ISOLATION OF URINARY PROTEOSE (an $alpha_1$-glycoprotein)*

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The isolation of a urinary proteose by acidification and boiling of urines to remove the bulk of the protein (Levine et al., 1959, and Leyton et al., 1964) leads to a product with a predictably low sialic acid content. In view of such a finding, a new approach was devised to isolate a more "native" proteose.

Essentially this procedure employed modifications of two methods; that of Hardwicke and de Vaux St. Cyr (1961), and of Tombs et al (1961). Twenty-four hour urines were collected frozen from acute nephritic patients, the volumes were measured and total proteins determined by the biuret procedure. Aliquots containing up to 5 gms. protein were then dialyzed versus 4 changes of an acetate buffer pH 4.55, 0.02 M., centrifuged to remove any precipitated material, and applied to a diethyl amino-ethyl cellulose (DEAE) column (1.4 X 30 cm) equilibrated with the same buffer.

The bulk of the proteins passed through allowing only the alpha globulins to exchange. The column was then washed with approximately 5 bed volumes of the equilibrating buffer followed by gradient elution with decreasing pH and increasing molarity. The peak eluted between pH 4.1

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and 3.9 (approximately 0.05M acetate) was found to be alpha$_1$-glycoprotein rich.

The peak was then dialzed versus three changes of an acetate buffer pH 4.55, 0.02M, and applied directly to a carboxymethyl cellulose column (1 X 15 cm) equilibrated with the same buffer. The peak that ran through was collected and the column was washed with an additional 3 bed volumes (approx. 50 ml) of equilibrating buffer and combined with the run through volume. This material after dialysis versus distilled water was shown to be electrophoretically homogenous on paper, cellulose acetate and in free moving boundary at pH 8.6, with a mobility of alpha$_1$-globulins.

In the isolation of urinary alpha$_1$-glycoproteins the advantages of this method are twofold; primarily one may handle large volumes of urine with high total proteins since the DEAE column binds mainly the alpha globulin fractions allowing the bulk of the protein to pass through without taxing the exchange capacity of the absorbant. Secondly, by employing the same equilibrating buffer in the second column, (CM) these exchange absorbents are employed in such a fashion that they oppose one another; material not bound by the first will be bound by the second. Thus, these two columns are used to their fullest advantage and a purification of urinary alpha$_1$-glycoproteins is accomplished quickly without having to resort to recycling through individual columns.

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