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A SIMPLE "ELECTROLYTIC" PREPARATION OF A ^{99m}Tc(Sn)-CITRATE RENAL SCANNING AGENT

Peter B. Schneider

Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts

A ^{99m}Tc(Sn)-citrate renal scanning agent can be prepared using the electrolytic release of Sn^{2+} from tin electrodes to serve as the reducing agent for $^{99m}TcO_4^-$. The materials needed for this method are commercially available in sterile and pyrogen-free form leading to a simple economical preparation.

A number of renal scanning agents based on various chelates of ^{99m}Tc have been described. The preparation of these agents requires an as yet undefined reduced form of ^{99m}Tc which can be derived from $^{99m}TcO_4^-$ by the use of several reducing agents of which Sn^{2+} as stannous chloride is a popular example (*1*). This communication describes the preparation of a ^{99m}Tc-citrate chelate suitable for renal scanning in which the reduction of TcO_4^- is accomplished by Sn^{2+} introduced into the solution by electrolytic release from metallic tin electrodes. This formulation permits the design of a "do-it-yourself" kit which has advantages of economy, stability with long shelf life, and low tin content.

METHODS AND MATERIALS

Electrolysis is carried out in 5-ml, or other suitable size, rubber-capped, sterile, pyrogen-free serum vials. The vials used for elution of $^{99m}TcO_4^-$ from generators are adequate. The electrodes consist of 0.02-in.-diam tin wire (99.999% purity, Alfa Inorganics, Beverly, Mass.) which is cut into 4-in. lengths and can be sterilized and packaged by any of the techniques used for surgical instruments. Only the anode need be tin, but to avoid possible confusion, tin should be used for both electrodes. The soft tin wire is introduced into the electrolysis vial with the aid of an 18-gage (or larger) sterile hypodermic needle. The needle is inserted into the vial until it just penetrates the rubber cap; the electrode wire is handled with sterile forceps or with a sterile sponge and is passed into the vial through the needle. The needle can then be withdrawn around the wire leaving sufficient wire outside the vial for connection of the power supply leads.

The reaction solution consists of generator eluate containing the desired activity of ^{99m}Tc plus about 0.05 *M* citrate at pH 5–7. A convenient, sterile, pyrogen-free source of citrate is acid citrate dextrose solution packaged in 10-ml amounts (ACD solution, Abbott Laboratories, containing 0.14 *M* citrate, pH 5). Two volumes of the $^{99m}TcO_4^-$ generator eluate plus one volume of the ACD solution are injected into the electrolysis vial, and the electrodes are attached with flexible leads to a power supply. The reduction is accomplished by passing a total charge of 0.05 coulombs/ml (i.e. amp-sec/ml) through the solution at a current of about 2 mA. This results in a tin concentration of about 25 μM (3 μg/ml). For the studies reported here, a constant current supply was used but, because of the permissible latitude of charge and current, cheaper supplies should serve. The simplest supply that will suffice is two 1.5-volt dry cells in series with a milliammeter and an adjustable 2,000-ohm resistor. Once the resistor is set for a given geometry of the electrolysis system, the current will be reasonably reproducible from one preparation to the next. Necessary adjustment of charge can be made by controlling the time for which current flows.

The efficiency of reduction of $^{99m}TcO_4^-$ was determined by chromatography on 0.25-mm-thin layers of cellulose (MN-300, Brinkmann) with 85% methanol as solvent. Radioactivity was located by radioautography and quantitated by counting bands

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For reprints contact: Peter Schneider, Beth Israel Hospital, 330 Brookline Ave., Boston, Mass. 02215.

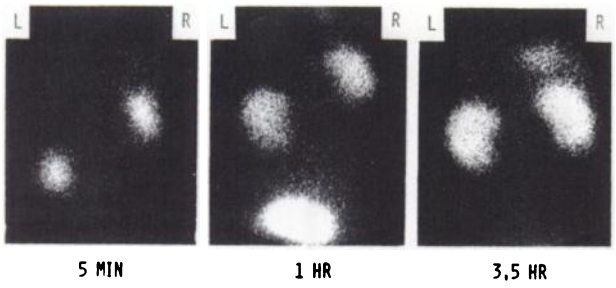

FIG. 1. Gamma camera images of kidneys of dog injected with 0.5 mCi of ^{99m}Tc(Sn)-citrate. Bladder is out of field of view on 5-min and 3.5-hr images. Accumulation of activity above right kidney which becomes just visible at 1 hr is gallbladder.

scraped from the plate. In this system $^{99m}TcO_4^-$ runs with a $R_f = 0.64$ while the ^{99m}Tc(Sn)-citrate runs at $R_f = 0.14$ though with some streaking.

Further evaluation of chelate formation was performed by column chromatography on Sephadex G-25 (*2*). A portion of the chelate appears in the effluent close to the void volume of the column and a variable portion remains adsorbed to the Sephadex but can be eluted after addition of 2-ml 0.5 *M* citric acid to the column. Both these fractions are distinctly separated from the $^{99m}TcO_4^-$ peak.

RESULTS

Injection of 0.5 mCi of the ^{99m}Tc(Sn)-citrate into a dog results in prompt visualization of the kidneys (Fig. 1). At 1 hr a trace of activity was observed above the right kidney which became more prominent over the next few hours. This was apparently due to activity in the gallbladder as demonstrated in Table 1 which shows the distribution of activity in another dog, sacrificed 2 hr after isotope administration. Although the gallbladder had relatively little total activity, the concentration is comparable to that of the kidneys. Similar renal images were recorded in rats, but since they do not have gallbladders, no focal suprarenal activity was seen. However, 1 hr or more after injection, rats showed variable amounts of activity in the gut (Table 1) and, since activity never appeared in the stomach, it is possible that the intestinal activity entered by way of the bile. Prior to 1 hr after injection, only the kidneys and bladder were visualized with no interference from other focal accumulations.

Recording of activity over the kidney showed a rapid rise followed by a fall-off parallel to the decrease in blood activity (Fig. 2). Direct sampling of blood over a prolonged period in a dog disclosed an effective blood half-life of ^{99m}Tc(Sn)-citrate radioactivity of 1.8 hr after an initial rapid decay with a half-life of 10 min.

The limits within which the factors of electrolysis could be safely altered were determined by observing the scintillation images in rats injected with various preparations of the chelate. The distribution of isotope and the quality of the kidney images were unchanged when citrate concentration ranged from 0.01 to 0.1 *M* and the charge ranged from 0.01 to 0.2 coulomb/ml except that the combination of the highest charge and lowest citrate concentration resulted in some liver accumulation of isotope, perhaps due to formation of an insoluble ^{99m}Tc-Sn(OH)$_4$ colloid. The pH of the preparation could range between 5 and 7. Varying the current from 1 to 10 mA made no difference. The preparation was stable for at least 4 hr after electrolysis even if air was bubbled through the solution during that time.

Under all of the above conditions, the amount of

TABLE 1. DISTRIBUTION OF ^{99m}Tc ACTIVITY 2 HR AFTER I.V. ADMINISTRATION OF ^{99m}Tc(Sn)-CITRATE CHELATE

	Dog		Rat (mean of two)	
Organ	% dose	% dose/gm	% dose	% dose/gm
Bladder (with urine)	55*	0.69	70†	—
Kidneys	8.2	0.057	11.8	5.30
Gallbladder (with bile)	1.4	0.033	—	—
Liver	3.1	0.007	1.2	0.11
Heart and lungs	0.8	0.002	0.25	0.08
Large intestine	0.2	0.002	0.17	0.02
Small intestine	1.0	0.002	3.0	0.18
Stomach	0.3	0.002	0.17	0.05
Spleen	0.04	0.001	0.01	0.02
Blood		0.006		0.18

* Complete urine collection.

† Urine not collected; urine activity calculated by difference of dose and recovered activity.

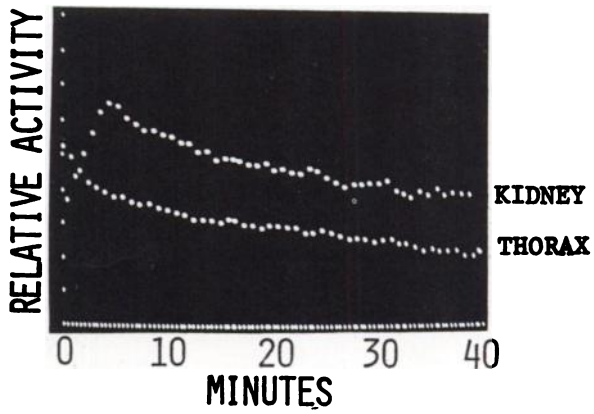

FIG. 2. Recording of radioactivity through gamma camera "regions of interest" set over thorax and left kidney of dog whose images are shown in Fig. 1.

free $^{99m}TcO_4^-$ as determined by thin-layer and column chromatography was less than 1% of the total activity.

A similar chelate could be prepared by substituting 0.05 *M* DTPA at pH 5.5 for citrate. The ^{99m}Tc activity in such a chelate was cleared by rat kidneys much more rapidly than the citrate chelate with the result that little activity remained in the kidney at 1 hr and that contrast between kidney and background was never as great as with the citrate product.

DISCUSSION

The electrolysis used in the production of this ^{99m}Tc(Sn)-citrate chelate presumably serves merely to release Sn^{2+} ions from the anode into solution where they reduce the $^{99m}TcO_4^-$, and the properties of this chelate should not be unique to the electrolytic method. There are, however, several advantages to introducing the Sn^{2+} into solution this way as opposed to the standard use of stannous chloride. The tendency of stannous chloride to undergo oxidation and hydrolysis requires strict attention to techniques with exclusion of air in the preparation of kits based on that ion (*1*). Metallic tin, however, is not oxidized readily, and the tin electrodes have an indefinite shelf life. Furthermore, there is no need for particular exclusion of air from the electrolysis solution. The electrolytic ^{99m}Tc(Sn)-citrate chelate appears to be much more stable to oxidation than a citrate chelate (*2*) or an ascorbate complex (*2,3*) prepared with ferrous reduction.

The amount of tin in the electrolytic preparation (about 3 μg/ml as Sn) is one tenth that present in a DTPA chelate based on stannous chloride reduction of the $^{99m}TcO_4^-$ (*1*). This lower tin requirement appears to be a function of the method rather than of the chelating agent since we have electrolytically prepared a ^{99m}Tc-DTPA chelate with the low tin content.

A major advantage of the method described here is that the chelate can be prepared by a closed aseptic technique from commercially available materials which are sterile and pyrogen free, thus eliminating the need for much of the on-site testing otherwise required for the use of this scanning agent in humans.

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