letters to the editor

[3H]Inulin as a Marker for Glomerular Filtration Rate

To the Editor: The renal clearance of inulin has for a long time been the gold standard for determination of the glomerular filtration rate (GFR) in experimental animals. Inulin is an uncharged polyfructose molecule with an average molecular weight of ~5,000, is not bound to plasma proteins, is filtered freely in glomeruli, and is neither reabsorbed nor secreted by the renal tubules. Alternative markers for GFR, e.g., clearances of [51Cr-EDTA or [99mTc-diethylthamninepentaacetic acid used routinely in human studies, have been developed and validated by comparison with inulin clearance.

However, the reliability of inulin as the gold standard is critically dependent on the method used for analysis of the polyfructose molecule. Although a number of chemical assays are available (1–3), these methods require relatively large samples of plasma and are therefore not suitable for experiments with small laboratory animals.

This problem is usually circumvented by use of radioactive tracers of inulin, which have the additional advantage that laboratory work is less time-consuming. Thus in our laboratory we routinely use the renal clearance of [3H]inulin for GFR measurement and the clearance of [14C]tetraethylammonium for measurement of renal plasma flow, both compounds being assayed simultaneously in 10–15 μl of plasma (4).

[3H]-labeled tracers are relatively inexpensive compared with [14C]-labeled tracers, but this advantage might be outweighed by lower stability and radiochemical decomposition. In 1991, we published in the American Journal Physiology-Renal Fluid Electrolyte Physiology chromatographic data showing that several batches of [3H]inulin from the worldwide commercial suppliers Amersham and New England Nuclear could be severely decomposed (5). Furthermore, we demonstrated by clearance studies in rats that the renal clearance of a decomposed [3H] isotope declines progressively with time during the experiment, compared with the renal clearances of unlabelled or [14C]-labeled inulin.

The decomposition of [3H]inulin appears unrelated to unfavorable storage conditions but has been detected on several occasions immediately after delivery from one of the suppliers (Amersham) during the past 12 years. Our routine procedure on delivery of a new batch of [3H]inulin is to dissolve the isotope in ice-cold water and to divide it into aliquots in glass ampules, then freeze-drying and storing the closed ampules at −20 or −80°C. Before a new batch is released for experiments, it is chromatographed together with genuine inulin (Sigma I-2255 from chicory root) at 4°C on a Sephadex G-25 column (90 × 2.5 cm). The column is eluted with 10 mM ammonium acetate (pH 6.85) at a rate of 0.5 ml/min, and 5-ml fractions are collected for determination of radioactivity and inulin. Inulin is determined spectrophotometrically by the method of Heyrowski (3).

We recently received supplies of [3H]inulin from both of the aforementioned suppliers, Amersham batch no. 188 and New England Nuclear lot no. 3499004. As shown in Fig. 1, both isotopes were decomposed, because the radioactive peaks were displaced to the right of the peak of genuine inulin. Because the larger molecules are eluted first on the Sephadex column, this chromatographic pattern indicates that the radioactivity is associated with polyfructose fragments with a lower molecular weight. Based on our experience (5), this will result in an underestimation of the GFR, probably due to tubular reabsorption and accumulation of low-molecular-weight fragments in the plasma.

To elucidate the magnitude of this problem, we searched in the PubMed database using the search term “inulin clearance and rats.” Eighteen papers appeared from the past 5 years in which [3H]inulin was used as a marker for GFR.

Fig. 1. Sephadex G-25 chromatograms of cold and [3H]-labeled inulin eluted together with 10 mM ammonium acetate (pH 6.85). Higher molecular weight molecules are eluted first.
REFERENCES


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