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Note

A method for the isolation of urinary proteins

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The human kidney retains albumin and proteins of higher molecular weight in the blood, but a number of polypeptides and proteins of molecular weight lower than *ca.* 50,000 could be excreted through the kidneys into the urine[1]. It has already been established[2] that even normal human urine contains tens of milligrams of proteins per litre of urine.

However, because of the low concentration of these proteins and compounds of a polypeptidic nature, their isolation is still a problem. For the concentration of dissolved compounds in urine a number of techniques have been used, but it is still not easy to work with this material.

The first step in the isolation of urinary proteins is always the concentration of the compounds in urine. Freeze-drying of normal urine seems to be the best process for this step. During freeze-drying, the sample loses not only its water content but also most odoriferous substances. The hygroscopic, yellowish powder obtained can be stored in a frozen state almost indefinitely. Moreover, the mildness of the freeze-drying process prevents the proteins in urine from being denatured.

The second step in the isolation of urinary proteins consists in their separation from low-molecular-weight compounds. One of the simplest methods of achieving this separation is the gel chromatography of the freeze-dried urinary powder, dissolved in a small amount of water, on Sephadex G-50.

The freeze-dried urinary powder is dissolved in distilled water to form an approximately 10% (w/v) solution. This solution is applied to a chromatographic column of Sephadex G-50, equilibrated with water and eluted with water. Tris–hydrochloric acid buffer, pH 7.4, 0.01 *M*, is also suitable for this purpose. No staining of the Sephadex column after repeated chromatographic runs was observed. When the absorbance at 280 nm of the effluent from the column is measured, two distinct peaks appear. The first, smaller peak shows the presence of high-molecular-weight compounds from the urine, consisting mainly of proteins, as established by its characteristic UV spectrum and a positive biuret reaction. The second, larger peak contains most of the low-molecular-weight compounds and is discarded.

The high-molecular-weight compounds isolated from the first peak after concentration by freeze-drying could be further purified by re-chromatography on the same Sephadex G-50 column, again eluted with water. A small amount of low-molecular-weight compounds is usually present in the preparation of substances from the first peak, but they are separated during re-chromatography.

The substances contained in the first peak after re-chromatography are again concentrated by freeze-drying. They form a brownish white, odourless powder, containing mainly low-molecular-weight proteins. As the first gel chromatography and the re-chromatography were carried out in distilled water, the powder obtained is virtually free from contaminating compounds. The urinary proteins obtained in this way are easily dissolved in water or buffers and can be used for further experiments.

REFERENCES

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