Release of pre-absorbed cadmium from cultured cells by the addition of copper ion

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Summary. The biological interaction between cadmium and copper has been studied. Cadmium uptake of KB cells was specifically inhibited by the addition of copper ions to the culture medium. Cadmium which had already been taken up by the cells was released following the addition of copper ions.

The natural biological half-life of cadmium in the human body has been estimated to be several tens of years[2,3]. Much interest exists in finding ways to achieve earlier release of cadmium from tissues[4]. The most notorious environmental disease which has been attributed to cadmium pollution is Itai-itai disease. Most of the patients are post-menopausal women who have had several deliveries[2]. It has been pointed out that the copper concentration in the serum of a pregnant woman increases more than twice as much as that for a non-pregnant woman[5]. The effect of high copper concentration on cadmium accumulation has been of interest with respect to Itai-itai disease as a trace-element interaction between cadmium and copper[6–11]. It has already been reported that cadmium is taken up consecutively and is accumulated in cultured cells[12–15].

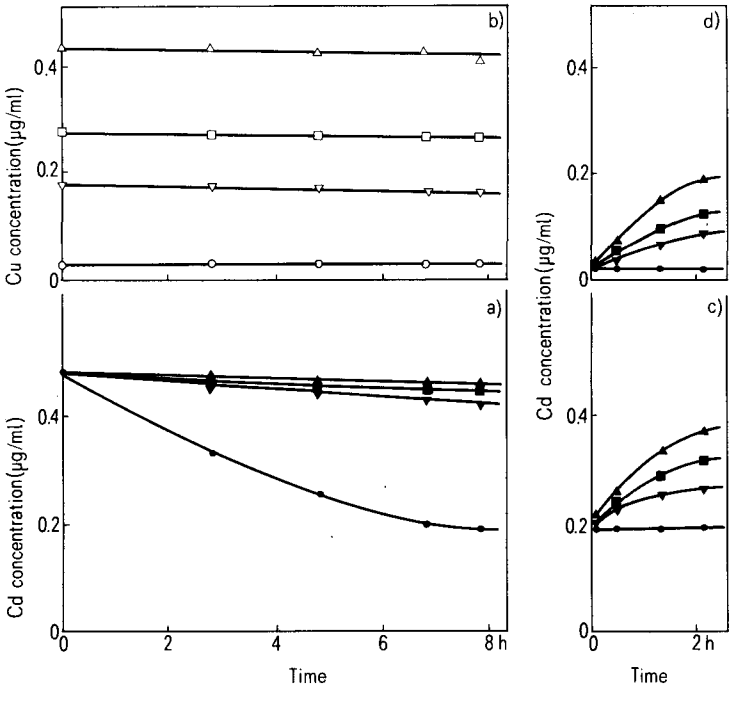
Materials and methods. KB cells were maintained in Eagle's MEM (Daigoeiyokagaku, Osaka) with 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 2% newborn calf serum (Microbiological Associates, Maryland). KB cells, 5×10^4, in 2 ml of culture medium were distributed in each tube. Incubation was performed by the stationary culture method with a silicon stopper at 37 °C. Cd was added 48 h after the inoculation of the cells, when the medium was changed. The concentrations of Cd and Cu were measured directly by flame atomic absorption spectrometer.

Results and discussion. The uptake of Cd in the presence of various essential metal ions is shown in the table. A specific inhibitory effect by Cu on the Cd uptake was observed. Other micro-essential metal ions did not inhibit the Cd uptake. The inhibition of the Cd uptake by Cu depended on the Cu concentration as shown in the figure, a. The concentrations of Cu in the culture medium did not change (fig., b). Then Cu was added to the culture medium after the Cd concentration in the culture medium reached a stationary level, and the changes of Cd and Cu concentrations in the culture medium were examined. Cd which had been taken up by the cells was released into the medium on the addition of Cu to the culture medium. No metal ions other than Cu caused such an effect. The release of Cd

Cadmium uptake by KB cells in the presence of various micro-nutrient metals

Metal ions	Concentrations[a] (µg/ml)	Cd uptake[b] (µg/tube)	Relative uptake (%)
Cd		0.60	100
Cd + Fe	0.36	0.55	92
Cd + Fe	0.60	0.49	82
Cd + Cu	0.51	0.04	7
Cd + Cu	0.89	0.05	8
Cd + Zn	0.60	0.55	92
Cd + Zn	1.04	0.58	97
Cd + Mn	0.39	0.57	95
Cd + Mn	1.10	0.58	97
Cd + Co	0.67	0.54	90
Cd + Co	2.13	0.52	87
Cd + Ni	0.69	0.51	85
Cd + Ni	1.32	0.55	92

[a] Concentrations of micro-nutrient metal ions added.
[b] Initial concentration of Cd was 0.52 µg/ml. Uptake amounts in 10 h of incubation were obtained from the decreases of Cd concentrations in the culture medium.



The time course of changes of cadmium and copper concentration in the culture medium due to the uptake or release by KB cells. In each pair of figures the plots (— ● —, — ▼ —, — ■ —, and — ▲ —) correspond to the plots (— ○ —, — ▽ —, — □ —, and — △ —) respectively, depending on the concentration of Cu. The former represent the Cd concentrations and the latter represent the Cu concentrations. *a* and *b* Cd and Cu were added to the culture medium simultaneously; *c* Cu was added to the culture medium 8 h after the addition of Cd; *d* Cu was added to the culture medium after the medium was replaced with fresh medium in the absence of Cd.

began soon after the addition of Cu. The initial release rate of Cd depended on the Cu concentration, as shown in the figure, c. The release rate of Cd was so rapid at 0.49 µg/ml of Cu concentration that 63% of the Cd taken up by the cells was released in 2 h. Cd was not released from the cells into the fresh medium without the addition of Cu, and the release of Cd was very slow even in the presence of EDTA[14]. After the uptake of Cd by the cells, the culture medium was replaced with fresh medium not containing Cd. The release of Cd was again observed on the addition of Cu, as shown in the figure, d. From the comparison between figures c and d it seemed likely that the rate of Cd release did not depend on the concentration of Cd outside the cells. The inhibition of Cd uptake by Cu may be explained by the relationship between the rate of the uptake of Cd and that of the release of Cd in the presence of Cu. The organ distribution of Cd and Cu may be explained by this kind of interaction of Cd and Cu[16-18].

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2 Friberg, L., Piscator, M., Nordberg, G., and Kjellstro, T.,, eds, in: Cadmium in the environment, p.44. Chemical Rubber Co., Cleveland 1974.
3 Goyer, R.A., and Cherian, M.G., in: Clinical chemistry and chemical toxicology of metals, p.89. Ed. S.S. Brown. Elsevier/North-Holland, Amsterdam 1977.
4 Cherian, M.G., Nature *278* (1980) 871.
5 Underwood, E.J., in: Trace elements in human and animal nutrition, 3rd edn, p.69. Academic Press, New York 1971.
6 Kirchgessner, M., Schwarz, F.J., Grassman, E., and Steinhart, H., in: Copper in the environment, part 2, Health effects, p.433. Ed. O.J. Nriagu. John Wiley, New York 1979.
7 Sandstead, H.H., in: Effects and dose-response relationships of toxic metals, p.511. Ed. G.F. Nordberg. Elsevier, Amsterdam 1976.
8 Mills, C.F., in: Trace element metabolism in animals, vol.2, p.79. Eds W.G. Hoekstra, J.W. Suttie, H.E. Ganther and W. Mertz. University Park Press, Baltimore 1974.
9 Petering, H.G., in: Trace element metabolism in animals, vol.2, p.311. Eds W.G. Hoekstra, J.W. Suttie, H.E. Ganther and W. Mertz. University Park Press, Baltimore 1974.
10 Evans, G.W., Majors, P.F., and Cornatzer, W.E., Biochem. biophys. Res. Commun. *40* (1970) 1142.
11 Suzuki, T., Iwanaga, R., Togo, C., Katsunuma, H., and Suzuki, S., Ind. Health *9* (1971) 46.
12 Meshitsuka, S., and Ishizawa, M., Toxic. appl. Pharmac. *46* (1978) 807.
13 Hidalgo, H.A., Koppa, V., and Bryan, S.E., Biochem. J. *170* (1978) 219.
14 Meshitsuka, S., Ishizawa, M., and Okamoto, M., Yonago Acta med. *22* (1978) 12.
15 Meshitsuka, S., and Ishizawa, M., Jap. J. ind. Health *22* (1980) 206.
16 Mills, C.F., and Dalgarno, A.C., Nature *239* (1972) 171.
17 Suzuki, K.T., Arch. environm. Contam. Toxic. *8* (1979) 255.
18 Suzuki, Y., Ind. Health *18* (1980) 19.

The effect of urethane on histamine-induced contraction of guinea-pig tracheal smooth muscle

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Summary. Urethane possesses a direct depressant action on histamine-induced contractions of guinea-pig tracheal smooth muscle both in vivo and in vitro.

Urethane anesthesia appears to potentiate indirectly histamine-induced broncho-constriction through a reduction of sympathetic bronchodilator tone[1]. Since urethane depresses contraction of vascular smooth muscle induced by vasoactive agents[2-6] it seemed worthwhile to determine whether or not it also has a direct inhibitory effect on histamine-induced contractions of guinea-pig tracheal smooth muscle as compared to a competitive (receptor) antagonist (i.e. chlorpheniramine)[7] of histamine in this preparation.

Materials and methods. In vitro experiments: Male albino guinea-pigs weighing 300-400 g were stunned and bled, and the whole trachea rapidly removed and placed in oxygenated (96% O_2+4% CO_2) Krebs solution of the following composition in mM: NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.5, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11. The trachea was carefully cleaned of adhering connective tissue. Five rings of tracheal tissue were cut and arranged to form a chain as described by Castillo and De Beer[8]. This chain was mounted in a 5-ml organ bath (heated at 37 °C by means of a Julabo Paratherm III water bath) under a constant load of 1 g and attached to an isometric force transducer (MARB 79 Tl). Contractile tone and its variations were delivered to a MARB 776 DC preamplifier and recorded on a Hewlett Packard 7402 A polygraph. After a stabilization period of 1 h concentration response curves (CRC) to histamine were constructed according to Van Rossum[7] at 15-min intervals, until 2 or more reproducible curves were obtained. The effect of urethane and chlorpheniramine were evaluated after a 15-min incubation period.

In additional experiments, after a 1-h stabilization period, tracheal chains were exposed to a high K^+ Ca^{++} free depolarizing solution (mM composition; NaCl 69, $NaHCO_3$ 25, KCl 54,7 $MgSO_4$ 1.5, KH_2PO_4 1.2, glucose 11, EDTA 0.77) for 1 h with successive washings with this solution every 15 min.

A cumulative CRC to $CaCl_2$ was then obtained at the end of the which $CaCl_2$ was washed out with normal Krebs solution, reincubated for 60 min and the high K^+ Ca^{++} free depolarizing procedure repeated again as described above.

A 2nd cumulative CRC for $CaCl_2$ was then obtained in the presence of urethane or chlorpheniramine added to the

Table 1. Effect of urethane aerosol on histamine-induced bronchospasm in conscious guinea-pigs

Treatment	No. of animals	Total dose nebulized (mg/kg)	Time of appearance for histamine-induced bronchospasm (sec, mean ± SE)
Controls	10	– –	62.7 ± 3.5
Urethane	8	10	83.5 ± 3.8[a]
Urethane	8	50	109.2 ± 6.7[b]
Urethane	8	100	139.0 ± 12.3[b]
Chlorpheniramine	8	0.35	504.8 ± 34.1[b]

[a] Significantly different from controls $p < 0.02$; [b] significantly different from controls $p < 0.01$.