righting reflex following the administration of hexobarbital Na (125 mg/kg i.p.). Only rats losing the righting reflex within 5 min after the hexobarbital injection were included in these experiments. In each experiment, sleep times for both control and cadmium-treated groups were determined simultaneously.

The data were analyzed using the Kruskal-Wallis H Test. Individual comparisons between groups were then performed using the Mann-Whitney U Test[4].

Results. In the first experiment, groups of rats were treated with varying doses of cadmium acetate (0.1–2.0 mg/kg, i.p.). Hexobarbital sleep times were measured 3 days later. The data in the Table show that the minimal effective dose of cadmium acetate required to significantly prolong hexobarbital sleep time was 2.0 mg/kg. This corresponds to a dose of 840 μg/kg cadmium ion.

In the second experiment, groups of rats were treated with a single dose of cadmium acetate (2 mg/kg, i.p.) and hexobarbital sleep times were measured at several time intervals (1 through 10 days) thereafter. The data in the Figure show that sleep times in all cadmium-treated rats (days 1 through 10) were significantly prolonged when compared to controls. The cadmium-induced potentiation of hexobarbital sleep time began on day 1 and peak activity extended from day 2 to day 5. Although the mean sleep time in the cadmium-treated rats appears to be of longer duration at day 2 than all other time periods, no statistical differences were found among days 2 through 5.

Discussion. A number of studies have reported that various heavy metal cations may influence drug response. RIBEIRO[5] reported that pretreatment of mice with soluble salts of arsenic, beryllium, lead, or mercury potentiated hexobarbital hypnosis. PEKKANEN and SALMINEN[6] reported that methyl mercury pretreatment significantly shortened hexobarbital hypnosis in rats which was subsequently shown to be the result of stimulation of liver drug metabolizing enzymes[7].



Peak activity of cadmium-induced potentiation of hexobarbital sleep time. Rats were treated with a single dose of cadmium acetate (2 mg/kg, i.p.) and hexobarbital sleep times were measured at the indicated times (day 1 through 10) thereafter. Each bar represents the mean sleep time of 4–6 rats. All mean sleep times in cadmium-treated rats differed significantly ($p < 0.01$; Mann-Whitney U Test) from controls.

[1] D. F. FLICK, H. F. KRAYBILL and J. M. DIMITROFF, Envir. Res. *4*, 71 (1971).
[2] D. B. LOURIA, M. M. JOSELOW and A. A. BROWDER, Ann. intern. Med. *76*, 307 (1972).
[3] W. M. HADLEY, T. S. MIYA and W. F. BOUSQUET, Toxic. appl. Pharmac., in press.
[4] B. J. WINER, *Statistical Principles in Experimental Design*. McGraw Hill, New York 1962).
[5] H. A. RIBERIO, Proc. West. pharmac. Soc. *13*, 13 (1970).
[6] T. J. PEKKANEN and K. SALMINEN, Acta vet. scand. *13*, 149 (1972).
[7] T. J. PEKKANEN and K. SALMINEN, Acta pharmac. toxic. *32*, *289* (1973).

Minimum effective dose of cadmium acetate required to potentiate hexobarbital sleep time[a]

Dose of cadmium acetate (mg/kg, i.p.)	(*n*)	Duration of hexobarbital sleep time (min ± S.E.)
0 (Control)	8	23.0 ± 2.8
0.1	6	25.4 ± 1.3
0.25	6	26.8 ± 1.3
0.5	5	26.9 ± 2.7
1.0	5	25.7 ± 0.7
1.5	5	25.4 ± 1.4
2.0	6	57.0 ± 7.4[b]

[a] Cadmium acetate was administered i.p. in the doses indicated to male rats. 3 days later the duration of sleep time induced by hexobarbital Na (125 mg/kg, i.p.) was measured. [b] Significantly different ($p < 0.01$, Mann-Whitney U Test) from control rats.

Cadmium is a toxic metal which is increasingly finding its way into our environment as an industrial pollutant[8]. Since absorbed cadmium is very slowly excreted, the body burden increases with age[9]. It is this cumulative nature of cadmium that makes it potentially toxic at low environmental concentrations. The results presented here indicate that cadmium potentiates sleep time induced by hexobarbital. The peak effect following cadmium treatment starts on day 2 and extends to day 5. The short-term nature of this peak effect may be related to the fact that cadmium is known to induce the synthesis of a cadmium-binding protein, metallothionine[10,11]. Conceivably, the presence of this protein could bind cadmium, thus rendering the metal inert. The minimal effective dose of cadmium ion required to potentiate the sleep time was 840 µg/kg which corresponds to 20% of the LD_{50} dose of cadmium ion as determined in our laboratory. In any study designed to link contaminant burdens with biological response and environmental exposure, it is of utmost importance to establish a dose-response relationship between the contaminant being examined and the specific changes induced in the biological system[12].

Zusammenfassung. Nachweis, dass Cadmium bei Ratten die Hexobarbital-Schlafdauer erhöht. Eine wirksame Reaktion war bereits bei einer Cadmium-Dosis von 840 µg/kg beobachtet, und die Schlafdauer wurde bereits nach einer einzigen Dosis vom 1. bis zum 10. Tage erhöht, wobei zwischen dem 2. und 5. Tage die Maximalwirkung beobachtet wurde.

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[8] H. A. Schroeder and J. J. Balassa, J. Chronic Dis. *14*, 236 (1961).
[9] L. Friberg, Am. med. Ass. Arch. Ind. Hyg. occup. Med. *5*, 30 (1952).
[10] J. H. R. Kagi and B. Vallee, J. biol. Chem. *236*, 2433 (1961).
[11] Z. A. Shaikh and O. J. Lucis, Fedn. Proc. *29*, 298 abs (1970).
[12] This research was supported by a grant from National Institutes of Health NES-00921-01 and a Purdue Research Foundation, David Ross Grant.

Toxic Metabolites of *Aspergillus candidus*

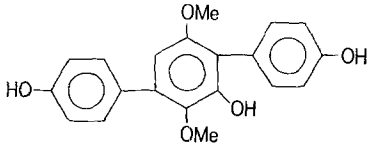
In our course of chemical and biological surveys on toxic mold metabolites, a strain (69-SA-156 = NHL 5107) of *Aspergillus candidus* Link was noticed by the production of new toxic metabolites[1,2]. The extracts of the mycelium and the filtrate on potato-dextrose medium, or of the culture on rice, were separated by chromatographic methods and monitored by cytotoxicity test using HeLa cells[1,2] and 2 new toxins, tentatively named toxin A and B, were isolated.

The toxin A, m.p. 244–245°, $C_{20}H_{18}O_5$, has apparently no effect on mice with a dose of 200 mg/kg by single oral or subcutaneous administration. It evokes, however, characteristic morphological changes on HeLa cells; that is, slightly enlarged cells with faintly stained cytoplasm, evenly distributed chromatin and relatively small nucleoli. 50% growth inhibitory dose is approximately 10 µg/ml. The growth is recoverable when the cells are treated for 24 h and placed in the control medium. No remarkable change is observed in the chromosome preparation of affected cells. Whereas incorporation of ^{3}H-thymidine and -uridine into the treated cells at 32 µg/ml is completely supressed, that of ^{3}H-leucine is retained as in control cells. It has been known that the similar characteristic change of cultured cells is produced by the administration of mycophenolic acid[2] and the effect is nulified by the concomitant administration of guanine[3]. In the case of the present compound, however, the normal growth cannot be restored by the simultaneous addition of any purines or pyrimidine nucleosides.

The toxin A forms the triacetate and the spectral data (especially NMR) indicated the presence of 2 methoxyls, 3 phenolic hydroxyls, 2 pairs of A_2B_2 type *o*-coupled aromatic protons, and 1 singlet aromatic proton in the toxin[4]. The methylation of toxin A with diazomethane gave a mixture of mono-, di-, and tri-methyl ethers. The oxidative degradation of toxin A and the dimethyl ether afforded *p*-hydroxylbenzoic acid and *p*-anisic acid respectively. Treatment of toxin A with boron trifloride gave an unstable polyphenol, the oxidation of which by ferric chloride or chromiun trioxide gave a quinone, $C_{18}H_{12}O_5$. The quinone forms the triacetate. The UV-spectra of the quinone ($\lambda_{max}^{dioxane}$ 255, 390 nm) and the acetate ($\lambda_{max}^{dioxane}$ 345 nm) suggested the *p*-terphenyl-quinone chromophore in the quinone[5]. The above evidence suggested the structures (**1**–**3**) for the toxin A.

The terphenyl metabolite by the fungus *A. candidus* reported recently by Marchelli and Vining[6] is assumed to be identical with the toxin A. They proposed the structure **1** for the compound but the evidence shown in

[1] M. Saito, T. Ishiko, M. Enomoto, K. Ohtsubo, M. Umeda, H. Kurata, S. Udagawa, S. Taniguchi and S. Sekita, Japan. J. exp. Med., *44*, 63 (1974).
[2] M. Umeda, T. Yamashita, M. Saito, S. Sekita, C. Takahashi, K. Yoshihira, S. Natori, H. Kurata and S. Udagawa, Japan. J. exp. Med., *44*, 83 (1974).
[3] T. J. Franklin and J. M. Cook, Biochem. J. *113*, 515 (1969).
[4] S. Natori, S. Sekita, C. Takahashi and K. Yoshihira, the paper presented at 24th IUPAC Congress, Sept. 1973, Hamburg, Abstracts of Papers, p. 188.
[5] J. Gripenberg, Acta chem. scand. *12*, 1762 (1958).
[6] R. Marchelli and L. C. Vining, J. chem. Soc. Chem. Commun. *1973*, 555.



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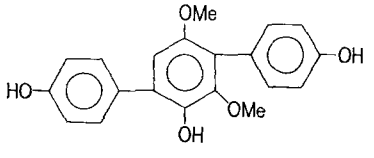

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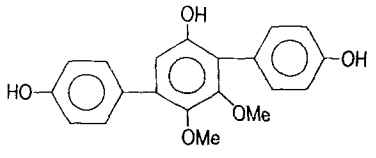

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