treated rats had no effect on the enzyme activities of normal rat urine.

Cadmium induces the synthesis of a low molecular weight protein (metallothionein) in the liver (Piotrowski et al., 1974). This protein is thought to act as a detoxifying agent by binding avidly to the cadmium species (Shaik & Tohyama 1984). The cadmium–metallothionein complex is then transported to the kidney, whereupon it is reabsorbed by the proximal tubule and sequestered into lysosomes (Nishizumi, 1972). The low activity therefore might be due to the fact that damaged structures such as mitochondria are engulfed by lysosomes, possibly leading to low excretion of GDH and β-NAG activity.

These mechanisms could be looked upon as possible adaptive responses to cadmium, and the significant increase in ALP which has also been reported by other workers (Nomiyama et al., 1979) suggests damage to the brush-border membrane. Chronic exposure to cadmium leads to cellular necrosis and proteinuria (Goyer et al., 1980) with enzymuria (Bomhard et al., 1984), but urinary ALP appears to be the most sensitive means for detecting the initial damage to the kidney caused by cadmium.


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Oxygen uptake and glutamate dehydrogenase release in isolated renal tubules from rat kidney

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The aminoglycoside antibiotics are important agents in the management of severe bacterial infections, but clinical and animal studies show that they can be nephrotoxic. High doses of the aminoglycosides cause tubular necrosis which is largely confined to the proximal convoluted tubules (Milton, 1974; Wellwood et al., 1976). The main target of these antibiotics is the lysosomes (Patel et al., 1975; Cotocel & Hook, 1983), although gentamicin does appear to affect the mitochondria by causing them to increase in size (Houghton et al., 1976; Morin et al., 1982). As part of a long-term study on the nephrotoxic action of the aminoglycosides, it was decided to examine the viability of isolated renal tubules by measuring the respiration and the leakage of enzymes from the cell since genatamicin causes an increased excretion of several urinary enzymes (Plummer et al., 1982, 1986).

Renal tubules were prepared from rat kidney cortex (Macdonald & Saggerson, 1977) and suspended in Krebs–Henseleit medium to a final protein concentration of about 4 mg/ml. The tubules were continuously gassed with O₂/CO₂ (95:5) and incubated at 37 °C in a shaking water bath.

The appearance of the tubules was examined by light microscopy under phase contrast and the viability of the tubules determined by measuring their respiration. This was carried out by removing samples at suitable time intervals and measuring their oxygen uptake in the oxygen electrode.

The integrity of the preparation was determined by following the release of glutamate dehydrogenase (GDH) and lactate dehydrogenase (LDH) from the tubules into the surrounding medium (Plummer et al., 1986). Aliquots of the tissue suspension were removed at suitable time intervals, centrifuged at high speed on a Micro Centaur centrifuge and the enzyme activity determined on the supernatant.

The oxygen uptake of the isolated tubules declined steadily with time and this fall in the respiration rate was the same whether glucose, succinate or pyruvate (all at 5 mM) was used as substrate. On the basis of this measurement, 60% of the tubules were still viable at 3 h.

The total enzyme activity in the renal tubules was determined by treating them with 1% (w/v) Triton X-100 and determinations of GDH and lactate dehydrogenase.

Abbreviations used: GDH, glutamate dehydrogenase; LDH, lactate dehydrogenase.

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their activity expressed as nmol of substrate converted per min per g wet weight of cortex. The percentage of GDH released into the supernatant was determined and a plot of respiration rate against the GDH released is shown in Fig. 1. An inverse relationship was found to exist between these two parameters with a high correlation coefficient \( r = 0.97 \). It is therefore clear that incubation of the tubules leads to a decline in the mitochondrial function and this is reflected by the fall in the respiration and the increased release of the mitochondrial enzyme GDH from the cells.

The leakage of the cytoplasmic enzyme LDH from the preparation of isolated cells is usually used to check the integrity of preparations and there is also a very good correlation between the release of this enzyme and the fall in respiration (Fig. 1). LDH is a cytoplasmic enzyme while GDH is present in the mitochondrial matrix, so the decline in viability is probably due to a number of cells becoming too damaged to respire.

Whatever the ultimate explanation, it is quite clear that any one of these parameters can be used to monitor the integrity of the renal tubules.

**Urinary enzyme excretion in humans**

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The determination of urinary enzymes is a rapid and non-invasive test for assessing kidney damage in experimentally induced toxic studies (Beck et al., 1977; Lockwood & Bosnar, 1979), screening for potential nephrotoxic drugs (Raab, 1978; Sack et al., 1978), and monitoring changes in the functional states of the kidney in transplant patients (Wellwood et al., 1973; Horpacsy et al., 1977). Activities of urinary enzymes, however, can only be identified as pathological if grossly elevated, since a wide range of activities is excreted by normal individuals. Although some investigations have dealt with urinary enzyme excretion in healthy subjects (Werner et al., 1970; Peters et al., 1972; Maruhn et al., 1976; Price, 1979), the possible dependence on various factors such as flow rate and urinary pH have not previously been investigated fully. In the present study the effect of flow rate on the activity of various urinary enzymes has been examined.

Twenty-one normal human subjects were prehydrated with 7.5 ml of distilled water/kg and 1 h later were hydrated with 15 ml of distilled water/kg. Hourly samples were collected both before and after hydration and their enzyme activity determined. The experiments were performed in the morning and no food or drink was taken during this period. The urine samples collected were stored in ice and assayed as soon as possible to avoid loss of activity. The volume and pH of the untreated urine were measured and the urine was centrifuged for 15 min at 3000 g. The supernatant was then removed for subsequent assays.

The urinary enzymes examined in this study were alkaline phosphatase (located in the brush border; Ngaha & Plummer, 1977); lactate dehydrogenase (cytoplasmic; Wright & Plummer, 1974; Wachsmuth et al., 1978); glutamate dehydrogenase (mitochondrial; Wright & Plummer, 1974); N-acetyl-β-D-glucosaminidase (lysosomes; Wellwood et al., 1974). The alkaline phosphatase activity was determined at 37°C and pH 10.1 by following the increase in A\(_{400}\) owing to the release of p-nitrophenol.

The lactate dehydrogenase and glutamate dehydrogenase activity were determined at 37°C and pH 7.4 by following the decrease in extinction at A\(_{400}\) as the oxidation of NADH is monitored with time. The substrates used for lactate dehydrogenase and glutamate dehydrogenase assays were sodium pyruvate and 2-oxoglutarate respectively.

The N-acetyl-β-D-glucosaminidase activity was determined by following the hydrolysis of the substrate 2-methoxy-4(2'-nitrovinyl)-phenyl-2-acetamido-2-deoxy-β-D-glucopyranoside which liberates 2-methoxy-4(2'-nitrovinyl) phenol. The sample was first diluted with 0.15 m-citric acid/disodium phosphates buffer, pH 4.5, then incubated with 1.5 mm-substrate at 37°C for 30 min. The reaction was stopped by the addition of 0.5 m-sodium carbonate/bicarbonate buffer, pH 10.0.

The enzyme activity was expressed in terms of μmol of substrate converted per minute (units) per ml of urine, and the enzyme excretion expressed as units excreted per hour.

Water loading of normal human subjects caused significant increases in the total urinary lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase and N-acetyl-β-D-glucosaminidase activities of the maximal-hydrated samples, as shown in Fig. 1(a) for alkaline phosphatase. By the third hour after hydration the total enzyme activity in each case was almost the same as that for the morning samples (Fig. 1a), except for alkaline phosphatase which still remained slightly high.

The change in flow rate (ml/min) with time is represented in Fig. 1(b), which shows that the total alkaline phosphatase activity and flow rate vary in close correspondence with time. If the alkaline-phosphatase activity is expressed as units/ml, then the activity of the maximal-hydrated samples is not significantly different from that of the other timed samples. However, this pattern was not observed with the other enzymes studied.

The high correlation \( r = 0.83 \) observed between total alkaline phosphatase activity and flow rate, together with the independence of alkaline phosphatase activity in terms of units/ml and flow rate, demonstrates that it is very useful enzyme for the diagnosis of kidney damage. One might speculate that normally a small and constant amount of alkaline phosphatase (a brush-border enzyme) is excreted in

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