ELECTROLYTIC COMPLEXATION OF \(^{99m}\text{Tc}\) AT CONSTANT CURRENT: ITS APPLICATIONS IN NUCLEAR MEDICINE

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A preliminary report on electrolytic complexation of technetium at a constant voltage of 2 volts has appeared in a previous paper by one of the authors (PPB) (1). Anodic dissolution of zirconium in the presence of, or followed by the addition of, \(^{99m}\text{Tc}\)-sodium pertechnetate and \(^{99m}\text{Tc}\)-human serum albumin (HSA) results in nearly quantitative binding of the technetium with the protein. The technetium-albumin (complex) seems to be highly stable for a pH range of 0.8–8. Upon coagulation of the protein, more than 99% of the radioactivity stays with the coagulum and could not be washed away by dilute acids or alkalies.

Zirconium in some form (probably cationic) seems to act as an intermediate complexing agent with both technetium and albumin acting as ligands. A plausible mechanism of the reaction is suggested.

In the previous report the rate of disappearance of this preparation from the blood stream in rabbits was given and possible clinical applications were suggested. Various organs have been scanned after administration of the labeled protein as either molecular suspension or as aggregates. These results are included in this report along with a recipe for producing the labeled protein for optimum results in clinical diagnosis. The constant-current electrolysis permits a better estimate of the dissolved zirconium. This is important in clinical studies.

EXPERIMENTAL METHODS

A constant current (dc) power supply (voltage range 0–10 volts) was used to supply the current. The cell consisted of a 25-ml zirconium crucible (Wa Chang Corp.) as the anode and a micro platinum electrode (Sargent S-30420) as the cathode. A Sargent synchronous rotator (S-76485, 600 rpm) was used to rotate the cathode. The electrolytic solution normally consisted of various amounts of 1 N HCl, HSA and 5 cc of NaTcO\(_4\)– from Neisler Neimotec* (\(^{99m}\text{Mo}\)-\(^{99m}\text{Tc}\) generator). A 1 N sodium bicarbonate solution was used for pH adjustments. The analysis of the unreacted pertechnetate from the bound technetium was performed by:

1. Descending paper (untreated, Whatman No. 1) chromatography in 85% methanol (2) where the \(R_f\) of free TcO\(_4^–\) was approximately 0.65.
2. Thin-layer chromatography on Gelman Type A (combination of a slurry of micro filaments of glass with alumina) using 85% methyl alcohol as eluent, where the \(R_f\) of TcO\(_4^–\) is approximately 1.
3. Dowex I-X2 anion exchange column chromatography with 1 N HCl as eluent where the free pertechnetate is efficiently retained on the column. At this low pH, albumin easily passes through the column.

The assay of technetium activity was performed with an ionization chamber, a well scintillation counter or a chromatogram scanner (Actigraph II, Nuclear-Chicago). Qualitative studies of the distribution of radioactivity on the paper and thin-layer media were done by autoradiography.

RESULTS AND DISCUSSION

The autoradiograms of paper chromatographs at different stages of the reaction are given in Fig. 1. Figures 1A and D represent acidified pertechnetate

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* Traces of oxidants like HClO and H\(_2\)O\(_2\) inhibit the reaction almost completely. Commercial isotope generators (\(^{99m}\text{Mo}\)-\(^{99m}\text{Tc}\)) using eluents containing these compounds must be eluted several times with normal saline so that the columns are washed free of any traces of the oxidizing agents. All elutions thereafter should be performed with normal saline.
(pH $\approx 1.3$) with and without albumin, respectively. Nearly all the technetium activity moves from the origin with an $R_f = 0.65$. Figures 1B and E refer to chromatographs taken immediately after electrolysis with and without albumin, respectively. In Fig. 1B practically all the radioactivity stays at the origin while in Fig. 1E (without albumin) approximately $40\%$ only of the initial activity stays at the origin. However, Fig. 1F shows the effect of addition of a drop of HSA ($25\%$). It seems to eliminate almost completely all the free pertechnetate. Figure 1C represents the same system as Fig. 1B except that the pH is raised to 8 using a saturated solution of sodium bicarbonate. It was observed that any pH adjustments after electrolysis without albumin (being present initially or added immediately after electrolysis) decreases the yield of zirconium-technetium complex drastically.

Table 1 shows the free activity towards the solvent front with $R_f = 0.65$ and the bound activity at the origin of a descending paper chromatograph as a percentage of the initial total activity. The details of chromatographic analysis are given in previous works (1,2). The electrolysis was done at low pH, 1–1.3, (without albumin) for varying times at a constant current of 100 mA and cell voltage of 5–6 volts. There was appreciable streaking of the radioactivity (5–10%) on the chromatograms as evidenced by autoradiography and counting of the paper strips.

In Figs. 2A and B the percentage yield of the bound pertechnetate is plotted against the number of coulombs and the estimated* amount of zirconium dissolved during electrolysis (3), respectively. Results with and without albumin are given in these figures. The effect of adding a drop of albumin solution to the reaction system is clearly seen. With about 4–4.2 coulombs, 95–98% of the initial activity stays bound to the albumin. In Fig. 3 the bound TeO$_4^-$ (percent initial activity) is plotted against albumin concentration (mg/ml) for 4.2 coulombs of electricity at pH 1.2–1.4. The yield of the bound TeO$_4^-$ reaches a maximum of 98% of initial activity at a concentration of about 0.9–1.2 mg/ml of albumin. These experiments are repeated at different pH values, and the results are plotted

* The number of faradays required to dissolve a mole of zirconium when it is made anodic is known to be about 3.9 over a series of current densities and varying electrolytes (3). Therefore the quantity of zirconium dissolved per coulomb of electricity is assumed to be 0.242 mg. The chemical corrosion of zirconium under the conditions of the experiment is assumed negligible.
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FIG. 2. A shows yield of Zr-Tc complex vs. quantity of electricity. B shows yield of Zr-Tc complex vs. estimated concentration of Zr.

in Fig. 4. With albumin present initially or added immediately after electrolysis the yield of the bound pertechnetate gradually decreases from about 98% at a cell pH of 0.9 to 90% at a cell pH of 3. For higher values of cell pH, the yield rapidly falls off. The electrolysis is done in each case with 4.2 coulombs at 5–8 volts. However, without the addition of albumin the percent yield of bound technetium gradually increases from about 30% at pH 0.9 to about 80% at pH 3 and then rapidly falls off as the cell pH is higher. The electrolysis in this case was done with 6 coulombs at 5.7–6 volts.

The parameters for optimum results in terms of percentage yield of strongly bound pertechnetate for a single run are as follows:

1. 5 cc of sodium pertechnetate solution (0.9% saline) from Neisler Neimotec (see Footnote, page 147).
2. 0.3–0.5 cc 1 N HCl (pH of electrolytic solution is about 1.3).
3. 0.05–0.1 cc of 25% human serum albumin (Hyland).
4. 4.2 coulombs of electricity (100 mA for 42 sec at 5–6 volts seems convenient).
5. pH adjusted to 7–8 with saturated solution of sodium bicarbonate after electrolysis.

FIG. 3. Yield of Zr-Tc-albumin complex vs. albumin concentration.

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scanning after adjusting the pH to 6–7 with sterile saturated NaHCO₃. Or it can be heated for 15–30 min in boiling water. After cooling the solution and adjusting the pH to 7 with NaHCO₃ this solution can be further heated for 10–15 min at 85°C in a thermostat to produce macroaggregates for liver scans. Or after the initial heating in boiling water and cooling the pH can be adjusted to 5.5 and heated again at 85–90°C for about 15 min. Centrifuge at 20,000 rpm for 15 min and separate the supernate. The macroaggregates obtained in this way are suspended in isotonic saline for intravenous injection. The particle size varies from 10 to 50 microns.

**DISCUSSION OF CHEMICAL AND ELECTROCHEMICAL REACTIONS**

Hydrous zirconia ordinarily begins to form at a pH of about 2.0 from solutions of moderate zirconium concentrations. An adsorption complex of an ion or molecule on hydrous zirconia can easily be mistaken for a genuine chemical compound. Only when a reaction of a zirconium compound takes place in a fairly strong acid environment at a pH of 1.5 or less can one be reasonably sure that the product is not an adsorption complex. As mentioned earlier (Figs. 1 and 2), at low pH (0.8–1.4) if sodium pertechnetate is added during or immediately following electrolysis some form of zirconium-technetium complex forms. On standing or on raising the pH to 7, however, most of the radioactivity appears as free TcO₄⁻ by chromatographic analysis. Addition of a few drops of 25% human serum albumin solution to the reaction system, at low pH, removes all free pertechnetate ions. This would indicate the occurrence of a chemical reaction and not chemisorption.

No galvanic corrosion* or any behavior reflecting an ionic (Nerst) solution pressure is known for zirconium (4,5). There is little effect of hydrochloric acid, concentrated or dilute, hot or cold, on solid zirconium metal. Anodized films of monoclinic zirconium dioxide (ZrO₂, baddeleyite) (6) which usually attains a limiting thickness (7,8) can be formed at metallic zirconium anode in electrolytic solutions containing oxyanions. The films do not form when anions such as chlorides only are present (8). When the particle size of ZrO₂ is less than 1 micron, slight

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* Zirconium is corroded by chloride solutions containing oxidizing agents Zr + 4(Cl⁻) → ZrCl₄ or Zr + 4 Cl⁻ → ZrCl₄ + 4 e⁻. The oxidizing ions make it possible for the reaction to proceed to the right. Since NaTcO₄ need not be present during electrolysis, this possibility seems to be of no importance. Dissolved oxygen may be expelled by nitrogen and the electrolysis performed while the nitrogen is still bubbling: there is no difference in the results.
solubilization in hydrochloric acid has been observed (8), apparently due to the action of the hydronium ion (9,10):

\[
\text{ZrO}_2 + H_2O^+ \rightarrow H_2O + \text{Zr OOH}^+. \tag{1}
\]

However, as reported earlier (1), for the labeling to take place sodium pertechnetate need not necessarily be present during electrolysis. Addition of the anion, NaTcO_4^−, and albumin may be done after electrolysis is performed with normal saline and HCl mixture (pH ~ 0.8–1.4) to obtain nearly the same yield of labeled protein.* Moreover, minute traces of oxidizing agents such as H_2O_2 and HClO almost completely inhibit the reaction (see footnote, p. 147). The mechanism of the reaction therefore does not appear to be through the oxide film formation. In the aqueous electrolysis, the pH rises in the vicinity of the cathode and almost an invisible layer of hydrous zirconia tends to be deposited over the cathode (11,12). For a quantitative yield of labeled protein the final cell pH does not exceed 1.5. Above pH 1.5, at which the formations of hydrous zirconia is more likely, the yield of bound technetium is very low. Moreover, ions and molecules which complex zirconium tend to inhibit or prevent formation of hydrous zirconia. A slight tendency in this direction is manifested by ClO_4^− ions since more base is needed to precipitate hydrous zirconia (13). In our reaction slight turbidity begins to appear when the pH after electrolysis is raised to 4–6 (with albumin present). The mechanism of the reaction through the formation of hydrous zirconia does not seem to be a likely possibility.

Furthermore, carbonated hydrous zirconia (32% TAM, National Lead, N.Y.) does not show any indication of strong binding with pertechnetate ions in HCl solution (with or without albumin). At low pH some chemisorption (30%) of TcO_4^− on a suspension of hydrous zirconia in 1 N HCl seems to take place. Albumin does not appear to enhance this reaction. Raising pH to 7 liberates the absorbed TcO_4^− almost quantitatively.

A plausible mechanism of the reaction appears to consist of the following stages:

1. Atomic attack by chlorine on zirconium.
2. Ionic addition of TcO_4^−, followed by . . .
3. . . . Molecular addition of albumin as a ligand.

* The order of addition of albumin and NaTcO_4^− seems important (1). If the albumin is added first followed by NaTcO_4^−, there is noticeable decrease in the percent yield of labeled protein. It would appear that the highest coordination number of zirconium is reached with albumin as its ligand so that there are less chances for the pertechnetate ions to react with the zirconium species.

These stages may be represented as follows excluding aquo groups:

Stage 1.† Zr + 4[Cl] + 2 H_2O \rightarrow Zr OOH^+ + 3 H^+ + 4Cl^− \tag{2a}

Zr + 4[Cl] + H_2O \rightarrow ZrO^{2+} + 2 H^+ + 4 Cl^− \tag{2b}

ZrOOH^+ + HCl \rightarrow ZrO^{2+} + 2 H_2O \tag{3}

Stage 2.‡ ZrOOH^+ + TcO_4^− \rightarrow ZrOOHTcO_4^− \tag{4}

ZrO^{2+} + 2TcO_4^− \rightarrow ZrO(TcO_4^−)_2 \tag{5}

Stage 3.|| Attainment of highest coordination number sterically possible for these zirconium species by: (1) hydration, (2) direct formation of coordinate covalent bond between the organic ligand and zirconium atom, (3) chelation due to vicinal functional groups of the albumin and (4) exchange of aquo groups by the organic ligand.

Applications in nuclear medicine. The stability of this ⁹⁹mTc-albumin complex over a wide range of pHs and temperatures makes it possible to obtain this material in different physical forms such as:

† Besides the monomer and the dimer, trimeric and tetrameric cations, Zr (OH)_n^+ and Zr^{n+} may be formed in the presence of a noncomplexing acid such as HCl, due to the action of the hydronium ions (14–18). This does not mean that a tetra positive zirconium ion ever exists. The highest positive charges are distributed over the sphere of coordinate aquo groups. The prevailing species is the monomer at low total acid concentration and the tetramer at high acid concentration. It has been observed that when the pH is brought low with 0.5 cc of 5 N HCl, the yield of the bound technetium is considerably lowered. The decrease in yield is also observed when the cell pH is brought to ~1.5 with only 0.05–0.1 cc of HCl. The optimum results is at pH ~ 1.3 with 0.5 cc of 1 N HCl.

‡ The principal ion in acid solution is generally assumed to be zirconyl ZrO^{2+}. However, Connick has concluded that the ion in acid solution is Zr^{4+}. For a perchlorate solution of ionic strength of 2, he has reported (19) Zr^{4+} + 3 H_2O + ClO_4^− = Zr (OH)_4ClO_4 (colloid) + 3H^+. Addition of potassium metaperiodate, KIO_3, to a zirconate nitrate solution gives 3 ZrO_2 \cdot (IO_4)^− \cdot xH_2O (x = 14 – 18). It seems to be quite stable and unaffected by boiling water. A structure similar to that of polysulfato polyzirconic acids has been suggested (20,21).

|| In this connection it may be pointed out that while zirconyl chloride, ZrOCl_2, and zirconium tetra chloride ZrCl_4, and other zirconium salts have no tanning effect on leather, the behavior of disulfato zirconic acid ZrO(HSO_4)_2 is significantly different. It hardens gelatin and is an excellent tanning agent. According to Lasserre (22) skins in which carboxyl groups are blocked by methylation show unimpaired combining capacity for zirconium as a sulfate complex (disulfato zirconic acid), indicating that probably it is the peptide not the carboxyl groups which are involved in the reaction (23). A similar scheme for pertechnetate complexes of zirconium seems feasible.
Depending upon the size of the particles, the \(^{99m}\text{Tc}\)-human serum albumin finds extensive use in nuclear medicine. In molecular suspension this can be used:

1. To visualize placenta and cardiac blood pool \((2)\).
2. To evaluate surgical and spontaneous cerebrospinal fluid shunts \((30)\).

In the form of microaggregates it is used in studying reticuloendothelial function in man \((26)\). In the macroaggregated form it can be used for:

1. Detection of pulmonary embolism by lung scan \((27, 28)\).
2. As a brain scanning agent when injected into specific arteries. In this case the hemispheres
may be scanned without the obscuring effect of muscle and bone activity (31).

Without describing the instrumental details and structural interpretations of the scans, several representative scans of the various organs visualized after i.v. administration of approximately 1 mCi of $^{99m}$Tc-albumin are given in Figs. 5–8.

Testing this material for over 2 years on several animals (white mice, rabbits and dogs) shows no evidence of any toxicity. For about 4 coulombs of electricity used in the electrolytic process of tagging albumin with technetium, the quantity of zirconium dissolved does not exceed 1 mg in the entire volume (5–6 ml) of the preparation (see Footnote, p. 148). Since the specific activity at the time of preparation is about 2–4 mCi/ml, the quantity of zirconium per milliliter will not exceed 0.2 mg. There is no toxicity at this level for several zirconium compounds administered to higher animals, orally, intratracheally, intravenously, intraperitoneally or topically (32–39).

**SUMMARY**

In an earlier report published elsewhere an electrolytic method of labeling human serum albumin with $^{99m}$Tc at constant voltage was described and possibilities for its clinical application were suggested. In the present paper these possibilities have been further explored. With constant-current electrolysis the quantity of the intermediate complexing agent dissolved at the anode was estimated with reasonable accuracy. Albumin may be used in any of the following forms after labeling:

1. Molecular solution.
2. Microaggregates.
3. Macroaggregates.

Prolonged testing of these materials on animals produced no ill effects. A recipe for obtaining quantitative yields of the labeled albumin without the use of any anion exchange column is given. Representative scans of the various organs of the human body are presented. A plausible reaction mechanism is suggested.

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**17th ANNUAL MEETING**

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Young Investigator’s Prize

The Scientific Program Committee will award a young investigator’s prize at the forthcoming Annual Meeting (Washington, D.C., July 6–12th, 1970). This will be given to the young investigator who presents the most outstanding paper at the special plenary session. The competition will be limited to one paper from each chapter of the Society. Each chapter will select its representative.

Interested investigators, who are 35 years of age or younger, are requested to contact their local chapter president for details concerning local selection procedures. The name of the local officers can be obtained from the Society of Nuclear Medicine, 211 East 43rd Street, New York, New York.