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A POSSIBLE ARTIFACT IN GEL CHROMATOGRAPHY OF SOME ^{99m}Tc-CHELATES

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Gel chromatography of the renal agent ^{99m}Tc-Sn-gluconate showed two fractions: a chelate fraction and a fraction retained in the column. When this agent was prepared and analyzed on four occasions, there was no significant variation in biological distribution in the rat, but retention of activity in the column varied from 10 to 60%. The biological behavior of the chelate fraction eluted from the column did not differ from that of the unfiltered preparation. The successive passage of the chelate through two columns resulted in retention of less than 5% by the first column and 65% by the second. No difference was found in the biological behavior of the unfiltered, filtered, and refiltered chelate.

The results indicate that the noneluted fraction shown by gel chromatography of this ^{99m}Tc-chelate is an artifact produced by the analytical procedure. In studying the chemistry of radiopharmaceuticals, the results of analytical separations must be correlated with the biological behavior of the separated fractions to establish the validity of the separation procedure.

Eckelman, Meinken, and Richards have suggested the use of gel chromatography for the analysis of ^{99m}Tc-labeled compounds because it is the only method that will separate labeled proteins, chelates, pertechnetate, and hydrolysis products (*1,2*). They used Sephadex G 25 gel chromatography columns to analyze a number of ^{99m}Tc-chelates (*1,2*) and have detected three separate ^{99m}Tc fractions: the chelate fraction, the pertechnetate fraction, and a fraction not eluted from the column. The authors state that this third fraction represents, "the hydrolized unchelated fraction of ^{99m}Tc".

Technetium-99m-Sn-gluconate is a renal-specific agent (*3–5*), which is prepared by the reduction of Tc(VII) with Sn(II) in the presence of gluconate by combining 1 ml gluconate solution (Ca or Na salt, 1–10% w/v), 1 ml 0.01% $SnCl_2 \cdot 2H_2O$ solution in water, and $^{99m}TcO_4$ in saline. Gluconate can be used to chelate many metal ions (*6*), and the compound formed is almost certainly a ^{99m}Tc-gluconate chelate.

No significant variation in the biological behavior of this compound has been found in animal experiments or in over 100 patient studies. In the rat, over 60% of the administered activity is excreted in the urine during the first hour after injection and 12–20% is retained in the renal cortex at 1 hr. In man, 40–60% is excreted within the first 2 hr. The renal cortex can be rapidly and clearly imaged up to 4 hr after injection, and renal scintiphotos have been obtained as long as 20 hr after administration of the isotope.

A series of experiments was conducted to study the relationship of the biological and gel chromatographic behavior of this radiopharmaceutical.

MATERIALS AND METHODS

Technetium-99m-Sn-gluconate was prepared as described, using a 10% Ca-gluconate solution that also contained 0.4% Ca-saccharate as the stabilizer.

Gel chromatography was performed using 25 × 1 cm Sephadex G 25 columns, eluting with 40 ml saline, and collecting 1-ml fraction. It was determined that ^{99m}Tc-Sn-gluconate was eluted in the fraction 15–18 ml and $^{99m}TcO_4^-$ in the fraction 30–33 ml.

Biological distribution studies were carried out in the rat. Animals were injected i.v. and sacrificed 1 hr after injection. The kidneys, liver, stomach, and

Received March 20, 1972; revision accepted Nov. 8, 1972.

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TABLE 1. BIOLOGICAL BEHAVIOR VS GEL CHROMATOGRAPHIC ANALYSIS OF ^{99m}Tc-Sn-GLUCONATE

Experiment No.	Material injected*	Distribution of activity in the rat 1 hr after i.v. injection†			
		Kidneys	Liver	Stomach	Bowel
1	A	13.6	1.3	0.9	6.1
	B (10%)	16.1	1.3	0.1	5.3
2	A	17.9	1.4	0.1	6.9
	B (20%)	21.3	1.6	0.2	8.0
3	A	18.2	0.8	0.2	2.8
	B (70%)	19.7	0.9	0.5	8.0
4	A	18.9	1.8	0.6	4.3
	B (20%)	18.4	1.2	0.2	3.9

* A. ^{99m}Tc-Sn-gluconate before gel filtration; B. Chelate fraction of filtrate, percent not eluted from gel in parenthesis.
† Percent of injected dose, average of 2 rats.

bowel were counted, and the percentage of the injected dose in each organ was determined by comparison with a standard.

In four experiments, ^{99m}Tc-Sn-gluconate was eluted through the column, and the eluted fractions were counted. The total activity eluted and the percentage retained on the column were calculated. Rat distribution studies were carried out on the compound before chromatography and on the chelate fraction of the eluate.

In one experiment the chelate was passed successively through two columns, and distribution in the rat was studied before and after each filtration. In addition, ^{99m}Tc-Sn-gluconate was mixed with Sephadex G 25 gel in a test tube and washed repeatedly with saline. The percentage of activity retained by the gel was measured after each washing.

RESULTS

In all experiments, a chelate fraction was eluted from the column, and no activity was detected in the 30–33-ml $^{99m}TcO_4$ fraction. The fraction of activity retained on the column, and the distribution of activity in the organs of the rats, are shown in Table 1. The fraction of activity removed from the ^{99m}Tc-Sn-gluconate during gel filtration varied from 10 to 70%. The removal of these varying fractions by filtration did not produce any alteration in biological distribution.

When ^{99m}Tc-Sn-gluconate was filtered through one Sephadex G 25 column, and the chelate fraction that emerged was eluted through a second column, less than 5% of the ^{99m}Tc was retained on the first column, but retention by the second column was 65%. No significant difference was found in the biological behavior of the unfiltered, filtered, and refiltered compounds.

In three experiments in which Sephadex was washed repeatedly after the addition of ^{99m}Tc-Sn-gluconate, a plateau was reached after three washings, and no further ^{99m}Tc activity could be removed. The fraction of retained activity was 45%, 49%, and 59%.

DISCUSSION

The biological distribution studies of ^{99m}Tc-Sn-gluconate before and after filtration indicate that the noneluted fraction detected on gel chromatography of this ^{99m}Tc chelate does not represent a nonchelated hydrolized fraction in the original preparation. Furthermore, the recycling experiment also suggests that the noneluted fraction is an artifact produced by the separation procedure.

It is likely that reduced ^{99m}Tc, complexed to gluconate or to some other relatively weak chelating agent, will dissociate to some extent when passing through the column. Sephadex is a polymerized polysaccharide, and we have shown that ^{99m}Tc(VII), reduced with Sn(II), will complex with a number of carbohydrates, including the disaccharides, sucrose, and lactose (*4*). Eckelman, Meinken, and Richards state that when Tc(VII) is reduced by $NaBH_4$ or concentrated HCl and HI, it will bind firmly to Sephadex (*1*). Therefore, it is possible that during gel-filtration some exchange of ^{99m}Tc occurs between weak chelating agents and the Sephadex gel. The persistence of activity on Sephadex gel after mixing with ^{99m}Tc-Sn-gluconate and repeated washing indicates either strong adsorption or actual chelation of ^{99m}Tc by the Sephadex.

Eckelman and Richards have shown the presence of a nonchelated reduced form of ^{99m}Tc in some preparations of ^{99m}Tc-DTPA and ^{99}Tc-HSA, which can be removed by gel filtration (*1,2*), and the above observations do not apply to the analysis of all ^{99m}Tc compounds. However, these observations do indicate that when gel chromatography is used to study weak chelates of ^{99m}Tc, an artifactual component may be produced by the analytical procedure. If the analytical results described here had not been correlated with animal distribution studies, it would have appeared that a large fraction of the ^{99m}Tc was present in nonchelated form in the original preparation. This study indicates that there are potential pitfalls in gel chromatographic analysis of some ^{99m}Tc compounds, and that the gel chromatographic behavior of any compound should always be correlated with biological distribution studies.

It is important to study the chemistry of radiopharmaceuticals, and analytical methods such as gel

chromatography are valuable tools for this purpose. However, the results of analytical separations must must be correlated with studies of the biological behavior of the separated fractions to establish the validity of the analytical technique.

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