Scintiphotos in Rabbits Made with Tc-99m Preparations Reduced by Electrolysis and by $SnCl_2$: Concise Communication

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[^{99m}Tc] pertechnetate was reduced electrolytically with a carbon cathode and platinum anode: a) in 1% DTPA at pH 4-5; b) in 4% sodium pyrophosphate at pH 7; c) in 1% HEDP at 7.3; and d) in 5% sodium gluconate at pH 9.2. Chromatography on Biogel P-10 showed that the various complexes were prepared in high yield. Rabbits were injected in pairs, one receiving the electrolyzed preparation, and the other receiving the conventional $SnCl_2$-ligand-$^{99m}TcO_4^-$ preparation. In the DTPA, pyrophosphate, and HEDP solutions, the two types of preparation gave similar scintiphotos, but decreased body background was seen with the electrolyzed solutions. With gluconate the technetium in both preparations went to the kidneys initially, but with the electrolyzed material the renal activity virtually disappeared within 30 min, whereas it persisted for at least an hour with the $SnCl_2$ preparation. The injection of electrolyzed Tc-gluconate, when followed within minutes by injection of a sodium gluconate-$SnCl_2$ solution, fixed the activity in the kidneys just as it did with the ordinary tin-technetium-gluconate kit, but with an hour between injections it produced the same effect as the electrolyzed solution alone. We suggest that tin compounds in the blood change the permeability of various membranes and cause the retention of technetium by the kidneys that is seen with the usual Tc-99m(Sn)glucoheptonate preparations. It is concluded that the reduction of pertechnetate by $SnCl_2$ is not necessary for the formation of the four labeled complexes that we studied, at least in relation to gel chromatographic analysis and scintigrams. The more general question of the existence of mixed tin-technetium complexes is briefly discussed.

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Recently, Russell reported that he had succeeded in labeling both tetracycline and EDTA with Tc-99m by the reduction of $^{99m}TcO_4^-$ at a mercury-pool cathode using a controlled potential (*1*). This followed his polarographic and voltametric studies of the reduction of $^{99}TcO_4^-$ at platinum, gold, glassy carbon, and mercury cathodes, in solutions of different pH. For each cathode he measured the range of potential available for the reduction of $^{99}TcO_4^-$ before the onset of hydrogen evolution, in the presence of a large number of potential ligands for the reduced technetium. These studies were then applied to the reduction of $^{99m}TcO_4^-$ in solutions of EDTA and tetracycline at the most negative applied potentials at which molecular hydrogen did not form. He concluded that $SnCl_2$ was not necessary

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for the formation of these two Tc-99m complexes.

We had started a more limited investigation of the electrolytic reduction of $^{99m}TcO_4^-$ before the publication of Russell's comprehensive article. Our immediate goals were the preparation by electrolysis of several Tc-99m radiopharmaceuticals, an analysis of their yields by gel chromatography, and comparison of rabbit scans made with the electrolyzed solutions and with $SnCl_2$-reduced radiopharmaceuticals. In agreement with Russell, we felt that inert cathodes with a high hydrogen overvoltage would be preferable to platinum, since hydrogen evolution on platinum would start virtually at the theoretical equilibrium potential (*2*) and might produce much smaller yields of reduced technetium. Electrolyses were not conducted at controlled potentials. A simple regulated voltage supply was used, and voltmeter readings were recorded for the applied voltage at which satisfactory yields of the various Tc-99m complexes were obtained. All but one of the experiments reported here were carried out with a graphite cathode.

MATERIALS AND METHODS

Equipment. A regulated power supply (range 0–8 volts) was the source of applied voltage and a multimeter was used to measure current. An electrolytic H-cell (a two-compartment cell with a sintered disc and an agar plug saturated with KCl between compartments) was the electrolysis vessel. The capacity of the cathode compartment was about 20 ml, and that of the anode compartment 30 ml. The cathodes were ¼-inch-thick graphite rods of moderate purity. The anode was a platinum basket and wire. For the chromatographic determinations, Tc-99m activity was measured in an automatic well counter. Chromatography was performed with a conventional saline and benzyl alcohol eluant, using Biogel P-10 (100–200 mesh) in a 0.9- by 30-cm glass column, and fractions collected. Activities injected into the rabbits were measured in a dose calibrator. Animal scans were done with an Anger camera.

Chemicals. 1,1 Hydroxyethylidene diphosphonate (HEDP) was obtained commercially. Mercury was triple-distilled and nitrogen gas was pre-purified. All other chemicals—including gluconolactone, DTPA, and sodium pyrophosphate—were of reagent grade. Pertechnetate-99m was eluted with normal saline from a generator and diluted as needed.

Animals. New Zealand White male rabbits, weighing 6–8 lb were used.

Procedures. Before electrolysis, the cathode compartment received 10 ml of a previously de-aerated solution of ligand and [^{99m}Tc] pertechnetate in normal saline that had been brought to the desired pH. Ten milliliters of normal saline were added to the anode compartment. The catholyte was de-aerated with nitrogen for 30 min before the electrolysis was started. A small magnetic bar stirred the solution, and nitrogen was passed through it during electrolysis. At the end, samples were withdrawn either for animal injection or for analysis by column chromatography. The saline eluant and the columns were kept under nitrogen.

Two rabbits of approximately equal size were injected i.v. for each run. One was injected in the ear with 1–2 ml of an electrolyzed solution, and the other with an equal volume of a solution containing 1 mg $SnCl_2$/ml, with the same ligand, saline, and Tc-99m concentrations as those of the electrolyzed solution. Virtually identical doses, from 250 to 500 μCi, were administered to the two animals. Each was strapped on a retaining board before injection, and scintigraphed with the camera. For this purpose, a small target area was selected, the time to reach 2000 counts was recorded, and all images of that particular rabbit were run with that exposure time. When bone-scanning agents were injected, scintigrams were taken 2 hr after injection. Imaging was started within 1 min after injection of the renal scanning agents.

RESULTS

Table 1 shows the operating conditions and analytical results of electrolyses with a carbon cathode and a platinum anode. In each of the four pairs of radiopharmaceuticals, the Biogel P-10 elution patterns were the same for the electrolytically reduced and the $SnCl_2$-reduced preparations. The currents were measured approximately. They averaged 4 mA at 3 volts, 8.6 mA at 4 volts, and 180 mA at 5 volts. Gas bubbles were evolved at each electrode, increasing in rate of formation with increasing current.

Figure 1 shows the scintiphotos of two rabbits injected with Tc-99m HEDP preparations (left = electrolytic, right = $SnCl_2$—see Table 1).

Images similar to those in Fig. 1 were obtained with Tc-99m pyrophosphate preparations.

Figure 2 shows scintiphotos of two rabbits injected with Tc-99m DTPA preparations (left = electrolytic, right = $SnCl_2$).

Figure 3 shows scintiphotos of two rabbits injected with Tc-99m gluconate preparations (left = electrolytic, right = $SnCl_2$).

If an electrolyzed Tc gluconate solution and $SnCl_2$ were injected into the same rabbit within 5 min of each other, regardless of the order of the injection, the scintiphoto made 1 hr later was indis-

TABLE 1. CHROMATOGRAPHIC ANALYSES OF ELECTROLYZED PERTECHNETATE IN VARIOUS LIGAND SOLUTIONS

Ligand	pH	Voltage	Time (min)	%complex	$\%TcO_4^-$
DTPA, 1%	4-5	3.0	30	94	-
$Na_4P_2O_7$, 4%	7.0	4.0	30	100	-
HEDP, 1%	7.3	5.0	45	95	9
Na gluconate, 5%	9.2	4.0	75	92	9
None	9.0	3.0	45	15	77

tinguishable from that of the $SnCl_2$-reduced preparation shown in Fig. 3. On the other hand, if an hour elapsed between the injections, regardless of the order of injection, the final image 1 hr after the second injection resembled the final image of the electrolyzed material in Fig. 3.

DISCUSSION

The data of Table 1 show that is is possible to reduce pertechnetate by electrolysis, using a carbon cathode, in the presence of suitable ligands. The overvoltage or overpotential—defined as the difference between the actual potential of an electrode while current is flowing, and the reversible electrode potential for the given reaction—is a function of the electrode, the concentration of the electrolyzed species, the current density, and other factors. The overvoltages for the liberation of hydrogen gas from 1 *M* sulfuric acid at a current density of 10 ma/cm² rank in the following order: Pt < Au < C < Pb < Hg (*2*). Graphite has a moderately high overvoltage, much lower than those for lead and mercury but apparently adequate for the required reduction of pertechnetate. The passage of current up to 180 milliamperes, at an apparent applied potential of 5 volts, is due in all probability almost entirely to hydrogen and oxygen evolution at the two electrodes. At the same time, this has caused no problem. Were platinum to be used, the overvoltage would be almost the same at any current density. With carbon and other inert electrodes, there is an actual gain in overvoltage with increased current (*2*). The true potential between the electrodes is less than the applied potential because of the IR drop between the electrodes and

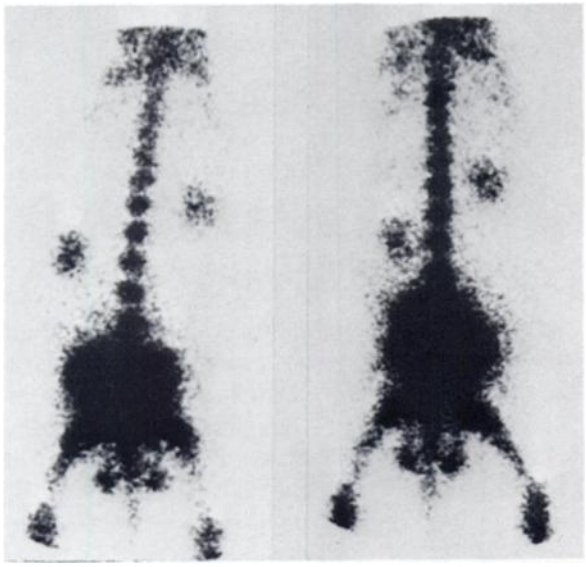

FIG. 1. Scintiphotos of two rabbits injected with Tc-99m HEDP preparations. The scan on the left indicates the electrolyzed preparation; The scan on the right the $SnCl_2$ preparation. Both were made 2 hr after injection.

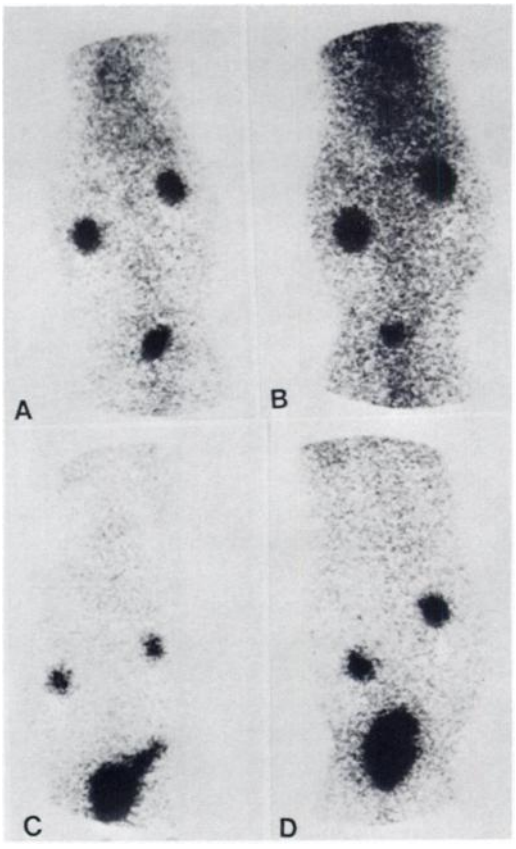

FIG. 2. Scintiphotos of two rabbits injected with Tc-99m DTPA preparations. A and C were made with the electrolyzed preparation; B and D with the $SnCl_2$ preparation. A and B were made 3 min after injection, and C and D after 28 min.

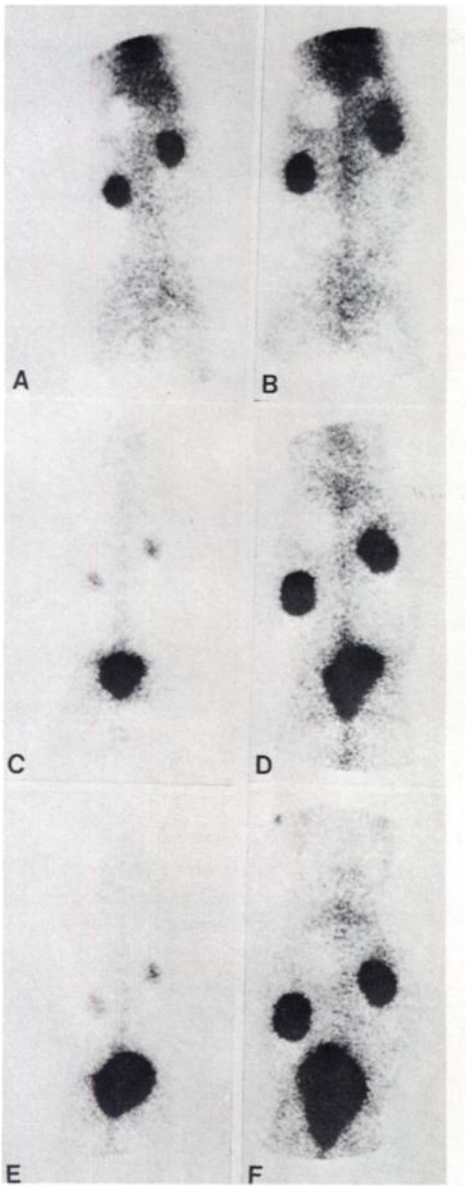

FIG. 3. Scintiphotos of two rabbits injected with Tc-99m gluconate preparations. A, C, and E were made with the electrolyzed preparation; B, D and F with the $SnCl_2$ preparation. A and B were made within 1 min after injection; C and D 30 min later; and E and F 1 hr after injection.

the back-EMF generated by the products of electrolysis. No attempt was made to determine this true potential.

A one-compartment cell cannot be used because oxygen, formed at the anode, will have to be reduced again at the cathode.

Figures 1 and 2 show that the scans made with electrolytically reduced Tc-99m compounds were like those of the usual $SnCl_2$-reduced preparations in the case of DTPA, pyrophosphate, and HEDP preparations, with one difference. The electrolyzed preparations yielded images that, at least visually, showed better target-to-nontarget ratios than those from tin. General body backgrounds were lower, and the scans appeared sharper and clearer. That is, soft-tissue uptake was more marked with tin.

With gluconate, however, the scintigrams made with the electrolytically generated Tc-99m complex were very different from those made with tin. Initially (i.e., 1 min after injection) there was little difference in kidney uptake. After ½ hr, most of the electrolytically reduced technetium had left the kidneys, whereas the tin-reduced compound still showed the same activity. After 1 hr, the difference between them was even more marked. This is evident in Fig. 3. If a $SnCl_2$ solution (containing gluconate) and an electrolytically reduced Tc-gluconate preparation were separately injected into the same animal within 5 min of one another, the scintiphoto showed strong activity in the kidneys 1 hr after the two injections, as with the $SnCl_2$-reduced technetium gluconate preparation (see Fig. 3F). The order of injection made no difference to the result. However, if 1 hr elapsed between an injection of $SnCl_2$ and an injection of electrolytically reduced technetium gluconate, the image made 30 min after the second injection showed very little activity remaining in the kidneys, like that shown in Fig. 3C. It is likely that the tin is producing this retention of technetium activity in the kidneys with the gluconate preparation. This may be related to the false-positive brain scans that are sometimes seen when pertechnetate is injected after a bone scan has been done (*3,4*). It is possible that tin compounds in the blood change the permeability of various membranes and that this is the cause of the retention of activity by the kidney that is seen with the usual Tc-99m(Sn)glucoheptonate preparations. If this is so, apparently the renal effect is operative within 5 min of the injection of the $SnCl_2$-gluconate solution, but has been either diluted out or nullified after an hour.

We conclude that reduction of pertechnetate by electrolysis on a carbon cathode produces radiopharmaceuticals that are equivalent to those formed by $SnCl_2$ reduction in the presence of the four ligands investigated. Reduction by $SnCl_2$ is not necessary for the formation of labeled compounds, at least from the point of view of gel chromatographic analysis and of scintigrams with the same solutions. This does not prove that $SnCl_2$ fails to form mixed complexes with Tc-99m in these preparations. It may do so, but if it is, these mixed complexes are not essentially different from the electrolyzed materials in the two respects listed above. The question of mixed-metal complex formation is a chemical question, and must ultimately

be answered in chemical terms for each technetium compound. In our view, it is extremely unlikely that such mixed complexes exist in all pertechnetate formulations with $SnCl_2$, at least in aqueous solution. Two tin-technetium complexes have been reported. One is a tin-technetium-99 complex of dimethylglyoxime that crystallized after long standing out of ethanol, not water (*5*). The other is a mixed Tc(III)-Tc(IV)-Sn(II) complex with orthophosphate, formed in aqueous solution (*6*). With respect to the ligands reported here, pyrophosphate in water forms a complex with Tc(III), by electrolytic reduction on mercury, whose spectrum is the same as that formed by $SnCl_2$ reduction (*6*). Gluconate forms a Tc(*V*) complex whose spectrum is the same whether formed by the addition of the stoichiometric quantity of $SnCl_2$ required for the reduction, or whether it is formed in the presence of an excess of $SnCl_2$ (*7*). If mixed-metal complexes had formed, one would have expected a shift in the spectrum. The question is still unresolved for the DTPA and HEDP complexes of Tc-99, although Russell's conclusions about the EDTA complex can almost certainly be applied to that of the DTPA complex (*1*).

The electrolytically formed gluconate and DTPA complexes were injected on several occasions at least 1 hr after they had been drawn up in the syringes. The scans were not different from those obtained with freshly prepared material. These solutions without excess reducing agent were more stable against air oxidation than one might have expected.

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