SUBCELLULAR DISTRIBUTION OF ^{239}Pu IN THE LIVER OF RAT, MOUSE, SYRIAN AND CHINESE HAMSTER

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It is well known that the biological half life of transuranium elements in the liver of mammalian species varies from a few days to many years (ref. s.1). However, the reasons for these differences are unknown. The aim of our studies is to elucidate the biochemical mechanisms responsible for these species differences as a part of our attempt to improve risk estimation following incorporation of transuranium elements into man through a profound understanding of the biochemistry of these elements. We have chosen rats and mice as models for a short and two hamster species as models for a long biological half life of Pu-239 in liver.

METHODS

The animals used were adult females of the following strains: Rats (Heiligenberg strain), mice (NMRI), Syrian hamsters (commercial strain) and Chinese hamsters (Breeding stock of Institute for Zoology, Darmstadt). Radiochemically pure Pu-239- and Fe-59-citrate injection solutions were injected i.v. (5-10 µCi/kg Pu-239, 50 - several hundred µCi/kg Fe-59). On the sixth day 750 mg/kg of the non-ionic detergent Triton WR1339 were given i.p. and sacrifice took place on the tenth day. Triton WR1339 causes a shift of the density of the lysosomes from $\sim$ 1.2 to $\sim$ 1.1 g/cm^3 and any lysosomally-associated material can be recognized by a parallel shift. Control animals received 0.9 % NaCl. After differential centrifugation of liver homogenates, a fraction designated MLP was obtained, which contained most of the formed cell elements except the nuclei. Aliquots of this MLP fraction were centrifuged in a linear sucrose density gradient for 4 or 16 hours at 88 000 av. g. Radioactivity and marker enzymes (s. Figures) were determined in all fractions obtained after isopycnic centrifugation. Details of the methods are described elsewhere (2).

RESULTS AND DISCUSSION

The MLP fraction contained more then three quarters of the total cellular radioactivity. The results obtained by isopycnic centrifugation of that fraction are shown in Figs. 1 and 2. Certain results are common to all species: The extent to which the profile of the lysosomal marker, acid phosphatase, is shifted to light densities by injecting Triton WR1339 is very similar. The profile of glutamate dehydrogenase (mitochondria) is not influenced by Triton WR1339 and

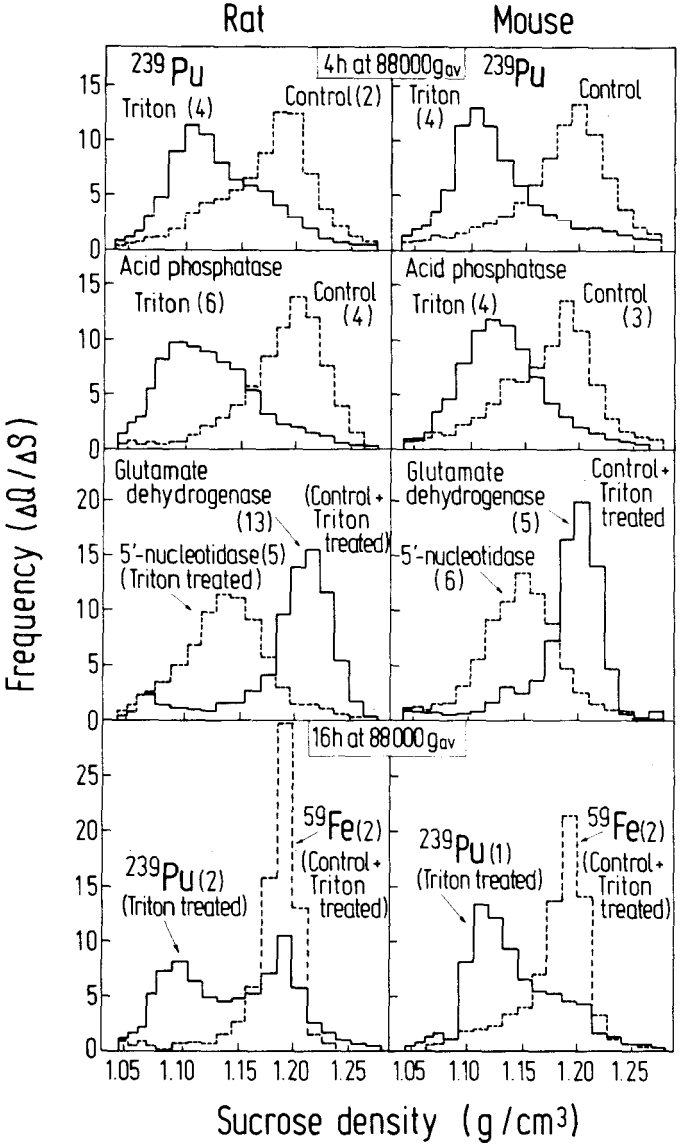

Figure 1. Distribution of radioactivity and marker enzymes (s.text) after centrifugation of the MLP fraction from liver cells in a sucrose density gradient. ΔQ: fractional amount of constituent found in that section; $\Delta\rho$: density increment from one fraction to the other. The area under each section is proportional to the fractional amount and the total area is one. Values in brackets represent number of experiments.

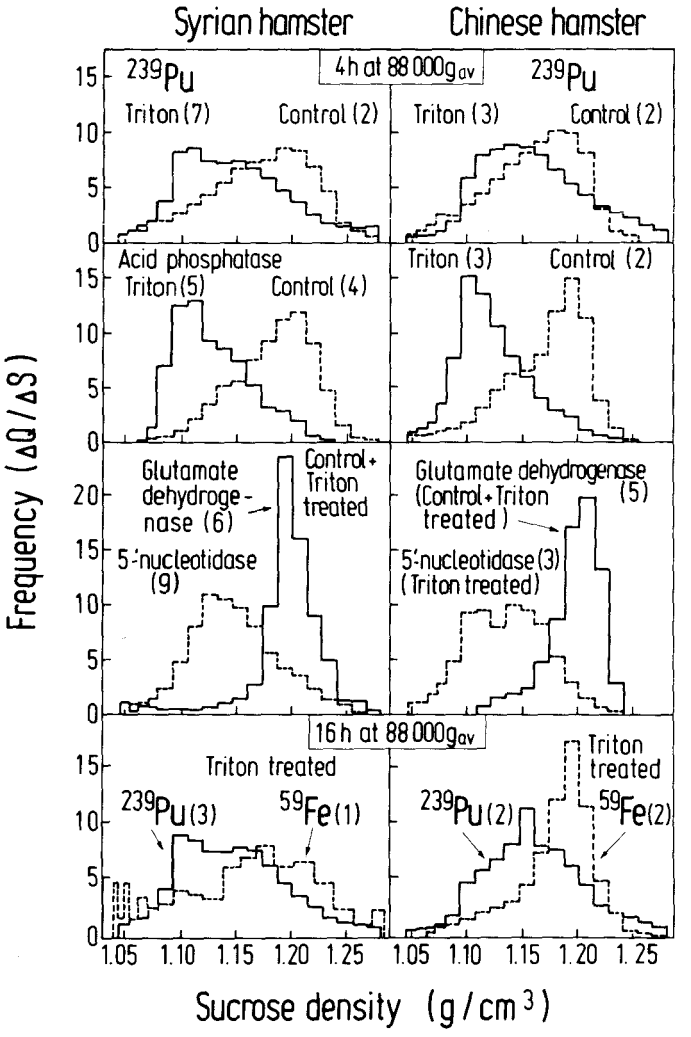

Figure 2. Distribution of radioactivity and marker enzymes after centrifugation of the MLP fraction from liver cells in sucrose density gradients. For presentation s. Fig. 1.

data for controls and treated animals could be combined. On the other hand, the Pu-profiles are considerably broader and the fraction which is unequivocally shifted is considerably smaller in both hamster species as compared to rats and mice (upper row of Figures). In Chinese hamsters, the peaks of Pu-239 and acid phosphatase after Triton treatment are not even congruent; a distinct shoulder at $\rho \sim 1.5$ g/cm^{3}is visible in the Pu profile for Triton treated Syrian hamsters. With a centrifugation time of 16 instead of 4 hours (lower row of Figures) the profiles of the marker enzymes remain at virtually the same density. Again clear discrepancies between the Pu-profiles of the various species occur. In rats, and to a lesser degree also in mice, a second peak develops at the same density at which Fe-59 equilibrates. The profile for Pu in Chinese hamsters becomes unimodal with a peak at ~ 1.5, where a minimum exists in the corresponding profile for rats. The clear relationship to the Fe-profile seen in rats was not observed in Chinese hamsters. At this point it should be mentioned that we have seen only minor, or even no, differences between the Fe-profiles from control or treated animals, which, therefore, could be combined for presentation. There is no reason to assume any important association of Pu with plasma membranes, represented by 5'-nucleotidase, in rats, mice and Syrian hamsters. However, the role of the plasma membrane needs to be clarified by further experiments, especially in Chinese hamsters.

If the parallelism between the shift of Pu-239 and acid phosphatase is taken as a measure for the extent of lysosomal binding, there is clear evidence for association with these organelles for rats, mice and to some degree, also for Syrian hamsters. However, the data for Chinese hamsters are equivocal, at least at the tenth day, and it is possible that neither lysosomes nor iron-binding proteins are major sites of fixation in this species. It is interesting to note that the indications for lysosomal association are unequivocal in both rats and mice, the species with rapid Pu elimination, whereas these organelles appear to become less important in the species with longer retention, Syrian and especially Chinese hamster. However, investigations of the changes with time in the subcellular distribution and on the exact nature of the binding components for Pu-239 in the livers of all species, but especially of Chinese hamsters, are needed before further conclusions can be drawn.

REFERENCES

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