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EFFECT OF TREATMENT WITH PHENOBARBITAL AND SPIRONOLACTONE ON ^{144}Ce LEVELS IN BLOOD, LIVER, URINE AND FAECES AT VARIOUS TIME PERIODS

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Abstract—The influence of pre-treatment with phenobarbital and spironolactone and pre- and post-treatment with phenobarbital on cerium concentration in liver, blood, urine and faeces after a sublethal intravenous injection of cerium chloride (5·5 mg/kg) was investigated in male and female mice at various time periods after the injection. About 70 per cent of the administered dose accumulated in the liver during the first day after the injection. More cerium accumulated in the livers of phenobarbital-treated males and females and spironolactone-treated males than in spironolactone-treated females and controls during the first hours after the injection. However, neither compound was found to alter the total amount of cerium accumulated in the liver on the first, second, third, sixth and fourteenth day after the injection. The amount of cerium accumulated in the livers of untreated animals at 6 hr and 1 day after the injection was higher in females than in males. Treatment with phenobarbital and spironolactone increased the liver weight and decreased the hepatic cerium concentration in treated animals. The decrease in hepatic cerium concentration was greater in phenobarbital-treated than in spironolactone-treated animals. The hepatic cerium concentration was higher in females than in males. The concentration of cerium in blood serum was lower in treated animals during the first 3 days after the injection. The amount of cerium excreted in the faeces during the first, second and third day after the injection was higher in phenobarbital- than in spironolactone-treated animals. Only small amounts of the injected cerium were excreted in the urine and no differences between treated and untreated animals were found.

CERIUM and certain other light lanthanons, when administered intravenously to rats, produce fatty infiltration and hepatic necrosis.[1–3] Magnusson[3] observed that about 70 per cent of an intravenous cerium injection localized in the rat liver within 3 hr and that the drop in the liver concentration of the cerium was slow. Magnusson also found that cerium caused changes in the structure of the endoplasmic reticulum of hepatic cells.

Phenobarbital[4] and spironolactone,[5–7] two known enzyme inducers, have been found to prevent the development of certain pathological lesions caused by various toxic compounds. In our laboratory we found that pretreatment with phenobarbital[8] for 11 days and with spironolactone[9] for 3 days decreased acute lethal toxicity in mice caused by cerium chloride, and that pre-treatment with these compounds decreased the hepatic fatty changes and necrosis induced by cerium chloride.

The present study aims at clarifying whether spironolactone pre-treatment and phenobarbital pre- and post-treatment affect the accumulation of cerium in the liver and if the excretion of cerium via urine and faeces is speeded up by these two drugs.

MATERIALS AND METHODS

Chemicals. The cerium isotope (^{144}Ce) used in this study was delivered by The Radiochemical Centre (Amersham) as an aqueous cerium chloride solution. Phenobarbital and spironolactone were obtained from Lääke Oy.

Treatment of animals. A total of about 270 male and female NMRI mice were used in the experiment. The initial weight for females was about 28 g and for males 32 g and the age about 3 months. The animals received normal laboratory pellet food and water *ad lib.* throughout the experiment.

Males and females were divided into four experimental groups. One received sodium phenobarbital in drinking water (0·05 per cent solution) for 11 days before the injection of cerium chloride. The second group received the same solution for 11 days before and then after the injection until decapitation. The third group received spironolactone (100 mg/kg) suspended in 2 per cent carboxymethylcellulose twice a day by gastric intubation for 3 days before the injection of cerium chloride. The fourth group served as a control.

All animals received a sublethal intravenous injection (5·5 mg/kg) of $CeCl_3$ in physiological saline solution as a single injection in the tail vein. The pH of the solution was 2·8–3·0 and the administered radioactivity about 1 μCi per animal.

The animals were decapitated 1 hr, 6 hr, 1 day, 2 days, 3 days, 6 days and 14 days after the injection of cerium and blood samples were collected from all animals. The livers were weighed and pieces of 100–200 mg were cut out from the central lobe for radio assay.

For urine and faeces collection male and female animals were kept, for the first, second, third, sixth and fourteenth day, in cages which permitted separate collection of faeces and urine. The faeces samples were dried at 105° for 48 hr and thoroughly homogenized. About 50 mg of the dried samples were taken for radio assay. For radio assay of urine, the cages were rinsed with 1 N HCl and the cage washing and the urine samples were combined.

Counting techniques. The liver and faeces samples were solubilized in 2 ml Protosol (NEN Chemicals) at 55° for 24 hr in counting vials. The counting vials were allowed to cool and 12 ml of a toluene-based scintillation mixture was added. The final counting solution contained 4·2 g/l of 2·5-diphenyloxazole (PPO) and 0·09 g/l of *p*-bis-(*o*-methylstyryl)-benzene (bis-MSB). Blood serum and urine (100–500 μl) were pipetted into counting vials and 10 ml Aquasol (NEN Chemicals) was added.

All samples were counted in a liquid scintillation counter (LKB-Wallac 8100) with the discrimination levels set at 2 and 2000 keV. Appropriate dilutions were made from the injection solutions and aliquots of these standards were used as internal standards when the samples were corrected for quenching.

RESULTS

The concentration of cerium in blood serum is shown in Figs. 1 and 2. The elimination of cerium from the blood is fast. One day after the injection the content of cerium in 200 μl serum is less than 1 per cent of the administered dose. According to Aeberthardt[10] the concentration of cerium in serum should be in close agreement with that found in whole blood. It can be seen that phenobarbital speeded up the elimination of cerium in both males and females. In spironolactone-treated animals

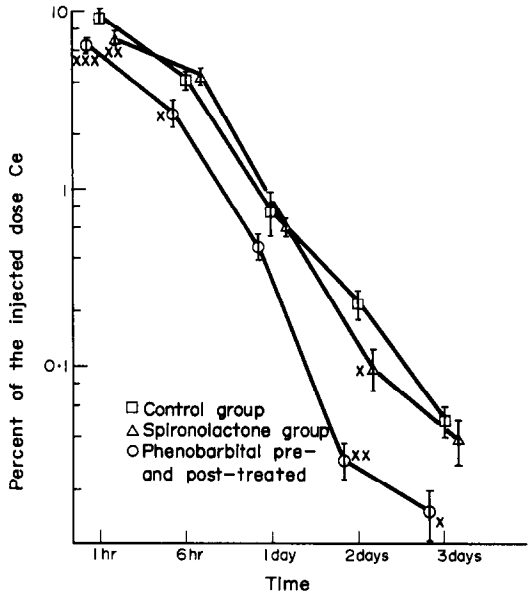

FIG. 1. Concentration of cerium as per cent of the given dose in 0·2 ml of serum in male mice. The results are given as mean ± S.E. (n = 4–6). Statistical analysis was carried out using Student's *t*-test. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

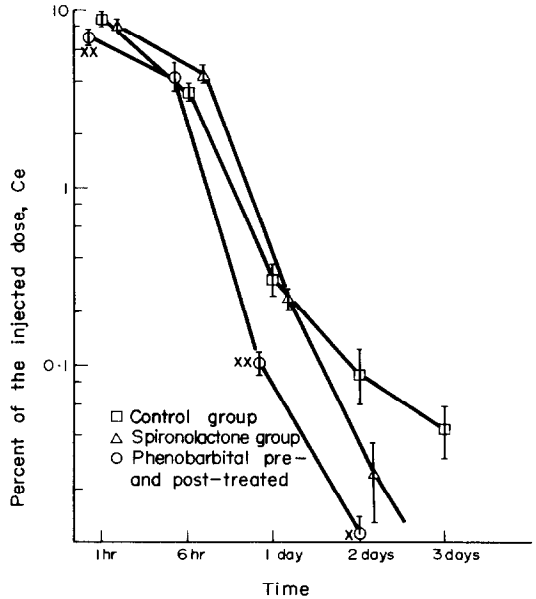

FIG. 2. Concentration of cerium as per cent of the given dose in 0·2 ml of serum in female mice. The results are given as mean ± S.E. (n = 4–6). Statistical analysis was carried out using Student's *t*-test. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

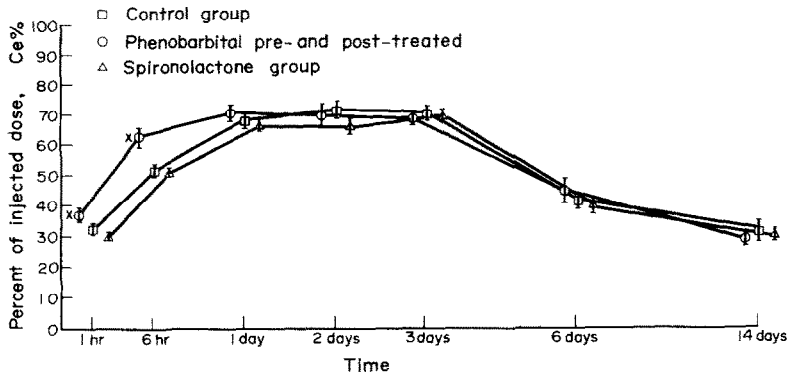

FIG. 3. Amount of cerium in the liver as per cent of the given dose at various time periods in male mice. Statistical analysis was carried out using Student's *t*-test. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

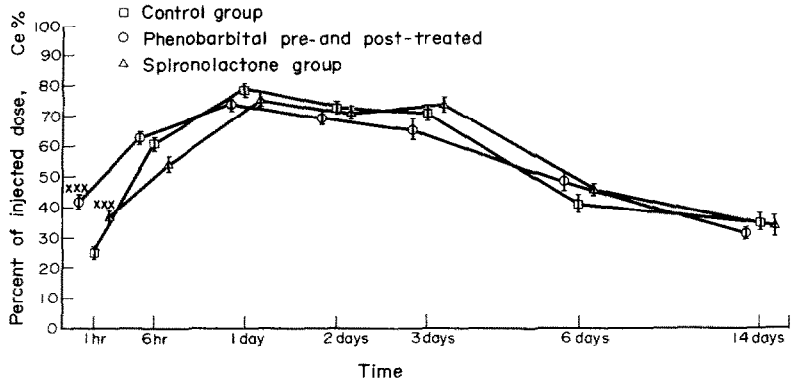

FIG. 4. Amount of cerium in the liver as per cent of the given dose at various time periods in female mice. Statistical analysis was carried out using Student's *t*-test. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

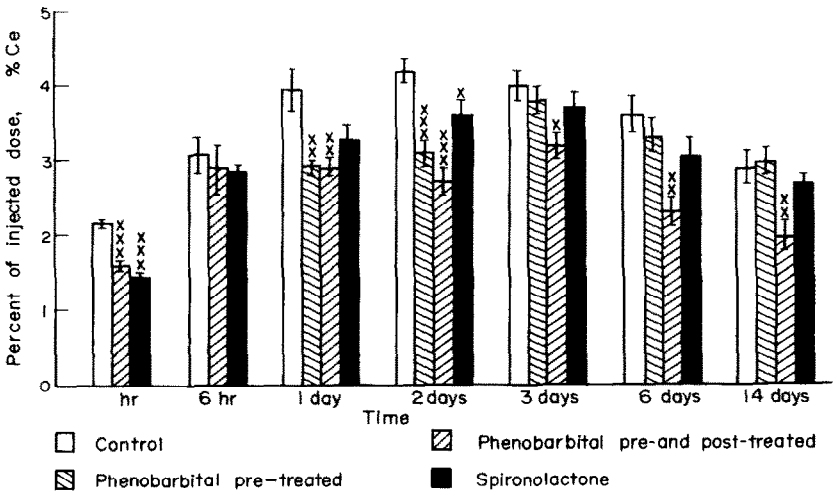

FIG. 5. Concentration of cerium in per cent of the injected dose, in 100 mg of male mice liver at various time periods. Statistical analysis was carried out using Student's *t*-test. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Bars at the top of each column indicate ±S.E.

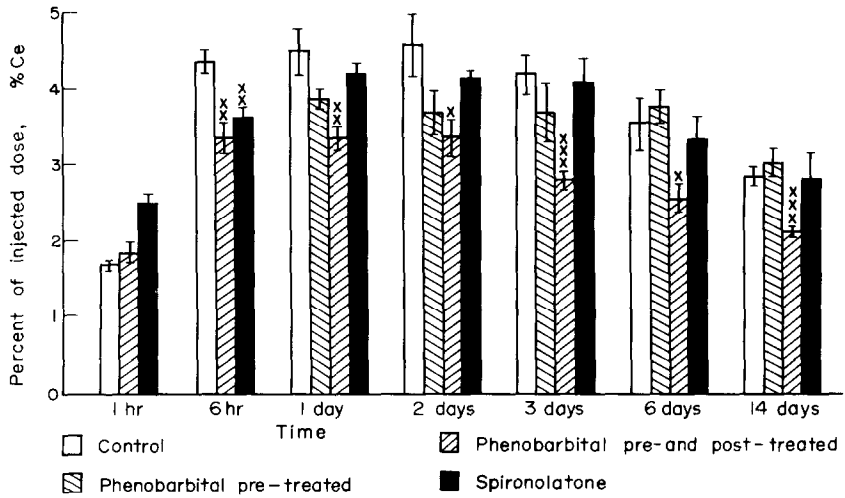

FIG. 6. Concentration of cerium in per cent of the injected dose, in 100 mg of female mice liver at various time periods. Statistical analysis was carried out using Student's *t*-test. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Bars at the top of each column indicate ± S.E.

a significantly lower blood concentration was found only during the first hour and second day after the injection in male mice, and during the third day in female mice.

Figures 3 and 4 show the amount of cerium in whole livers. Phenobarbital pre-treated animals did not differ from phenobarbital pre- and post-treated animals and are not presented in the figures. The livers of phenobarbital-treated males and phenobarbital- and spironolactone-treated females contained more cerium during the first hour after the injection. However, there were no differences between treated and untreated animals at 1–14 days after the injection.

The hepatic cerium concentration at the various time periods is shown in Figs. 5 and 6. The concentration of cerium in the liver tissue of phenobarbital pre- and post-treated males and females was lower than in control animals throughout the experiment. Only pre-treatment with phenobarbital decreased the cerium concentration but to a lesser extent than pre- and post-treatment. Pre-treatment with spironolactone also decreased the hepatic cerium concentration but the drop in concentration was smaller than in pre-treatment with phenobarbital.

Because phenobarbital and spironolactone increased the liver weights, the differences in hepatic cerium concentration were looked at in view of the changes of the liver weight. Figures 7 and 8 show the liver weights and the hepatic cerium concentration of treated animal's compared to controls. It can be observed that an increase of the liver weight is paralleled by a corresponding decrease in hepatic cerium concentration. It can further be seen that the relations between the hepatic cerium concentration of pre-treated and untreated animals alter during the experiment so that the concentration at the end of the experimental period is the same in pre-treated and untreated animals. However, the animals receiving phenobarbital throughout the experiment had a lower hepatic cerium concentration throughout the experiment.

The excretion of cerium via faeces is given in Figs. 9 and 10. Phenobarbital treatment increased the faecal excretion of cerium, the effect being more pronounced in the pre-

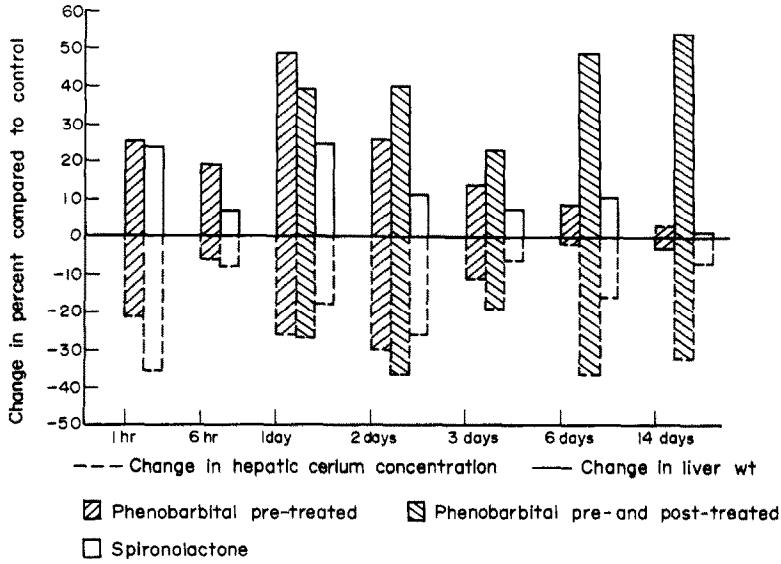

FIG. 7. The liver weight and hepatic cerium concentration in treated male mice compared to control animals. The calculation is based on the means of the groups and values are expressed as per cent of respective control group.

and post-treated groups. Spironolactone had no effect on the faecal excretion of cerium. This suggests that they may act as liver inducers in a different way.

Only small amounts of the injected dose were excreted in the urine, less than 1 per cent of the given dose during the first three days after the injection. It cannot be excluded that part of this amount represents contamination from feces. No differences in the urinary output of cerium were observed between the various groups.

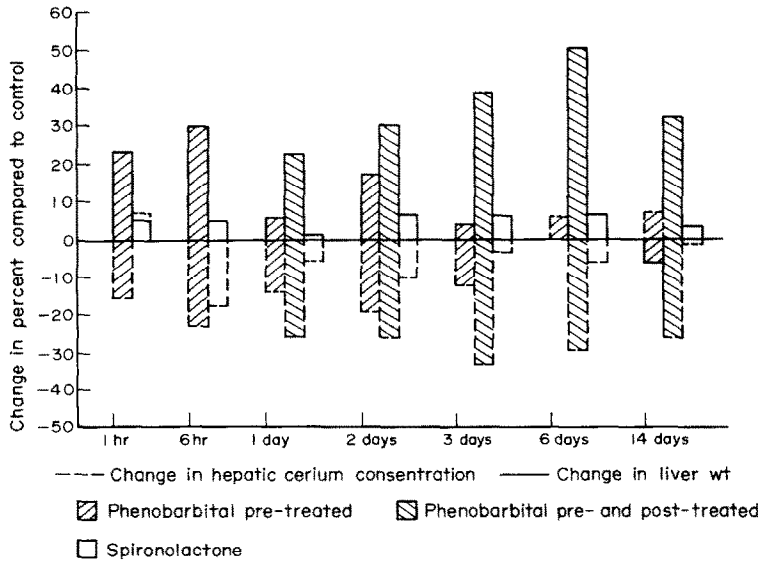

FIG. 8. The liver weight and hepatic cerium concentration in treated female mice compared to control animals. The calculation is based on the means of the groups and values are expressed as per cent of respective control group.

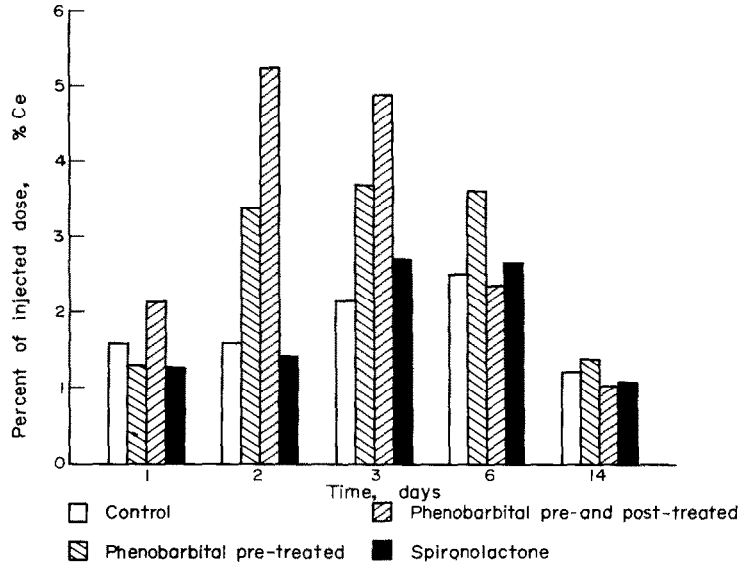

FIG. 9. Faecal excretion of cerium in per cent of the injected dose at various time periods in male mice. Faeces was collectively collected from 4 to 6 animals.

Sex differences in toxicity of cerium to rats have been reported by several authors[1,3,12] who found that fatty degeneration of the liver is confined to females. In mice fatty degeneration has been observed in both sexes, but to a lesser extent in males when the same dose (7·5 mg/kg $CeCl_3$) was given to males and females.[8] Comparisons between males and females in this study yield several significant differences. The amount of cerium accumulated in the livers of untreated animals 6 hr and 1 day after the injection was higher in females than in males. Females had a lower

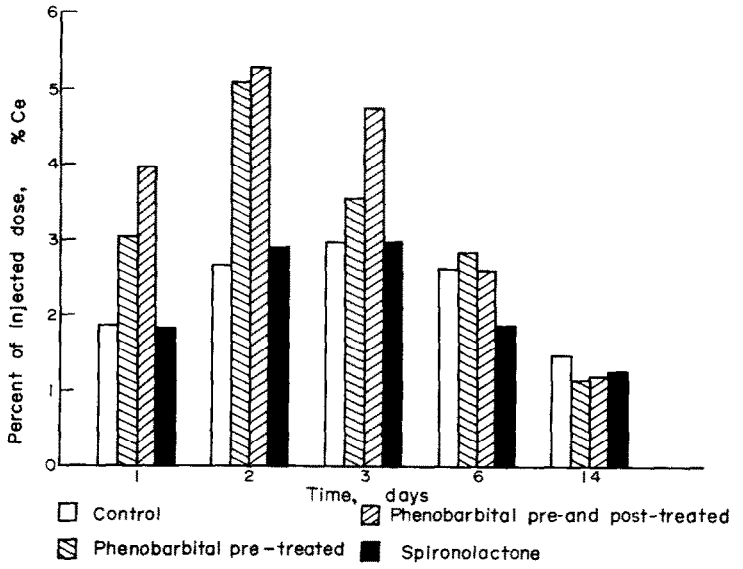

FIG. 10. Faecal excretion of cerium in per cent of the injected dose at various time periods in female mice. Faeces was collectively collected from 4 to 6 animals.

hepatic cerium concentration than males. The concentration of cerium in blood was lower in females on the first, second, third, and sixth day after the injection.

DISCUSSION

The major deposition site after an intravenous injection of cerium is the liver. According to previous studies[2,3] about 70 per cent of an intravenous injection is deposited in the liver during the first day after the injection. In large doses cerium causes fatty infiltration and necrosis in the liver. A change in the structure of the endoplasmic reticulum of liver cells has been observed[2] and an inhibition of drug metabolism in rat liver has been established by Arvela and Kärki[12] who found that phenobarbital impairs the inhibitory effect of cerium on drug metabolism.[13] In our previous studies we have observed that both phenobarbital and spironolactone decrease the acute toxicity of cerium and liver damage in mice.[8,9] In this study we were interested in finding whether this effect was due to a decreased accumulation of cerium in the liver.

Both phenobarbital and spironolactone increased the uptake of cerium by the livers of females during the first hour after the injection. In males this effect was observed only in phenobarbital treated animals. In phenobarbital treated animals the increased uptake by the liver was parallel to a decrease in concentration of cerium in blood. Pretreatment with phenobarbital increased the liver weight. At the time of the injection there was a rise of 20–30 per cent in the liver weight in phenobarbital pretreated animals. This may, perhaps, reflect the greater amount of cerium initially accumulated in the livers of treated animals. However, during the rest of the experimental period there were no differences between the total amount of cerium accumulated in the livers of treated and untreated animals though the rise in the liver weights of phenobarbital pre- and post-treated animals persisted.

Phenobarbital, but not spironolactone, increased the faecal excretion of cerium. Phenobarbital treated animals had a lower concentration of cerium in blood during the first, second and third day after the injection and it is possible that this is due to the increased excretion. However, the increase in the faecal excretion is not sufficient to link the decrease in toxicity of cerium to phenobarbital treated animals.

Treated animals had a lower concentration of cerium in the liver tissue. It was found that with an increase in the liver weight caused by phenobarbital and spironolactone there was a corresponding decrease in the hepatic cerium concentration. It cannot be decided from this study if this decrease in cerium concentration actually is due to an influence of the drugs on the binding of cerium in liver tissues. It is possible that a part of the weight increase, induced by the drugs, is passive in respect of cerium binding. If this is the case it would declare the inverse relationship between liver weight and hepatic cerium concentration.

In previous investigations we found that phenobarbital and spironolactone decreased the liver damage caused by cerium[8,9] and that cerium was more toxic to female mice.[11] In this study it was found that treated animals had a lower hepatic cerium concentration and that the concentration was higher in females than in males, but it cannot be determined from this study whether this decrease in hepatic cerium *per se* is responsible for the decrease in toxicity. Earlier investigations have shown that cerium causes changes in the liver endoplasmic reticulum[3] and impairs the activity of the drug-metabolizing enzymes.[14] Arvela and Kärki[14] suggest that phenobar-

bital has a protective effect against cerium-induced alternations of the endoplasmic reticulum and that this protective effect is due to an increase of the phospholipid content in liver microsomes. Spironolactone has been shown to increase the hepatic microsomal enzyme activity[1,5] and to increase the smooth endoplasmic reticulum in liver cells.[2] Cerium is known to be more toxic to female rats, and Magnusson[3] found in distribution studies of liver cell fractions a higher amount of cerium in the microsomal fraction in female rats. It is thus tempting to presume that phenobarbital and spironolactone decrease the toxicity of cerium by effecting the binding of cerium to the endoplasmic reticulum in liver cells. Studies to test this assumption are currently in progress in our laboratory.

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