

## 2D -NEPHGE

Two-dimensional non-equilibrium pH gel electrophoresis (2D-NEPHGE) analysis was used to evaluate the effects of dietary protein depletion on the protein composition of mouse liver cytosol. Analysing the cytosol from both normal and protein depleted liver, the position in gels of more than three hundred protein spots was determined. After 5 days of protein depletion, about 20% of the spots either increased or decreased more than 2 fold. Five spots of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were recognised by specific antibodies. The glutathione S-transferase (GSTs) subunits Ybl, Yc and Yf were identified by the simultaneous analysis of both glutathione-binding cytosolic proteins and the corresponding standards. As estimated by internal optical density (IOD) of spots, the changes caused by protein depletion in GAPDH and GST subunit contents were similar to those obtained by other methods. By means of mass spectrometric analysis of tryptic peptides generated from spots and/or comparison of two-dimensional gel electrophoretic patterns, carbonic anhydrase III (CAIII), Cu, Zn superoxide dismutase (CuZnSOD) and a cytochrome P450 cytosolic protein (cyt P450) were identified. These three proteins, as well as GSTs, are related with intracellular detoxification and free radical scavenging systems. Their contents were regulated by dietary protein restriction in a manner indicative of diminished liver defence against oxidising agents.