Chromatographic Quality of Three ^{99m}Tc Bone-Imaging Agents

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Increased uptake by stomach, thyroid, and liver incidental to bone scanning suggests that ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) and reduced hydrolyzed technetium-tin colloid [$^{99m}Tc(OH)_4 \cdot Sn(OH)_2$] are radiochemical impurities present in Sn-diphosphonate (EHDP) and Sn-pyrophosphate (PP). The amount of uptake in stomach, salivary glands, and thyroid is due to the presence of pertechnetate and is related to the time interval between preparation and administration. An evaluation of the bone agents ^{99m}Tc-EHDP and ^{99m}Tc-PP showed increasing amounts of $^{99m}TcO_4^-$ during a 5-h interval, while ^{99m}Tc-EHDP with stabilizer was unchanged. In contrast, the colloid concentration was the same in all agents tested and did not change with time. Administration of ^{99m}Tc-EHDP or ^{99m}Tc-PP within 2 h after preparation will reduce the amount of free pertechnetate and improve bone-imaging quality. Our data suggest that a stabilizer should be used when the interval from preparation to administration is expected to exceed 2 h.

Bone scans performed using either Sn-diphosphonate (^{99m}Tc-EHDP) or Sn-pyrophosphate (^{99m}Tc-PP) will occasionally demonstrate increased activity in organs such as liver, thyroid, or stomach, in addition to the normal uptake in the skeleton, kidneys, and soft tissue. This has been observed by several investigators (*1, 2*), and suggestions were made that pertechnetate ion ($^{99m}TcO_4^-$) and reduced hydrolyzed technetium-tin colloid [$^{99m}Tc(OH)_4 \cdot Sn(OH)_2$] may be impurities and be responsible for this unusual organ uptake (*3–5*). Because these compounds do not label bone, they lead to unnecessary radiation and degrade the quality of the image. In this report, the radiochemical purity of the bone-scanning agents ^{99m}Tc-EHDP, ^{99m}Tc-PP, and ^{99m}Tc-EHDP with stabilizer (ascorbic acid) is evaluated in an attempt to learn more about the nature and control of these impurities.

Materials and Methods

A total of 500 bone scans were reviewed to determine the occurrence of abnormal organ uptake and to estimate the magnitude of the problem. The $^{99m}TcO_4^-$ and the colloid were then prepared to demonstrate their biologic distribution in rabbits. The $^{99m}TcO_4^-$ was obtained from a commercial ^{99}Mo-^{99m}Tc generator and diluted with isotonic saline solution. The colloid was prepared by adding, to the effluent of the same technetium generator, a 0.01% solution of $SnCl_2 \cdot 2H_2O$ in isotonic saline. The final preparation contained 2 mCi of ^{99m}Tc and 0.1 mg of $SnCl_2 \cdot 2H_2O$ per milliliter. Activity of ^{99m}Tc in both preparations was kept equal on a volume basis. One to 3 mCi of either $^{99m}TcO_4^-$ or colloid were injected iv into adult albino rabbits (3 to 4 kg). One hour after injection, with the rabbits anesthetized, imaging was performed in the anterior projection, using a Searle Radiographics scintillation camera with parallel hole collimator. Images were recorded on Polaroid film.

In the third part, a screening method was established to evaluate preparations of commercially available bone-scanning agents for the presence of pertechnetate and colloid, using chromatography on Whatman No. 1 paper and impregnated glass fiber media (Gelman ITLC type SG). Autoradiographs were prepared by exposing the chromatograms to Kodak RP-L14 medical x-ray film for 1 h.

With paper chromatography, quantitation of the different compounds was achieved by applying the sample 3 cm from the bottom of a 20-cm paper strip and developing the strip in 85% methanol by the ascending method. The R_f for ^{99m}Tc-EHDP was less than 0.20, and for $^{99m}TcO_4^-$ the range of R_f was 0.43 to 0.54. Paper strips were cut at R_f 0.30, and each section was counted in an NaI (Tl) well counter. The amount of $^{99m}TcO_4^-$ was calculated using the formula

$$^{99m}TcO_4^- (\%) = \frac{\text{count rate } (> 0.30\ R_f) - \text{background}}{\text{total strip count rate} - \text{background}} \times 100.$$

The reproducibility of measuring TcO_4^- by this method was ±1.2% (coef. var.) for a sample containing 14% $^{99m}TcO_4^-$. On paper strips in isotonic saline, the colloid had an R_f of about 0 and the $^{99m}TcO_4^-$ had an R_f of 1.0. Strips were cut at R_f 0.50, for separating these two compounds, and the percentage of the colloid was calculated by the same formula.

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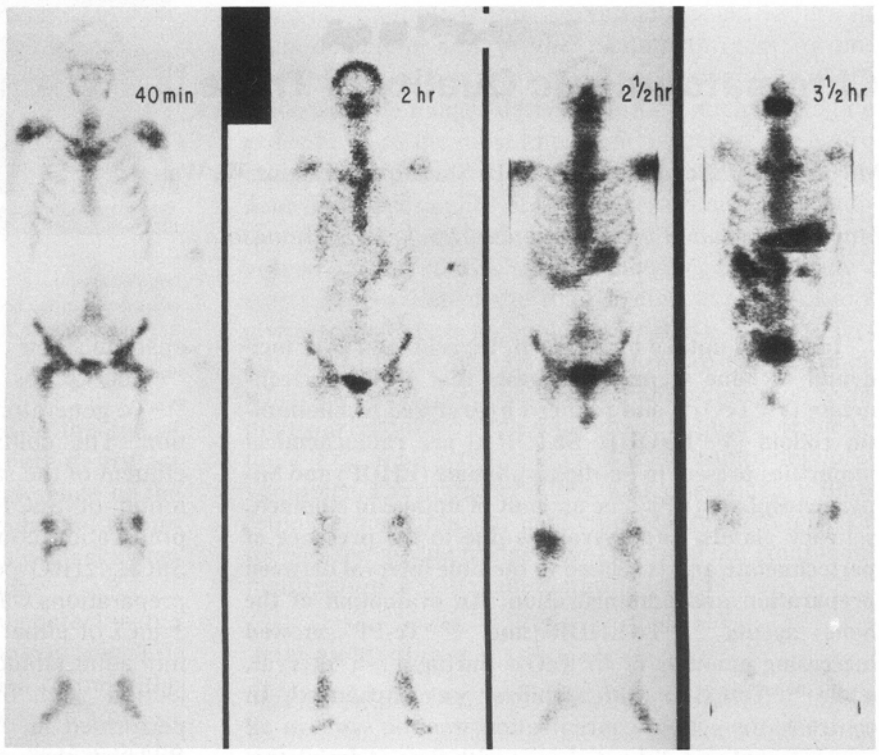

FIG. 1. Bone scans performed with ^{99m}Tc-EHDP suggesting significant amounts of $^{99m}TcO_4^-$. All scans were performed with same preparation of radiopharmaceutical but on different patients. Time elapsed between preparation and injection differed and is noted in upper right-hand corner of each scan.

For glass fiber chromatography, impregnated glass fiber media chromatography was performed using acetone (100%) for the separation of $^{99m}TcO_4^-$ from labeled phosphates. The R_f values were 0 for the ^{99m}Tc-phosphates and 1.0 for $^{99m}TcO_4^-$. Using isotonic saline, the R_f values for the colloid were 0 and 1.0 for both $^{99m}TcO_4^-$ and ^{99m}Tc-phosphates. Quantities of the radiochemical impurities were determined in the manner described for paper media.

Results

Abnormal organ uptake in bone scans occurred in less than 5% of the cases reviewed. However, when it did occur, it was seen in several consecutive patients. Its occurrence appeared unpredictable.

Bone scans demonstrating the presence of unusual organ uptake are shown in Fig. 1. The four bone-imaging procedures were performed on the same day. All factors regarding dosage of ^{99m}Tc-EHDP were identical except the time interval from dose preparation to administration, which ranged over 4 h. Significant quantities of pertechnetate are suggested by the uptake in the stomach, thyroid, and salivary glands. More uptake in these organs is seen with the time elapsed between preparation and injection of the radiopharmaceutical agent. Another bone scan performed with ^{99m}Tc-PP demonstrated uptake in the region of the liver, suggesting the presence of colloid (Fig. 2). One series of patients demonstrating uptake in stomach and thyroid region is described, together with the pertinent radiopharmaceutical data (Fig. 3).

FIG. 2. Bone scan performed with ^{99m}Tc-pyrophosphate showing uptake of technetium-tin colloid in liver.

The biologic distribution of pertechnetate and the colloid in rabbits is shown in Fig. 4. The distribution pattern of pertechnetate shows greatest activity in the bladder, kidneys, thyroid, stomach, and heart (blood pool). The distribution of colloid is in the liver and spleen, as expected for a colloidal pharmaceutical.

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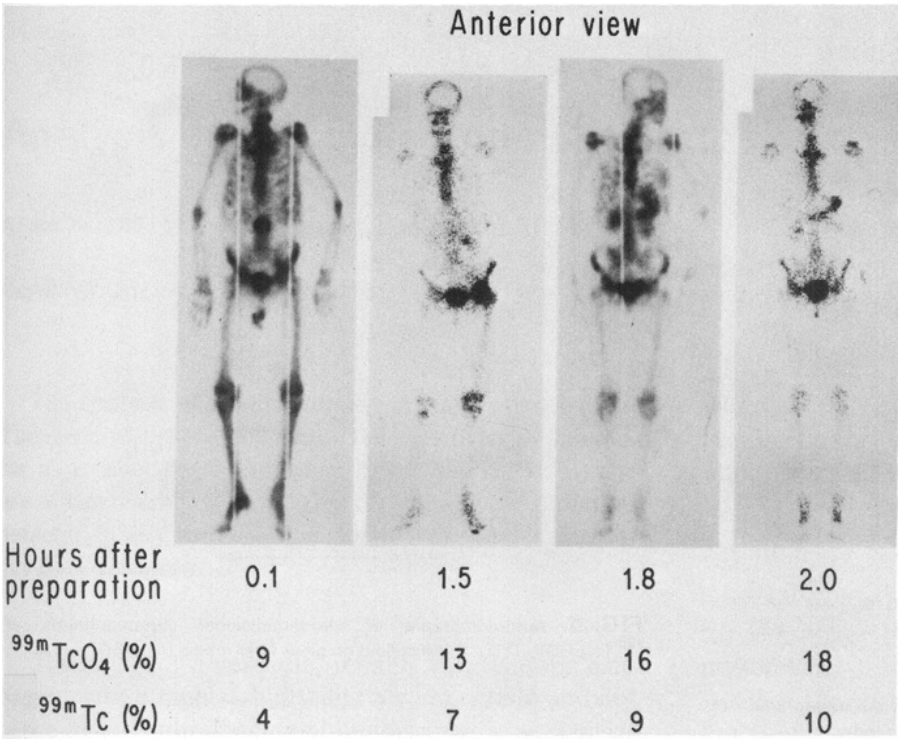

FIG. 3. Bone scans made with ^{99m}Tc-EHDP showing quantities of $^{99m}TcO_4^-$ and $^{99m}Tc(OH)_2 \cdot Sn(OH)_2$ colloid as impurities. Stomach activity was noted at about 15% of $^{99m}TcO_4^-$. Up to 10% of colloid did not result in liver image on scan.

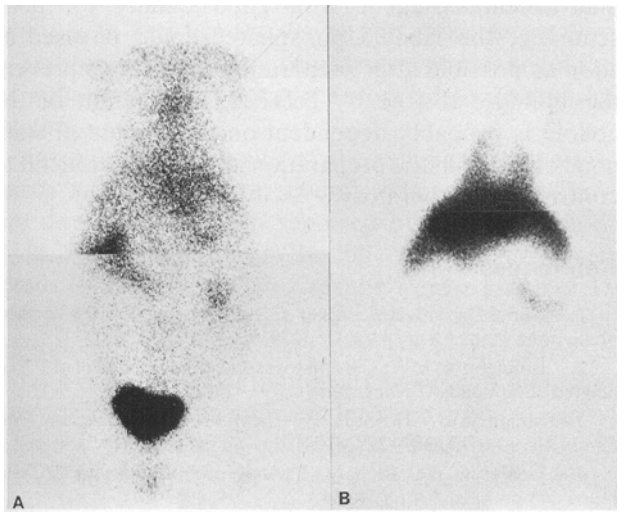

FIG. 4. (A) Distribution of $^{99m}TcO_4^-$ in rabbit is predominantly in thyroid, heart (blood pool), stomach, kidney, and bladder. (B) Distribution of colloid in rabbit is in liver and spleen.

colloid are summarized in Tables 1 and 2. Technetium-99m-EHDP and ^{99m}Tc-PP showed increasing amounts of free $^{99m}TcO_4^-$ with the time interval studied, while ^{99m}Tc-EHDP with stabilizer remained unchanged, attesting to the effectiveness of the stabilizer. Technetium-99m-EHDP and ^{99m}Tc-PP showed the same amount of $^{99m}TcO_4^-$ buildup. In general, colloid remained relatively stable over 5 h in all three agents. Comparison of both impurities showed that the colloid was present initially in greater amounts than pertechnetate in ^{99m}Tc-EHDP until about 4 h after preparation, and was present in greater amounts in ^{99m}Tc-PP until about 2 h after preparation.

TABLE 1. $^{99m}TcO_4^-$ in Three Bone-Scanning Agents at Different Times After Preparation*

Agent	Time elapsed after preparation (h)				
	1	2	3	4	5
^{99m}Tc-diphosphonate (20 examinations)	4.7 (±3.3)	7.8 (±6.1)	11.2 (±10.9)	16.0 (±13.4)	18.9 (±16.7)
^{99m}Tc-pyrophosphate (7 examinations)	4.8 (±6.3)	5.5 (±5.2)	11.4 (±8.0)	21.0 (±13.5)	27.6 (±16.9)
^{99m}Tc-diphosphonate with stabilizer (5 examinations)	1.4 (±2.5)	1.8 (±2.6)	1.3 (±1.6)	1.3 (±1.5)	0.4 (±0.2)

**Values are given as $^{99m}TcO_4^-$ percent of total ^{99m}Tc present (mean ± 1 sd).*

TABLE 2. $^{99m}Tc(OH)_4 \cdot Sn(OH)_2$ Colloid in Three Bone-Scanning Agents at Different Times After Preparation*

Agent	Time elapsed after preparation (h)				
	1	2	3	4	5
^{99m}Tc-diphosphonate (5 examinations)	11.6 (±5.3)	10.4 (±3.8)	13.5 (±5.1)	12.1 (±6.7)	6.6 (±4.4)
^{99m}Tc-pyrophosphate (6 examinations)	7.2 (±2.9)	6.0 (±4.0)	5.8 (±4.0)	4.6 (±4.2)	4.8 (±4.0)
^{99m}Tc-diphosphonate with stabilizer (5 examinations)	6.7 (±11.4)	7.6 (±13.0)	6.2 (±9.2)	6.0 (±10.3)	6.0 (±10.3)

**Values are given in colloid percent of total ^{99m}Tc in the preparation (mean ± 1 sd).*

Chromatographs of the potential radiochemical impurities $^{99m}TcO_4^-$ and $^{99m}Tc(OH)_4 \cdot Sn(OH)_2$ colloid

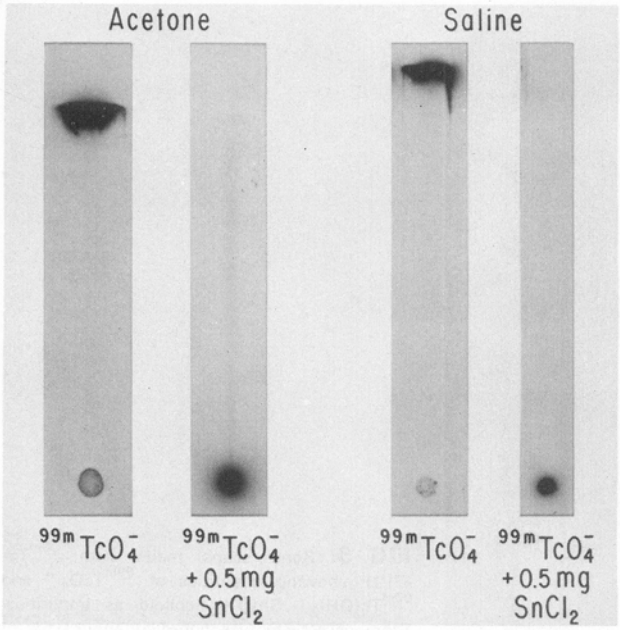

FIG. 5. Autoradiographs of one-dimensional chromatograms of $^{99m}TcO_4^-$ and colloid on glass fiber (type SG media) strips.

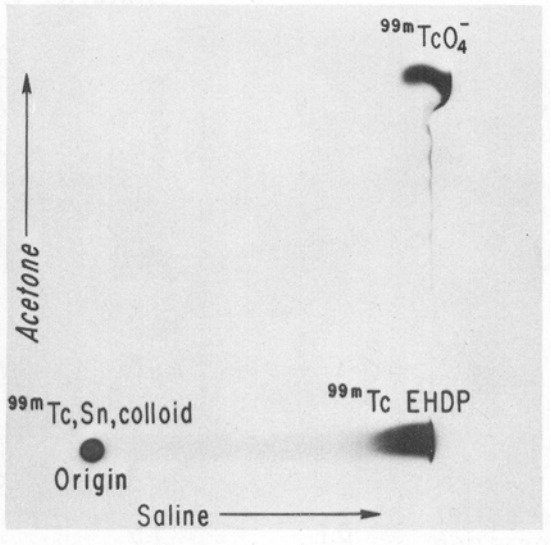

FIG. 6. Autoradiographs of two-dimensional chromatograms of ^{99m}Tc-EHDP, TcO_4^-, and colloid on glass fiber media (type SG).

are demonstrated in Fig. 5. A two-dimensional chromatogram of ^{99m}Tc-EHDP and the impurities is shown in Fig. 6.

Discussion

Our experience with the bone-seeking radiopharmaceuticals ^{99m}Tc-EHDP and ^{99m}Tc-PP prepared from commercially available kits shows that, despite efforts for quality control on behalf of the commercial supplier and the house staff, poor results are occasionally obtained.

Free technetium and a tin-technetium colloid were the principal impurities in these preparations. The two compounds have a different biologic behavior in the experimental animal, and a bioassay procedure could easily be set up for their recognition. None of the three preparations tested was free of these impurities, but the presence of a stabilizer significantly reduces the buildup of free $^{99m}TcO_4^-$ with time. To obtain a radiopharmaceutical agent with good quality for bone scanning, the labeled phosphate should be used as soon as possible after preparation in order to prevent the buildup of free $^{99m}TcO_4^-$. The amount of the colloid is probably dependent on the amount of stannous chloride in the preparation and is more difficult to control in the laboratory.

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