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原　　報

PARAQUAT ENHANCES NEPHROCALCINOSIS UNDER MG RESTRICTION IN THE DIET

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マグネシウム制限食餌下でのパラコート投与による腎臓石灰化の促進

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Summary

This study has been undertaken to determine which organs are damaged by herbicide paraquat (PQ) under restriction of Mg and other minerals. Experiments were made with osteogenic disorder Shionogi rats, which lack the ability of synthesizing vitamin C like humans. The dietary Mg or mineral-mixture was given to the rats according to either the concentration or half of it recommended by American Institute of Nutrition. The dose of PQ was either 483 or 965 nmol/g diet. The concentrations of PQ, vitamin C radical and kininogen in tissues or plasma were measured to assess the severity of injury. Tissue levels of several metals, such as Mg, Ca, Fe, Cu and Zn, were also quantitated, because severe PQ toxicity appeared under Mg restriction or the mineral-mixture restriction showing the close relation between PQ toxicity and metals. The most notable change in metal levels caused by the synergistic effect of restriction of either Mg or the mineral-mixture plus dosing of PQ appeared for kidney Ca; the concentration of kidney Ca increased 10 times the control value under Mg restriction and 28 times the control value under the mineral-mixture restriction. The kidney PQ level of the Mg restricted or the mineral-mixture restricted rats was higher than that of the rats dosed with larger amounts of PQ under sufficient mineral supply. These results indicate that the kidney is the target organ of PQ under Mg restriction or mineral-mixture restriction, and suitable amounts of minerals in the diet are useful to reduce chronic PQ toxicity.

Key words: Paraquat; Magnesium restriction; Calcinosis; Kidney; Lung; Osteogenic disorder Shionogi rats

Introduction

Induction of Parkinson's disease by 1-methyl-4-phenyltetrahydropyridine, which resembles paraquat (PQ) structurally, has renewed the interest in the role of environmental pollution by PQ [1,2]; the usage of herbicides PQ and diquat (DQ) as well as other pesticides is increasing year by year worldwide. Tissue metal levels were influenced greatly by taking PQ and DQ [3-5]. Previously, we reported that the restriction of either Mg or the mineral-mixture (M) recommended by American Institute of Nutrition (AIN)-76 enhanced the toxicity of PQ in rats [6]. The present work has aimed to find out which organs are damaged by PQ under such restriction conditions.

Herbicide PQ is known as a substance to induce oxidative injuries [7] mainly in the lung [6,8,9]. Mg deficiency is also known to induce oxidative injury of rat brain and kidney [10]. The modern food manufacturing techniques frequently cause considerable loss of Mg from the diet [11,12].

Several drugs, such as diuretics, digitalis, amphotericin B and cisplatin, cause the urinary loss of Mg [11]. Excess dietary intakes of Ca and fat also reduce the absorption of Mg [11]. Moderate mineral deficiency seems much more common in humans than severe one.

In this study the condition of the restriction of Mg or M in the diet has been tentatively determined as half the recommended level by AIN-93 in which the concentration of Mg is the same as that of AIN-76; PQ dose has been lowered to 483 nmol/g diet which does not induce any effect for 14 days [6], although the dose is 3.6 times higher than the non-toxic dose resulting in survival for two years, *i.e.*, 135 nmol/g diet [13]. In spite of such mild conditions, nephrocalcinosis has been unexpectedly found in this study.

Experimental

Animals and feeding protocol

M and vitamin mixture were made according to AIN-93 . Supplemented amount of vitamin C was 300 μg per g diet. Osteogenic disorder Shionogi (ODS) unable to synthesize vitamin C like humans were used. Sixty one male mature rats weighing 180 ± 10 g were divided into 7 groups by changing concentrations of minerals and PQ as shown in Table 1. The concentration of Mg or

Table 1. List of groups, number of rats, mineral condition, paraquat (PQ) dose, and means ± SD of PQ, kininogen and vitamin C (VC) radical

group	1	2	3	4	5	6	7
number of rats	10	5	6	10	10	10	10
mineral condition	M-A	Mg-R	M-R	M-A	Mg-R	M-R	M-A
PQ dose (nmol/g diet)	0	0	0	483	483	483	965
PQ in the kidney (pmol/g)	0	0	0	< 27	590 ±220	1,350 ±540	480 ±160
PQ in the lung (pmol/g)	0	0	0	< 27	160 ±50	710 ±300	920 ±320
kininogen in plasma(unit/ml)[a]	0.80 ±0.11	0.80 ±0.10	0.80 ±0.08	0.81 ±0.09	1.86* ±0.65	4.28* ±0.37	3.17* ±0.50
VC radical in the lung (pmol/g)[a]	500 ±50	500 ±60	510 ±45	520 ±60	800* ±150	990* ±140	1,140* ±130

[a] The significance of the difference between the control value in group 1 and that of other groups was determined for kininogen and VC radical by Student's *t*-test, and *P*-value less than 0.05 was indicated by*.

M was either that of AIN-93 written as A (adequate) or half the amount written as R (restricted) in Table 1. The amounts of starch and sucrose were increased in accordance with the decrease of minerals. The groups were arranged from 1 to 7 according to the possible order of toxicity severity, since the toxicity may depend primarily on PQ doses. The feed and distilled water were given freely. The rats were housed in individual cages in a temperature controlled room (22℃) under 12 h light-dark cycle. The feed consumption of each rat was measured every day. The rats in group 6 were sacrificed on day 6, because they stopped eating on day 5, and the rats in other groups on day 8, by cardiac puncture under light nembutal anesthesia. The livers, kidneys, lungs, hearts, spleens and plasma were collected, weighed and stored at - 80℃ until analysis.

Analyses

About 0.2 g each of the five tissues was wet-ashed by conc. nitric acid and used for quantitation of metals. The concentrations of Mg, Ca, Fe and Zn were measured using a Shimadzu flame atomic absorption spectrometer (AA6200); EDTA was added in the cases of Mg and Ca to prevent interference by other substances. The concentration of Cu was measured by the ESR method [5]. Kininogen in plasma, vitamin C radical in the lung and PQ in the lung and kidney were quantitated as reported previously [6,9]. Nitric acid and standard metal solutions were of atomic absorption grade, and other reagents were of analytical grade. Ultra-pure water having specific resistance of 18 MΩ cm was used. The significance of the difference was determined by the Student's *t*-test [14], and the *P*-value less than 0.05 was considered significant.

Results and discussion

The rats in groups 2－4 did not show any differences from the rats in group 1 in either appearance, autopsy examination or several biochemical values listed in Table 1, and in mineral levels as shown in Figs. 1－5. The food intake of groups 1－4 increased day by day up to about 120% from day 1 to day 7 in group 1. The mean total food intakes per animal for 7 days were 112, 110, 105 and 98 g, in groups 1－4, respectively. The symptoms of PQ intoxication, such as anorexia, hypokinesia, diarrhea and epistaxis, were observed from day 3 in rats of group 6, and from day 4 in groups 5 and 7. The severity of PQ toxicosis was also reflected by kininogen levels, showing the highest one in group 6 in Table 1. The food intake in groups 5－7 decreased day by day, and it became down to 0 on day 5 in group 6 and on day 7 in groups 5 and 7. The mean total food intake for 5 days was 33 g in group 6; for 7 days, 56 g in group 5; for 7 days, 53 g in group 7. The total amounts of PQ ingested per rat could be calculated as 47, 27, 16 and 51 μmol in groups 4－7, respectively. As listed in Table 1, the levels of PQ found in the kidney and lung

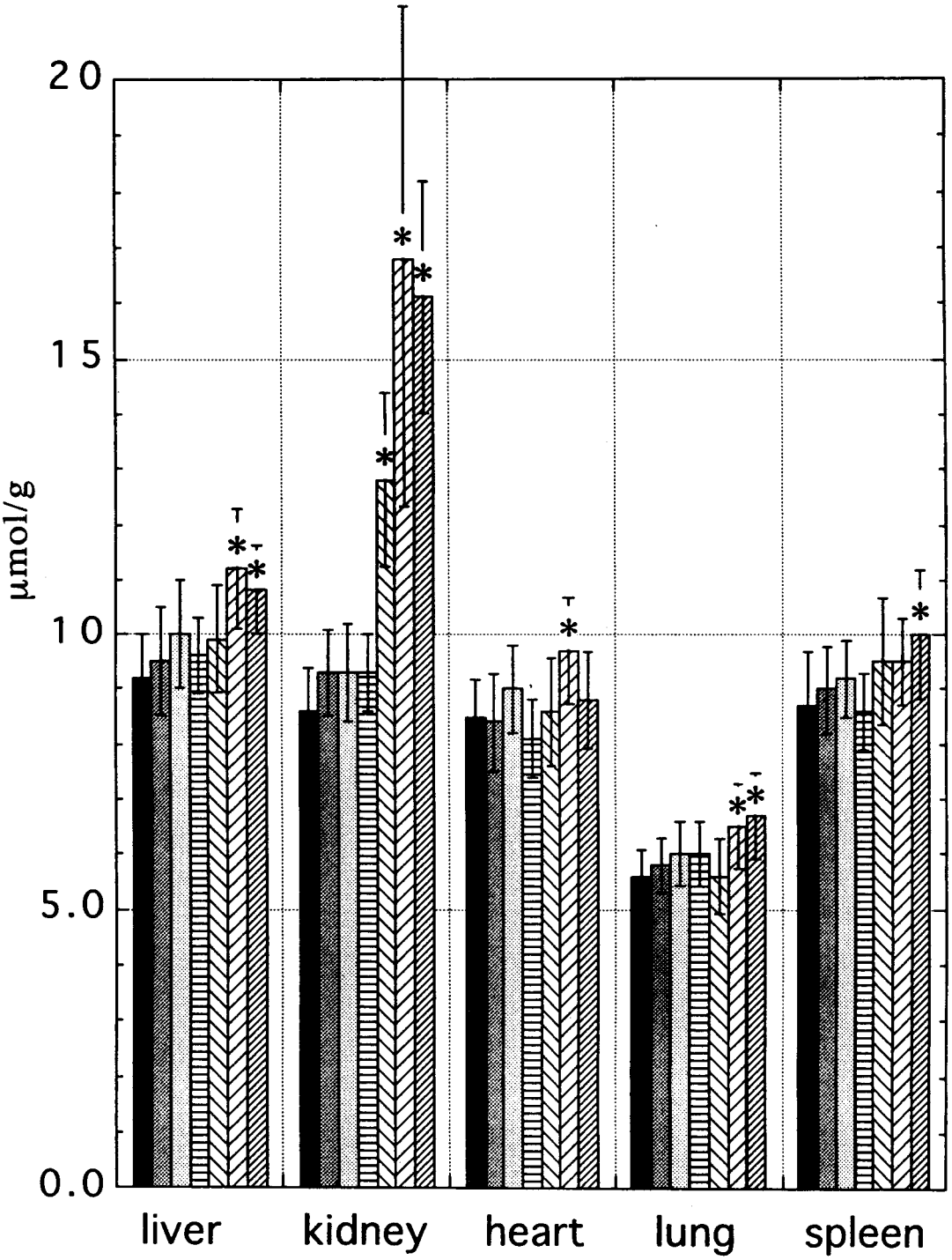

Fig. 1. Concentrations of Mg in tissues of ODS rats after treatments according to the protocol listed in Table 1. The columns of mean values with SD are arranged, from left to right, for groups 1−7. The value significantly different ($P<0.05$) from that in group 1 is indicated by *.

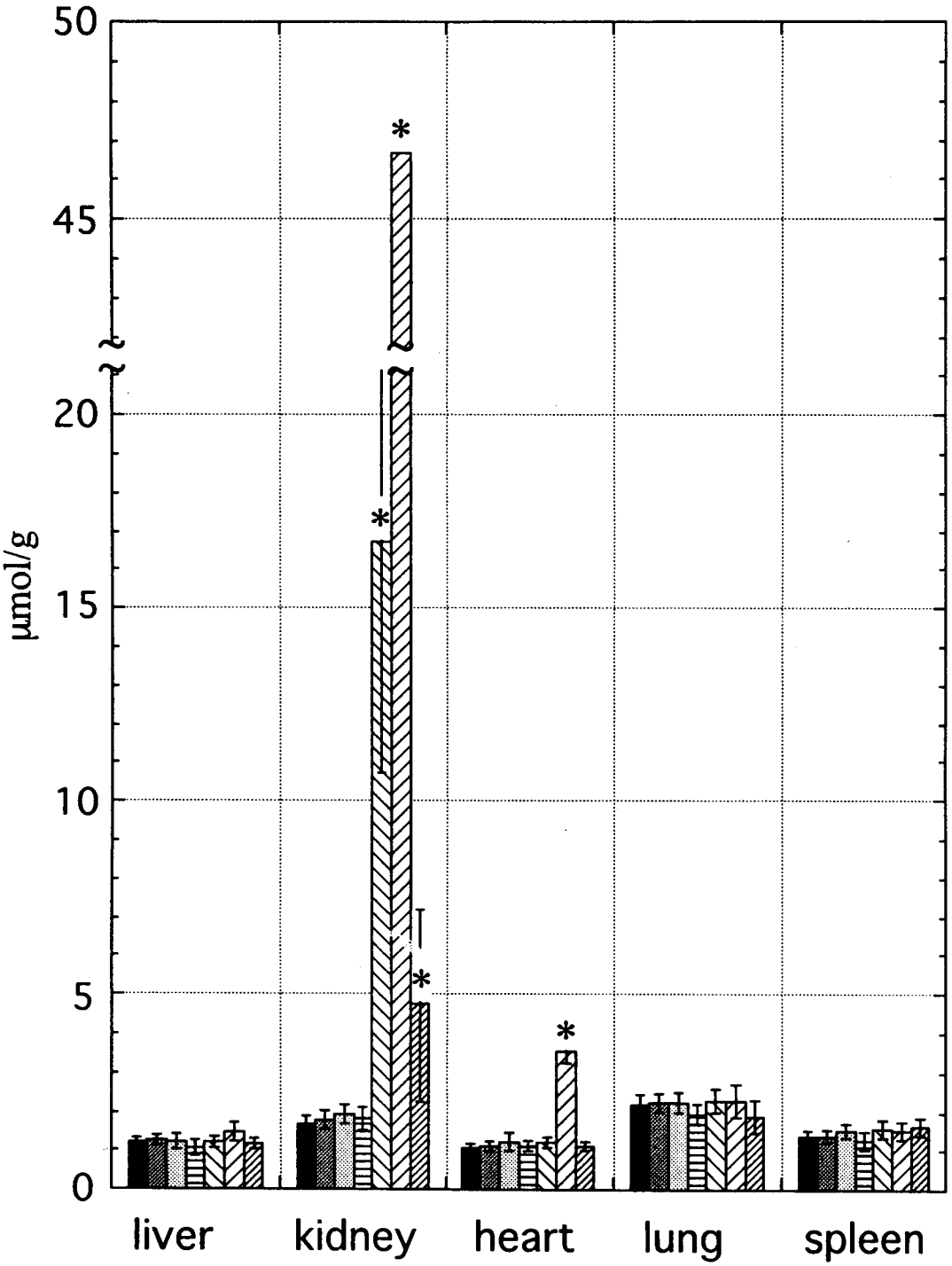

Fig. 2. Concentrations of Ca in tissues of ODS rats after treatments. The explanations are the same as specified in Fig. 1. The concentration of kidney Ca (46,660 ± 27,940 nmol/g) was so high that the column was cut and the SD bar was not shown in group 6.

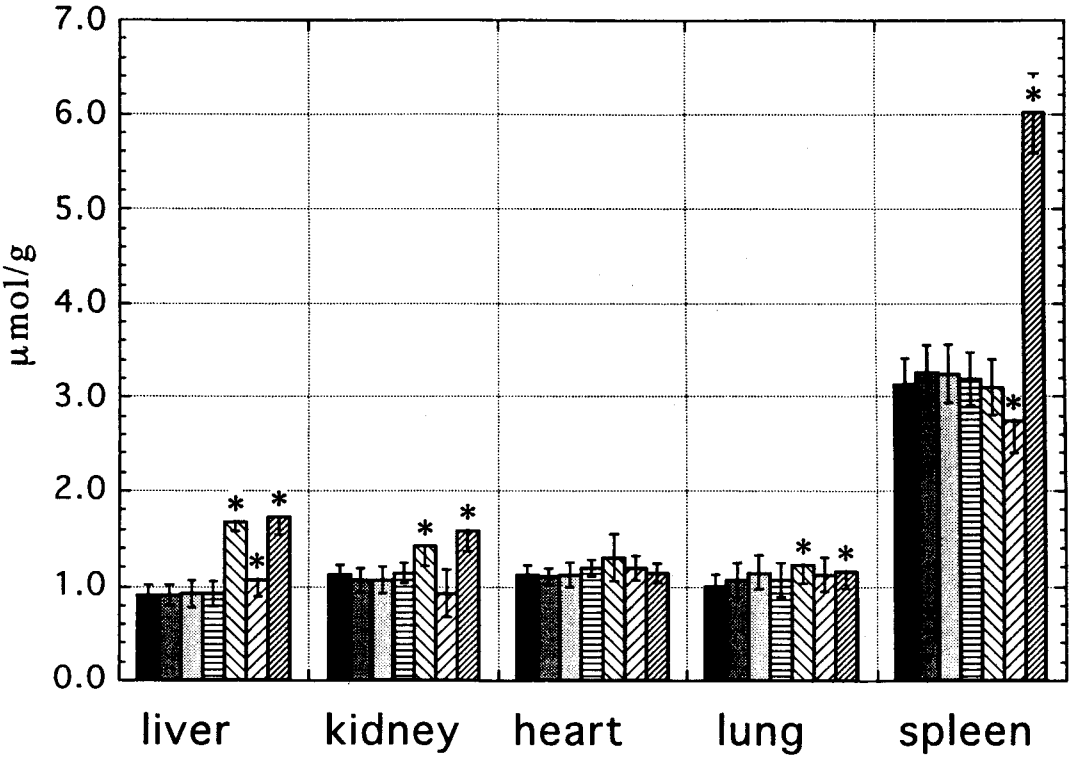

Fig. 3. Concentrations of Fe in tissues of ODS rats after treatments. The explanations are the same as specified in Fig. 1.

were not proportional to the total amounts of PQ ingested; although the rats in group 4 ingested the secondly largest amounts of PQ, the PQ levels in the kidney and lung were below the detection limit (<0.27 pmol/g). Although the rats in groups 6 and 5 ingested the smallest and secondly smallest amounts of PQ, the kidney PQ levels were highest and secondly highest, respectively, among 4 groups treated with PQ. Granular kidney degeneration was observed in groups 5 and 6 at autopsy. These results indicate that PQ under Mg or M restriction damaged the kidney more seriously than the lung. On the contrary, the main target organ of PQ was the lung, when mineral conditions were adequate [4,6], as indicated in PQ and vitamin C radical levels in group 7 listed in Table 1. At autopsy, severest hemorrhage and congestion of the lung were observed in group 7 and secondly in group 6. Only petechiae of the lung were observed in group 5.

The concentrations of tissue Mg in groups 2, 3, 5 and 6 were not lowered as shown in Fig. 1, although these groups received the diet in which the concentration of Mg was half of that in other groups. The concentrations of liver and kidney Mg were even increased in the intoxicated groups.

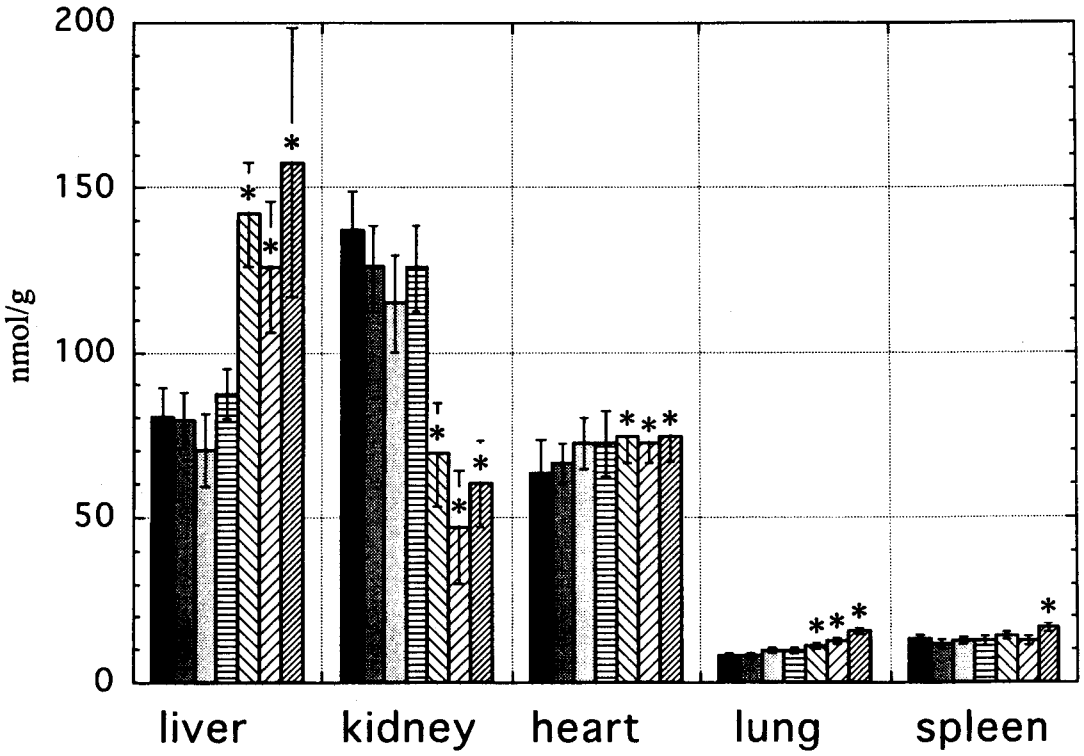

Fig. 4. Concentrations of Cu in tissues of ODS rats after treatments. The explanations are the same as specified in Fig. 1.

Changes in the concentration of Ca were largest among five metals examined as shown in Fig. 2. The levels of kidney Ca in groups 5－7 increased 10-, 28- and 3-fold, respectively, and heart Ca level in group 6 increased 3-fold of the control value. The concentrations of Ca of other three organs in intoxicated groups remained almost at the same level as in the control group. Nephrocalcinosis was reported to be a typical symptom of Mg deficiency [15]; it took more than 35 days to induce 10-fold increase in kidney Ca by feeding a diet covering half of the required Mg. The concentrations of Ca of the rats fed with Mg or M restricted diet for 8 days in groups 2 and 3 remained at the same level as in the control group. The addition of PQ treatment, however, induced 10-fold increase in kidney Ca within 8 days in group 5. Severer nephrocalcinosis was observed in M restricted rats in group 6.

The changes in concentration of Fe in the intoxicated groups were observed in the liver, kidney and spleen as shown in Fig. 3. Although kidney Fe increased in groups 5 and 7, it decreased slightly in group 6 in which dietary Fe concentration was half of that in groups 5 and 7.

The elevation of Cu level was observed in the liver and lung, and the decrease was observed in the kidney in all intoxicated groups (Fig. 4).

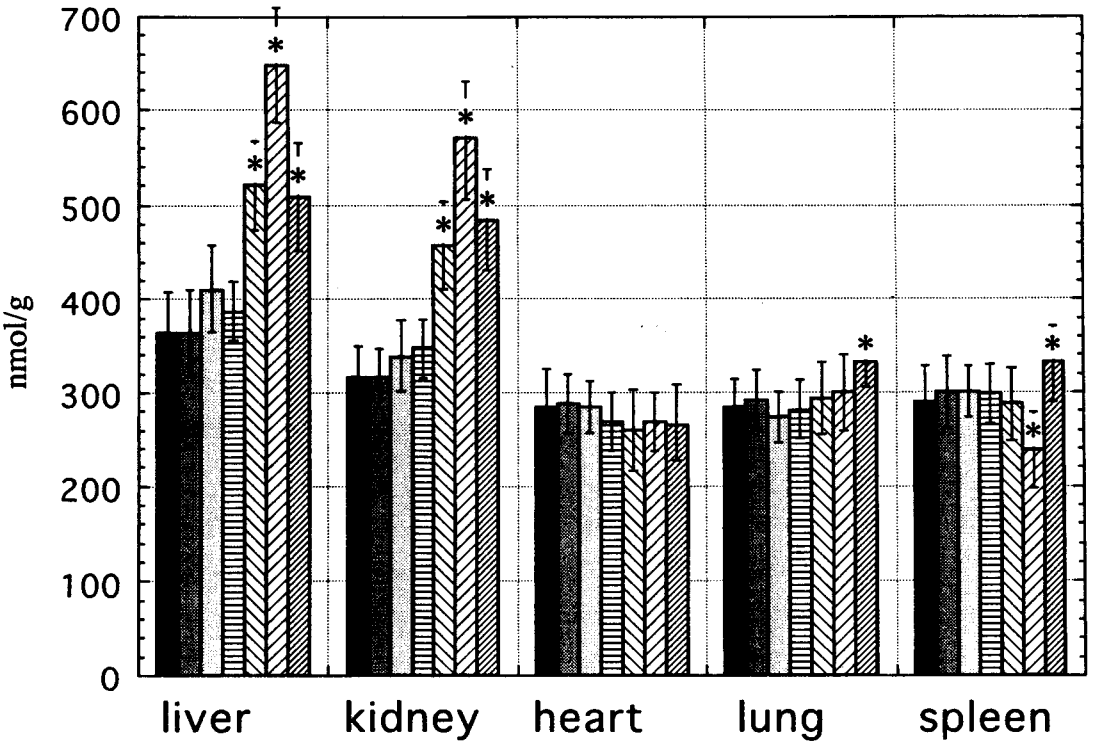

Fig. 5. Concentrations of Zn in tissues of ODS rats after treatments. The explanations are the same as specified in Fig. 1.

The largest changes of Zn levels in the kidney and liver were only less than 1.8 times the control value, as shown in Fig. 5.

The effect of vitamins on PQ toxicity was found not so marked or almost undetectable. For example, vitamin E deficiency had no influence on chronic PQ toxicity [16]. Neither restriction nor over-supply of vitamin C influenced the onset of PQ toxicosis [6]. The restriction of a set of vitamin mixture did not enhance the toxicity [17].

There are several works dealing with the effects of metals on PQ toxicity; the addition of Cu and Fe enhanced PQ toxicity in mice [18] and in *E. coli* [19]. The enhanced effect by Cu on PQ toxicity in E. coli could be reduced or prevented upon addition of 10- or 50- fold excess of Zn over Cu [20]. Surprisingly, the PQ dose at 483 nmol/g under M restriction in group 6 resulted in severer toxicity than the PQ dose at 965 nmol/g in group 7; the concentrations of PQ in the kidney, kininogen in plasma (Table 1), Ca in the kidney and heart in group 6 were higher than those in group 7 (Fig. 2). These results may indicate that the toxicity of PQ can be lowered when a mineral status (especially Mg status) is adequate as in group 4. The protection by minerals against PQ toxicity was also reported on plants [21]; the plants grown in tap water were much more resistant to

herbicide PQ than those grown in distilled water.

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