

EFFECT OF DIFFERENT TECHNIQUES ON THE DISTRIBUTION OF TISSUE
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Several techniques can be used to extract and chromatograph copper (Cu) binding proteins from liver and kidney samples. The tissue sample may be stored at -20°C for varying periods of time before extraction and chromatography. Alternatively the tissue can be extracted immediately, the extract then being stored at -20°C before chromatography. A reducing substance such as 2-mercaptoethanol (ME) may also be added to the extracting buffer. Preliminary investigations showed that the profile of Cu-containing fractions obtained with sephadex G-75 chromatography was affected by the extraction procedure, and the stage at which samples were frozen. A series of experiments were thus undertaken to quantitate these effects and to examine the mechanisms involved.

Fresh liver or liver stored at -20°C was homogenised in 2.3 to 3.0 volumes of 10 mM tris-acetate pH 7.4. In some cases 1% ME or ^{35}S -labelled metallothionein (MT) was added to the homogenising buffer. Supernatant (SN) extracts were obtained by centrifugation of the homogenate at 105,000 xg for 1 h. Sephadex G-75 chromatography was performed using an eluant of 10 mM tris-acetate pH 7.4 as described by Allen and Gawthorne (1985). ^{35}S -labelled MT was prepared by sephadex G-75 chromatography of a SN extract derived from the liver of a rat which had been injected with Cu and ^{35}S -cysteine. The concentration of Cu in column fractions was measured directly by atomic absorption spectrophotometry and the radioactivity was determined by liquid scintillation counting.

Three Cu-containing peaks were observed in chromatographs, namely a high MW peak, a medium MW peak possessing Cu-superoxide dismutase activities and a low MW peak containing Cu-MT. Radioactivity was predominantly located in the MT peak but a minor peak of radioactivity was also observed in the high MW peak.

In the case of liver samples stored at -20°C prior to homogenisation a greater proportion of Cu was extracted into the SN. A greater proportion of this Cu was present in the high MW peak and a reduced proportion in MT. The specific radioactivities of the high MW peak and of MT indicated that the extra amount of Cu present in the former peak was not due to complex formation with ^{35}S -labelled Cu-MT.

Freezing of the SN extract prior to chromatography resulted in a slightly lower extraction of Cu in the final SN because a precipitate containing Cu and radioactivity formed during thawing. However the chromatographic distribution of Cu was not markedly affected. When ME was added to the homogenising buffer the degree of extraction of Cu was greater and a large proportion of this Cu was MT. The effect may be attributed to decreased oxidation of thiol groups in MT. The oxidation of such groups has been associated with reduced affinity for Cu (Geller and Winge 1982). Alternatively, ME may have enhanced the reduction of Cu to Cu(I) a process which is thought to be necessary for incorporation of Cu into MT (Suzuki and Maitani 1981).

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