clearly resolvable. The other resonances arise from the methylphosphonate (MeP) and from 2,3-bisphosphoglycerate (DPG), which is converted to inorganic phosphate and ATP in glycolytic reactions.

While both the decline in substrate and increase in product resonance can be measured, conservation of peak area (substrate + product = constant) is not observed, because the inorganic phosphate is consumed and released in a variety of endogenous reactions (e.g., ATPase activity, production of glucose 6-phosphate for glycolysis and the pentose phosphate pathway, phosphorolytic reactions, etc.). Hence, an accurate estimate of P5N activity may only be obtained by observing the decline in CMP resonances. The P5N activities (μmol/L of packed cells/min) obtained for red cells of patients and normal controls were 36 ± 2.9 and 32 ± 2.7, respectively. A t-test for unequal sample sizes[20] showed that the two sample populations were not significantly different at the level of $p < 0.005$. As expected, cytosine and cytidine each inhibited the P5N, as can be seen by comparing figure 2A with figures 2B and 2C.

The quantitative analysis of the assay using ^{1}H-NMR spectroscopy did not yield good results for the following reasons. The ^{1}H spectrum exhibits greater complexity and is less resolved than the ^{31}P spectrum. This arises from the J-coupling phase modulation in spin-echo spectra[21], the larger number of proton nuclei present and the inherent small chemical-shift difference between substrate and product resonances. In the aromatic region, the C5 and C6 protons of the pyrimidine ring appear at 5.890 ppm and 7.828 ppm, respectively. Product resonances appear at slightly lower frequencies and are partially obscured by nicotinamide and hemoglobin-histidyl spectral lines. This complicates considerably the computer integration of the peaks and subsequently the determination of P5N activity.

The ^{31}P NMR method would be suitable for use in lower field, less expensive, spectrometers. Further, it considerably simplifies and expedites in situ assay of P5N and hence the diagnosis of P5N deficient subjects.

Although anomalies in P5N activity have been reported in other myelin associated disorders, there is no apparent change in the red blood cells of multiple sclerosis patients.

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2 Paglia, D.E., Valentine, W.N., and Dahlgren, J.G., J. clin. Invest. *56* (1975) 116.
3 Valentine, W.N., Fink, K., Paglia, D.E., Harris, S.R., and Adams, W.S., J. clin. Invest. *54* (1974) 886.
4 Vives-Corrons, J.L., Monserrat-Costs, E., and Rozman, C., Hum. Genet. *34* (1976) 285.
5 Lieberman, J.E., and Gordon-Smith, E.C., Br. J. Haemat. *44* (1980) 425.
6 Torrance, J.D., Whittaker, D.A., and Beutler, E., Proc. natn. Acad. Sci. USA *74* (1977) 3701.
7 Torrance, J.D., Whittaker, D.A., Enzyme *26* (1981) 64.
8 Torrance, J.D., Wulfsohn, M.S., and Mills, W., Analyt. Biochem. *126* (1982) 235.
9 Zimmerman, T.R., and Cammer, W., Biochim. biophys. Acta *760* (1983) 377.
10 Torrance, J.D., West, C., and Beutler, E., J. Lab. clin. Med. *90* (1977) 563.
11 Buc, H., and Kaplan, J.C., Clinica chim. Acta *85* (1978) 193.
12 Ellims, P.H., Bailey, L., and van der Weyden, M.B., Clinica chim. Acta *88* (1978) 99.
13 Paglia, D.E., and Valentine, W.N., J. biol. Chem. *250* (1975) 7973.
14 Kuchel, P.W., and Chapman, B.E., Experientia *41* (1985) 53.
15 Swanson, M.S., Angle, C.R., Stohs, S.J., Wu, S.T., Salhany, J.M., Eliot, R.S., and Markin, R.S., Proc. natn. Acad. Sci. USA *80* (1983) 169.
16 Beilharz, G.R., Middlehurst, C.R., Kuchel, P.W., Hunt, G.E., and Johnson, G.F.S., Analyt. Biochem. *137* (1984) 324.
17 Krebs, H.A., and Henseleit, K., Z. Physiol. Chem. *210* (1932) 33.
18 Stewart, I.M., Chapman, B.E., Kirk, K., Kuchel, P.W., Lovric, V.A., and Raftos, J.E., Biochim. biophys. Acta, in press (1985).
19 Becker, E.D., Ferretti, J.A., and Gambhir, P.N., Analyt. Chem. *51* (1979) 1413.
20 Huntsberger, D.D., Elements of Statistical Inference, 2nd Edn, pp. 296–302. Allyn and Bacon, Boston 1967.
21 Rabenstein, D.L., and Nakashima, T.T., Analyt. Chem. *51* (1979) 1465A.

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Chronic hydrogen peroxide intake and peroxide metabolizing enzyme activities in some tissues of mice and rats

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Summary. Chronic daily intake of 0.5% H_2O_2 in drinking water decreased Se-dependent glutathione peroxidase (Se-GSHPx) activity in rat skeletal muscle, kidney and liver. Non-Se GSHPx activity decreased in kidney. Deprivation of drinking water decreased Se-GSHPx activity in kidney and non-Se GSHPx activity in kidney and liver. H_2O_2 intake decreased activity of catalase in rat skeletal muscle. H_2O_2 intake or water deprivation caused no changes in these enzyme activities in mice.

Key words. Hydrogen peroxide intake; glutathione peroxidase; catalase; lipid peroxides; antioxidants.

Certain chemicals e.g. CCl_4, ethanol[2] or phenobarbital[3] may cause the formation of lipid peroxides and other organic hydroperoxides in biological tissues. It is observed that H_2O_2[4] or cumene hydroperoxide[5] can initiate lipid peroxidation in vitro. Glutathione peroxidase (GSHPx) and catalase are the two main enzymes responsible for the enzymatic decomposition of H_2O_2. The GSHPx activity can be divided by different substrates into two activities[6]: H_2O_2 is the substrate for the selenoenzyme, Se-dependent GSHPx only, and cumene hydroperoxide is the substrate for both the Se-dependent and the Se-independent (non-Se) GSHPx activities. The non-Se activity arises from a group of glutathione S-transferases[7]. The detoxification of H_2O_2 is not affected by the antioxidant vitamin E[8].

In rat liver, lipid peroxidation increases as a result of an excess of dietary iron. This increase is potentiated by a deficiency of Se and/or vitamin E[9]. The increase in lipid peroxidation is reflected by a shift in the two activities of GSHPx; the Se-dependent GSHPx activity decreases with the increase of iron concentration. The decrease is additionally enhanced by the simultaneous deficiency of Se or vitamin E. This effect is partially compensated by an increase of the non-Se activity. Lee et al.[9] also observed the increase of liver catalase activity as a response to the increase of dietary iron, which increase also was potentiated by the deficiency of Se and/or vitamin E. The purpose of this study was to find out whether oral H_2O_2 intake could provoke an oxidative stress in some tissues of mice and rats as reflected by changes in the levels of catalase and glutathione peroxidases. Knowledge of possible enzymatic adaptations would be useful in studies on the role of scavenger systems in tissue injuries.

Materials and methods. Male NMRI mice (1 month old at the beginning of the experiment) and male Wistar rats of the same age were used in the experiment. The experimental animals were housed under similar conditions with free access to food pellets (Hankkija, Finland). Eight mice and eight rats received 0.5% (v/v) H_2O_2 added to the drinking water. Equal numbers of freely-water-drinking animals served as controls. All animals were weighed weekly during the experiment and the daily consumption of drinking water or the H_2O_2 solution was measured. The loss of H_2O_2 from the water was about 15% during a day. The solutions were prepared daily.

After 1 week it was evident that the animals in the H_2O_2-supplied group drank less than the control animals. Therefore we established a new group of animals, to which the same volume of pure water was given as the H_2O_2-supplied group had drunk the day before. These water-deprived animals were of the same age, strain and sex as the others. The mice were killed 40 days and the rats 56 days after the beginning of the experiment. The heart, the proximal red part of the quadriceps femoris muscle (MQF), the kidneys and the liver were removed for biochemical analysis. The activities of catalase[10], Se-dependent[11] and non-Se[6] GSHPx and β-glucuronidase (β-GU)[12] were measured. The protein contents of the tissue homogenates and supernatants were measured by the method of Lowry et al.[13]. The significance of the differences between the group means was measured using Student's t-test.

Results and discussion. The growth of the rats was delayed in the water-deprived and in the H_2O_2-supplied groups as seen in figure 1A. The most probable cause for this effect was the smaller consumption of drinking solutions in the H_2O_2-supplied and water-deprived groups compared with the control group (fig. 1B). The phenomenon was similar with the mice, but the group differences were not statistically significant. In the mice the only significant ($p < 0.05$) change due to H_2O_2 administration was an increase in the weight of the kidneys (117 ± 5 mg in the H_2O_2 group compared to 99 ± 3 mg in the control group). Catalase, Se-dependent GSHPx and β-GU activities were unaffected (data not shown). In the H_2O_2-supplied rats, the activity of catalase in MQF was 0.90 ± 0.05 $U \cdot g^{-1}$, which is significantly lower ($p < 0.001$) than that of the control group (1.59 ± 0.16 $U \cdot g^{-1}$). The other tissues showed no changes with respect to catalase activity. The results concerning GSHPx activity are given in figure 2. The Se-dependent activity in the rat MQF decreased in the H_2O_2-supplied group. A decrease was also observed in kidney and in liver but not in heart. The non-Se GSHPx activity decreased strikingly in kidney, but was unaffected in other tissues (fig. 2).

Surprisingly, water deprivation also caused a decrease in the activity of Se-dependent GSHPx in the kidneys of rats, but not in other tissues, and a decrease in the activity of non-Se GSHPx in kidney and liver but not in muscular tissues (fig. 2). The activity

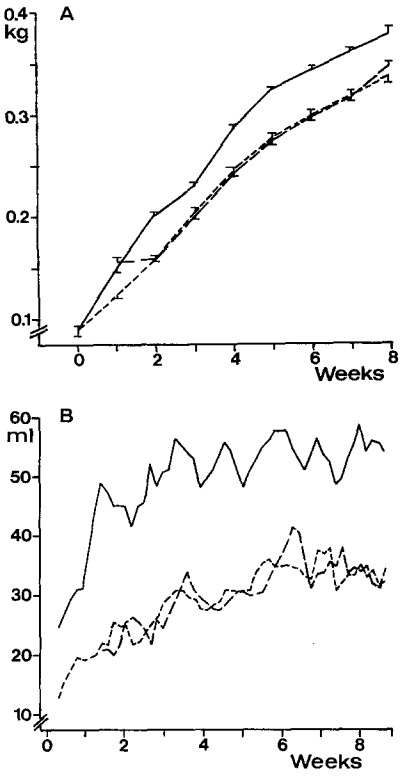

Figure 1. *A* Weight of rats during experiment. Values are means for 8 rats ± SE. *B* Amount of water or H_2O_2 solution consumed per rat per day. Values are 3-day moving averages. Continuous line (control group), broken line (water-deprived group) and dotted line (H_2O_2-supplied group). After 2 weeks, values for H_2O_2-supplied and water-deprived groups differed significantly ($p < 0.001$) from those of control group.

of β-GU in rat liver decreased ($p < 0.05$) in the water-deprived group (38.6 ± 0.8 $\mu kat \cdot kg^{-1}$ compared to that of the control 42.4 ± 1.2 $\mu kat \cdot kg^{-1}$). The β-GU activity of the H_2O_2-supplied group was unaltered (41.6 ± 1.1 $\mu kat \cdot kg^{-1}$). The protein content of the rat liver was higher ($p < 0.05$) in the water-deprived group (184 ± 3 $g \cdot kg^{-1}$) than in the control group (174 ± 3 $g \cdot kg^{-1}$). The protein content was lower ($p < 0.05$) in the hearts of the H_2O_2-supplied group (192 ± 2 $g \cdot kg^{-1}$) than in the comparable control group (199 ± 3 $g \cdot kg^{-1}$). The protein contents of the other tissues were unchanged.

The catalase and GSHPx results of the present experiment show a discrepancy from those of Matkovics and Novak[14]. They found that the catalase activity increased significantly in heart, kidney and liver with response to the H_2O_2-intake. The only similarity with our results was the decrease in the catalase activ-

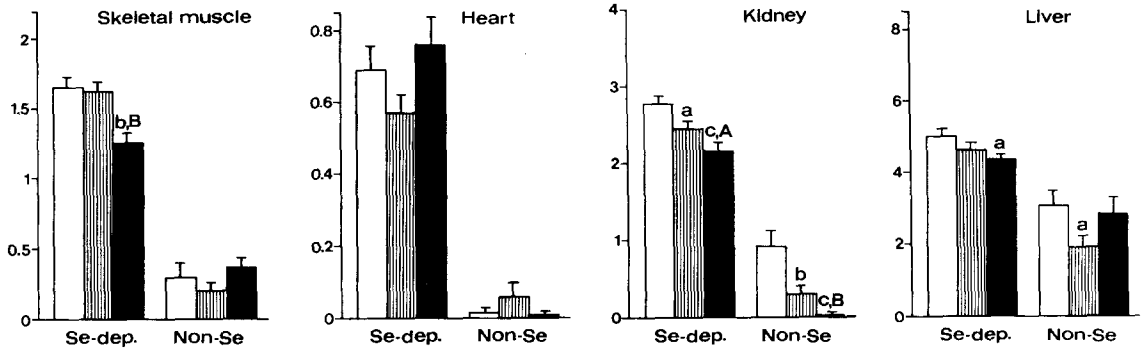

Figure 2. Activities of Se-dependent and Se-independent (non-Se) glutathione peroxidase (GSHPx) in rat MQF, heart, kidney and liver at end of experiment. Activities of GSHPx are given as $mkat \cdot kg^{-1}$ supernatant protein. Values are means of 8 rats ±SE. White columns (control group), lined columns (water-deprived group) and black columns (H_2O_2-supplied group). a: $p < 0.05$, b: $p < 0.01$ and c: $p < 0.001$ compared with respective control group values and A: $p < 0.05$ and B: $p < 0.01$ compared with water-deprived group values.

ity of the skeletal muscle. Matkovics and Nowak[14] also found that the activity of the peroxidase assayed (EC 1.11.1.7) was higher in the H_2O_2-supplied group in kidney and skeletal muscle whereas our results from the assay for Se-dependent GSHPx (EC 1.11.1.9) showed a decrease in the enzyme activity of kidney, liver and skeletal muscle. The experimental design of Matkovics and Novák[14] was similar to ours but they used the CFY-strain of rats and they had no water-deprived group similar to ours.

The GSHPx inhibits lipid peroxidation in vitro[15]. Selenium is necessary for the H_2O_2 decomposing activity of Se-dependent GSHPx and various organs contain different amounts of Se and Se-dependent GSHPx activities. For example, rat kidney contains Se 1.45 $mg \cdot kg^{-1}$, liver 1.29 $mg \cdot kg^{-1}$, heart 0.37 $mg \cdot kg^{-1}$ and muscle 0.16 $mg \cdot kg^{-1}$ [16]. The activity of Se-dependent GSHPx changes logarithmically in several tissues of rat with respect to the Se concentration in the diet[17]. The liver and kidneys are the organs most susceptible to degeneration induced by Se deficiency, whilst abnormalities in heart are rare[18]. H_2O_2 as an oxidizing agent could have an effect on the oxidization of the biologically effective organic selenium compounds of the food to inorganic derivatives which are not so bio-available[18]. This reduces the Se intake and causes a decrease in the activity of Se-dependent GSHPx. Smith et al.[19] found a reduced activity of GSHPx (cumene hydroperoxide as substrate) in kidney, red cells and liver in Se deficiency. The effects of Se deficiency, as well as those of oral H_2O_2-supply, are highly selective in various tissues, which suggests tissue differences in antioxidant regulation.

1 Matkovics, B., Novák, R., Szabó, L., Marik, M., and Zsoldos, T., Gen. Pharmac. *9* (1978) 329.
2 Valenzuela, A., Fernandez, N., Fernandez, V., Ugarte, G., and Videla, L.A., FEBS Lett. *111* (1980) 11.
3 Hahn, H.K.J., Barak, A.J., Tuma, D.J., and Sorrell, M.F., Biochem. Pharmac. *26* (1977) 164.
4 Ursini, F., Maiorino, M., Ferri, L., Valente, M., and Gregolin, C., J. inorg. Biochem. *15* (1981) 163.
5 Kulkarni, A.P., and Hodgson, E., Int. J. Biochem. *13* (1981) 811.
6 Lawrence, R.A., and Burk, R.F., J. Nutr. *108* (1978) 211.
7 Jakoby, W.B., in: Advances in Enzymology, Vol. 46, p. 383. Ed. A. Meister. John Wiley & Sons, New York 1978.
8 Hoekstra, W.G., Fedn. Proc. *34* (1975) 2083.
9 Lee, Y.H., Layman, D.K., Bell, R.R., and Norton, H.W., J. Nutr. *111* (1981) 2195.
10 Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J.W., Fowler, S., and DeDuve, C., J. Cell Biol. *37* (1968) 482.
11 Paglia, D.E., and Valentine, W.N., J. Lab. clin. Med. *70* (1976) 158.
12 Barrett, A.J., in: Lysosomes, A Laboratory Handbook, p. 46. Ed. J.T. Dingle. Elsevier/North Holland, Amsterdam 1972.
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
14 Matkovics, B., and Novák, R., Experientia *33* (1977) 1574.
15 Burk, R.F., Trumble, M.J., and Lawrence, R.A., Biochim. biophys. Acta *618* (1980) 35.
16 Behne, D., and Wolters, W., J. Nutr. *113* (1983) 456.
17 Chow, C.K., and Tappel, A.L., J. Nutr. *104* (1974) 444.
18 Schwarz, K., Fedn. Proc. *20* (1970) 666.
19 Smith, P.J., Tappel, A.C., and Chow, C.K., Nature *247* (1974) 392.

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Altered K^+ movement in liver mitochondria from alloxan diabetic rats

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Summary. Potassium movements were monitored in liver mitochondria from control and alloxan diabetic rats with a cationic electrode. There was net accumulation of K^+ after Ca^{2+} addition to the mitochondria with the diabetic but not with the control.
Key words. Diabetes; K^+; mitochondrial permeability; energy metabolism.

Liver mitochondria from alloxan diabetic rats were used to assess cellular energy production in the diabetic state[1,2]. Stimulation of respiration by addition of adenosine diphosphate (ADP) and Ca^{2+} to the mitochondria and the production of ATP or accumulation and retention of Ca^{2+} by mitochondria are standard methods for evaluating mitochondrial energy production[3]. The diabetic mitochondria are less efficient in energy production than mitochondria from untreated rats[1] and the accumulation and retention of Ca^{2+} in the diabetic mitochondria is lower than in normal mitochondria[2]. Changes in the ionic composition of the medium helped to elucidate these differences and indicated that a description of the interaction of K^+ with the mitochondria could help explain the altered pattern in the diabetic mitochondria[2].

In an earlier study the ionic content of the mitochondria was measured after a set incubation time using a flame photometer[2]. In that study changes in respiration were followed continuously and indicated that a continuous monitoring of changes in mitochondrial K^+ would be informative. Consequently the studies reported here were performed with continuous monitoring of net K^+ flux in mitochondria with a K^+ electrode. The patterns of K^+ flux in normal and diabetic mitochondria before and after Ca^{2+} stimulation are reported here. The purpose of these studies was to determine the pattern of K^+ movement in the diabetic state compared to control animals and to correlate these, if different from normal, to changes reported in the diabetic animal.

Methods. Care and preparation of the rats and preparation of the mitochondria (in 0.25 M sucrose) were described previously[1]. Potassium movements were monitored continuously with a Beckman cationic electrode (No. 39137) and an Orion 701 digital pH meter and recorded on a strip chart. The response of the electrode to various incubation medium components and to pH changes was evaluated to confirm that the changes monitored were those due to K^+ levels only. Mitochondria were incubated in 6 ml of medium containing: 100 mM choline chloride, 50 mM Tris HCl (tris(hydroxymethyl)aminomethane), 5 mM Tris succinate, 4 mM KCl, and 6-7 mg of mitochondrial protein/ml with a total osmolarity of 300 mosmoles. After equilibration, 0.5 mM $CaCl_2$ (80 nmoles/mg protein) was added and continuous recordings made for at least 5 min to monitor the changes in medium K^+ after Ca^{2+} addition. It should be noted that *net* K^+ movements are monitored in this system and increased influx and efflux of K^+ is measured only if there is a *net* change in medium content of K^+ due to these movements.

Volume changes in mitochondria as reflected by changes in absorption[4], which have been described as phase 1 or low amplitude changes, were monitored with a Perkin-Elmer Spectrophotometer at 520 nm and recorded on a strip chart. The incubation medium of 3 ml contained 225 mM sucrose, 20 mM Tris HCl, 5 mM succinate and 1 mg mitochondrial protein/ml. These volume changes occur as a normal part of the cycle seen in stimulated and resting mitochondira and are reversible[3]. After equilibration calcium or phosphate was added and the changes moni-