

1,9-DIMETHYLMETHYLENE BLUE TESTS ARE UNRELIABLE TO QUANTIFY URINARY GLYCOSAMINOGLYCANS

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The determination of glycosaminoglycans in biological fluids is gradually gaining importance in the literature. Nevertheless, the results obtained by different methods vary widely. The aim of the present study was to evaluate 1,9-dimethylmethylen blue (DMB) dye-binding assays for quantification of urinary glycosaminoglycans, in comparison to densitometry after agarose gel electrophoresis. Urinary glycosaminoglycans from different mammalian species were quantified by three different DMB dye-binding assays. The results were compared to those obtained by densitometry after agarose gel electrophoresis of glycosaminoglycans isolated from urine samples by ion exchange chromatography. Densitometry after agarose gel electrophoresis has shown glycosaminoglycan urinary concentrations of 1-20 mg/l, and glycosaminoglycan/creatinine ratios of 2-25 x 10⁻³, for all the mammalian species here studied. A decrease with age was observed for humans, cats and horses. In comparison, DMB assays gave much higher results – up to 200 mg/l and 500 x 10⁻³ glycosaminoglycan/creatinine ratios. These values were greatly reduced after 4-h dialysis, suggesting that low molecular weight compounds do interfere. Furthermore, urinary anions such as sulfate, phosphate and citrate, react with metachromatic dyes, such as Toluidine Blue and DMB/DMB assays, although rapid and simple, are not appropriate to quantify urinary glycosaminoglycans in normal mammals, since other urinary components interfere with the reactions.

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