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**Isolation of mouse isometallothioneins : A comparison of  
isometallothioneins in growing cells  
and post-mitotic cells.**

Shizuko KOBAYASHI and Junko SUZUKI

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As we were interested in the relationship between cell growth and MT synthesis, we carried out an analysis of isoMTs in neonatal-mouse liver, and in Zn-, Cd- or glucocorticoid-treated mouse tumour cells, using an anion-exchange h.p.l.c. column and the results were compared with those for metal-treated adult liver. In the present paper, we describe the findings obtained, which suggest that MT-2 has a relationship with glucocorticoid in growing cells.

The h.p.l.c. instrument consisted of a chromatograph (Shimadzu HPLC CL-6A Gradient System; Shimadzu Co., Kyoto, Japan) and an anion-exchange chromatography column (Asahipak ES-502N,  $13 \pm 0.5 \mu\text{m}$  particle size;  $7.6 \text{ mm} \times 100 \text{ mm}$  column; Asahi Chemical Industries Co., Kawasaki, Japan). A  $10 \mu\text{l}$  portion of the concentrated MT fraction obtained from gel filtration, containing  $0.1\text{--}1.0 \mu\text{g}$  of metal, was applied to the column and eluted with  $4 \text{ mM}$ -potassium phosphate buffer, pH 7.5, at a flow rate of  $0.5 \text{ ml/min}$ . Subsequently, the sample was eluted with the same buffer for 10 min and then with a linear gradient of  $4\text{--}52 \text{ mM}$  potassium phosphate buffer, pH 7.5, for 30 min at  $29 \text{ C}$ , and the  $A_{220}$  was determined with a Shimadzu SPD-6A u.v. detector. The peak fractions detected by the  $A_{220}$  were collected with a Frac-100 (Pharmacia Fine Chemicals, Uppsala, Sweden) by switching them from the u.v. monitor, and heavy-metal concentrations were determined by atomic-absorption spectrometry.

Mouse metallothioneins (MTs) were separated into three isoforms by an anion-exchange h.p.l.c. column; conventionally isolated MT-1 and MT-2 showed a single peak (MT-1-1) and two peaks (MT-2-1 and MT-2-2), respectively. In growing cells, developing hepatocytes and growing tumour cells, MT-1/MT-2 ratios were less than 0.6, irrespective of the type of MT inducer, whereas adult liver post-mitotic cells had a ratio of more than 1.0. A large amount of the MT-2-2 subfraction was found in dexamethasone-treated FM3A cells; 90% of MTs was MT-2-2, suggesting that glucocorticoid hormone mainly induces MT-2-2 in tumour cells.