A CRITICAL EVALUATION OF $^{99m}$Tc-Fe-ASCORBIC ACID COMPLEX AS A RENAL SCANNING AGENT


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Chlormerodrin labeled with $^{197}$Hg leaves something to be desired as a renal scanning agent. Since the photon energy of $^{197}$Hg is less than 80 keV, tissue absorption becomes a problem with only one-half of the disintegrations which occur in the kidney being available for interaction with the detector crystal. Because of the renal radiation dose (about 1 rad/100 $\mu$Ci) (1), one can give only small amounts of this compound. This precludes doing perfusion studies with $^{197}$Hg since at least 10 mCi are necessary for such a study. Iodine-131-orthoido-Hippuran is useful for visualizing kidneys in severe renal disease if continuously infused; however, when used with the single-injection technique, it introduces artifact by causing areas of increased activity due to drainage phenomena. Unfortunately, no outstanding alternative has arisen to replace these radiopharmaceuticals. It would be advantageous to use a $^{99m}$Tc-labeled agent because of the favorable characteristics of this nuclide for gamma-camera scintiphotography. The $^{99m}$Tc-Fe-ascorbic acid complex, while it is not a perfect radiopharmaceutical for renal evaluation, appears to have certain advantages over the mercury-labeled compounds. A considerable amount of data has been accumulated on the clinical aspects of $^{99m}$Tc-Fe-ascorbic acid complex (2), but little is known about its protein binding, red-cell binding, or its renal clearance compared with inulin clearances. In the following paper we present both clinical and basic data on this radiopharmaceutical.

MATERIALS AND METHODS

The $^{99m}$Tc-Fe-ascorbic acid complex was made by the method of Stapleton et al (3), incorporating 15 mCi of $^{99m}$TcO$_4^-$ from an Abbott molybdenum generator. The radiopharmaceutical was administered to patients as a bolus following positioning of the patient under the Anger camera. Polaroid prints were pulled manually at 4-sec intervals until eight exposures were obtained. Fifty milliliters of Warner-Chilcott inulin was then immediately administered intravenously. Four urine samples were collected on seven patients, each of 20-min duration, and one clearance on an eighth patient. Blood was drawn midway during each collection period. The patients were mildly hydrated to insure adequate urine flow rates. The single-injection technique for the clearances was chosen over continuous-infusion studies because the usual method of administering the radiopharmaceutical is by single-injection techniques (3). Clearance values did not vary by more than 20% in any patient. Static scintiphotography of the kidneys was performed at hourly intervals for 3 hr. For each scintiphoto 200,000 counts were collected.

Heparinized blood samples were centrifuged within 30 min after collection; the plasma was then removed, and an aliquot of washed, packed red blood cells, plasma, and urine was counted in a Baird-Atomic well counter. An aliquot of each sample of plasma was then precipitated with 20% trichloroacetic acid and centrifuged for 15 min at 3,500 rpm following a thorough mixing on a vortex mixer. All samples were precipitated at the same time. The precipitate and the supernatant were counted, with care being taken to keep the geometry constant. Plasma and urine inulin determinations were performed by the method of Roe et al (4). Chromatography was performed in Gaffney’s solution (butanol 4:acetic acid 1:water 1 (v/v) on Whatman No. 1 paper, then placed on photographic film overnight. The areas on paper corresponding to the radioactivity were cut out and counted. Clearances were performed for total radioactivity and for nonplasma protein-bound radioactivity using the standard UV/P formula. Five other patients with serum creatinine concentrations.

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greater than 3 were studied, and the quality of these scans was correlated with this test. Inulin and $^{99m}$Tc-Fe-ascorbic acid clearances were not performed on these patients.

**RESULTS**

**Clinical studies.** Perfusion studies performed with $^{99m}$Tc-Fe-ascorbic acid complex are identical to those performed with $^{99m}$TcO$_4^-$ (Fig. 1A).

Scintiphotos of good quality (Figs. 1B and 1C) were obtained in eight patients with serum creatinine concentration below 3.0. In five other patients (clearances not included in this study) who had serum creatinine concentrations above 3.0, a variable degree of resolution was obtained (Fig. 1D). In all cases, particularly those with poor renal function, scintiphoto quality was improved with delayed imaging. Optimal results were usually obtained by 3 hr postinjection.

**Ureteral visualization.** In many instances the ureters could be visualized; however, the results were not reliable enough to warrant use of this radiopharmaceutical for this purpose.

**Organ interference.** Liver activity was invariably present on all scintiphotos, and the spleen was occasionally visualized. In cases of poor renal function (Fig. 1D) this always presented a problem in interpretation.

**Effect of time on the $^{99m}$Tc-Fe-ascorbic acid complex.** Figure 2 shows the effect of time on the $^{99m}$Tc-Fe-ascorbic acid complex. The radiopharmaceutical was freshly made and chromatographed. Following this it was allowed to stand in the container and was chromatographed at intervals of 1 hr. This experiment was repeated four times with similar results in each case. The change in the complex is striking, with a great deal more radioactivity moving from

![Image](https://example.com/image1.png)

![Image](https://example.com/image2.png)

**FIG. 1.** A shows normal perfusion study; B, normal gamma camera scintiphoto; C, normal rectilinear scan (note uptake in liver); and D, delayed scan in patient with poor renal function showing increased uptake in liver.
the origin to the \(99\text{mTcO}_4^-\) area. There is also an increase in activity toward the solvent front.

**Chromatographic study of the radioactivity excreted in the urine.** Figure 3 is the chromatogram of pertechnetate, \(99\text{mTc}-\text{Fe}-\text{ascorbic acid complex, and the radioactivity excreted in the urine following the injection of the } 99\text{mTc}-\text{Fe}-\text{ascorbic acid complex. Chromatography was performed immediately after collection of the urine. Since the quantity of radioactivity excreted does not fog the film well in the appropriate places, lines have been drawn to show the areas counted in the well counter. They correspond in } R_t \text{ to the injected material spotted beside them. This experiment was performed on the urine of five of the ten patients studied, and the results were consistent.}

The preinjection material contains more radioactivity at the origin and in the tail of its chromatogram than the urine that was chromatographed, while the urine has a decidedly higher percentage of \(99\text{mTcO}_4^-\) than the preinjection material.

**Renal clearances of \(99\text{mTc-Fe-ascorbic acid complex compared with inulin.}** Multiple clearances of inulin and \(99\text{mTc}-\text{Fe-ascorbic acid complex were performed on seven of the eight patients (one patient had only one clearance) and are presented in Table 1. The clearance of the radioactivity from whole plasma is much lower than that of inulin with a CTC/CIN ratio being only 0.36. If Patient 6 were eliminated from the group, the ratio would have been 0.43. The free radioactivity clearance is nearly identical to the clearance of inulin, the CTC/CIN ratio being 0.91. Again, if Patient 6 were eliminated, the free radioactivity clearance would be 1.03, very close to unity.

**Red blood cell and plasma protein binding.** Table 2 shows conclusively that very little \(99\text{mTc}\) in the vascular compartment is bound to the red cells but that a great deal of the radioactivity is associated with the plasma proteins. When one washes the protein precipitate with trichloroacetic acid, another 10% of the radioactivity can be recovered in the supernatant. Thus, at least half of the radionuclide is associated with the plasma proteins.

**DISCUSSION**

Since its introduction in 1966 by Harper et al (5), several institutions including our own have studied patients using \(99\text{mTc}-\text{Fe-ascorbic acid complex and found it to be useful for renal evaluation. It fails to be a perfect renal scanning agent because the kidney image is degraded in moderate-to-severe renal disease by excessive liver background and its continuous breakdown into compounds other than the original complex.}

In order to obtain high-quality scans the background radioactivity should be much lower than that of the organ being scanned. In the case of \(99\text{mTc}-\text{Fe-ascorbic acid complex, the radioactivity is distributed in many body pools, and when coupled with renal excretion, which is the chief mechanism of its}
elimination in the first 24 hr, one achieves the desired target organ-to-background ratio. The target-to-background ratio reaches a level in the kidneys sufficient for scanning to be performed in about 1 hr postinjection, and autoradiographs show the isotope concentration to be in the renal cortex.

Formal studies of the renal excretion of any compound require one to know its purity, the quantity bound in the vascular compartment, and the clearance of the compound compared with inulin which is the basic measuring agent for glomerular filtration rate. As is shown in Fig. 2, the compound is certainly not "pure" and furthermore it changes its form on standing to a compound that migrates at a different Rf from the original substance. This is important since the kidney may excrete different technetium compounds at different rates. The clearance of pertechnetate ion is only 13.5% that of inulin (6) in the human; therefore the background remains high following its injection, and renal scanning cannot be done well using this agent. Since one of the products of degradation of 99mTc-Fe-ascorbic acid complex is 99mTcO₄⁻, one should use 99mTc-Fe-ascorbic acid complex as quickly as possible after it is made to avoid the formation of pertechnetate which clears so slowly.

Our data show that in each milliliter of blood, 10% of the radioactivity is bound to the red cells, which is approximately the percentage RBC binding noted by Burdine and Legeay (7). The remaining radioactivity in the blood specimen is in the plasma, and 56% of this is precipitable with the plasma proteins. Vigorous washing procedures will remove another 10%. However, it is probable that this radioactivity was actually loosely bound to the proteins. Plasma protein binding limits the clearances of 99mTc-Fe-ascorbic acid complex to substantially less than that of inulin, as shown in Table 1. It is noteworthy, however, that while the clearances are less than those of inulin, they are substantially higher than those of 99mTcO₄⁻ reported by Dayton et al (6). It would appear, therefore, that more than one form of technetium has to be excreted, and the chromatogram of the urine shows this to be true. About 30% of the radioactivity excreted in the urine migrated as 99mTcO₄⁻ in Gaffney's solution with slightly more than this remaining at the origin. When the injected dose was chromatographed, only half as much radioactivity migrated at the 99mTcO₄⁻ area as did the radioactivity from the urine. It is probable that some of the reduced technetium is oxidized by the body to 99mTcO₄⁻, thereby increasing the percentage of the VII valence state in the urine. This would be in keeping with the chemistry of technetium as described by Lederer (8).

Of interest are the free radioactivity clearances

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**TABLE 1. RENAL CLEARANCE OF 99mTc-Fe-ASCORBIC ACID COMPLEX AND ITS PROTEIN-FREE MOIETY COMPARED WITH INULIN CLEARANCE**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Urine flow (cc/min)</th>
<th>CTC</th>
<th>CIN</th>
<th>CTC/CIN</th>
<th>C free TC</th>
<th>C free TC/CIN</th>
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<tbody>
<tr>
<td>1</td>
<td>4.2</td>
<td>17</td>
<td>90</td>
<td>0.19</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>4.25</td>
<td>22</td>
<td>54</td>
<td>0.40</td>
<td>52</td>
<td>0.96</td>
</tr>
<tr>
<td>3</td>
<td>10.58</td>
<td>21</td>
<td>58</td>
<td>0.36</td>
<td>60</td>
<td>1.03</td>
</tr>
<tr>
<td>4</td>
<td>2.28</td>
<td>45</td>
<td>95</td>
<td>0.47</td>
<td>112</td>
<td>1.18</td>
</tr>
<tr>
<td>5</td>
<td>1.58</td>
<td>30</td>
<td>73</td>
<td>0.41</td>
<td>79</td>
<td>1.08</td>
</tr>
<tr>
<td>6†</td>
<td>1.4</td>
<td>2</td>
<td>22</td>
<td>0.10</td>
<td>4</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
<td>44</td>
<td>89</td>
<td>0.49</td>
<td>90</td>
<td>1.01</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>11</td>
<td>23</td>
<td>0.49</td>
<td>21</td>
<td>0.91</td>
</tr>
<tr>
<td>Average</td>
<td>3.99</td>
<td>24</td>
<td>63</td>
<td>0.36</td>
<td>-60</td>
<td>+0.91</td>
</tr>
</tbody>
</table>

* Cannot be calculated since plasma proteins were not precipitated.
† Renal failure.
‡ Average excludes Patient 1.

**TABLE 2. RED BLOOD CELL AND PLASMA BINDING OF 99mTc-Fe-ASCORBIC ACID COMPLEX IN VASCULAR COMPARTMENT**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Plasma protein bound</th>
<th>Plasma free</th>
<th>RBC bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>not performed</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>58</td>
<td>35.3</td>
<td>24.7</td>
</tr>
<tr>
<td>3</td>
<td>64.7</td>
<td>39.5</td>
<td>8.0</td>
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<tr>
<td>4</td>
<td>60.5</td>
<td>37.7</td>
<td>8.9</td>
</tr>
<tr>
<td>5</td>
<td>62.3</td>
<td>50.6</td>
<td>9.2</td>
</tr>
<tr>
<td>6</td>
<td>49.4</td>
<td>49</td>
<td>6.6</td>
</tr>
<tr>
<td>7</td>
<td>51.0</td>
<td>52.6</td>
<td>5.4</td>
</tr>
<tr>
<td>8</td>
<td>47.4</td>
<td>43.8</td>
<td>10.7</td>
</tr>
</tbody>
</table>

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which suggest that the protein-free moiety of $^{99m}$Tc-Fe-ascorbic acid complex is handled by glomerular filtration. Without further studies it is not possible to be certain that this is true, nor is it possible to determine the extent to which glomerular clearance of $^{99m}$Tc-Fe-ascorbic acid complex contributes to the scan image. If this were the only method of excretion of this compound, one would suspect that very poor scans would result in patients with impaired glomerular filtration since background would remain high giving a poor target organ-to-background ratio. Until further information regarding its excretion is available, it would not be wise to use $^{99m}$Tc-Fe-ascorbic acid complex as a measure of glomerular filtration rate.

While it is less than optimal as a kidney scanning agent for reasons mentioned, the $^{99m}$Tc-Fe-ascorbic acid complex does have some advantages over the mercury compounds. The efficiency of the 140-keV photon makes it more suitable for the Anger camera, and it penetrates tissues better than $^{197}$Hg. The low radiation dose allows large amounts to be given, thereby enabling the investigator to perform perfusion studies. If Anger-camera studies are performed, the imaging will be quite rapid, reducing respiratory artifact often present with mercury. In severely curtailed renal function one can get a better image with delayed scanning; unfortunately liver background distorts the $^{99m}$Tc scintiphoto to about the same degree that it does the mercury scintiphoto.

CONCLUSION

Our data show $^{99m}$Tc-Fe-ascorbic acid complex to be an adequate but not outstanding renal scanning agent. Problems of resolution arise when serum creatinine levels rise above 3.0 mg%. These problems can be partially compensated for by delaying scanning time for 3 hr. Perfusion studies using this radiopharmaceutical are identical to those obtained when pertechnetate ion is used, thereby obviating the need for multiple isotope procedures. The radiopharmaceutical apparently oxidizes upon standing and should be used immediately. There is 10% red blood cell and 56% plasma protein binding. The clearance ratio of $^{99m}$Tc-Fe-ascorbic acid/inulin is approximately 0.36, and the clearance ratio of free $^{99m}$Tc-Fe-ascorbic acid/inulin is 0.91, suggesting that the proteins free moiety is excreted by glomerular filtration.

REFERENCES