# Potassium Bromate-Induced Hyperuricemia Stimulates Acute Kidney Damage and Oxidative Stress

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Acute exposure of mice to potassium bromate (KBrO<sub>3</sub>), which is a major disinfection by-product of ozonation and/or chlorination of bromide-containing raw waters, causes serious kidney failure and neuropathological disorders. We observed significant elevations of serum uric acid levels and xanthine oxidase activity by KBrO<sub>3</sub> administration (1.2 mmol/kg) with elevating relative kidney weight, serum creatinine levels and renal oxidative stress. Therefore, allopurinol was administered to KBrO<sub>3</sub>-treated mice to examine if the elevation of blood uric acid levels causes acute kidney damage and renal oxidative stress. These KBrO<sub>3</sub>-induced elevations were significantly prevented by intraperitoneal administration of allopurinol (10 or 50 mg/kg) and significant correlation between kidney damage and uric acid levels was observed. Reduction of catalase activity in the kidney of KBrO<sub>3</sub>-treated mice, which results in the accumulation of hydrogen peroxide, was also restored by allopurinol. There were significant correlations between catalase activity and uric acid levels or kidney damage. Furthermore, in in vitro experiment, catalase activity was reduced in the presence of physiological concentration of uric acid (approximately 0.3 mM or more). These results suggest that the reduction of catalase activity by the elevation of blood uric acid levels is a major cause of KBrO<sub>3</sub>-induced acute kidney damage. Allopurinol also suppressed KBrO<sub>3</sub>-induced increases of renal thiobarbituric acid reactive substances levels and renal protein carbonyl levels of mice. Furthermore, significant correlation between oxidative stress and blood uric acid levels was observed. Therefore, KBrO<sub>3</sub> seems to cause hyperuricemic status which in turn brings about acute kidney damage and oxidative stress with reducing catalase activity.

Key words — potassium bromate, hyperuricemia, kidney damage, oxidative stress, allopurinol

#### INTRODUCTION

Bromate is a major disinfection by-product generated from bromide-containing raw waters that undergo ozonation and chlorination, and is frequently detected in tap water and bottled water.<sup>1,2)</sup> Bromate is currently regulated in treated drinking water at a maximum contaminant level of 10  $\mu$ g/l in the U.S.A. and Europe.<sup>3)</sup> Although potassium salt of bromate is classified as a category I group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer, similarly as Trp-P-1 and Trp-P-2, *etc.*,<sup>4,5)</sup> it is commonly used as an available oxidizing agent for the treatment of wheat flour and as a constituent in cold-wave hair solutions. Acute exposure to bromate causes not only kidney failure<sup>6,7)</sup> but also neuropathological disorders such as vertigo,<sup>8)</sup> tinnitus<sup>9)</sup> and irreversible deafness.<sup>3,10</sup> Methemoglobinemia and cyanosis in the incipient stage of renal damage are also caused by the bromate ingestion.<sup>3)</sup> Bromate brings about serious oxidative modification of lipid, protein and DNA in the kidney, which has been thought to contribute to renal carcinogenesis.<sup>3,11,12</sup> Previously we suggested that potassium bromate (KBrO<sub>3</sub>)-induced renal oxidative stress and kidney damage are attributable to the remarkable reduction of cytoplasmic glutathione peroxidase (GPx) activity, probably caused by the enhanced production of nitric oxide (NO) and peroxynitrite (ONOO<sup>-</sup>).<sup>13)</sup> Our recent preliminary results suggest that KBrO<sub>3</sub> ingestion increases blood uric acid levels and xanthine oxidase (XOD) activity simultaneously with renal oxidative stress and acute kidney damage in rodents. Therefore, it is pos-

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sible that an increase of superoxide anion resulting from the accelerated purine metabolism is one of the causes of bromate-induced renal oxidative stress. Moreover, bromate may induce hyperuricemia and gout, because blood uric acid levels are controlled by the production and excretion thereof. Hyperuricemia leads to the accumulation of uric acid in the body causing gouty arthritis and uric acid nephrolithiasis.<sup>14)</sup> However, hyperuricemia and gout resulting from bromate ingestion have not been reported. Since accumulation of uric acid in kidney tissue is postulated to cause serious kidney damage,<sup>15)</sup> a remarkable increase of XOD activity seems to accelerate the bromate-induced acute kidney damage. In order to clarify the correlation between bromate-induced hyperuricemia and kidney damage, we investigated the effect of allopurinol, an XOD inhibitor, on KBrO<sub>3</sub>-induced acute kidney damage, oxidative stress, and hyperuricemia, which has been used in the treatment for hyperuricemia and gout patients to lower their blood uric acid levels.

# MATERIALS AND METHODS

**Chemicals** — Glutathione reductase from bakers yeast (EC 1.6.4.2), catalase from bovine liver (EC 1.11.1.6), glutathione peroxidase from bovine erythrocyte (EC 1.11.1.9), xanthine oxidase from buttermilk (EC 1.1.3.22), cumene hydroperoxide and reduced glutathione (GSH) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 2,4-Dinitrophenylhydrazine (DNPH) was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). Potassium bromate, potassium cyanide, sodium hydrosulfite, xanthine, hypoxanthine, bovine serum albumin (BSA) and nitroblue tetrazolium (NBT) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Other chemicals of the highest grade were obtained commercially.

Animals and Treatments — Five-week-old male ddY mice purchased from Tokyo Experimental Animal Supply Co. (Tokyo, Japan) were given MF pellet basal diet (Oriental Yeast Co., Tokyo, Japan) and tap water freely, and were used after 1 week of acclimation. Mice were housed in an air-conditioned room with temperature of  $23 \pm 1^{\circ}$ C, humidity of  $50 \pm 3\%$ , and a 12 hr light and dark cycle. They were administered orally 1.2 mmol/kg body weight of KBrO<sub>3</sub><sup>13</sup> dissolved in 0.9% (w/v) NaCl or 0.9% (w/v) NaCl 15 min before intraperitoneal administration of allopurinol. Allopurinol (10 mg/kg as low dose and 50 mg/kg as high dose<sup>16)</sup>) suspended in 0.9% (w/v) NaCl or 0.9% (w/v) NaCl was injected to the abdominal cavity of the mice. All administrations were quickly and carefully carried out without anesthesia. The mice were sacrificed 3 hr after the KBrO<sub>3</sub> administration. Kidneys were immediately excised after decapitation, weighed, washed and homogenized in ice-cold 10 mM phosphate buffered saline (pH 7.4). The homogenate was used for the measurement of protein carbonyl, thiobarbituric acid reactive substances (TBARS) and enzyme activity. Whole blood removed by decapitation was used for the measurement of serum creatinine levels, uric acid levels and XOD activity. This experimental design was approved by the Animal Experimental Committee of Hoshi University and the mice were cared for in accordance with the Guidelines Concerning the Care and Use of Laboratory Animals.

Measurement of Serum Uric Acid Concentration — Measurement of uric acid concentration in deproteinized serum of mice was carried out using a Hitachi HPLC apparatus (pump, L-6000; UV detector, L-4000; chromato-integrator, D-2500; Hitachi Co.) according to the method of Akaike *et al.*<sup>17)</sup> The detection limit of uric acid in this method was 1  $\mu$ M or more.

## Measurement of Enzyme Activity -----

Glutathione Peroxidase: Cumene hydroperoxide dissolved in distilled water was used as the substrate to eliminate the effect of catalase contamination in the sample solution on the measurement of GPx activity in the kidney.<sup>18)</sup> Enzyme activity was calculated from the decrease in NADPH concentration. One unit of enzyme catalyzes the oxidation by cumene hydroperoxide of 1  $\mu$ mol of GSH to oxidized glutathione per min.

*Catalase*: Catalase activity in the cytosol fraction of mice kidney cells was measured as previously described.<sup>13)</sup> Enzyme activity was calculated from the decrease in hydrogen peroxide concentration. One unit of enzyme decomposes 1.0  $\mu$ mol of hydrogen peroxide per min.

*Xanthine Oxidase*: Xanthine oxidase activity in the cytosol fraction of kidney cells and in the serum was assayed by the method of Akaike *et al.*<sup>16)</sup> Detection of uric acid formation was carried out using HPLC (Hitachi Co.). One unit of enzyme converts

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Group	Body weight	Kidney weight	Relative kidney	Creatinine
	(g)	(g)	weight	(mg/l)
Control	$36.1\pm2.7$	$0.675 \pm 0.057^{a)}$	$1.87\pm0.08^{a)}$	$0.572 \pm 0.038^{a)}$
KBrO <sub>3</sub>	$37.4\pm1.4$	$0.893 \pm 0.018^{b)}$	$2.39\pm0.05^{b)}$	$1.080 \pm 0.068^{b)}$
KBrO <sub>3</sub> + allopurinol (10 mf/kg)	$37.0\pm4.1$	$0.732 \pm 0.110^{a)}$	$1.98\pm 0.12^{a)}$	$0.696 \pm 0.111^{a)}$
KBrO <sub>3</sub> + allopurinol (50 mg/kg)	$38.8 \pm 1.4$	$0.738 \pm 0.032^{a)}$	$1.89\pm0.04^{a)}$	$0.611 \pm 0.074^{a)}$

Table 1. Body Weight, Kidney Weight, Relative Kidney Weight and Serum Creatinine Concentration of Mice

Potassium bromate was orally administered to ddY male mice 15 min before allopurinol administration. Allopurinol was administered intraperitoneally to mice. All mice were sacrificed 3 hr after KBrO<sub>3</sub> administration. Kidney was removed after phrebotomy and weighed. Relative kidney weight was calculated as follows, kidney weight  $\times$  100/body weight. Values are expressed as mean  $\pm$  S.D. (n = 4). a, b) Values not sharing a common letter are significantly different at p < 0.05 (ANOVA with Duncan's multiple-range test).

## 1.0 $\mu$ mol of xanthine to uric acid per min.

Superoxide Dismutase: Copper and zinc-containing superoxide dismutase (Cu/Zn-SOD) and manganese-containing superoxide dismutase (Mn-SOD) activities were measured by the method of Beauchamp and Fridovich.<sup>19)</sup> One unit of enzyme is defined as the amount of enzyme required for 50% inhibition of the NBT reduction.

Measurement of Protein Carbonyl Group — Measurement of protein carbonyl group in a mouse kidney was carried out using the method of Reznick *et al.* using DNPH.<sup>20</sup> Finally, absorbance at 370 nm was measured and carbonyl concentration was calculated as follows; carbonyl concentration ( $\mu$ M) = OD<sub>370</sub> × 45.45.<sup>20</sup>

Measurement of Thiobarbituric Acid Reactive Substances — Kidney homogenate was heated with thiobarbituric acid, and the red pigment that appeared was extracted with a mixture of *n*-butyl alcohol and pyridine (15 : 1; v/v).<sup>21)</sup> The absorbance at 535 nm of dehydrated organic solution was measured and the molar extinction coefficient of malondialdehyde ( $\varepsilon = 1.56 \times 10^2 \text{ mM}^{-1}\text{cm}^{-1}$ ) was used to calculate TBARS concentration as described elsewhere.<sup>21)</sup>

Measurement of Protein Concentration — Measurement of protein concentration in each sample solution was carried out by the method of Lowry *et al.*, using bovine serum albumin as the standard protein.<sup>22)</sup>

**Statistics** — Data were expressed as the mean  $\pm$  S.D. A one-way analysis of variance (ANOVA) was used to determine any significant differences (p < 0.05) between means. When significant differences were found, Duncan's multiple-range test was used to determine the exact nature of the difference.

## RESULTS

In all experimental groups, the body weight of mice was not affected by the treatment (Table 1). On the other hand, both kidney weight and relative kidney weight (RKW; kidney weight  $\times$  100/body weight) of KBrO<sub>3</sub>-treated mice increased, indicating kidney damage. Post-KBrO<sub>3</sub> administration of allopurinol significantly suppressed these increases. Serum creatinine concentration, a parameter of kidney damage, of KBrO<sub>3</sub>-treated group increased, and allopurinol also significantly reduced this increase (Table 1). These suppressive effects of allopurinol show that XOD plays an important role in KBrO<sub>3</sub>-induced kidney damage.

Serum uric acid concentration was remarkably elevated about 6-fold by the KBrO<sub>3</sub> administration (Table 2). In the allopurinol high dose group, KBrO<sub>3</sub>induced elevation of serum uric acid concentration was significantly prevented (Table 2). Table 2 also shows the increase in serum XOD activity in KBrO<sub>3</sub>treated mice. Allopurinol reduced KBrO<sub>3</sub>-induced increase of XOD activity in both low and high dose groups. Significant correlations between serum uric acid levels and XOD activity (correlation coefficient = 0.752 ; p < 0.01) and between serum creatinine levels and uric acid levels (correlation coefficient = 0.745 ; p < 0.01) were observed (Fig. 1). Thus, the elevation of serum uric acid concentration is likely to be related to KBrO<sub>3</sub>-induced kidney damage.

Levels of TBARS and protein carbonyl in the kidney of KBrO<sub>3</sub>-treated mice increased to 155% of control and to 134% of control, respectively (Table 2). These increases indicated the occurrence of KBrO<sub>3</sub>-induced renal oxidative stress. Post-KBrO<sub>3</sub> administration of allopurinol significantly prevented both increases to the control level (Table 2). These suppressive effects of allopurinol show that super-oxide generation during purine metabolism contrib-

Group	Uric acid	XOD activity	TBARS	Protein carbonyl
	(nmol/ml)	(unit/ml)	$(\mu mol/g kidney)$	$(\mu mol/g kidney)$
Control	$10.8\pm3.1^{a)}$	$1.96\pm0.24^{a)}$	$0.299 \pm 0.027^{a)}$	$0.406 \pm 0.025^{a)}$
KBrO <sub>3</sub>	$61.7\pm8.5^{b)}$	$3.52\pm0.85^{b)}$	$0.463 \pm 0.014^{b)}$	$0.544 \pm 0.078^{b)}$
KBrO <sub>3</sub> + allopurinol (10 mg/kg)	$51.9\pm2.8^{c)}$	$2.76 \pm 0.77^{b}$	$0.402 \pm 0.027^{c)}$	$0.431 \pm 0.020^{a)}$
KBrO <sub>3</sub> + allopurinol (50 mg/kg)	$18.8\pm4.7^{a)}$	$2.08\pm0.23^{a)}$	$0.352 \pm 0.014^{d)}$	$0.415 \pm 0.024^{a)}$

Table 2. Blood Uric Acid Concentration, Blood XOD Activity, Renal TBARS and Renal Protein Carbonyl of Mice

Each measurement was performed as described in MATERIALS AND METHODS. Values are expressed as mean  $\pm$  S.D. (*n* = 4). *a*, *b*, *c*, *d*) Values not sharing a common letter are significantly different at *p* < 0.05 (ANOVA with Duncan's multiple-range test).



Fig. 1. Correlation between Serum Uric Acid Concentration and XOD Activity, and Serum Uric Acid and Creatinine Concentrations Correlation coefficient of A and B are 0.752 (p < 0.01) and 0.745 (p < 0.01), respectively. The least squares equations are Y = 21.1X – 19.1 (A) and Y = 80.5X – 23.6 (B).

Table 3. Catalase, GPx and Cu/Zn-SOD Activities in the Kidney of Mice

Group	Catalase activity	GPx activity	Cu/Zn-SOD activity
	(unit/mg prot.)	(munit/mg prot.)	(unit/mg prot.)
Control	$406\pm77^{a)}$	$35.0\pm 6.3^{a)}$	$36.6\pm8.7$
KBrO <sub>3</sub>	$127\pm50^{b)}$	$7.8\pm4.0^{b)}$	$40.5\pm5.0$
KBrO <sub>3</sub> + allopurinol (10 mg/kg)	$289\pm 64^{c)}$	$11.8\pm1.2^{b,c)}$	$34.9\pm2.3$
KBrO <sub>3</sub> + allopurinol (50 mg/kg)	$351\pm 43^{a,c)}$	$16.2\pm3.9^{c)}$	$38.6\pm5.4$

Each measurement was performed as described in MATERIALS AND METHODS. Values are expressed as mean  $\pm$  S.D. (n = 4). a, b, c) Values not sharing a commom letter are significantly different at p < 0.05 (ANOVA with Duncan's multiple-range test).

utes to the KBrO<sub>3</sub>-induced renal oxidative stress.

Although no significant change of Cu/Zn-SOD activity in the mouse kidney was observed in all experimental groups, KBrO<sub>3</sub> ingestion remarkably reduced catalase and GPx activities in the kidney to 31.3% of control and to 22.3% of control, respectively (Table 3). Accumulation of reactive oxygen species (ROS) due to the reduction of such antioxidant enzyme activity would induce oxidative stress. Table 3 shows a suppressive effect of allopurinol on these KBrO<sub>3</sub>-induced reductions of antioxidant enzyme activities. Furthermore, a significant correlation between catalase activity and the serum uric acid

level (correlation coefficient = 0.792; p < 0.01) was also observed (Fig. 2).

In the *in vitro* experiment, catalase activity was inhibited by the addition of physiological concentration of uric acid to the reaction mixture (Fig. 3). In contrast, uric acid did not affect GPx activity (data not shown).

#### DISCUSSION

As bromate is rapidly absorbed from the gastrointestinal tract, at least in part unchanged, its tox-



Fig. 2. Correlation between Catalase Activity and Serum Uric Acid Concentration

Correlation coefficient is 0.792 (p < 0.01). The least squares equation is Y = -0.153X + 80.5.



Fig. 3. In Vitro Effect of Uric Acid on Catalase Activity Catalase activity, commercial (A) and extracted from mouse kidney

(B), was measured as described in MATERIALS AND METHODS. Enzyme activity was calculated from the decrease in hydrogen peroxide concentration.

icity appears in a few hours after ingestion.<sup>3)</sup> Therefore, symptoms of kidney damage, oxidative stress and hyperuricemia were examined 3 hr after KBrO<sub>3</sub> ingestion.

Elevated kidney weight, RKW and serum creatinine levels of KBrO<sub>3</sub>-treated mice indicate that the KBrO<sub>3</sub> ingestion caused acute kidney damage similarly to those previous noted.<sup>13)</sup> Therefore, the experimental design chosen here seems to be appropriate for the acute kidney damage model of mice.

In KBrO<sub>3</sub>-treated mice, the serum uric acid levels significantly increased, simultaneously with acute kidney damage. At the same time, serum XOD activity that is responsible for the alteration of blood uric acid levels also increased. Blood uric acid levels are controlled by not only the rates of production and excretion but also the rate of both enzymatic and non-enzymatic degradation thereof.<sup>23)</sup> Since blood uric acid is frequently used for the elimination of excess ROS,<sup>23,24)</sup> elevation of uric acid levels in our experiments seems to be a response to KBrO<sub>3</sub>-induced oxidative stress for the enhancement of antioxidant capacity of mice.<sup>24)</sup> However, KBrO<sub>3</sub>induced serious augmentation of blood uric acid levels would result in hyperuricemia and gout, and uric acid is apt to accumulate in kidney tissue causing kidney failure.<sup>15)</sup> Since oxidative stress causes elevations of hypoxanthine and uric acid derived from ATP,<sup>25)</sup> it is possible that KBrO<sub>3</sub> also results in the enhancement of ATP degradation. As this has yet to be reported that KBrO<sub>3</sub> ingestion elevates blood uric acid levels, this data is the first such one relating to KBrO<sub>3</sub>-induced hyperuricemia.

Xanthine oxidase activity is increased in ischemia-reperfusion injury and inflammation, which is regarded as a physiological response to increase superoxide anion.<sup>26)</sup> In our experiment, allopurinol prevented both KBrO<sub>3</sub>-induced kidney damage and an increase in the serum uric acid levels through the reduction of serum XOD activity. Since significantpositive correlations between serum creatinine and uric acid levels were observed, the uric acid level elevation resulted from the increase of XOD activity and is responsible for the KBrO<sub>3</sub>-induced acute kidney damage. That is, acceleration of superoxide anion generation seems to promote KBrO<sub>3</sub>-induced acute kidney damage.

As TBARS increases according to the accumulation of ROS in several tissues,<sup>21,27)</sup> it is utilized as an available parameter of oxidative stress. Since carbonyl group in oxidatively modified proteins increases with the accumulation of ROS, it has been used as a bona fide indicator of oxidative stress.<sup>28-30)</sup> We previously reported the contribution of ONOOto KBrO<sub>3</sub>-induced oxidative stress.<sup>13)</sup> Peroxynitrite can be formed much faster than the reaction of NO with hem compounds, and the rate is comparable to that at which superoxide anion reacts with SOD.<sup>31)</sup> Suppressive effects of allopurinol on TBARS and protein carbonyl in our experiment strongly support the significant correlation between XOD activity and KBrO<sub>3</sub>-induced oxidative stress. Generation of superoxide anion during the purine metabolism catalyzed by XOD and the acceleration thereof also may have resulted in the accumulation of ONOO<sup>-</sup>. Thus, these results indicate that superoxide anion accumulation due to increased XOD activity is likely to be a cause of KBrO<sub>3</sub>-induced oxidative stress.

We previously reported that ROS accumulation resulted from the significant reductions of cytosolic catalase and GPx activities seem to promote KBrO<sub>3</sub>induced oxidative stress.<sup>13)</sup> And the increase of NO and ONOO- is indicated to attenuate cytosolic GPx activity.<sup>32,33)</sup> However, it was not identified in the previous paper what reduced catalase activity. Suppressive effects of allopurinol and the significant negative correlation between catalase activity and serum uric acid levels indicate that the elevation of uric acid level and/or superoxide anion level in kidney tissue seems to reduce catalase activity. In the in vitro experiment, the rate of hydrogen peroxide degradation based on the catalase activity was delayed by uric acid in dose-dependent manner. Although catalase activity is inhibited by azide, cyanide, and hydroxylamine, etc.,<sup>34)</sup> there is no previous report on the inhibitory effect of uric acid. Furthermore, change of catalase activity in hyperuricemia and gout patients have not been reported. Although the elevation of the blood uric acid level was suspected to be a response to reduce oxidative stress,<sup>24)</sup> we expect that its elevation is a cause of oxidative stress through the reduction of catalase activity. In contrast, uric acid scarcely affected GPx activity in in vitro experiments (data not shown). In fact, restoration of GPx activity by allopurinol in the kidney of KBrO<sub>3</sub>-treated mice was insufficient. Therefore, KBrO<sub>3</sub>-induced reduction of GPx activity seems to have resulted from the elevation of ONOO<sup>-</sup> but not the accumulation of uric acid. In brief, cellular hydrogen peroxide may have promoted KBrO<sub>3</sub>-induced oxidative stress and acute kidney damage in concert with uric acid.

In conclusion, KBrO<sub>3</sub> was found to cause hyperuricemia owing to the acceleration of purine metabolism by the increase of XOD activity. And the elevation of blood uric acid level was also confirmed to be responsible for KBrO<sub>3</sub>-induced acute kidney damage and oxidative stress. Then, it was suppressed by the inhibition of XOD activity. In contrast to kidney damage, cyanosis, irreversible deafness and kidney cancer induced by KBrO<sub>3</sub>,<sup>3)</sup> hyperuricemia and gout having resulted from KBrO<sub>3</sub> ingestion has not been reported. Thus, our data is the first one relating to the KBrO<sub>3</sub>-induced hyperuricemia which may be a cause of KBrO<sub>3</sub>-induced oxidative stress resulting from severe reduction of catalase activity.

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#### REFERENCES

- Weinberg, H. S., Delcomyn, C. A. and Unnam, V. (2003) Bromate in chlorinated drinking waters: occurrence and implications for future regulation. *Environ. Sci. Technol.*, **37**, 3104–3110.
- van Dijk-Looijaard, A. M. and van Genderen, J. (2000) Levels of exposure from drinking water. *Food Chem. Toxicol.*, **38**(Suppl. 1), S37–S42.
- U.S. Environmental Protection Agency (2001) EPA toxicological review of bromate in support of summary information on the integrated risk information system, Washington, D.C., U.S.A.
- Kurokawa, Y., Maekawa, A., Takahashi, M. and Hayashi, Y. (1990) Toxicity and carcinogenicity of potassium bromate-a new renal carcinogen. *Environ. Health Perspect.*, 87, 309–335.
- 5) De Angelo, A. B., George, M. H., Kilburn, S. R., Moore, T. M. and Wolf, D. C. (1998) Carcinogenicity of potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N rats. *Toxicol. Pathol.*, 26, 587–594.
- 6) Gradus, D., Rhoads, M., Bergstrom, L. B. and Jordan, S. C. (1984) Acute bromate poisoning associated with renal failure and deafness presenting as hemolytic uremic syndrome. *Am. J. Nephrol.*, 4, 188–191.
- 7) De Vriese, A., Vanholder, R. and Lameire, N. (1997) Severe acute renal failure due to bromate intoxication: report of a case and discussion of management guidelines based on a review of the literature. *Nephrol. Dial Transplant.*, **12**, 204–209.
- Young, Y. H., Chuu, J. J., Liu, S. H. and Lin-Shiau, S. Y. (2001) Toxic effects of potassium bromate and thioglycolate on vestibuloocular reflex systems of Guinea pigs and humans. *Toxicol. Appl. Pharmacol.*, 177, 103–111.
- Yei, C. C., Fuh, J. J., Su, S. Y. and Horng, K. Y. (1993) Acute renal failure, neuropathy and deafness due to sodium bromate intoxication: report of one case. *J. Formos Med. Assoc.*, **92**(Suppl. 4), S262– S266.
- 10) Sashiyama, H., Irie, Y., Ohtake, Y., Nakajima, K., Yoshida, H., Sakai, T. and Okuda, K. (2002) Acute renal failure and hearing loss due to sodium bromate poisoning: a case report and review of the literature. *Clin. Nephrol.*, 58, 455–457.
- 11) Murata, M., Bansho, Y., Inoue, S., Ito, K., Ohnishi, S., Midorikawa, K. and Kawanishi, S. (2001) Requirement of glutathione and cysteine in guaninespecific oxidation of DNA by carcinogenic potassium bromate. *Chem. Res. Toxicol.*, 14, 678–685.
- 12) Chipman, J. K., Davies, J. E., Parson J. L., Nair, J., O'Neill, G. and Fawell, J. K. (1998) DNA oxidation by potassium bromate; a direct mechanism or

linked to lipid peroxidation? *Toxicology*, **126**, 93–102.

- Watanabe, S., Yoshimura, Y. and Fukui, T. (2001) Contribution of glutathione peroxidase and nitric oxide to potassium bromate-induced oxidative stress and kidney damage in mice. *J. Health Sci.*, 47, 565– 570.
- 14) Kong, L., Zhou, J., Wen, Y., Li, J. and Cheng, C. H. K. (2002) Aesculin possesses potent hypouricemic action in rodents but is devoid of xanthine oxidase/ dehydrogenase inhibitory activity. *Planta Med.*, 68, 175–178.
- 15) Kang, D. H., Nakagawa, T., Feng, L., Watanabe, S., Han, L., Mazzali, M., Truong, L., Harris, R. and Johnson, R. J. (2002) A role for uric acid in the progression of renal disease. *J. Am. Soc. Nephrol.*, 13, 2888–2897.
- 16) Fields, M., Lewis, C. G. and Lure, M. D. (1996) Allopurinol, an inhibitor of xanthine oxidase, reduces uric acid levels and modifies the signs associated with copper deficiency in rays fed fructose. *Free Radic. Biol. Med.*, **20**, 595–600.
- 17) Akaike, T., Ando, M., Oda, T., Doi, T., Ijiri, S., Araki, S. and Maeda, H. (1990) Dependence on O<sub>2</sub><sup>-</sup> generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. *J. Clin. Invest.*, 85, 739–745.
- Lawrence, R. A. and Burk, R. F. (1976) Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun.*, 71, 952–958.
- Beauchamp, C. and Fridovich, I. (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, 44, 276– 287.
- Reznick, A. Z., Kagan, V. E., Ramsey, R., Tsuchiya, M., Khwaja, S., Serbinova, E. A. and Packer, L. (1992) Antiradical effects in L-propionyl carnitine protection of the heart against ischemia-reperfusion injury: the possible role of iron chelation. *Arch. Biochem. Biophys.*, **296**, 394–401.
- Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95, 351–358.
- 22) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- 23) Child, R. B., Wilkinson, D. M., Fallowfield, J. L.

and Donnelly, A. E. (1998) Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. *Med. Sci. Sports Exerc.*, **30**, 1603–1607.

- 24) Chevion, S., Moran, D. S., Heled, Y., Shani, Y., Regev, G., Abbou, B., Berenshtein, E., Stadtman, E. R. and Epstein, Y. (2003) Plasma antioxidant status and cell injury after severe physical exercise. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 5119–5123.
- 25) Meyer, W., Kretschmer, M. and Harisch, G. (1989) Regional histochemical aspects of xanthine oxidase activity in ischemic and reperfused small intestine of the rat. *Cell. Mol. Biol.*, **35**, 357–365.
- 26) Till, G. O., Guilds, L. S., Mahrougui, M., Friedl, H. P., Trentz, O. and Ward, P. A. (1989) Role of xanthine oxidase in thermal injury of skin. *Am. J. Pathol.*, **135**, 195–202.
- Yagi, K. (1976) A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem. Med.*, 15, 211–216.
- 28) Dean, R. T., Fu, S., Stocker, R. and Davies, M. J. (1997) Biochemistry and pathology of radical-mediated protein oxidation. *Biochem. J.*, **324**, 1–18.
- 29) Stadtman, E. R. (1993) Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. *Annu. Rev. Biochem.*, 62, 797–821.
- 30) Chevion, M., Berenshtein, E. and Stadtman, E. R. (2000) Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic. Res.*, **33**, S99–S108.
- 31) Halliwell, B. and Gutteridge, J. M. C. (1999) Free Radicals In Biology and Medicine third edition, Oxford University Press Inc., New York, pp. 95–100.
- 32) Asahi, M., Fujii, J., Suzuki, K., Seo, H. G., Kuzuya, T., Hori, M., Tada, M., Fujii, S. and Taniguchi, N. (1995) Inactivation of glutathione peroxidase by nitric oxide. Implication for cytotoxicity. *J. Biol. Chem.*, **270**, 21035–21039.
- 33) Padmaja, S., Squadrito, G. L. and Pryor, W. A. (1998) Inactivation of glutathione peroxidase by peroxynitrite. *Arch. Biochem. Biophys.*, 349, 1–6.
- 34) Hochman, A. and Shemesh, A. (1987) Purification and characterization of a catalase-peroxidase from the photosynthetic bacterium *Rhodopseudomonas capsulata*. J. Biol. Chem., 262, 6871–6876.