

# Determination of endogenous middle molecules in normal and uremic body fluids

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## Abstract

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Methods have been developed which allow the separation and quantitation of middle molecules in biological fluids. The methods involve a newly developed high speed gel filtration technique (HSGF) combined with gradient ion exchange chromatography (GEC). The solutes were detected at 254 and 206 nm. The molecular weight range of the fractions isolated by HSGF was assessed with standards of known molecular weight. By the HSGF method normal and uremic plasma and urine were separated into 10 to 11 peaks based upon differences in molecular size. One of these peaks, no.7, which was present in uremic plasma but not detected in non-uremic plasma contained middle molecules (mol

wt 1000-2000). Amino-acid analysis before and after acid hydrolysis showed this peak to contain a mixture of peptides. Using the GEC method peak 7 was further separated into 7-8 new peaks (7a,b,c, etc.). Plasma from six normal subjects and six non-uremic patients with various diseases yielded only two peaks, 7f and g, whereas urine from normal subjects, and plasma and urine from uremic patients contained all or most of these peaks. Amino-acid analysis of peak 7c, which was frequently found prominent in severely uremic patients indicates that it consists of a small peptide chain containing 8-10 amino acids.

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