URINALYSIS FOR DETECTION OF CHEMICALLY INDUCED RENAL DAMAGE (1)

Changes in urinary excretions of enzymes and various components caused by mercuric chloride and gentamicin –

Hisayuki OHATA, Kazutaka MOMOSE, Atsushi TAKAHASHI* and Yoshihito OMORI*

Department of Pharmacology, School of Pharmaceutical Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo 142
* National Institute of Hygienic Sciences, Kamiyoga, Setagaya-ku, Tokyo 158

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Abstract In order to establish sensitive methods of detecting minor renal damage, changes of enzymes, tubular cell counts, and creatinine in the urine were investigated in rats that had been given nephrotoxic chemicals. Daily administration of mercuric chloride (HgCl₂) dose-dependently increased urinary excretions of lactate dehydrogenase (LDH), aspartate aminotransferase (GOT), alkaline phosphatase (ALP), leucine aminopeptidase (LAP), lysozyme (LZM), N-acetyl-β-D-glucosaminidase (NAG), and acid protease together with increased counts of tubular cells in the urine. The increase in tubular cell counts and the change in urinary LDH isoenzyme profile preceded the changes in the other enzymes.

Daily administration of gentamicin (GM) increased urinary excretions of LDH, GOT, LZM, NAG, acid protease and tubular cell counts in a dose-dependent manner, but did not increase γ-glutamyl transpeptidase (γ-GTP) and ALP excretions. The urinary isoenzyme profiles of LDH in rats treated with GM were different from those with HgCl₂. The increase in acid protease excretion outlasted those in LDH and GOT in the high dose group.

It was concluded that the severity of renal damage can be readily detected by periodic determinations of the following urinary parameters: tubular cell counts, LDH isoenzyme, acid protease, LZM and NAG, in
addition to either LDH or GOT and one of the enzymes ALP, LAP or γ-GTP. Furthermore, the site of renal damage can be presumed from these results.

**Key words:** Urinalysis, renal damage, urinary enzyme excretion, mercuric chloride, gentamicin.

**INTRODUCTION**

The kidney is a susceptible organ to the toxic action of various drugs and chemicals because, as the kidney is a main excretory pathway of wastes, they become concentrated there. In particular, heavy metals and antibiotics are potent nephrotoxic agents (Hook, 1980; Porter, 1981). The chemically induced renal damage has been verified by renal function tests such as various clearance tests and biochemical analyses of blood, especially of blood urea nitrogen and serum creatinine, as well as determination of urinary protein and electrolytes in urinalysis (Diezi and Biollaz, 1979; Berndt, 1981). Histological examination of the affected kidney is the most useful method for detecting the chemically induced renal damage, and is an important route for determining the site of renal damage. However, this technique is not suitable as a routine method for detecting nephrotoxic potentials of many drugs because of its complicated procedure and lack of quantitative evaluations of the histological changes.

There are some reports demonstrating that the increase in urinary enzyme excretion precedes the increase in urinary protein, decrease in creatinine clearance, elevated level of serum creatinine and blood urea nitrogen, and that the increased urinary excretions of enzymes correlate well with the onset of histological changes in renal damage caused by gentamicin, cephaloridine, mercuric chloride (HgCl₂) or cadmium (Raab, 1972; Piperno, 1981; Price, 1982; Takahashi and Ohata, 1982). Therefore, urinary enzymes are suggested to be good indicators of renal damage. On the other hand, Cottrell et al. (1976) reported that increases in urinary excretions of lactate dehydrogenase (LDH), alkaline phosphatase and leucine aminopeptidase are transient in the rats treated with HgCl₂ or p-aminophenol, while renal histological changes are persistent. It was also reported that celluria is more sensitive than enzymuria in detecting nephrotoxicity induced by HgCl₂ (Prescott and Ansari, 1969). Thus, there is some debate as to the usefulness of enzymuria for the detection of renal damage.

In this study, in order to establish methods of detecting renal damage in an early phase and estimating the damaged site, changes in some enzymes, LDH isoenzyme patterns and tubular cell counts of urine were investigated in rats treated with the tubulotoxic chemicals HgCl₂ and gentamicin.
MATERIALS AND METHODS

Materials
Mercuric chloride (HgCl₂) was obtained from Iwai Chemical Co., and gentamicin (GM) was obtained from Shionogi Seiyaku Co. All other chemicals were of commercially available analytical grade.

Animals
Male Wistar rats (9–10 weeks of age) were used throughout this study. The animals were housed in a temperature (23–25°C), humidity (50–70%), and light-cycle (12 h light, 12 h dark) controlled room with free access to standard rat chow and water.

Treatments
The chemicals were dissolved in saline and injected (2 ml/kg body weight) as follows: HgCl₂ was injected s. c. for 7 days in doses of 0.25 mg/kg/day (HgCl₂-L), 0.5 mg/kg/day (HgCl₂-M), or 1.0 mg/kg/day (HgCl₂-H). GM was injected twice daily s. c. for 13 days in doses of 15 mg/kg/day (GM-L), 30 mg/kg/day (GM-M), or 60 mg/kg/day (GM-H). In some experiments in which lactate dehydrogenase isoenzyme and tubular cell counts were estimated, GM was injected once daily s. c. for 15 days in doses of 15 mg/kg/day (GM-L), 30 mg/kg/day (GM-M), or 60 mg/kg/day (GM-H). Control animals were injected s. c. with saline (2 ml/kg/day) in each experiment.

Urine collection
The rats were housed singly in metabolic cages (Nippon Clea) with free access to standard rat chow and water. The urine was collected in tubes cooled by Cool nit (CML-III, Taiyo Scientific Industrial Co.) to 4–8°C. In the groups subcutaneously treated with HgCl₂ for 7 days, the urine samples were collected during the periods of 0–10 and 10–24 h after the first injection, and then 24 h urine samples were collected on days 2, 3, 5 and 7. In the GM-treated groups, 24 h urine samples were collected on days 1, 3, 5, 7, 9, 11, 13 and 15.

Measurement of urinary tubular cell counts
After measurement of urine volume, the urine was passed through a nylon mesh and was centrifuged at 1,500 r. p. m. for 10 min. The supernatant was discarded and 0.5 ml of deionized water was added to the sediment. The tubular cell counts in the sediment were determined using the differential diaminofluorene-peroxide-phloxine staining method of Prescott and Brodie (1964) in a Thoma counting chamber.

Enzyme assays
Urine for enzyme assays was dialyzed against over 50 volumes of deionized water at 4°C for 3 h. The dialyzed urine samples were stored at 4°C and the enzyme activities were assessed within two days.

Lysozyme (LZM, EC 3.2.1.17): LZM activity was measured according to the method of Parry et al. (1965) using Micrococcus Luteus (Miles Lab. Inc.) as its substrate. One ml of dialyzed urine was incubated at 37°C for 10 min with 2 ml of
the substrate solution which contained 50 mg of substrate in 100 ml of 1/15 M phosphate buffer (pH 6.2). The difference between initial and final turbidity at 640 nm was measured. LzM activity was expressed as a μg equivalent to egg white LzM. There was no difference in LzM activities between before and after dialysis of the urine.

N-Acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30): NAG activity was measured using p-nitrophenyl-2-acetamide-2-deoxy-β-D-glucopyranoside as its substrate according to the method of Patel et al. (1972). The dialyzed urine in a volume of 0.05 ml was incubated at 37°C for 15 min with 0.95 ml of the substrate solution (pH 4.2). After the addition of 2 ml of a 2 M ammonia-HCl buffer (pH 10.7), absorbance at 420 nm was measured. NAG activity was expressed as μg of p-nitrophenol/min.

Acid protease (EC 3.4.23.-): Acid protease activity was measured using 14C-hemoglobin as its substrate according to the method of Peters et al. (1972). 14C-hemoglobin was labeled according to the method of Roth et al. (1971). The dialyzed urine in a volume of 0.05 ml was incubated at 37°C for 30 min with 0.35 ml of 0.2 M lactate buffer (pH 3.2) and 0.1 ml of 14C-hemoglobin solution (200,000 dpm/mg hemoglobin/0.1 ml). The reaction mixture was centrifuged at 3,000 r. p. m. for 10 min after the addition of 0.5 ml of 1% casein solution and 1 ml of 10% trichloroacetic acid solution in ice/water. The supernatant in a volume of 0.5 ml was added to 10 ml of a scintillation cocktail (4 g of 2, 5-diphenyloxazole and 0.2 g of 1, 4-bis (5-phenyl-2-oxazolyl benzene) in 1,000 ml of toluene and 424 ml of Triton X-100) and the radioactivity was counted with a liquid scintillation spectrometer.

Lactate dehydrogenase (LDH, EC 1.1.1.27), aspartate aminotransferase (GOT, EC 2.6.1.2), alkaline phosphatase (ALP, EC 3.1.3.1), and leucine aminopeptidase (LAP, EC 3.4.11.1) activities were measured using LDH-UV Test Wako, GOT-UV Test Wako, Alkaline Phospha K-Test Wako and LAP C-Test Wako, respectively. γ-Glutamyl transpeptidase (γ-GTP, EC 2.3.2.2) was measured using γ-GTP test pack AK (Sankyo Co.).

LDH isoenzyme: Urine for the LDH isoenzyme assay was dialyzed against over 50 volumes of deionized water at 4°C for 15 h. The dialyzed urine was lyophilized and dissolved in a small volume of deionized water so as to adjust the LDH concentration to over 100 IU/ml. Electrophoresis of LDH isoenzyme was performed on a Sephaphore III strip (Gelman Sciences Inc.) at 200 V for 20 min. The Sephaphore III strips were stained with LDH Isoenzyme Substrate Set (Gelman Sciences Inc.) and the ratio of LDH isoenzymes was obtained on the densitometer.

Other analytical methods

Creatinine: Creatinine concentration in nondialyzed urine was measured by the Jaffe reaction (Bonsnes and Taussky, 1945).

Urinary excretions of each parameter were expressed as activity or as amount per h per kg of body weight.
RESULTS

1. Changes in urinary parameters from HgCl₂ treated rats

1-1) Urine volume, creatinine and tubular cell counts

In the HgCl₂-H group, urine volume and creatinine excretion were maximally increased to 3.0 and 1.9 times the control values during the 10-24 h period, respectively, and then declined. In the HgCl₂-M group, urine volume and creatinine excretion were increased to 2.7 and 1.5 times the control values on day 2, respectively (Fig. 1-A, B). Tubular cell counts in the HgCl₂-M and the HgCl₂-H groups began to increase during the 0-10 h period. In the HgCl₂-H group, tubular cell counts were maximally increased to 130 times the control value during the 10-24 h period. In the HgCl₂-M group, tubular cell counts were maximally increased to 93 times the control value on day 2. In the HgCl₂-M and the HgCl₂-H groups, tubular cell counts maintained a higher level on days 5-7. In the HgCl₂-L group, tubular cell counts began to increase on day 2 and reached to 115 times the control value on day 7 (Fig. 1-C).

![Graphs A, B, and C showing changes in urine volume, creatinine, and tubular cells over time.](image)

Fig. 1. Effects of mercuric chloride (HgCl₂) on various parameters in rat urine. Each point is mean ± SE of five animals. ○-○: control, □-□: HgCl₂ 0.25 mg/kg/day s.c. for 7 days, ●-●: HgCl₂ 0.5 mg/kg/day s.c. for 7 days, ■-■: HgCl₂ 1.0 mg/kg/day s.c. for 7 days. A: urine volume, B: creatinine, C: tubular cells, *, **: significantly different (*: p<0.05, **: p<0.001) from the control group.
1–2) Urinary enzymes

Changes in excretions of LDH, GOT, ALP, LAP, LzM, NAG and acid protease in the urine from HgCl₂ treated rats are shown in Fig. 2. All the enzyme excretions were not increased during the 0–10 h period in the HgCl₂-H group, during the 0–24 h period in the HgCl₂-M group and on days 1–3 in the HgCl₂-L group. Excretions of investigated enzymes excluding LzM and acid protease, reached their peaks during the 10–24 h period in the HgCl₂-H group and on day 2 in the HgCl₂-M group. In the HgCl₂-L group, NAG and LzM excretions were not increased, while other enzyme excretions reached the peaks on day 5. In the HgCl₂-M group, LDH and GOT excretions were maximally increased to 44 and 25 times the control values, respectively (Fig. 2-A, B). However, the increases in excretions of both enzymes were transient. The values of both enzymes on days 5 and 7 were only slightly higher (3–6 times) than the control values. ALP and LAP excretions were maximally increased to 25 and 43 times the control values, respectively, in the HgCl₂-H group and then declined to the control values (Fig. 2-C, D). In the HgCl₂-H group, LzM excretion was increased to 12.6 ± 4.3 and 17.0 ± 4.0 µg/kg/h during the 10–24 h period and day 2, respectively. In the HgCl₂-M group, LzM excretion was increased to 11.1 ± 2.1 µg/kg/h only on day 2 (Fig. 2-E). The peak value of NAG excretion was lower than that of other enzymes and the values in the HgCl₂-M and the HgCl₂-H groups were 3.8 and 4.5 times the control value, respectively (Fig. 2-F). Acid protease excretion in the HgCl₂-H and the HgCl₂-M groups were maximally increased to 7.8 and 6.0 times the control value on days 2 and 3, respectively. Then, acid protease excretion declined gradually, although the values were significantly higher than the control values on days 3–5 in the HgCl₂-H group and on days 5–7 in the HgCl₂-M group (Fig. 2-G). After day 2, the values of LDH, GOT ALP and LAP in the HgCl₂-M group were higher than those in the HgCl₂-H group.

1–3) LDH isoenzymes

In normal rat urine, LDH1 and LDH5 predominated and each of them accounted for about 35% of total LDH excretion, respectively (LDH5 > LDH1 > LDH2 > LDH4 > LDH3). In the HgCl₂ treated groups, LDH5 predominated. These increases in the HgCl₂-H and the HgCl₂-M groups were observed already during the 0–10 h period when the total LDH excretion was not increased. Also in the HgCl₂-L group, the increase in LDH5 excretion was also observed already on day 3 (Fig. 3).

2. Changes in urinary parameters from GM treated rats

2–1) Urine volume and creatinine

Urine volume and creatinine excretion were not increased in the GM-L and the GM-M groups (Fig. 4-A, B). In the GM-H group, urine volume began to increase on day 5 and reached the peak of 4.4 times the control value on day 11. Creatinine excretion was increased to 1.7 times the control value on days 11 and 13 only in the GM-H group.
Fig. 2. Effects of mercuric chloride (HgCl₂) on various enzymes in rat urine. Each point is mean ± SE of five animals. ○——○: control, □——□: HgCl₂ 0.25 mg/kg/day s. c. for 7 days, ●——●: HgCl₂ 0.5 mg/kg/day s. c. for 7 days, ■——■: HgCl₂ 1.0 mg/kg/day s. c. for 7 days, A: LDH, B: GOT, C: ALP, D: LAP, E: LZM, F: NAG, G: acid protease, *, **: Significantly different (*: p<0.05, **: p<0.001) from the control group.
Fig. 3. Effects of mercuric chloride (HgCl₂) on pattern of LDH isoenzymes in rat urine. Each value is mean ± SE of two to five animals. A: control, B: HgCl₂ 0.25 mg/kg/day s.c. for 7 days, C: HgCl₂ 0.5 mg/kg/day s.c. for 7 days, D: HgCl₂ 1.0 mg/kg/day s.c. for 7 days.

Fig. 4. Effects of gentamicin (GM) on various parameters in rat urine. Each point is mean ± SE of five animals. ○——○: control, □——□: GM 7.5 mg/kg twice daily s.c. for 13 days, ●——●: GM 15 mg/kg twice daily s.c. for 13 days, ■——■: GM 30 mg/kg twice daily s.c. for 13 days, A: urine volume, B: creatinine, *: significantly different (p<0.05), **: p<0.001) from the control group.
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2-2) Urinary enzymes

LDH and GOT excretions began to increase on day 3 and reached to 6-7 times the control values on days 9-13 also in the GM-L group. In the GM-M group, LDH and GOT excretions began to increase on days 3 and 1, respectively, and reached the peaks of 47 and 36 times the control values on day 11, respectively. In the GM-H group, LDH and GOT excretions began to increase on day 1 and reached the peaks of 95 and 98 times the control values on day 7, respectively. However, LDH and GOT excretions in the GM-H group declined after day 9 and revealed the values of 27 and 22 times the control values on day 13, respectively. These values were lower than those of the GM-M group (Fig. 5-A, B). ALP and \( \gamma \)-GTP excretions were not increased (Fig. 5-C, D). LAP excretion was slightly increased to less than 2 times the control value in the GM-M and the GM-H groups (Fig. 5-E). LZM excretion in the GM-M group began to increase on day 7 and reached to 16.4±6.9 \( \mu \)g/kg/h on day 13, while LZM excretion in the GM-L group was not increased. In the GM-H group, LZM excretion began to increase on day 5 and reached the peak of 842±97 \( \mu \)g/kg/h on day 11 and then declined (Fig. 5-F). NAG and acid protease excretions were increased to less than 2 times the control value on days 5-13 in the GM-L group. In the GM-M group, NAG and acid protease excretions began to increase on day 5 and reached to 5.7 and 4.6 times the control values on day 13, respectively. In the GM-H group, excretions of both enzymes began to increase on day 1, and then acid protease excretion was gradually increased to 16 times the control value on day 13, while NAG excretion declined after reaching the peak of 10 times the control value on day 9 (Fig. 5-G, H).

The pattern of changes in excretions of LDH, \( \gamma \)-GTP, LZM, NAG and acid protease in the GM-L, the GM-m and the GM-h groups almost coincided with those in the GM-L, the GM-M and the GM-H groups, respectively.

2-3) LDH isoenzymes

In the GM-L and the GM-m groups, excretion of each isoenzyme was increased and indicated almost equal activities in each group on day 5, and then LDH1 and LDH2 predominated on day 9. In the GM-h group, LDH1 and LDH2 predominated on days 3, 5 and 7 (Fig. 6).

2-4) Tubular cell counts

Tubular cell counts in the GM-L, the GM-m and the GM-h groups were dose-dependently increased from day 3, and reached the values of 4.4, 35 and 43 times the control value on day 7, respectively. Then tubular cell counts gradually declined in the GM-m and the GM-h groups but the values on day 15 were significantly higher than the control value (Fig. 7).
Fig. 5. Effects of gentamicin (GM) on various enzymes in rat urine. Each point is mean ± SE of five animals. ○ ○ : control, □ □ : GM 7.5 mg/kg twice daily s. c. for 13 days, ● ● : GM 15 mg/kg twice daily s. c. for 13 days, ■ ■ : GM 30 mg/kg twice daily s. c. for 13 days, A : LDH, B : GOT, C : ALP, D : γ - GTP, E : LAP, F : LZM, G : NAG, H : acid protease, * , ** : Significantly different (* : p<0.05, ** : p<0.001) from the control group.
Fig. 6. Effects of gentamicin (GM) on pattern of LDH isoenzymes in rat urine. Each value is mean ± SE of five animals. A: GM 15 mg/kg/day s.c. for 15 days, B: GM 30 mg/kg/day s.c. for 15 days, C: GM 60 mg/kg/day s.c. for 15 days.

Fig. 7. Effects of gentamicin (GM) on tubular cells in rat urine. Each point is mean ± SE of five animals. ○ control, □ GM 15 mg/kg/day s.c. for 15 days, ● GM 30 mg/kg/day s.c. for 15 days, ■ GM 60 mg/kg/day s.c. for 15 days. *, **: Significantly different (*: p<0.05, **: p<0.001) from the control group.
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DISCUSSION

Urine can be easily collected and contains various components, changes of which indicate abnormalities in renal function. In particular, urinary enzymes are very sensitive indicators of renal damage (Raab, 1972; Price, 1982; Takahashi and Ohata, 1982). On the other hand, urinary enzymes have been criticized as useful markers because of their transience (Piperno, 1981). In this study, in order to establish sensitive methods of detecting renal damage in animal experiments, changes in some enzymes, LDH isoenzyme, tubular cell counts and others in urine from rats treated with nephrotoxic chemicals were investigated.

Daily administration of mercuric chloride (HgCl$_2$) and gentamicin (GM) dose-dependently increased tubular cell counts in urine. In the HgCl$_2$ administration groups, the increases in tubular cell counts preceded those in the seven enzymes measured, and maintained the higher level until day 7. From these results, it is concluded that the tubular cell count can be a sensitive indicator in the renal damage induced by HgCl$_2$ and GM.

In the HgCl$_2$-M and the HgCl$_2$-H groups, LDH, GOT, ALP and LAP excretions declined after reaching the peak values on days 1 or 2, while the increase in tubular cell counts maintained the higher level until day 7. These results were in agreement with those of Cottrell et al. (1976). Therefore, we suppose that LDH, GOT, ALP and LAP are sensitive indicators of renal damage. However, periodic determinations of these enzymes are needed for the assessment of nephrotoxicity by urinalysis, because these increases are transient.

When the degree of increase in each enzyme excretion was compared between the HgCl$_2$-H and the GM-H groups, some differences were observed. Namely, GM increased LzM, NAG and acid protease excretions more markedly than HgCl$_2$, and the increases in $\gamma$-GTP, ALP and LAP excretions by GM were slight whereas HgCl$_2$ markedly increased ALP and LAP excretions. It seemed that the marked increases in LzM, NAG and acid protease excretions caused by GM were due to its strong effect on lysosomes. Namely, in regard to LzM, Cojocel and Hook (1983) reported that GM increased the glomerular sieving coefficient of LzM and reduced tubular reabsorption and renal lysosomol catabolism of LzM, and that these results reflected proteinuria. Further, Inaba et al. (1984) reported that aminoglycosides bind to the lysosomal membrane and increases the membrane’s fluidity, leading to the release of NAG and acid phosphatase from the lysosomes. It seems that the increases in NAG and LzM excretions by GM in the present study were caused by these mechanisms. In regard to acid protease, it seems that this activity includes pepsin and lysosomal enzymes such as cathepsin D and cathepsin E, while it is unclear whether urinary acid protease is any of these enzymes. We suppose that at least a portion of acid protease is either cathepsin D or cathepsin E, because the specific inactivator of pepsin, 2,4'-dibromoacetophenone, had almost no effect on this enzyme activity (unpublished observation).
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In regard to the site of damage caused by GM or HgCl₂, Koseki et al. (1980) reported that HgCl₂ might damage S₂ and S₃ in proximal tubules and that the toxic action of GM may be localized in the S₁ portion on the basis of the distribution of γ-GTP, ALP and LAP in the rat nephron and urinary excretions of these enzymes. In a histological study on HgCl₂, Gritzka and Trump (1968) reported that HgCl₂ damaged proximal straight tubes, and Kempson et al. (1977) reported that fragmentation of the microvilli of the brush border occurs within 3 h of administration of HgCl₂. On the other hand, Kosek et al. (1974) reported that GM damage resulted in the formation of numerous lysosomal cytosegresomes, many of which contained prominent myeroid bodies, in proximal convoluted tubular cells. Kojima and Suzuki (1984) reported that GM damaged proximal tubular epithelial cells in the renal outer cortex and that HgCl₂ damaged tubular epithelial cells in the renal inner cortex. From all these considerations, it seems that our results show that the two chemicals damage different sites in the proximal tubules. Namely, it seems that HgCl₂ damages mainly the proximal tubular brush border membranes in the renal inner cortex, in which ALP and LAP are localized, but GM damages proximal tubules in the renal outer cortex. Therefore, we suppose that the site of renal damage can be estimated by simultaneous determinations of a battery of these urinary enzymes.

In the HgCl₂-H and the GM-H groups, the increase in acid protease excretion persisted but was lower than those in LDH, GGT, γ-GTP, ALP and LAP excretions. Kojima and Suzuki (1984) reported that severe necrosis of proximal tubular cells was observed also on day 10 in the rats treated with GM (80 mg/kg/day for 15 days). Therefore, we suppose that the change in acid protease excretion reflected renal histological change more truly than other enzymes and that it is a useful marker of renal damage. Baricos and Shah (1984) reported that puromycin aminonucleoside increased urinary acid protease activity and that this acid protease might be cathepsin D because of optimum pH (3.0) and inhibition by pepstatin, whereas a role for other acid proteases, such as uropepsinogen, in this urinary enzyme can not be ruled out. Thus it seems that there are some acid proteases in urine and, therefore, we suppose that the importance of urinary acid protease is promoted if increased enzymes in those by renal damage can be ascertained.

LDH₁ and LDH₅ predominated in normal rat urine. HgCl₂ mainly increased LDH₅ and LDH₄ excretions. The increase in LDH₅ excretion in the HgCl₂-M group was observed already during the 0–10 h period when total LDH excretion had not yet increased. Therefore, we suppose that LDH isoenzyme is a very sensitive indicator and useful for detecting renal damage in an early stage. In contrast to HgCl₂ increased LDH₅ and LDH₄ excretions, GM increased LDH₁ and LDH₂ excretions. It is reported that the distribution of LDH isoenzymes in the rat kidney is not homogeneous, and LDH₁ and LDH₂ predominate in the renal cortex whereas LDH₄ and LDH₅ predominate in the renal inner medulla (Ringoir, 1970 ; Cestonarono et al., 1979). These results also suggest that there is a difference in damaged sites in
proximal tubules between the two chemicals. Therefore, we suppose that the site of damage can be presumed by analysis of urinary LDH isoenzymes if their distribution in the nephron is ascertained.

From these results, it was concluded that the severity of renal damage can be readily detected by periodic determinations of the following urinary parameters: tubular cell counts, LDH isoenzyme, acid protease, LZM and NAG in addition to either LDH or GOT and one of the enzymes ALP, LAP or \( \gamma \)-GTP. Moreover, the site of renal damage can be presumed from these results.

**REFERENCES**


