In summary, the reported results show that addition of a perfluorotributylamine containing fluorocarbon emulsion to rat microsomes does not severely affect a number of parameters that are related to microsomal drug oxidation. This is also true when the polymeric detergent Pluronic 68 alone is employed in concentrations not surpassing 4 mg/ml. In spite of their relative inertness the use of fluorocarbon emulsions as oxygen reservoirs for artificially prolonged reaction periods is very limited by the fact that within reasonable proportions they cannot carry sufficient oxygen to maintain metabolic formation clearly longer and more linear with time than under normal incubation conditions.

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Dose dependent formation of zinc-thionein in livers and kidneys of rats and mice by zinc injection

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The induction of zinc-thionein, a metallothionein, in the livers of rats exposed either parenterally or perorally to zinc is well documented, as are some distinct properties that distinguish zinc-thionein from cadmium-containing metallothioneins, such as short half-lives of the apoprotein and the metal [1–10]. Zinc-thionein is also reported to be induced in the livers of rats by physiological alterations caused by starvation [1], infection [11], and stress [12].

Although the induction of zinc-thionein in the intestine of rats by zinc loading is also reported along with liver zinc-thionein [4, 13–15], there are conflicting reports concerning the induction of zinc-thionein in the kidneys; one research group reported that zinc-thionein was induced in the kidneys by feeding with excess zinc [2, 3, 6], while another group reported that zinc-thionein was not induced in the kidneys of rats injected with zinc [4, 16, 17], although both groups reported induction in the livers.

The reports concerning the induction of zinc-thionein have been restricted only to rat, and they contrast with the reports for the induction of cadmium-containing metallothioneins in other kinds of animal.

In this study the following four questions were examined; (i) whether or not zinc-thionein is induced in the kidneys by intraperitoneal injection of zinc, (ii) whether or not there are any dose–response relationships for the amount of induced zinc-thionein, (iii) whether or not there are any effects of zinc loading on the copper content and distribution, especially in the kidneys, as observed by cadmium loading [18, 19], and (iv) whether or not zinc-thionein is induced by zinc injection in liver and kidney of the mouse.

Experimental. Female rats of the Wistar strain (9-weeks-old, mean body weight ± S.D.; 196 ± 8.4 g) and female mice of the ICR strain (8-weeks-old, mean body weight ± S.D.; 27.3 ± 1.5 g)(Clea Japan, Tokyo) were fed standard laboratory chow (Clea, Japan) and distilled water *ad lib.* Zinc acetate (purest grade, Wako Pure & Chemical Industries,

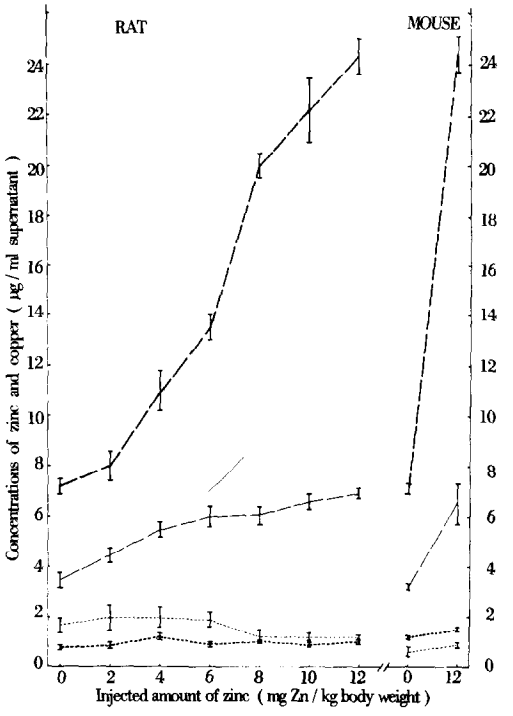

Fig. 1. Changes of zinc and copper contents in the liver and kidney supernatants of rats and mice injected with zinc. Female rats and mice were injected intraperitoneally with zinc once and sacrificed 18 hr after the injection. The livers and kidneys were homogenized in three times the volume of 0.1 M Tris buffer solution and centrifuged at 105,000 *g* for 75 min. — —, Zn in liver supernatants; — —, Zn in kidney supernatants; – – – –, Cu in liver supernatants; and – – – –, Cu in kidney supernatants. The values were mean ± S.D. (for rats, n = 4: for mice, five mice/group and n = 4).

Tokyo) was dissolved in 0.9% NaCl solution to obtain 0, 2, 4, 6, 8, 10, and 12 mg Zn/0.5 ml for rats and 12 mg Zn/0.2 ml for mice.

Zinc was injected intraperitoneally into rats and mice once and 18 hr after the injection the animals were sacrificed by exsanguination under light ether anaesthesia. The liver and kidneys of each rat were homogenized in three times the volume of 0.1 M Tris buffer solution (pH 7.4) containing 0.25 M glucose, using a Polytron homogenizer with ice-water cooling under nitrogen gas. The livers and kidneys of five mice were pooled and homogenized in the same way. A 0.5 ml portion of each homogenate was digested with mixed acids (0.2 ml $HClO_4$ and 1 millilitre HNO_3, acids for metal analysis; Wako). The homogenates were centrifuged at 105,000 *g* for 75 min at 2–4°. A 0.5 ml portion of each supernatant was retained for metal analysis.

2.5 ml of each supernatant were pooled to obtain a 10 ml supernatant in each group. The supernatant (10 ml) was applied to a Sephadex G-75 column (2.6 × 90 cm), eluted with 1 mM Tris buffer solution (pH 8.6) and collected (5 ml/tube). The zinc and copper contents of each eluate were determined by a Hitachi 508 atomic absorption spectrophotometer and absorbances at 254 and 280 nm in each eluate were determined by a Hitachi 170-40 spectrophotometer. The zinc and copper contents in the acid digested homogenates and supernatants were determined using a Hitachi 508 atomic absorption spectrophotometer after adjusting the solutions to 5 ml with doubly distilled water.

Results and discussion. The amounts of zinc found both in the homogenates (data not shown) and supernatants (Fig. 1) increased with the increase of the amount of zinc injected. Although the increased amounts of zinc above the control value in the kidney supernatants of rats are much lower than those in the liver supernatants at any injected dose, those in the kidney supernatants increased proportionally with the increase of injected amount of zinc as shown in Fig. 1. The copper contents in the rat liver supernatants increased only slightly at any injected dose. On the other hand, the copper contents in the rat kidney supernatants increased at lower injected doses and decreased at higher injected doses (Fig. 1).

The distribution profiles of the kidney (Fig. 2) and the liver supernatants (data not shown) of rats on a Sephadex G-75 column showed that the increased amounts of zinc were attributable to the metallothionien fraction at any injected dose. The changes of copper contents in the rat supernatants were also related to those in the metallothionein fraction. Although the control supernatant of rat kidneys contained zinc and copper in relatively high amount in the metallothionein fraction as shown in Fig. 2 (RK-O), the control supernatant of rat livers contained zinc and copper at levels less than the atomic absorption detection limits (data not shown).

As for mice, only control mice and mice injected with highest amount of zinc injected into rats were examined. The increased amounts of zinc in the liver and kidney supernatants of mice were comparable to those of rats injected with the same amount of zinc. Contrary to the control supernatant of rat kidney, that of mouse kidney did not contain zinc and copper in the metallothionein fraction (Fig. 2 MK-0). The control supernatant of mouse liver also did not contain zinc and copper in the metallothionein fraction (data not shown). The increased amounts of zinc and copper in the supernatants of liver (data not shown) and the kidney (Fig. 2 MK-12) of mice injected with zinc were also found in the metallothionein fraction for Sephadex G-75 column chromatography.

These observations taken together clearly indicated that zinc-thionein is induced not only in the liver but also in the kidneys of rats and mice by the injection of zinc, and the induced amounts of zinc-thionein in both organs are dependent on the injected amount. Although the extent of inducement was less than that for cadmium injection [18, 19], injection of zinc was found to affect the amount of copper in the metallothionein fraction on a Sephadex G-75 column.

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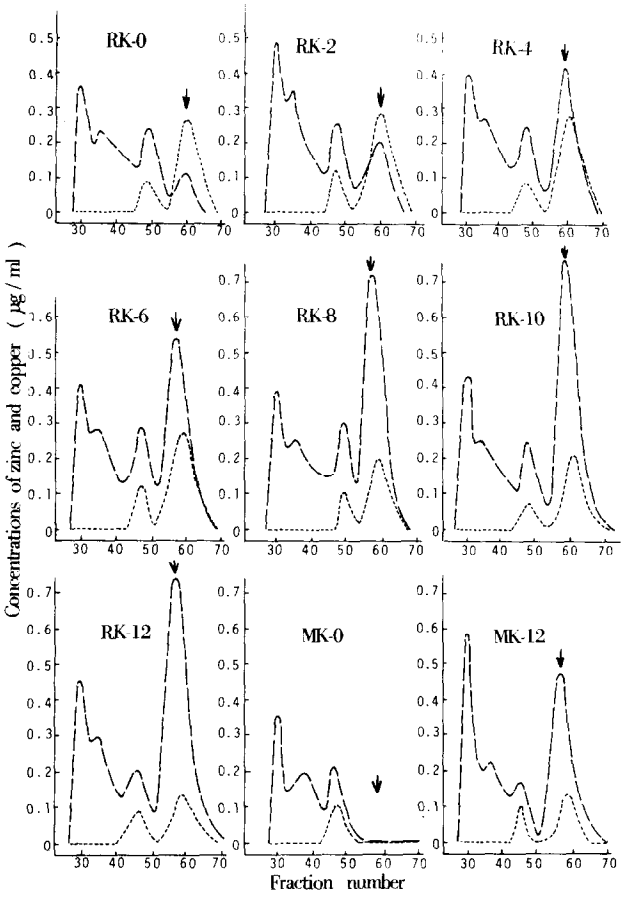

Fig. 2. Sephadex G.75 elution profiles of kidney supernatants of rats and mice injected with zinc. The kidney supernatants (10 ml/column) were applied to a Sephadex G-75 column, eluted with 1 mM Tris buffer solution (pH 8.6), and collected (5 ml/tube). — —, Zn; and – – – –, Cu. The arrow indicates the metallothionein fraction. RK and MK are abbreviated forms of rat kidney and mouse kidney, respectively, and 0–12 indicate injected amounts of zinc.

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Convulsant drug action on GABA and taurine synthesis

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Among convulsant drugs, allylglycine and several pyridoxal phosphate antagonists have been shown to inhibit cerebral glutamic acid decarboxylase activity (L-glutamate carboxy lyase, EC 4.1.1.15, GAD) and reduce brain GABA concentration [1–5]. Taurine has inhibitory actions on neuronal firing [6, 7]; the concentration of taurine is reduced in some forms of focal epilepsy [8] and an antiepileptic action of taurine has been demonstrated experimentally [9, 10]. As a key cerebral enzyme synthesising taurine, cysteine sulphinate decarboxylase (EC 4.1.1.29, CSAD) requires pyridoxal phosphate as a cofactor; it is possible that a reduction in taurine synthesis contributes to the genesis of seizures observed after these convulsant drugs. We have therefore investigated the activity of GAD and CSAD and the concentration of GABA and taurine in mouse brain at the time of seizure onset following the systemic administration of allylglycine