KINETICS OF ^{99m}Tc-LABELED PYROPHOSPHATE
AND POLYPHOSPHATE IN MAN

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The kinetics of ^{99m}Tc-labeled pyrophosphate were compared with those of polyphosphate in ten patients in a combined study. Both agents cleared from the blood in a biexponential fashion. The clearance half-time of Exponent I was the same for both and was shorter than the clearance half-time of Exponent II. Urinary excretion of both agents was the same during the first hour but during the next 3 hr Tc-pyrophosphate cleared at a slightly more rapid rate, resulting in lower blood background radioactivity. Both agents were bound loosely to plasma proteins, mainly to globulin fractions. The sensitivity of lesion detection was similar for both. Excellent bone images were obtained with both agents. The images with Tc-pyrophosphate were consistently superior owing to the low blood background and they took less time to accumulate an identical number of counts from identical regions. With the amount of ^{99m}Tc-complex used, no hpyocalcemia or tetany was noted, nor was there any significant effect on 1-hr serum levels of inorganic phosphorus and alkaline phosphatase. Four hours after injection, 9.5% of the dose of Tc-pyrophosphate was circulating in blood, 31.7% was excreted in urine, and the remaining 58.8% was taken up by bone and other tissues. The corresponding values with Tc-polyphosphate were 12.5% in blood, 29.0% in urine, and 58.5% in bone and other tissues. Among the soft tissues, the genitourinary system is most consistently visualized. It is concluded that both Tc-pyrophosphate and Tc-polyphosphate are excellent skeletal-imaging agents and that Tc-pyrophosphate appears slightly superior to Tc-polyphosphate.

these ^{99m}Tc-labeled phosphate complexes have been shown to be superior to the previously popular agents (6–8). We have undertaken a continuum study in which we have serially evaluated clinically all newer skeletal-seeking agents as they were introduced. In our first study, we compared the kinetics of ^{18}F with those of ^{99m}Tc-polyphosphate (6,7); in the second, we compared the kinetics of ^{99m}Tc-labeled diphosphonate with those of ^{99m}Tc-labeled polyphosphate (9). Recently ^{99m}Tc-labeled pyrophosphate has been introduced as another new skeletal-imaging agent (10). It is not clear whether there exists clinically any real difference between these agents. In this communication we report the results of a study comparing the kinetics of ^{99m}Tc-labeled pyrophosphate with those of ^{99m}Tc-labeled polyphosphate.

MATERIALS AND METHODS

The procedure was identical with the methods used in our previous studies (6,7,9). Ten patients with suspected bone lesions were chosen from a list of patients on whom bone images were requested by the clinician. Written informed consent was obtained in compliance with the code of ethics of the World Medical Association (11). Studies were done with 15 mCi ^{99m}Tc-pyrophosphate, then repeated within 2–14 days with 15 mCi of ^{99m}Tc-polyphosphate. (The pyrophosphate kit was supplied by Mallinckrodt/Nuclear; the polyphosphate kit was obtained from New England Nuclear.) The radiopharmaceutical was prepared according to the manufacturer's instructions and used within 3 hr of preparation. No special patient preparation was called for before injection, and ad libitum fluid intake was permitted. The urinary bladder was emptied before in-

Technetium-99m-labeled polyphosphate (1,2) and diphosphonate (3–5) have replaced ^{18}F, ^{85}Sr, and ^{87m}Sr for bone scanning. Both in animals and in man

jection. Blood was collected at 10 and 30 min and at 1, 2, 3, and 4 hr after injection. Radioactivity in whole blood, plasma, and red blood cells was determined and the results were expressed as a percentage of the injected dose per liter. The red blood cells were washed with saline three times, the radioactivity remaining in the cells after the first wash being considered 100% and the reduced radioactivity following subsequent washes being expressed as a percentage loss after each wash.

The radioactivity in plasma and that bound to the protein and to each protein fraction was determined as described in detail elsewhere (6).

Urine was collected as hourly samples for 4 hr and counted with an aliquot of the injected dose. The results were expressed as a percentage of the administered dose excreted per hour. The 4-hr cumulative excretion was calculated.

Serum calcium, alkaline phosphatase, and total protein levels were measured in an autoanalyzer SMA-12-60 (Technicon Instrument Corp.) before and 1 hr after injection of Tc-polyphosphate and Tc-pyrophosphate. Serium ionized calcium was calculated from the relationship between calcium and total protein by the original McLean and Hastings formula (12):

$$\text{Ionized serum calcium}/100 \text{ ml} = \frac{6\,\text{Ca} - (\text{p}/3)}{\text{p} + 6}$$

where Ca is the total calcium in mg/100 ml and p is the total protein in mg/100 ml.

Skeletal images of identical regions were obtained with both agents 4 hr after injection, using an Anger scintillation camera fitted with a Divcon Collimator (No. 820-822017). The time taken to accumulate 500,000 counts for each view from identical regions of the body was noted. In addition to these ten patients who had bone skeletal images and in vivo kinetics analysis with both agents, only skeletal images were obtained in 600 patients with Tc-polyphosphate and 75 patients with Tc-pyrophosphate. The images were assessed subjectively for sharpness, number, and resolution of the bone lesions.

RESULTS

The blood clearance of Tc-pyrophosphate and Tc-polyphosphate during the 4 hr of study was biexponential (Fig. 1). The clearance half-time of Exponent I—13.6 min for both Tc-pyrophosphate and Tc-polyphosphate—was relatively shorter than that of Exponent II. Exponent II had a mean blood clearance half-time of 380 min with Tc-pyrophosphate and 512 min with Tc-polyphosphate. Exponents I and II represent decreasing blood radioactivity caused, respectively, by bone uptake and
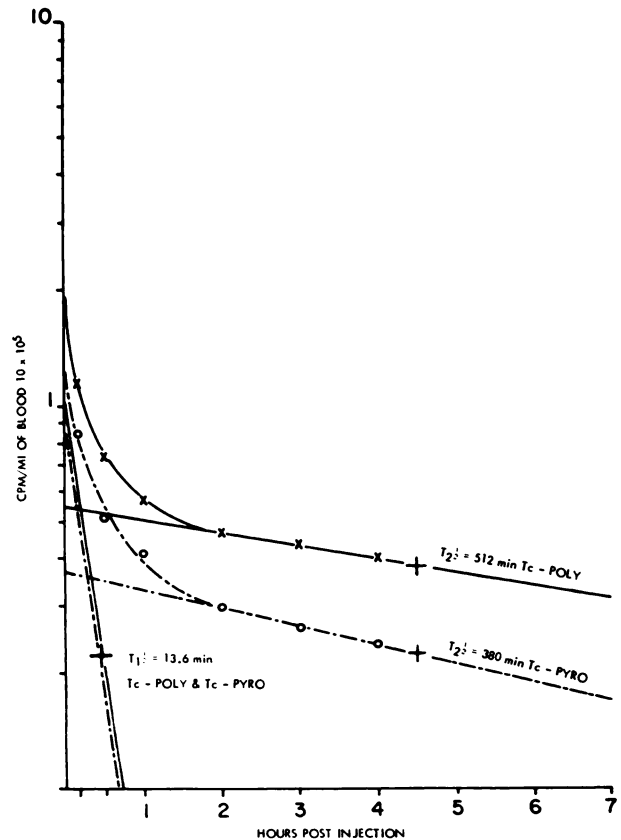

FIG. 1. Clearance of ^{99m}Tc-polyphosphate (Tc-poly) and ^{99m}Tc-pyrophosphate (Tc-pyro) from blood. Clearance is biexponential. Exponent I (T_1 ½) has shorter clearance half-life with both agents (13.6 min) and represents bone uptake. Exponent II (T_2 ½) has relatively longer clearance half-life with Tc-polyphosphate (512 min) than with Tc-pyrophosphate (380 min). Exponent II represents mainly renal excretion (mean of ten patients).

urinary excretion of Tc-pyrophosphate and Tc-polyphosphate.

Tc-pyrophosphate clears from the blood relatively faster than Tc-polyphosphate, giving a significantly lower blood background radioactivity (Fig. 2). At the end of 4 hr, a liter of blood had a mean radioactivity of 2.5% of the injected dose with Tc-polyphosphate and 1.9% with Tc-pyrophosphate (p < 0.05).

At 1 hr after injection, a mean of 84.3% of plasma radioactivity was protein-bound with Tc-pyrophosphate and 73.9% with Tc-polyphosphate. The remaining radioactivity was free in plasma (not bound to protein). Figure 3 shows the distribution of Tc-pyrophosphate and Tc-polyphosphate radioactivity among different protein fractions. Most of the radioactivity was associated with globulin fractions. Both Tc-pyrophosphate and Tc-polyphosphate were bound to red blood cells; Tc-pyrophosphate binding was relatively less firm than that of Tc-polyphosphate. Tc-pyrophosphate activity could be

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washed from the cells at a rate of 4–12% during each saline wash in contrast to Tc-polyphosphate where only 2–4% could be cleared from the cells after each wash.

The clearance of Tc-polyphosphate and Tc-pyrophosphate from the blood and plasma is shown in Table 1. The serum-ionized calcium mean value was 4.06 mg% before and 4.05 mg% 1 hr after injection of Tc-polyphosphate. The corresponding values with Tc-pyrophosphate were 4.09 mg% before and 4.04 mg% 1 hr after injection. These values were not statistically significant ($p < 0.05$). Serum-ionized calcium was calculated from the relationship between total calcium and total protein by the above formula. There was no significant effect of the injection of Tc-polyphosphate or Tc-pyrophosphate on serum levels of inorganic phosphate, total protein, or alkaline phosphatase.

The mean urinary excretion of Tc-pyrophosphate and Tc-polyphosphate was almost identical during the first hour after injection (16.4% for Tc-pyrophosphate and 15.8% for Tc-polyphosphate). During the next 3 hr Tc-pyrophosphate was excreted slightly more rapidly ($p < 0.1$) than Tc-polyphosphate) (Fig. 4). At the end of 4 hr, a mean of 31.7%
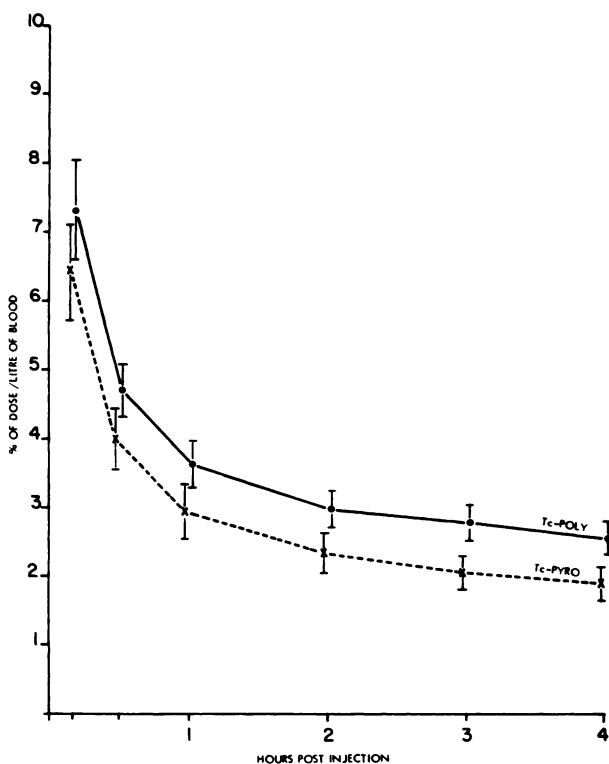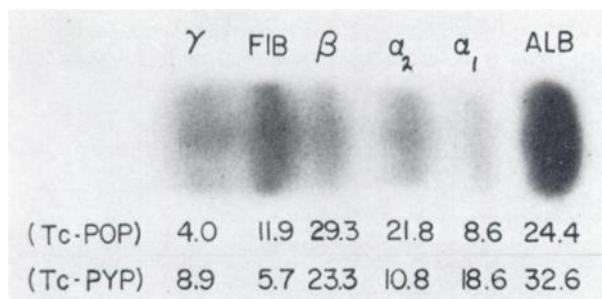

FIG. 3. In vivo plasma protein binding of ^{99m}Tc-polyphosphate (Tc-pop) and ^{99m}Tc-pyrophosphate (Tc-pyp). Note that major binding is to globulin fractions with both agents.

of the dose of Tc-pyrophosphate and 29.0% of the dose of Tc-polyphosphate had been excreted in urine. Skeletal images obtained with Tc-pyrophosphate took less time than Tc-polyphosphate to accumulate a 500,000 count from an identical region of the body (Fig. 5). Bone images obtained with Tc-pyrophosphate were qualitatively sharper and had better resolution than similar images obtained with Tc-polyphosphate. There was no difference between the agents in sensitivity of lesion detection.

DISCUSSION

Both polyphosphate and pyrophosphate have phosphorus-oxygen-phosphorus (P-O-P) linkage in their molecules and both are biodegradable (Fig. 6). In contrast to polyphosphate, which has a variable chain length, pyrophosphate has a discrete chain length with two atoms of phosphorus in each molecule. In the case of polyphosphate, the number of phosphorus atoms in a molecule can vary from 3 to 46. Pyrophosphate with a molecular weight of 250 is a smaller molecule than polyphosphate, which has a molecular weight of 300–4,000.

Like other bone-seeking agents (6,7,9), Tc-pyrophosphate and Tc-polyphosphate during the 4-hr study show a biexponential type of blood clearance. The blood was collected 10 min after injection to allow sufficient time for complete mixing, thus avoiding the influence of mixing on the shape of the exponential curves, which is due primarily to the biologic clearance of Tc-polyphosphate and Tc-pyrophosphate from the blood. We hypothesize that the Exponent I represents decreasing blood radioactivity due mainly to bone uptake. The clearance half-time of Exponent I is only 13.6 min with both agents, suggesting that the bone uptake of Tc-pyrophosphate and Tc-polyphosphate is a very rapid process. A similar type of exponential curve was noted in our previous studies using [18]F (6) and ^{99m}Tc-labeled diphosphonate (9). The clearance half-time of Exponent I is influenced very little by urinary excretion

FIG. 2. Blood clearance of ^{99m}Tc-polyphosphate (Tc-poly) and ^{99m}Tc-pyrophosphate (Tc-pyro) in man. Tc-pyrophosphate clears from blood at significantly faster rate ($p < 0.05$) than Tc-polyphosphate, and results in lower background radioactivity (mean ± s.e. of ten patients).

TABLE 1. CLEARANCE OF ^{99m}Tc-POLYPHOSPHATE AND PYROPHOSPHATE FROM BLOOD AND PLASMA (PERCENT DOSE PER LITER)

Radiopharmaceutical		Time after injection											
		10 min		30 min		1 hr		2 hr		3 hr		4 hr	
		B	P	B	P	B	P	B	P	B	P	B	P
Tc-polyphosphate	mean (N = 10)	7.3	11.5	4.7	6.9	3.6	5.2	2.9	3.6	2.8	3.1	2.6	2.7
	s.e.	0.7	1.0	1.2	0.6	0.3	0.5	0.3	0.4	0.3	0.3	0.2	0.3
Tc-pyrophosphate	mean (N = 10)	6.4	10.5	4.0	6.4	2.9	4.5	2.3	3.2	2.1	2.5	1.9	2.1
	s.e.	0.6	0.9	0.4	0.6	0.4	0.6	0.3	0.4	0.3	0.3	0.2	0.3

B, blood; P, plasma; N, number of patients.

during this 13.6-min interval since less than 4% of the injected dose was excreted in urine during this period (16% during the first hour). In previous studies (6,9), the clearance half-time of Exponent I with Tc-polyphosphate was 30 min, in contrast to the 13.6 min noted in this study. In previous studies, only two out of ten patients had abnormal bone images, and no patient had more than five lesions. In contrast, in the present study three of ten patients had abnormal bone images and each patient had at least ten lesions (Fig. 5). With more pathologic sites, the bone uptake of Tc-polyphosphate and Tc-pyrophosphate may be relatively more rapid, accounting for the shorter clearance half-time (13.6 min) of Exponent I.
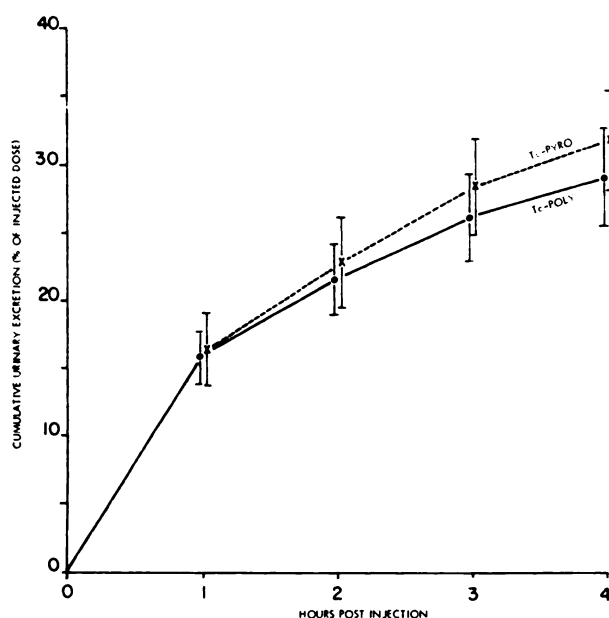

FIG. 4. Urinary excretion of ^{99m}Tc-polyphosphate (Tc-poly) and ^{99m}Tc-pyrophosphate (Tc-pyro) in man (mean ± s.e. of ten patients). Excretion is identical during first hour. During next 3 hr Tc-pyrophosphate is excreted at slightly faster rate (p < 0.1).

It has been documented in studies of animals sacrificed at different time intervals that the uptake of Tc-polyphosphate is maximal within 1 hr and does not increase to any significant level up to 24 hr after injection (2). (The animals were not sacrificed, however, at shorter time intervals than 1 hr to test whether maximal bone uptake can occur sooner.) In our clinical studies, the clearance half-time of Exponent I is always less than 30 min (6,7,9); and it can be as little as 13.6 min as shown in the present study suggesting that the maximal bone uptake may occur within 30 min.

We hypothesize that the Exponent II represents decreasing blood radioactivity due to urinary excretion. The blood clearance half-time of Exponent II with Tc-pyrophosphate is shorter (380 min) than that of Tc-polyphosphate (512 min). At 4 hr the radioactivity in blood was significantly lower with Tc-pyrophosphate than with Tc-polyphosphate. The lower blood radioactivity associated with Tc-pyrophosphate (Table 1 and Fig. 2) was probably due to relatively more rapid urinary excretion. The 4-hr cumulative mean urinary excretion was 31.7% with Tc-pyrophosphate and 29.0% with Tc-polyphosphate. Because of wide variations in urinary excretion from patient to patient, the difference at 4 hr in cumulative urinary excretion between the two agents was statistically significant only at a p value of 0.1.

By adding the radioactivity in blood (5 liters) and that in urine excreted in 4 hr and subtracting the sum from the total injected dose, the quantity taken up by the bone and other tissues was calculated. It was noted with both agents that an identical net amount was left for bone and other tissue uptake (Fig. 7). In the ten patients in this study, the kidneys were the only soft tissue that were consistently visualized and the uptake by other soft tissues was insignificant. By this it can be inferred that most of

FIG. 5. Skeletal images obtained with ^{99m}Tc-polyphosphate (A) and ^{99m}Tc-pyrophosphate (B) in one patient with cancer of prostate. Note multiple bone metastasis. Sensitivity is same for both agents. Time taken to accumulate 500,000 counts for images of identical regions of body was considerably shorter with Tc-pyrophosphate than with Tc-polyphosphate (number in lower right-hand corner of each image denotes time, in seconds, required to accumulate 500,000 counts. Intensity and imaging interval after injection were kept constant).
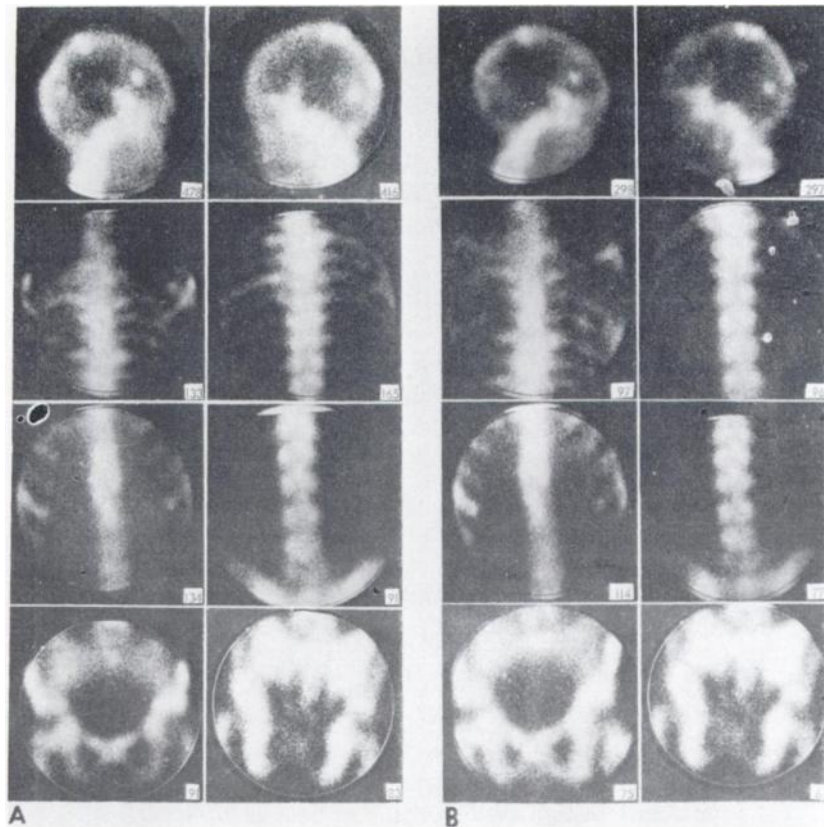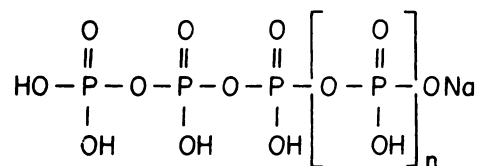
the radioactivity left behind was taken up by bone alone. This finding is again supported by studies done in animals where the rabbit kidneys retained only 4.3% of the dose per 1% body weight of Tc-polyphosphate at 3 hr after injection and other soft tissues did not show any significant concentration. Similar results were obtained by Hosain in m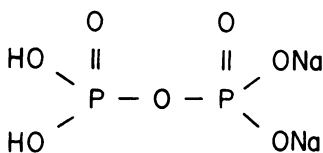ice where kidneys showed 2.6% dose per gram at 2 hr after injection of Tc-pyrophosphate and other soft tissues had less than 0.6% dose per gram. Bone radioactivity was 12.9% dose per gram (*13*). Figure 7 depicts the compartmental distribution of Tc-pyrophosphate and Tc-polyphosphate in man. It should be noted that the number marked over the upper end of the femur also includes the uptake of soft tissues, if any, including the kidneys.

Like other ^{99m}Tc-labeled skeletal-seeking phosphate complexes (*6,7,9*), most of plasma radioactivity of Tc-pyrophosphate is bound to the plasma proteins (84.3%). The bonding appears to be quite loose and dissociates easily from the proteins, allowing rapid uptake by bone. Most of plasma-protein radioactivity is associated with globulin fractions (Fig. 3).

POLYPHOSPHATE (MONOSODIUM POLYPHOSPHATE) HYDROLYZABLE, NON-DISCRETE CHAIN LENGTH

PYROPHOSPHATE (DISODIUM PYROPHOSPHATE) HYDROLYZABLE, DISCRETE CHAIN LENGTH

FIG. 6. Comparative chemical structure of polyphosphate and pyrophosphate.

Excellent skeletal images were obtained with bone agents (Fig. 5). Since all the skeletal lesions seen with one agent were also seen with the other, no difference in sensitivity can be claimed between the two agents. The skeletal images obtained with Tc-pyrophosphate were considerably better with less background radioactivity than those with Tc-polyphosphate. The time taken to accumulate 500,000 counts for each view of an identical region, although variable, was considerably less with Tc-pyrophosphate. Figure 5 shows the time required to obtain the images of identical regions in one patient. It was the subjective opinion that the Tc-pyrophosphate
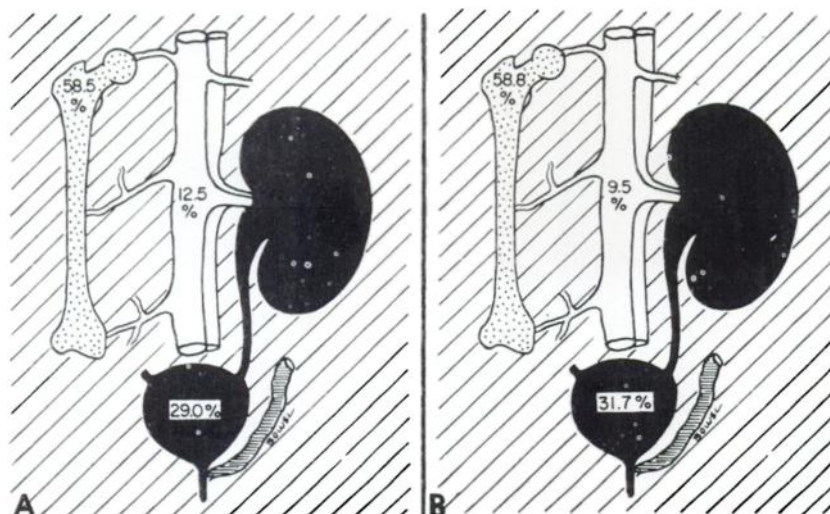
FIG. 7. Body distribution of Tc-polyphosphate (A) and Tc-pyrophosphate (B) at 4 hr after injection. With Tc-polyphosphate 12.5% circulates in blood, 29.0% is excreted in urine, and remaining 58.5% is taken up by bone and other tissues. Corresponding values with Tc-pyrophosphate are: 9.5% in blood, 31.7% in urine, and 58.8% by bone and other tissues.

images were slightly better than the images obtained with Tc-polyphosphate.

Since phosphate complexes are injected intravenously, there is at least a theoretical possibility that these agents have a hypocalcemic effect. We obtained the serum levels of total calcium, inorganic phosphorus, alkaline phosphatase, and proteins just prior to and 1 hr after injection of Tc-pyrophosphate and Tc-polyphosphate. Because it is difficult to measure ionized serum calcium directly, we calculated the ionized serum calcium from its relationship to total protein. There was no hypocalcemic effect with either Tc-pyrophosphate or Tc-polyphosphate. It has been shown in rats that serum total and ionized calcium concentration fall when these animals are injected with large doses of pyrophosphates, the hypocalcemic effect beginning at a dose of approximately 20 mg/kg. Stevenson, et al found hypocalcemia to be associated with tetany, which responded well to intravenous injection of calcium chloride (*14*). The hypocalcemic tetany was seen prior to the determination of LD_{50} dose effect, suggesting that the clinical manifestation appears well before the LD_{50} effect. It was also determined that in rats the LD_{50} (5 min) was 41.0 mg/kg body weight with Tc-pyrophosphate and 29.4 mg/kg with Tc-polyphosphate. Thus, based on LD_{50} (5 min) in rats, Tc-polyphosphate was found to be 1.4 times more toxic than Tc-pyrophosphate. Pre- and 1-hr postinjection serum levels of inorganic phosphorus, total protein, and alkaline phosphatase in this study did not show a statistically significant difference.

In the polyphosphate kits available commercially, each vial contains 40 mg of sodium polyphosphate (New England Nuclear) and 1 mg of stannous chloride. In the pyrophosphate kit (Mallinckrodt Nuclear), each vial contains 15.4 mg of stannous pyrophosphate. Each vial is used on the average

for 3–4 patients. Many times, however, a single vial may be used for one patient. Hence, the maximum amount of phosphate compound that a patient would receive at any time is 40 mg of sodium polyphosphate or 15.4 mg of stannous pyrophosphate. In our clinical study, over 600 patients have received Tc-polyphosphate and over 75 patients have received Tc-pyrophosphate. In no patient was there any clinical tetany attributable to hypocalcemia. For an adult weighing 70 kg, the contents of a single vial of polyphosphate (40 mg) would amount to 0.6 mg/kg, and those of a vial of pyrophosphate (15.4 mg) would amount to 0.2 mg/kg. These levels are well below the dose (20 mg/kg) required to produce hypocalcemia in rats (*14*).

It has been shown in man that the injection of a sufficient amount of phosphate reduces the serum calcium level mainly because of a rise in serum parathormone (*15*). In our study such effects were not seen either in the form of clinical tetany or of hypocalcemia. We conclude that the amount of polyphosphate and pyrophosphate present in the kits is well within the safety margin. It would appear beneficial if even the present low levels of these phosphate complexes could be reduced as long as the efficiency is not compromised.

The introduction of ^{99m}Tc-labeled phosphate complexes has given the nuclear medicine physician a wide choice of agents for skeletal imaging. Both polyphosphate and pyrophosphate are biodegradable and have P-O-P linkage (Fig. 6). Because of the complex nature of the molecule, one cannot be certain of the chain length in any given batch of polyphosphate. In contrast, ^{99m}Tc-labeled diphosphonate (*3–5*) has a discrete chain length and P-C-P linkage. Concern is expressed in the literature about the importance of exact chain length and biodegradability. One author has suggested waiting until more

is known about the toxicity of diphosphonate before using it in man (4). Another author replies that biodegradability should not be equated with toxicity (16). In our previous study we concluded that there was no reason to be concerned about toxicity with the amount of diphosphonate used for skeletal imaging (9). Now the introduction of technetium-labeled pyrophosphate by Perez, et al (10) should satisfy both protagonists for it has a discrete chain length and is biodegradable.

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