32

1510

URINARY METABOLITES OF SELENIUM IN THE RAT FOLLOWING LOW AND HIGH (^{75}Se)-SELENOMETHIONINE, (^{75}Se)-SELENOCYSTINE AND (^{75}Se)-SELENITE ADMINISTRATION. A. Nahapetian*, M. Janghorbani*, and V. R. Young. Massachusetts Institute of Technology, Cambridge, MA 02139.

Earlier studies have suggested that trimethylselenonium ion (TMSe) is a major metabolite of selenium (Se) in urine. To test this hypothesis, labeled test compounds were administered orally to adult male rats in low (6 ug Se) or high (600 ug Se) doses. Urine was collected for 48 hours. Urinary metabolites were separated by ion exchange chromatography. Mean percent recovery of radioactivity in urine for low Se and high Se test doses were 39% and 32%, respectively. For all the test compounds in the study under high Se intake, 50-60% and 30-40% of total radioactivity in the urine was recovered in TMSe and selenite fractions, respectively. In contrast, under low Se intake, 80-90% of urinary radioactivity was recovered in the selenite fraction, while only 6-7% of the total activity was found in the TMSe fraction. The data suggest that within the physiological range of Se intake, the major urinary metabolite of Se is not TMSe under the conditions of the present study. However, under toxic doses of the trace mineral, TMSe is formed as a means of detoxification.

1511

TRANSMISSION ELECTRON MICROSCOPY AND SELENIUM CONCENTRATIONS IN SELENIUM-INDUCED CATARACT. N.J. Russell*, J.L. Britton* and T.R. Shearer. Departments of Biochemistry and Pharmacology, Oregon Health Sciences University, Portland, OR 97201.

Selenium-induced cataracts are of interest because they provide a convenient animal model for the study of the basic mechanisms of cataract formation. Nuclear cataracts were easily induced by daily injections of 0.50 or 0.75 mg of Se/kg, as Na_2SeO_3, to suckling rats. However, the underlying mechanism for selenium-induced cataracts is unknown, and the purpose of the experiments to be described was to provide a histologic description of selenium-induced cataracts at the ultrastructural level. Preliminary results of transmission electron microscopy of the selenium-induced cataracts revealed extensive vacuolization of the cytoplasmic portion of the nuclear lens fibers. The cytoplasmic matrix appeared to be aggregated along the cell membrane. Cells maintained close apposition to one another, whereas the cytoplasm had lacy appearance. Selenium concentrations in the lens at 8 weeks post partum were approximately 0.5 ppm Se compared to control levels of 0.3 ppm. These results may indicate that excess selenium causes cataracts by water hydration, but the total lens selenium levels do not seem high enough to cause such changes by direct enzyme inhibition. (Partially supported by USPHS Grant #EY-03600.)

1512

CYTOTOXIC ACTIVITY OF SELENIUM COMPOUNDS AND GLUTATHIONE PEROXIDASE ASSESSED BY SCANNING ELECTRON MICROSCOPY. J.E. Spallholz, R. Freitas*, M.L. Hu* and J.H. Whitcomb*. Department of Food and Nutrition, Texas Tech University, Lubbock, TX 79409 and the Forrest C. Shaklee, Sr. Research Laboratories, Shaklee Corporation, Hayward, CA 94540.

Recognition of the cytotoxic property of some selenium compounds and glutathione peroxidase (GSHPx) prompted a visual assessment of cellular damage by scanning electron microscopy (SEM; Hitachi Model-450). Rat erythrocytes (RBC), Saccharomyces cerevisiae (Sc), Bacillus subtilis (Bs) and Escherichia coli (Ec) were incubated for 3 hrs @22°C in PBS buffer pH 7.4 with glucose, PBS with glucose and glucose oxidase (H_2O_2 system), and with sodium selenite (0.8 mM), selenocystine (0.2 mM), or GSHPx (42 ug/ml; Toyobo, Japan) each containing the H_2O_2 system. After 1, 2 and 3 hrs, cells were directly fixed in Saline containing 5% sucrose and 2% glutaraldehyde. After 3 days @4°C, cells were air dried on glass cover slips, dehydrated in ethanol-amylacetate solutions, dried in liquid CO_2, fixed to aluminum studs and gold sputtered (160°A) for SEM. Electron micrographs reveal little damage to control RBC but progressive damage to cells exposed to H_2O_2. Progressive and extensive cellular degradation is noted for RBC exposed to H_2O_2 along with selenocystine and GSHPx but not sodium selenite. The same pattern of progressive cellular damage was observed for Bs, Ec and to a lesser extent Sc cells. (Supported by the Robert A. Welch Foundation, Grant No. D-845, The Shaklee Corporation and in part by Toyobo NY, Inc)

1513

CHARACTERIZATION AND PROPERTIES OF A NEW SELENIUM-INDEPENDENT GLUTATHIONE PEROXIDASE (GSH-Px) FROM MOUSE CARDIAC MITOCHONDRIAL INTERMEMBRANE SPACE. Aspandiar Katki*[1], Rolf Zeisler*[2], and Charles Myers*[1]. (SPON: K.W. Kohn). [1]CPB, NCI, Bethesda, MD 20205, and [2]IARD, CAC, NBS, Washington, DC 20234.

Previously we reported the presence of a membrane-bound GSH-Px which is independent of dietary selenium. This new Se-independent GSH-Px can be purified using blue sepharose followed by DE-52 ion exchange chromatography. Purified material elutes from DE-52 column at ~2.1 M NaCl (fraction H) and absorbs only at 230 nm and not at 260 nm or 280 nm, and thus has many characteristic properties of a histone. It is free of Se as determined by neutron activation analysis and uses H_2O_2, linoleic hydroperoxide, DNA-hydroperoxide, RNA-hydroperoxide, and thymine hydroperoxide in addition to cumene and t-butyl hydroperoxides. Thus, it appears to differ from the glutathione-S-transferase. Fractions B, C and D eluted from DE-52 column at ~0.02-0.03 M NaCl had 260nm:280nm ratios suggesting 12-14% nucleic acid, show hyperchromic shift, heat stability 100°, and show increased enzymic activity when incubated with DNAase and trypsin. Iso-electric focusing of the pooled fraction shows the activity is at pI 9-9.5. On SDS-polyacrylamid gels, fractions B, C, D and fraction H, which contains just the enzyme, yield identical patterns with 2 bands (~70,000 and ~75,000 daltons). This histone-like protein not only is located in the membrane but is able to bind nucleic acids and utilize metabolically occurring hydroperoxides, thus controlling free radical damage.

1514

SELENIUM AND SELENIUM DEPENDENT GLUTATHIONE PEROXIDASE IN SICKLE CELL ANEMIA. Danny Chiu, Irene Byrne, and Bertram Lubin. Bruce Lyon Mem. Res. Lab., Children's Hospital Med. Ctr., Oakland, CA 94609.

We previously reported that glutathione peroxidase (GSH-PX) activity was elevated in sickle red cells (J. Lab. Clin. Med. 94:542, 1979.). However, the selenium (Se) status in patients with sickle cell disease has not been determined. Using a modified fluormetric method with 2,3-diaminoaphthalene, we determined Se status in sickle cell patients. Plasma Se levels in 52 sickle cell patients (90±17 ng/ml, mean ± S.D.) were significantly lower than that of controls (107 ± 10, n = 17). In contrast, red cell Se levels were significantly higher in sickle cell patients (361 ng/ml) than in controls (278 ng/ml). The elevated RBC Se levels in sickle cell patients was accompanied by an increased GSH-Px activity sickle erythrocytes. Only Se-dependent GSH-Px was detected in both normal and sickle RBC's. When sickle erythrocytes were separated into top (recticulocyte rich), middle (matured RBC) and bottom (irrevibly sickled cells) fractions, no significant difference in GSH-Px activity was observed between these subpopulations thus suggesting that elevated GSH-Px activity in sickle erythrocytes is not an age-dependent phenomenon. We interpret that elevated Se level and Se-dependent GSH-Px activity in sickle RBC's are a compensatory mechanism to enhanced peroxidative stress in these cells. (Supported in part by a grant-in-aid from Hoffmann-LaRoche, Inc.)

1515

Effects of Sodium Selenite and Selenomethionine on Tissue Selenium and Glutathione Peroxidase Activity in Hamsters. A.D. Julius and D.F. Birt (SPON: J.L. Smith). Eppley Institute for Research in Cancer, Univ. Nebraska Med. Ctr., Omaha, NE 68105.

The relative effects of sodium selenite (SS) and selenomethionine (SM) on blood and tissue selenium and glutathione peroxidase (GSH-Px) activity in hamsters were compared. Six groups, consisting of five males and five females, were fed torula yeast-based diets supplemented with either 0.1, 5.0, or 10.0 ppm Se as either SS or SM for three weeks. Blood and tissue Se concentrations increased with increasing dietary Se for all tissues measured except for heart muscle. No differences in Se concentrations between SS and SM fed hamsters occurred in blood, pancreas, muscle or heart. However, tissue Se concentrations were increased in liver, kidney, and lungs of hamsters fed SM supplemented diets. GSH-Px activity was not effected by the source or level of Se in the plasma or pancreas. Erythrocyte GSH-Px activity increased with increasing dietary Se with no difference between SS and SM groups. Liver GSH-Px activity was increased in SM fed hamsters but did not increase with increased dietary Se. These results suggest a difference by Syrian hamsters in utilization and metabolism between Se-containing compounds. (Supported by grant RO1 CA24549-03 from the National Cancer Institute)

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Abstracts 1–3477

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32 REF