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Chemistry of Technetium as it is Applied to Radiopharmaceuticals*

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The use of technetium-99m as a tracer in medicine was originally suggested because of its optimum nuclear properties (1). Since that time it has become the nuclide of choice in many different types of imaging operations, and many different chemical formulations have been developed. The study of the chemistry of this element, particularly in water solutions, should lead to a better understanding of the use of radiopharmaceuticals which contain it, and to a more rapid development of additional useful formulations.

The chemistry of technetium resembles that of rhenium, and, to a lesser extent, that of manganese. The most stable form of the metal is the pertechnetate ion, TcO_4^- , which resembles permanganate ion, MnO_4^- , but is a much weaker oxidant. Compounds of technetium are known in all valence states from +7 to -1, but in water the most stable are TcO_4^- (+7) and the insoluble TcO_2 (+4) (2).

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In radiopharmaceutical preparations the 6.0-hour half-life of technetium-99m requires either a molybdenum-technetium generator (3) or a ready supply of instant technetium, which is usually obtained by solvent extraction of pertechnetate from strong alkali (4,5,6). In generators there are three major chemical problems: The breakthrough of molybdenum, the breakthrough of aluminum (6), and the existence of some of the technetium in a lower-valent non-extractable form. The latter problem has been met by various methods such as either incorporating an oxidizing agent like hypochlorite in the eluting saline, or allowing the Al_2O_3 column to dry in the presence of air before milking .

When technetium-99m is injected intravenously in the pertechnetate form, there are no chemical problems (apart from those in the preparation). It would be interesting to know to what extent this anion is bound to blood proteins (especially to albumin), but there has been no detailed study of the kind reported for halide ions (7). There is one account in the recent literature which indicates that it is bound more strongly than iodide, and that it is displaced from albumin binding by perchlorate (8), but the number of binding sites and the quantitative strength of binding in different sites are not yet known.

Most of the technetium-based radiopharmaceuticals contain the nuclide in a reduced form. A large number of reducing agents have been used for this purpose, but stannous salts (and the chloride in particular) are the practical choices in the majority of clinically used preparations.

Practically nothing is known about the valence state of the technetium in these systems. A direct determination of the chemistry of technetium-99m is out of the question because of its low concentration (10^{-9} M or less in most formulations). A determination of the valence state of technetium-99 (so-called carrier technetium) is possible, at least in principle, at concentrations of the order of 10^{-4} - 10^{-5} M. While the chemical behavior of technetium at the trace level may well be very different from that at the more conventional concentrations, nevertheless measurements in the latter solutions may shed some light on the behavior of the more dilute solutions.

A study of the reactions of SnCl_2 with $^{99}\text{TcO}_4^-$ in the presence of various complexing agents is almost completed, and will be reported in greater detail elsewhere (9). Some of the results are given below, in a citrate buffer, in DTPA solution, and in HCl. A six-fold molar ratio of SnCl_2 to TcO_4^- was used in each solution. The pertechnetate concentration was approximately 3.4×10^{-4} M, and that of the stannous chloride was 0.020 M. After mixing, each solution was analyzed for unreacted SnCl_2 , and the valence state of the reduced technetium was calculated from the observed stoichiometry. In most cases the excess Sn(II) was determined polarographically from the height of the oxidation wave of the $\text{Sn(II)} \rightarrow \text{Sn(IV)}$ reaction. In each medium the EMF and the height of the wave were measured with known solutions of SnCl_2 . Another aliquot of the reaction mixture was potentiometrically titrated with a standard solution of I_2 , using a platinum electrode and a saturated

calomel reference electrode. This yielded the quantity of unreacted SnCl_2 and in some cases some of the reduced technetium as well. The results of the iodine titrations and the polarographic determinations were then compared.

A large excess of stannous chloride with respect to pertechnetate more closely approximates the conditions in radiopharmaceuticals than does a direct titration. In radiopharmaceuticals the ratio of SnCl_2 to $^{99\text{m}}\text{TcO}_4^-$ may be as high as 10^8 to 10^9 . A larger ratio than 6 in the experiments described below would not have changed matters very much from the point of view of the reduction potential of the solution. In commercially available $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ there will be at least 5% Sn(IV) (10), and the content of oxidized tin will increase on standing (11). The reduction potential of the solution is determined by the ratio Sn(IV)/Sn(II), and in this particular case the Sn(IV) content is not due to the reaction of Sn(II) with $^{99\text{m}}\text{TcO}_4^-$, but rather to its unavoidable presence as a relatively concentrated impurity in the original reagent. This argument is based on the absence of mixed-metal complexes, which may exist, but which have not yet been investigated.

A number of direct titrations were also carried out, in which 0.024 M SnCl_2 in 0.12 M HCl was added from a micro-burette to a solution containing the ligand and $(3.4 \times 10^{-4} \text{ M})$ $^{99}\text{TcO}_4^-$. The end-point was determined potentiometrically with a platinum indicating electrode and a saturated calomel reference electrode. Since the Sn(IV)-Sn(II)

couple does not register a Nernst potential on platinum (12), apparently because of very small exchange currents (13), and perhaps because tin metal deposits on the platinum (12), these titrations were of the bimetallic electrode variety (14). A comparison of these with the polarographic determinations yielded additional information which is described below.

Table 1 shows the results of the analyses of the SnCl_2 - $^{99}\text{TcO}_4^-$ reaction in a 0.4 M sodium citrate solution buffered at pH 7. The column entitled "n" shows the number of electrons gained by the TcO_4^- anion; these were calculated from the equation

$$n = 2 \times \frac{(\text{moles SnCl}_2 \text{ reacted})}{\text{moles TcO}_4^- \text{ initially present}} \quad (1)$$

The term " $\text{SnCl}_2 + \text{NaTcO}_4$ " in the second column refers to a mixture of the two reagents with a six-fold molar ratio of SnCl_2 to NaTcO_4 .

In the presence of excess SnCl_2 the $^{99}\text{TcO}_4^-$ anion in a citrate buffer is reduced to the Tc(IV) state. Since the polarographic and iodometric titrations are in agreement, it can be concluded that I_2 does not oxidize this complex or does so very slowly. The direct titration of pertechnetate with SnCl_2 in the same medium apparently produces a Tc(V) complex, which also is resistant to oxidation by I_2 , or else reacts very sluggishly with it. The formation of the Tc(IV) citrate complex in the presence of excess SnCl_2 , rather than the Tc(V) complex formed by direct titration, can be explained by the difference in reduction potential, with the added hypothesis that the Sn(IV)

citrate complex is much more stable than the Sn(II) complex. This latter situation would make Sn(II) citrate a much more powerful reducing agent than SnCl_2 .

A Tc(V) citrate complex of $^{99\text{m}}\text{Tc}$ has already been reported by Yeh and Kress (15). They treated $^{99\text{m}}\text{TcO}_4^-$ with thiocyanate in strongly acidic solution in the presence of a ferric salt and ascorbic acid. This is the analytical method which was developed by Howard and Weber for determining macroscopic quantities of ^{99}Tc ; they reported that the $^{99}\text{Tc(V)}$ thiocyanato complex was quantitatively extracted into butyl acetate so that its absorbance could then be measured in a spectrophotometer (16). Yeh and Kress simply added an aliquot of the strongly acid solution containing $^{99\text{m}}\text{Tc}$ and thiocyanate to a large excess of a citrate buffer, and assumed that the Tc(V) citrate complex had formed by simple ligand exchange. Whether a simple ligand exchange will occur is not known; Crouthamel reported that the Tc(V) thiocyanate complex resisted oxidation by strong oxidants like Br_2 or ceric sulfate in sulfuric acid, and was not extracted from butyl acetate into water. It was, however, easily reduced by excess stannous sulfate (17). It is possible, therefore, that further reduction took place (perhaps by ascorbic acid, catalyzed by the ferric salt at the buffer pH) before ligand exchange occurred, and that the procedure of Yeh and Kress yielded a mixture of the two Tc-citrate complexes reported above.

Table 2 shows the results of analyses of the SnCl_2 - $^{99}\text{TcO}_4^-$ reaction in a 0.4 M DTPA solution maintained at pH 4.

One can conclude from these results that a Tc(III)DTPA complex is formed from TcO_4^- in the presence of excess SnCl_2 . In the direct titration of TcO_4^- with SnCl_2 , the electron transfer number is 3.5, which is most simply interpreted to mean that a mixture of Tc(IV) and Tc(III) complexes of DTPA were formed. The observed n value (3.5) is not a kinetic artifact, since raising the temperature of the direct titration from 25° to 60° did not produce any significant change in the course of the reaction. In addition, the formation constant of a Tc(IV) complex of EDTA (which resembles DTPA) has been reported (18). It is about 10^{19} .

The identity of n values from the polarographic determination of excess SnCl_2 , and the iodometric titration of aliquots of the same solution means that I_2 reacted only with the excess SnCl_2 . The failure of the iodine to oxidize either the Tc(III) or the Tc(IV) complexes may be due to a very slow rate of reaction, or to a high thermodynamic stability of both complexes. At present it is not possible to distinguish between these two possibilities. The provisional conclusion can be drawn that the radiopharmaceutical preparation of $^{99\text{m}}\text{Tc-DTPA}$ forms the Tc(III) complex.

Similar experiments which are not reported here in detail show that the n value is 3 in the direct titration of $^{99}\text{TcO}_4^-$ with SnCl_2 in HCl solutions whose concentrations vary from 0.1 M acid to 2 M acid. Hence Tc(IV) is formed. Excess SnCl_2 did not change this significantly.

Work is in progress on various phosphate complexes of technetium.

We have encountered kinetic complications which have not yet been resolved.

A second research in progress is concerned with the complexing of reduced pertechnetate by polyhydric alcohols and acids (19). The reduction of pertechnetate by sodium borohydride in the presence of mannitol produced a radiopharmaceutical which tended to concentrate in the kidneys of a rabbit (20). Reduction of pertechnetate by SnCl_2 in the presence of sodium gluconate has also been reported to yield a renal scanning agent (21). One method of analyzing the presumed reduced technetium-mannitol complex was by elution with 0.15 M NaCl solution from a Sephadex G-25M column. In this case, virtually all the technetium remained on the gel. A similar effect was noted with a presumed gluconate-technetium complex, and has been reported by others (22).

Sephadex gels are cross-linked dextrans. The dextrans are branched-chain water-soluble polysaccharides (23). The cross-linking renders this polymer insoluble. In gel chromatography, the peak volume of the eluted material is given by the sum of the void volume (the volume of solvent outside the beads) and the volume of solvent inside the beads, the latter term being multiplied by a distribution coefficient (24):

$$V_{\text{eluate}} = V_{\text{void}} + V_{\text{interior}} \times K_d \quad (2)$$

Large particles like colloids and macromolecules will appear in the void volume ($K_d = 0$). Somewhat smaller particles can diffuse into the

beads, but with some difficulty ($K_d < 1$) and quite small particles will move without difficulty through the beads, and hence will appear late ($K_d = 1$). The assumption which underlies the general use of Sephadex gels is that there is no specific interaction between the solute and the gel ($K_d \gg 1$). Brookhaven Laboratory introduced the use of Sephadex gels in the analysis of radiopharmaceutical products with the expectation that there was no interaction between the various useful technetium-99m compounds and the Sephadex matrix (25). However, it became evident from time to time that some form of reduced technetium was strongly adsorbed by Sephadex, and required oxidation with H_2O_2 to the pertechnetate stage before it could be eluted (26). This substance was thought to be a hydrolyzed and possibly polymerized Tc(IV) compound, because K_2TcCl_6 at neutral pH behaved in this way (27).

In the case of the presumed mannitol and gluconate complexes under investigation, it was hypothesized that they were weak complexes of technetium, and that in competition with the Sephadex they lost the metal ion to the column. It was further hypothesized that the Sephadex retained the reduced technetium because of the high density of alcohol groups in the gel. If so, the adsorption might be overcome by eluting the column with a sufficiently concentrated solution of mannitol rather than with sodium chloride solution. It was found that with a 1% mannitol (0.055 M) solution a large part of the activity was stripped from the column, and appeared in the elution volume which is characteristic of technetium complexes.

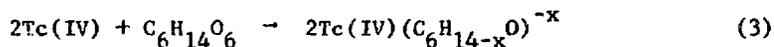
This result suggested the following method of estimating the relative complexing abilities of a group of chemically similar ligands: ^{99m}Tc pertechnetate was reduced with sodium borohydride or with stannous citrate in the presence of a polyhydroxylic compound, an aliquot of the mixture was placed on the column. Elution was performed usually with 70 ml of a 0.055 M solution of the complexing material in a saline-benzyl alcohol aqueous medium. That is, the column and the complexing substance were in competition for the technetium. This approach is limited to fairly weak complexes, and perhaps to chemically related ones.

Some results obtained with this technique are shown in Table 3.

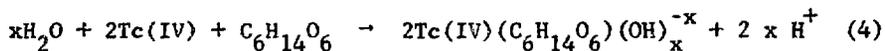
It appears that sugars and other ring compounds do not complex the reduced technetium efficiently, and that a minimum of five neighboring hydroxyl groups in a linear array must be present for successful column stripping. This is shown by the failure of inositol to elute any activity. At the same time, the failure of 1,6-hexanediol to remove technetium from the column indicates that both an appropriate chain length and some minimum number of hydroxyl groups are necessary for complexation. This suggests that the reduced technetium in the complex is in the form of a dimer; the metal atoms are probably linked together by an oxygen or hydroxyl bridge. Otherwise, ethylene glycol or glycerol should be capable of forming a stable complex with a single technetium atom, as they do with other transition metal ions (28).

Paper electrophoresis in 10% mannitol showed that the complex is

negatively charged. This means either that the technetium forms a complex with the mannitol by proton displacement:



or else that the complex adds hydroxyl ions from the water:



At concentrations of the order of 10^{-4} M, in 0.5 M mannitol solution, both ReO_4^- and $^{99}\text{TcO}_4^-$ on treatment with SnCl_2 gave brown-black colloidal solutions which then precipitated. The precipitates were presumably ReO_2 and TcO_2 . This meant that the valence state of technetium in the mannitol complex could not be investigated at a macroscopic concentration level by reaction stoichiometry.

The detailed structure of the complex which is formed is difficult to establish at the present time because in addition to our lack of knowledge of the valence state of the technetium, there is also a stereochemical effect which is involved. That is, both the number of hydroxyl groups and the configurations of the optically active carbon atoms in the molecules are important. This is shown in Table 4. In this table the configurations are shown in the conventional sense (29): The horizontal bars stand for the hydroxyl groups.

The three available hexitols are very much alike in their behavior, whereas there is a marked difference between ribitol, which complexes practically no technetium, and xylitol, which is fairly effective.

Similar effects, both with regard to chain length and to configuration, are seen in the hydroxy-acid series shown in Table 5.

The number of configurational isomers required for a complete evaluation of the stereochemical effect shown in Table 5 is quite large, and only a few have been tried. It can be concluded at this time that the technetium complexes are composed of dimers or higher aggregates of the metal, and that the limit has not yet been reached in complexing ability in the 5-, 6- and 7-carbon atom sequence of the polyhydroxy acids. Experiments at the 10^{-4} M level of concentration with $^{99}\text{TcO}_4^-$ showed no precipitate formation on the addition of sodium gluconate and SnCl_2 . Hence the gluconate complex is more stable than the mannitol complex, which at the 10^{-4} M $^{99}\text{TcO}_4^-$ level of concentration could not prevent the formation of a brown-black precipitate (presumably TcO_2).

The behavior on Sephadex of the weak polyol complexes of reduced technetium raises the more general question of the testing of technetium-containing radiopharmaceuticals for radiochemical purity. Throughout the earlier radiopharmaceutical literature one finds that quality control was attempted through passage of the acidified solution through an anion-exchange resin, or by means of paper chromatography on Whatman No. 1 paper in 85% methanol, or in aqueous saline, particularly (but not exclusively) in the case of tagged human serum albumin. The idea which lay behind all three techniques was that unreduced pertechnetate was the only radiochemical impurity of importance, and that all technetium which was not in the pertechnetate form was to be found in the desired compound. Pertechnetate is very strongly taken up by anion-

exchange resins, whereas various reduced technetium preparations, including anionic complexes, evidently are not. In paper chromatography in 85% methanol, the R_f of pertechnetate is 0.63 to 0.68, permitting ready detection (30). In aqueous saline solution its R_f is 0.75 (31). As Eckelman and Richards have pointed out, however, the matter is more complicated for both HSA and DTPA preparations (32). The complication arises from the formation of reduced, uncomplexed technetium, which they called hydrolyzed technetium. If an anion-exchange resin is used for quality control, one certainly measures pertechnetate, but the effluent will contain both the technetium complex and reduced, hydrolyzed uncomplexed technetium, and they will be counted together. In paper chromatography with 85% methanol as the solvent, pertechnetate will migrate, but the albumin will be denatured, will not move from the origin, and the hydrolyzed technetium may be trapped with it. In any event, they will be counted together. In paper chromatography with aqueous saline solution, albumin moves with the solvent front, and can be differentiated from pertechnetate, which moves more slowly, but the hydrolyzed technetium will remain at the origin. Richards, et al. (3) had previously introduced the use of Sephadex gel chromatography into the analysis of radiopharmaceutical preparations, and Eckelman and Richards (25) compared the elution with saline from Sephadex of Tc-HSA and Tc-DTPA with paper chromatography and with anion-exchange resin chromatography. Saline elution with both paper and Sephadex clearly showed the presence of hydrolyzed reduced technetium;

the latter did not move from the origin in paper, and remained adsorbed on the gel. This is not surprising, since both matrices are composed of polysaccharides. On the other hand, the anion-exchange resin treatment and paper chromatography simply differentiated between pertechnetate and the other forms of technetium. In a recent article Billinghamurst extended this work considerably, including paper electrophoresis as well as thin-layer chromatography on three different solid media using aqueous saline, 85% methanol, butyl acetate and acetone (33). He treated Tc-HSA, Tc-DTPA, TcO_4^- , two commercial preparations of Tc-polyphosphate, and a technetium-iron-ascorbic acid reaction mixture. He concluded that paper chromatography with saline solution was difficult to use because of air oxidation, and that Sephadex columns might be too expensive either in equipment cost or in technician's time. He suggested the use, as solvents, of acetone and butyl acetate on silica gel in thin-layer chromatography, and of acetone on paper in order to practice quick simple assays.

Another subject of interest in the radiopharmaceutical chemistry of technetium-99m is the mechanism of labeling of human serum albumin with this nuclide. One unsolved problem is the valence state of the technetium in the labeled protein. There is general agreement that the technetium is in a reduced state, since such reducing agents as stannous chloride (34) and a ferric chloride-ascorbic acid mixture (35,36) have been used. There is, however, the preparation described by Benjamin (37) in which the electrolysis of $^{99m}\text{TcO}_4^-$ in dilute HCl in the presence of albumin is conducted in a zirconium crucible which serves as the anode, with a

platinum cathode. The mechanism which has been put forth for the albumin labeling starts with the production of chlorine atoms at the anode. These oxidize zirconium metal to the +4 state, the latter forms a stable inner complex with pertechnetate, which remains unreduced, and the stable inner complex then attaches itself to the albumin through the (unsaturated) zirconium cation. We have advanced another explanation for the labeling process. Zirconium is an extremely powerful reducing agent according to its standard reduction potential (38). It is usually protected against oxidation by a very thin, self-healing oxide layer. This layer is penetrated and pitted by chloride ions when a positive potential is applied to the metal (39). Furthermore, hydrofluoric acid in the absence of an applied potential should produce the same effect as dilute HCl with a potential, since fluorides are known to strip the protective zirconium oxide coating very efficiently (40), because of the formation of a very stable zirconium fluoride complex (41). The exposed zirconium metal is then free to react with water, with acid, and with pertechnetate. Some of the experimental results obtained by means of this approach are shown in Table 6 (42). The electrolyses were conducted with an applied potential of 2-3 volts, a current of 0.1 ampere was passed for 42 seconds, and the electrolyte consisted of 0.1 M HCl which contained 0.4% HSA, and some $^{99m}\text{TcO}_4^-$ (37). The container was a zirconium crucible which also served as the anode, and the cathode was a platinum wire. In the zirconium metal-HCl-HF experiment, 100 mg of reactor-grade metal powder was treated under nitrogen for two minutes

with 4 ml of the test solution, the supernatant was decanted, and albumin was added. The pH was raised to 7 with 3 M NaOH, and an aliquot was chromatographed on Sephadex G-25M.

Similar results were obtained when DTPA was substituted for albumin. It was also found that HCl (0.1 M) electrolyzed in the zirconium crucible contained a reducing substance, which was capable of reducing the dye methylene blue. On the basis of these findings and others, it was concluded that the pertechnetate is reduced to some lower-valent state by the activated zirconium metal, and that it is the reduced technetium which binds to the albumin.

The opinion has been expressed that the technetium must be in the +5 state in order to bind to serum albumin (35,43). The basis for this conclusion is somewhat obscure. It appears to be associated with the early discovery that in 2.5 M HCl a mixture of ferric chloride and ascorbic acid serves to reduce ^{99m}Tc-pertechnetate. The role of the ferric chloride was thought to be either complexation with the technetium and ascorbic acid, or operation as a redox buffer to stop the reduction of Tc(VII) at the Tc(V) stage, and prevent further reduction [to Tc(IV)]. Probably the original source of this view is the paper by Howard and Weber (15). They developed a Tc(V) thiocyanate extraction method for the analysis of technetium-99, and used the FeCl₃-ascorbic acid mixture to obtain a quantitative yield of this particular thiocyanate complex. They thought that the ferric salt did not permit the reduction of Tc(VII) to Tc(IV); the latter forms a non-extractable thiocyanate complex,

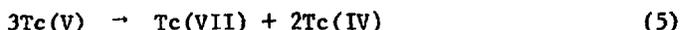
unlike Tc(V) (17). If this is the basis of the conclusion that technetium in tagged albumin is in the +5 state, it is not justified. The end-product of an oxidation-reduction reaction will depend on the nature and the stability of the possible products, as well as on the characteristics of the reagents. Thus, permananganate in neutral solution usually oxidizes Mn(II) to MnO₂; but because of the high stability of the pyrophosphate complex of Mn(III) in the presence of pyrophosphate the oxidation of Mn(II) and the reduction of MnO₄⁻ both stop at the trivalent manganese stage (45). The presence in the iron-ascorbic acid mixture of thiocyanate, which forms an extremely inert complex with Tc(V) and a less stable one with Tc(IV) (16) is of great importance in determining the oxidation level of the technetium. The ferric chloride in this particular system may very well be serving as a redox buffer since Tc(V) thiocyanate is easy to reduce, although difficult to oxidize (17). If there is an especially stable complex of Tc(V) with albumin, this argument will hold for it too. But one cannot simply quote from stability of the thiocyanate complex in order to justify the existence of the supposed Tc(V)-albumin. In addition, the iron may be doing quite different things in the two systems. Howard and Weber extracted the Tc(V) thiocyanate from a very acid solution (2.5 - 3.5 M), whereas it is necessary to make the iron-ascorbate-technetium mixture either practically neutral (35) or alkaline (36) before adding albumin and acidifying it. In the albumin labeling preparation the iron after reduction may form an ascorbate complex

and it may also be acting as a catalyst for the oxidation of ascorbate. Ascorbic acid increases its effectiveness as a reducing agent with increasing pH, but above pH 5 the product of oxidation -- dehydroascorbic acid -- is itself irreversibly oxidized. In addition, ascorbic acid oxidation is usually sluggish. In any event, the chemical behavior of the ferric chloride - ascorbic acid - pertechnetate system may be very different in very acidic thiocyanate solution and in neutral or alkaline solution, and the valence state of the technetium in labeled albumin is not necessarily pentavalent.

Williams and Deegan synthesized $^{99m}\text{Tc(V)}$ by mixing 99m -pertechnetate with dilute HCl, and evaporating the mixture to dryness (43). They then added a solution of human serum albumin, and obtained up to 30% labeling. More precisely, since they used the anion-exchange resin method of analysis, they obtained up to 30% reduction of the added pertechnetate. That is, they assumed that all the reduced technetium was associated with the protein. On mixing ascorbic acid with the albumin before adding the mixture to the dry Tc(V) deposit, lowering the pH to 2.5 and incubating, they obtained a labeling efficiency of up to 90% (i. e. 90% of the $^{99m}\text{TcO}_4^-$ was reduced). They explained their results as follows: albumin in the native state has approximately 26 active groupings or binding sites (46,47). On acidification the protein molecule expands, and the original sites are replaced by approximately 100 weaker groupings, which were hidden -- i. e. not available for reaction in the original protein (48). Ascorbic acid added to the

protein occupies a large number of these sites, and forces the Tc(V) to those sites where it can be strongly held. The localization of the ascorbic acid on the protein also prevents the acid from reducing the Tc(V) to a lower valence state, which would not bind to the protein.

The fate of Tc(V) in dilute acid or water is disproportionation (49), a fact recognized by the authors:



They believe that the disproportionation is minimized by "immediate adjustment to pH 2.5 after addition of the mixture of ascorbic acid and albumin" (43). This may not be sufficient to minimize the disproportionation.

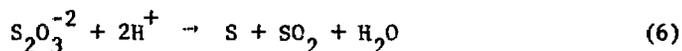
The role of the ascorbic acid in the labeling process is explained by an affinity of ascorbic acid for albumin, based on the work of Shamrai et al. (50). These workers showed that there was a stabilization by albumin of ascorbic acid against auto-oxidation, and reported in addition that in polarography with a solid platinum electrode the half-wave potential of the oxidation of ascorbic acid was changed in the presence of the protein. Neither of these reported effects justifies the conclusion that ascorbic acid is bound to albumin at any of the 100 sites exposed by acidification. In the native protein the nature of the 26 binding sites is not known, but they bind anions, and are thus thought to be positively charged; they are probably substituted ammonium ions. The binding appears to be almost entirely nonspecific differing not in kind but in extent from anion to anion (46), if one restricts the

generalization to small anions-halides, thiocyanate, etc. These, according to Scatchard (46) bring out three types of binding sites in albumin: one strong one, 8 weaker ones, and perhaps 20 still weaker ones. Presumably the strong binding site in Scatchard's preparations had a matching strong site which was blocked by stearic acid (47). It is not clear that ascorbic acid, which at pH 2.5 is a molecular compound, in a lactone form, will be bound at these same positively charged sites. The increase in the number of binding sites on acidification of the albumin solution which is referred to above was found after addition of an anionic detergent sodium dodecyl sulfate (48). These particular binding sites seem to have become available because the hydrophobic chain of the detergent was in direct contact with hydrophobic groups in the protein which previously had been hidden in the folded native protein. It does not seem reasonable from a chemical point of view that these sites, which are certainly positively charged and evidently near hydrophobic groups, should be blocked by the polyhydroxylic (and hence hydrophylic) uncharged ascorbic acid molecules. In this connection, if the same or similar sites are used by the Tc(V), this means that it is an anion. If so, it is difficult to understand why it should be bound more firmly than pertechnetate itself to these sites. The order of binding of small anions to albumin resembles their order of binding to an anion-exchange resin; yet pertechnetate is bound very strongly to anion-exchange resins, and this reduced technetium is not. It appears more reasonable to ascribe the binding of the reduced

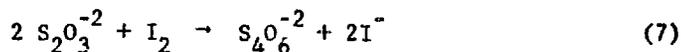
technetium to some specific groups in the protein molecule -- that is, to the formation of a stable complex. In this connection, the ascorbic acid may well be acting, at least in part, as a reducing agent towards any pertechnetate which may have been re-formed by the reaction shown in equation (5). Preliminary experiments with a mixture of HSA, ascorbic acid and $^{99m}\text{TcO}_4^-$ held at pH 2.5 for 30 minutes and at the given concentrations (44), showed by Sephadex gel fractionation with saline after restoration to pH 6.0, that more than 50% of the technetium emerged in the void volume, and that perhaps one-third remained as pertechnetate (51).

The hypothesis that the technetium in labeled albumin is in the +5 state becomes more difficult to sustain when reducing agents other than HCl or ascorbic acid-ferric chloride are used. Stannous chloride has been shown to be effective as a reducing agent for technetium with respect to albumin, either at pH 2.5 (34) or at pH 6 (52). Our own experience with pertechnetate and stannous chloride in dilute and concentrated hydrochloric acid shows that it is reduced to the +4 state, or (if there is insufficient acid) to colloidal TcO_2 . If technetium in the +5 state forms a particularly stable complex with albumin, this state will be stabilized regardless of the reducing agent. This, however, is a particular effect for a particular complex; the arguments advanced for the existence of Tc(V)-albumin rest essentially on the nature of the reducing agent, and not on the nature of the protein complex. We conclude that the actual valence of technetium in labeled albumin is not yet known.

The last topic which will be considered here is the probable chemical nature of technetium in the familiar technetium-sulfur colloid. The first formulation of this radiopharmaceutical, which was carried out by Richards at Brookhaven and later evaluated by Harper, et al. (53), called for acidification of $^{99m}\text{TcO}_4^-$ and the passage of H_2S through the solution. Air oxidation of dissolved H_2S produced colloidal sulfur, and the colloid was stabilized with gelatin. This colloidal preparation is used where small particle size is desired. In this preparation the technetium-99m is probably in the form of the heptasulfide, Tc_2S_7 , since carrier pertechnetate (^{99}Tc) in acid solution will precipitate Tc_2S_7 on the addition of H_2S (54). The method of producing the colloid which is more widely used at present is based on the reaction of thiosulfate with acid in the presence of a colloid stabilizer like gelatin (55,56). This reaction is quite complex. The principal products are sulfur and sulfur dioxide:



However, the reaction goes through the formation of thiosulfuric acid, $\text{H}_2\text{S}_2\text{O}_3$, and this acid, like polythionic acids generally, can also form H_2S (57). The question is whether the pertechnetate-99m may be reduced during the acidification process. Thiosulfates are known to act as reducing agents under some conditions. Thus, the well-known analysis of iodine depends on the oxidation of thiosulfate to tetrathionate:



Thiosulfates can be oxidized to other products as well (58). Thio-sulfate ion can reduce pertechnetate, which is a mild oxidizing agent. Quite recently Cifka and Besely reported that $^{99m}\text{TcO}_4^-$ in solution with $\text{Na}_2\text{S}_2\text{O}_3$ is partially reduced by the latter to a Tc(IV) compound if the mixture is allowed to stand; 10% of the original activity is in the Tc(IV) state after an hour (59). On the other hand, if $\text{Na}_2\text{S}_2\text{O}_3$ is added to an acidified solution of pertechnetate, there appears to be no reduction, and all the activity is found in the sulfur colloid. In this connection, pre-formed Tc(IV) added to TcO_4^- and $\text{Na}_2\text{S}_2\text{O}_3$ and acids does not become incorporated into the sulfur colloid. There is not, however, any direct demonstration that at the carrier-free level Tc_2S_7 is formed when the thiosulfate-pertechnetate mixture is acidified.

There is an argument by analogy which may apply to this case. Briscoe et al., in developing the chemistry of rhenium compounds, found that they could precipitate Re_2S_7 quantitatively (together with sulfur) from an excess of an acidified thiosulfate solution containing ReO_4^- (60). This suggests that the mode of decomposition of the thiosulfuric acid may depend in part on the presence of metallic cations which can form insoluble sulfides. It also suggests that the insolubility of the metal sulfide may stabilize the metal cation in a higher-valent state, and minimize or eliminate its reduction.

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TABLE 1. VALUES OF n IN THE TcO_4^- - SnCl_2 REACTION IN 0.4 M CITRATE AT pH 7

<u>Solution Titrated</u>	<u>Titrant</u>	<u>Method</u>	<u>n</u>
$\text{SnCl}_2 + \text{NaTcO}_4$	-	Polarographic	2.9 ^a
$\text{SnCl}_2 + \text{NaTcO}_4$	I_2	Potentiometric	3.1 ^b
NaTcO_4	SnCl_2	Potentiometric ^c	2.0±0.1 ^d

^aAfter 135 minutes

^bAfter 30 minutes

^cSeven samples

^dSephadex G-25M analysis: 82% complex, 9.5% TcO_4^- , 7.3% on column;
Spectrum: band at 299nm.

I_2 added at end-point produced an immediate jump in potential.

TABLE 2. VALUES OF n IN THE TcO_4^- - SnCl_2 REACTION IN 0.4 M DTPA AT pH 4

<u>Solution Titrated</u>	<u>Titrant</u>	<u>Method</u>	<u>n</u>
$\text{SnCl}_2 + \text{NaTcO}_4$	-	Polarographic	3.9 ± 0.08^a
$\text{SnCl}_2 + \text{NaTcO}_4$	-	Polarographic	4.0 ± 0.06^b
$\text{SnCl}_2 + \text{NaTcO}_4$	I_2	Potentiometric	4.2 ± 0.2^c
NaTcO_4	SnCl_2	Potentiometric	3.5 ± 0.1^d
NaTcO_4 at 60°C	SnCl_2	Potentiometric	3.4

^aSix samples. No trend in n values from 3 to 90 minutes after mixing.

^bFour samples. Doubled TcO_4^- concentration.

^cFive samples. No trend in n values from 15 to 120 minutes after mixing.

^dSix samples. Sephadex analysis showed 99% in complex fraction. Addition of I_2 at end-point produced a large jump in potential.

TABLE 3. ELUTION OF REDUCED TECHNETIUM FROM SEPHADEX G-25M COLUMNS
BY 0.055 M COMPLEXONES: POLYOLS AND SUGARS

<u>Eluant</u>	<u>Structure</u>	<u>Percent Complex</u>
Ethylene glycol	$\text{CH}_2\text{OH}-\text{CH}_2\text{OH}$	~0
Glycerol	$\text{CH}_2\text{OH}-\text{CHOH}-\text{CH}_2\text{OH}$	~0
Erythritol	$\text{CH}_2\text{OH}-(\text{CHOH})_2-\text{CH}_2\text{OH}$	~0
Xylitol	$\text{CH}_2\text{OH}-(\text{CHOH})_3-\text{CH}_2\text{OH}$	50-60
Mannitol	$\text{CH}_2\text{OH}-(\text{CHOH})_4-\text{CH}_2\text{OH}$	60-70
Perseitol	$\text{CH}_2\text{OH}-(\text{CHOH})_5-\text{CH}_2\text{OH}$	50-60
Inositol	$(\text{CHOH})_6$ (Ring System)	~0
1,6-hexanediol	$\text{CH}_2\text{OH}-(\text{CH}_2)_4-\text{CH}_2\text{OH}$	~0; trailing
d-Mannose	$\text{CHO}-(\text{CHOH})_4-\text{CH}_2\text{OH}$	~0
Sucrose	fructose-0-dextrose	~0

TABLE 4. ELUTION OF REDUCED TECHNETIUM FROM SEPHADEX COLUMNS
 BY 0.055 M SOLUTIONS OF POLYHYDRIC ALCOHOLS:
 CONFIGURATIONAL EFFECTS

<u>Eluant</u>	<u>Structure</u>	<u>Configuration</u>	<u>Percent Complex</u>
Xylitol (meso)	$\text{CH}_2\text{OH}-(\text{CHOH})_3-\text{CH}_2\text{OH}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	50-60
Ribitol (meso)	$\text{CH}_2\text{OH}-(\text{CHOH})_3-\text{CH}_2\text{OH}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	~0
d-Mannitol	$\text{CH}_2\text{OH}-(\text{CHOH})_4-\text{CH}_2\text{OH}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	60-70
Sorbitol (d-glucitol)	$\text{CH}_2\text{OH}-(\text{CHOH})_4-\text{CH}_2\text{OH}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	60-70
Galactitol (meso)	$\text{CH}_2\text{OH}-(\text{CHOH})_4-\text{CH}_2\text{OH}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	60-70

TABLE 5. ELUTION OF REDUCED TECHNETIUM FROM SEPHADEX COLUMNS
BY 0.055 M SOLUTIONS OF NEUTRALIZED HYDROXY-ACIDS

Eluant	Structure	Configuration	Percent Complex
Lactic Acid	$\text{CH}_3\text{-CHOH-COOH}$	-	7
Glyceric Acid	$\text{CH}_2\text{OH-CHOH-COOH}$	-	3
d-Ribionic Acid	$\text{CH}_2\text{OH-(CHOH)}_3\text{-COOH}$	$\begin{array}{c} \text{COOH} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	~0; trailing
d-Xylonic Acid	$\text{CH}_2\text{OH-(CHOH)}_3\text{-COOH}$	$\begin{array}{c} \text{COOH} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	22
d-Gluconic Acid	$\text{CH}_2\text{OH-(CHOH)}_4\text{-COOH}$	$\begin{array}{c} \text{COOH} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	82
l-Galactonic Acid	$\text{CH}_2\text{OH-(CHOH)}_4\text{-COOH}$	$\begin{array}{c} \text{COOH} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	~0
d-Glucoheptonic Acid	$\text{CH}_2\text{OH-(CHOH)}_5\text{-COOH}$	$\begin{array}{c} \text{COOH} \\ \\ \text{---} \\ \\ \text{CH}_2\text{OH} \end{array}$	94

TABLE 6. SEPHADEX CHROMATOGRAPHY OF Tc-LABELED ALBUMIN PREPARED BY TWO METHODS

<u>Method</u>	<u>Percent Tc in Albumin Fraction</u>	<u>Percent Tc in Complex</u>	<u>Percent TcO₄⁻</u>	<u>Percent Adsorbed</u>	<u>Percent Recovered</u>
Electrolysis (38)	62.4	-	24.0	9.8	96.2
Electrolysis (38)	58.9	-	29.5	8.0	98.4
Zr-HCl-HF (1 M HCl, 1 M HF)	62.6	9.5	8.2	15.7	96.0

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