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Measurements of Macromolecule-Bound and Ultra-Filtrable Polyamines in Rat Liver Homogenized without Buffer

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Ultra-filtrable and macromolecule-bound polyamines in rat liver homogenates, made without buffer, were determined, using Potter-Elvehjem homogenizer and commercially available, pressure-aided ultrafiltration device with a membrane pore size that allows passage of particles of molecular weight no larger than 5000. About 90% of polyamines in the liver were shown to be equilibrated with externally added ^{15}N-labeled polyamines, based on the difference in the ratio of the natural to ^{15}N-labeled polyamine in the liver homogenate and the ultrafiltrate. The entire amount of ultrafiltrate in the homogenized liver, required for calculation of the amounts of ultra-filtrable and macromolecule-bound polyamines, was estimated to be about 0.25 g in one gram of the homogenate, using a limited dilution curve of spermine in the ultrafiltrate with phosphate buffered saline and distilled water. With this value, ultra-filtrable polyamines in normal rat liver homogenate were calculated as about 25%, 8%, and 2% of the total amount of putrescine, spermidine, and spermine, respectively. The method was then used to measure ultra-filtrable and macromolecule-bound polyamines in regenerating rat liver homogenates, to examine possible changes of polyamines during cell growth. The method was also applied to measure other ultra-filtrable compounds such as amino acids and inorganic ions in rat liver homogenate.