TECHNETIUM-99m-METHYLENE DIPHOSPHONATE—A SUPERIOR AGENT FOR SKELETAL IMAGING: COMPARISON WITH OTHER TECHNETIUM COMPLEXES

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Methylene diphosphonate (MDP) was formulated as a complex of ^{99m}Tc for skeletal imaging. This agent was compared with three other bone-seeking technetium agents: ethane-1-hydroxy-1, 1-diphosphonate (EHDP), pyrophosphate, and polyphosphate. In tissue radioassay experiments in rodents, the technetium complexes of MDP and EHDP were similar, but skeletal concentration with both of these agents was higher than that with pyrophosphate or polyphosphate.

The total-body retention of MDP and EHDP complexed with ^{95m}Tc was studied in beagle dogs for 35 days by excretion measurements and total-body counting and compared with polyphosphate and pertechnetate. The long-term retention was greater for MDP. The 5-day cumulative fecal excretion of ^{95m}Tc was low when administered as EHDP or polyphosphate complexes and negligible when administered as MDP complex.

In six human volunteers the blood clearance of ^{99m}Tc-MDP was similar to that of ^{18}F and significantly faster than that of ^{99m}Tc-EHDP. Pyrophosphate cleared from the blood much faster than polyphosphate but slower than the diphosphonates. The urinary excretion of the MDP complex was greater than for EHDP within the first 2–3 hr after injection. The 24-hr urinary excretion of pyrophosphate and polyphosphate complexes was not as complete as for the diphosphonates.

All four ^{99m}Tc complexes proved satisfactory for clinical imaging studies. The MDP complex produced images of superior quality as early as 2 hr after administration, attributable to its more rapid clearance from the blood and soft tissues. On the contrary, a longer interval of 3–4 hr after injection was usually needed for ^{99m}Tc-EHDP; pyrophosphate and polyphosphate complexes regularly required a waiting period of 4 hr.

Comparative radiation dose estimates were made based on the available biologic distribution data for these ^{99m}Tc skeletal-localizing agents.

In the field of nuclear medicine condensed phosphates labeled with ^{32}P were used first by Kaplan and Fels (*1*) for therapy of osseous metastases. They demonstrated an increased concentration in the involved bone by autoradiography. Subramanian, et al demonstrated that linear polyphosphates formed a stable complex with ^{99m}Tc in the presence of stannous ions and after intravenous injection localized in skeletal lesions to a higher degree than in normal bone. Tripolyphosphate first was used for skeleton imaging (*2*) followed by longer chain linear polyphosphate (*3*). Perez, et al (*4*) proposed the ^{99m}Tc-Sn complex of pyrophosphate for skeletal imaging. Yano, et al (*5*) first reported the use of ^{99m}Tc-Sn-EHDP for this purpose. Since then these polyphosphates and EHDP (*6–8*) have been widely used both experimentally and clinically for the detection of skeletal metastases and other focal bone lesions.

The ^{99m}Tc-Sn complex of methylene diphosphonate (MDP) was proposed as a skeletal-imaging agent in 1973 (*9*). In the present report, the method of preparing and distributing ^{99m}Tc-MDP in animals and man is compared with that using other ^{99m}Tc complexes. Although many of its characteristics are similar to those of the other ^{99m}Tc bone-seeking complexes, ^{99m}Tc-MDP appears to offer significant advantages.

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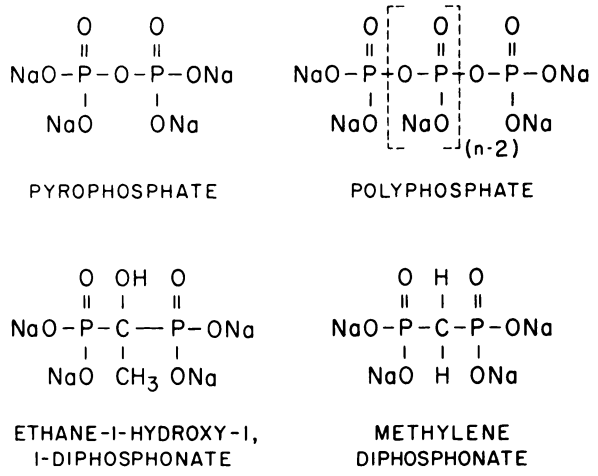

FIG. 1. Chemical formulas of bone-seeking phosphates and diphosphonates forming complexes of ^{99m}Tc. Although these are shown as tetrasodium salts, in solution at neutral pH they are probably disodium salts.

MATERIALS AND METHODS

The chemical structural formulas of the four phosphate compounds used in this study are shown in Fig. 1. The four phosphorus compounds were obtained either as acids or sodium salts from commercial sources*. "Instant" freeze-dried kits were prepared for the animal studies as stannous chelates in the weight ratios of compound to $SnCl_2 \cdot 2H_2O$ of:— 200:1 for polyphosphate (100 mg), 100:1 for pyrophosphate† (50 mg), and 20:1 for the diphosphonates (5–10 mg).

For clinical use sterile freeze-dried kits were formulated in 10-ml vials containing the following quantities of chemicals. EHDP and MDP, 5 mg each (10:1 weight ratio of compound to stannous chloride); polyphosphate, 100 mg (100:1 ratio); and pyrophosphate, 15.4 mg (37.5:1, commercial kit, from Mallinckrodt, Inc.). Details of the preparation of these freeze-dried kits are described elsewhere (*8*). The vials were either evacuated or purged with nitrogen. For labeling with ^{99m}Tc, the required activity of ^{99m}Tc in 2–5 ml of generator eluate (Radiopharmaceutical Div., New England Nuclear, North Billerica, Mass.) was simply added to the kit vial. The labeling yield was better than 95% for all compounds. Very little free TcO_4^- was detectable by paper chromatography in 85% methanol. The final pH of these compounds ranged from 6.5 to 7.0. In all cases a clear solution was obtained which quantitatively passed through a sterile 0.22-micron membrane filter.

The organ distribution of these compounds was studied in New Zealand adult albino rabbits (more than 6 months old; average age 9 months and average weight, 3–4 kg) after an intravenous injection of 50–200 μCi of ^{99m}Tc and compared with 10–20 μCi of ^{85}Sr chloride administered simultaneously. The chemical amounts injected into each rabbit were as follows: polyphosphate, 5 mg/kg; pyrophosphate, 0.5 mg/kg; and diphosphonates, 0.05–0.1 mg/kg. These animals were sacrificed for tissue radioassay at various time intervals from 15 min to 24 hr after injection. The methods of tissue assay used were described previously (*8*).

Because of the short physical half-life of ^{99m}Tc, carrier-free ^{95m}Tc (New England Nuclear Corp., Boston, Mass.) (half-life 60 days) was used to study the long-term retention of technetium-labeled polyphosphate, EHDP, and MDP in 1–7-year-old beagle dogs (9–18 kg body weight) (*10*). After the beagles were adapted to metabolism cages, they were injected intravenously with 10–20 μCi of ^{95m}Tc containing either 1 mg EHDP, 1 mg MDP, or 11 mg polyphosphate. Five dogs were injected with each compound and an additional group of five was given $^{95m}TcO_4^-$ for comparison. Blood clearance was determined by counting multiple serial blood samples. Urinary and fecal radioactivity was measured at 12 hr and daily for 5 days after injection. The total-body retention was determined also by serial measurements in a 4π total-body liquid scintillation counter for 35 days for the diphosphonates and 26 days for polyphosphate.

The acute toxicity of EHDP and MDP (omitting ^{99m}Tc) was determined after intravenous injection in both adult mice and New Zealand albino rabbits.

Blood and urinary clearances of these four compounds were measured in normal volunteers. First, ten volunteers were studied with ^{99m}Tc-polyphosphate containing 500 μCi of ^{99m}Tc and 15–20 mg of polyphosphate. Of this group, six normal volunteers were again studied with ^{99m}Tc-pyrophosphate containing 3–5 mg pyrophosphate and 0.5–1.0 mCi of ^{99m}Tc. At other times the same six volunteers were restudied with both ^{99m}Tc-MDP and ^{99m}Tc-EHDP; 1 mCi of ^{99m}Tc and 1–3 mg of each diphosphonate were used. Intravenous blood samples were withdrawn at intervals from 3 min to 24 hr after injection. The percent dose in the whole blood, plasma,

* Pyrophosphate kit was obtained from Mallinckrodt, Inc., St. Louis, Mo. Polyphosphate and EHDP were gifts from P. H. Ralston, Calgon Corp., Pittsburgh, Pa. The average linear polyphosphate chain length was 50 units; approximate mol wt 4,000 (acid form) 39% phosphorus or mol wt 5,000 (sodium salt). With EHDP, $C_2H_nP_2O_6$ mol wt was 191 (acid form) 32% phosphorus. EHDP (chemical structural formulas are shown in Fig. 1) has also been called HEDP and HEDSPA in the literature. MDP was obtained from Sigma Chemical Co., St. Louis, Mo. $CH_6P_2O_6$ mol wt 176 (acid form) 35% phosphorus.

† $Na_4P_2O_7 \cdot 10\ H_2O$ sodium pyrophosphate, J. T. Baker Chemical Co., Phillipsburg, N.J. mol wt 446 (hydrated tetrasodium salt) 14% phosphorus.

TABLE 1. DISTRIBUTION OF ^{99m}Tc BONE AGENTS IN RABBITS*

	MDP	EHDP	Pyrophosphate	Polyphosphate
No. of animals	12	12	12	12
Sacrifice time	2 hr	2 hr	2 hr	3 hr
Organ		% Dose/% body wt		
Blood	0.193 ± 0.093	0.188 ± 0.005	0.600 ± 0.206	0.482 ± 0.157
Liver	0.124 ± 0.038	0.192 ± 0.163	0.245 ± 0.142	0.438 ± 0.148
Kidney	2.810 ± 2.280	2.360 ± 1.100	3.020 ± 1.090	4.090 ± 1.130
Muscle	0.028 ± 0.013	0.022 ± 0.012	0.066 ± 0.048	0.066 ± 0.016
Marrow	0.166 ± 0.075	0.191 ± 0.121	0.184 ± 0.045	0.512 ± 0.316
Femur	8.810 ± 1.390	7.890 ± 1.990	3.610 ± 0.969	5.450 ± 0.640
Tibia	6.290 ± 2.550	8.280 ± 2.330	3.140 ± 0.788	2.970 ± 0.483
Pelvis	9.430 ± 1.900	9.210 ± 2.350	6.100 ± 0.872	7.930 ± 1.260
Spine	7.510 ± 1.380	7.020 ± 1.210	5.330 ± 1.010	5.240 ± 1.340
Avg. bone	8.000 ± 1.180	8.100 ± 1.650	4.940 ± 0.999	5.460 ± 0.823
	^{99m}Tc/^{85}Sr concentration ratios			
Blood	0.371 ± 0.182	0.362 ± 0.061	0.703 ± 0.160	1.480 ± 0.700
Liver	0.568 ± 0.169	0.954 ± 0.961	0.779 ± 0.394	2.610 ± 0.825
Kidney	3.930 ± 1.870	3.530 ± 1.760	2.810 ± 0.841	9.460 ± 3.390
Muscle	0.192 ± 0.066	0.172 ± 0.055	0.365 ± 0.251	0.623 ± 0.089
Marrow	0.617 ± 0.127	0.814 ± 0.465	0.726 ± 0.135	2.950 ± 1.490
Femur	0.820 ± 0.134	0.796 ± 0.079	0.710 ± 0.111	0.826 ± 0.112
Tibia	0.730 ± 0.143	0.798 ± 0.088	0.694 ± 0.118	0.660 ± 0.135
Pelvis	0.926 ± 0.213	0.913 ± 0.151	0.918 ± 0.082	1.010 ± 0.174
Spine	0.806 ± 0.132	0.801 ± 0.108	0.827 ± 0.101	0.820 ± 0.112
Avg. bone	0.833 ± 0.158	0.832 ± 0.099	0.812 ± 0.076	0.870 ± 0.141
	^{99m}Tc concentration ratios			
Bone/muscle	342 ± 137	385 ± 172	100 ± 61	85 ± 16
Bone/blood	51 ± 26	48 ± 19	9 ± 4	13 ± 4
Bone/marrow	57 ± 24	62 ± 45	26 ± 8	13 ± 7

* Mean and standard deviations.

and protein-bound fraction was calculated by counting samples of whole blood, aliquots of plasma, and plasma protein precipitated with trichloroacetic acid compared with a standard, allowing for decay correction.

Clinical scans and camera images were obtained using 15 mCi of ^{99m}Tc in over 200 patients with ^{99m}Tc-EHDP and 400 patients with ^{99m}Tc-MDP. In more than 40 patients comparative scans were obtained with both agents and compared also with several hundred other studies performed with polyphosphate and more than 40 with ^{99m}Tc-pyrophosphate. On each patient, a total-body scan was obtained using an Ohio-Nuclear dual 5-in. rectilinear scanner fitted with a 5-in. focal length collimator (55035L) followed by 15–30 images obtained in both posterior and anterior projections using a Searle Radiographics HP scintillation camera with a 140-keV high-sensitivity collimator.

RESULTS

Biologic distribution in animals. The tissue radioassay data in rabbits for the four ^{99m}Tc skeletal agents are summarized in Table 1 and each agent is compared with ^{85}Sr (used as individual biologic standard) simultaneously injected for calculation of ^{99m}Tc/^{85}Sr concentration ratios. The data shown for the two diphosphonate complexes and pyrophosphate were obtained by sacrificing the animals 2 hr after intravenous injection whereas the polyphosphate data were obtained at 3 hr. The data for each complex was compared with those of the others by Student's group t-test. No statistically significant difference was found in the distribution of MDP and EHDP in rabbits. The absolute average bone concentration was significantly higher for both diphosphonates than for either pyrophosphate or polyphosphate.

Considerable variation was found in the concentration of each of these agents in different parts of the skeleton. It was invariably highest in the pelvis and lowest in the tibia for all agents except EHDP with intermediate values for the lumbar spine and femur. In searching for a possible carrier effect, four groups of rabbits, six animals each, were given increasing intravenous doses of MDP, and a similar experiment was carried out for EHDP. Within the dosage levels administered (0.01, 0.05, 0.1, and 0.5 mg/kg body weight), no significant difference in blood, soft tissue, or bone concentrations or in urinary excretion was found at 2 hr.

A comparison of the long-term biologic fate of

TABLE 2. COMPARISON OF LONG-TERM BIOLOGIC FATE OF ^{95m}Tc SKELETAL COMPLEXES IN DOGS

	MDP		EHDP		Polyphosphate	
Component	% Dose	$T_{1/2}$ (hr)	% Dose	$T_{1/2}$ (hr)	% Dose	$T_{1/2}$ (hr)
			Blood clearance			
1st	75.3	0.053	79.0	0.064	85.8	0.063
2nd	24.0	0.42	17.2	0.86	11.4	2.41
3rd	0.74	13.3	3.76	10.2	2.68	91.2
			Whole-body retention			
1st	57.7	0.2	64.1	0.3	67.3	0.3
2nd	11.5	5.8	23.8	1.1	16.9	1.5
3rd	32.1	115.5	10.1	72.2	15.5	29.0
			Cumulative excretion (% dose)			
	Urine	Feces	Urine	Feces	Urine	Feces
1 day	55.2	0.4	65.2	2.8	54.1	2.1
2 days	57.8	0.7	72.8	7.8	57.8	4.8
5 days	61.7	1.7	78.3	11.9	62.4	8.4

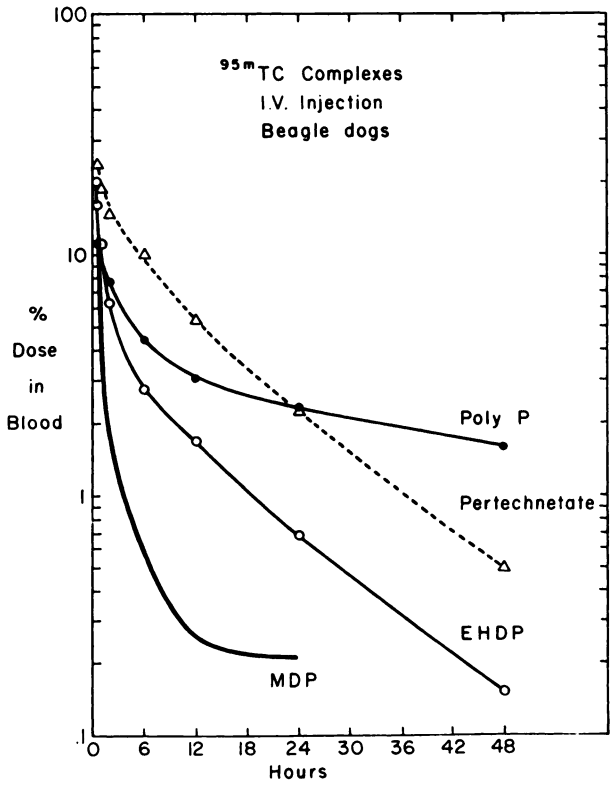

FIG. 2. Blood clearance of methylene diphosphonate (MDP) in dogs compared with two other skeletal agents and pertechnetate (corrected for physical decay), assuming blood volume was 8% of body weight.

^{95m}Tc-labeled MDP, EHDP, and polyphosphate in beagle dogs is summarized in Table 2 and illustrated in Figs. 2, 3, and 4. All dogs in this series were fully grown and at least 1 year old (epiphyseal closure complete at 9 months). MDP had the fastest blood clearance and EHDP had a faster clearance than polyphosphate. At 2 hr the mean blood level for MDP was only 1.5% of the administered activity compared with 6% for EHDP. During the first 24 hr the blood clearance of polyphosphate was faster than that of pertechnetate but beyond 24 hr the remaining 2–3% of the polyphosphate blood level cleared very slowly. The blood clearance curves for the three skeletal agents were separated by multi-exponential analysis on a PDP-11 computer into three components. All three of these skeletal agents had a fast initial component with a biologic half-time less than 6 min for 75% or more of the injected

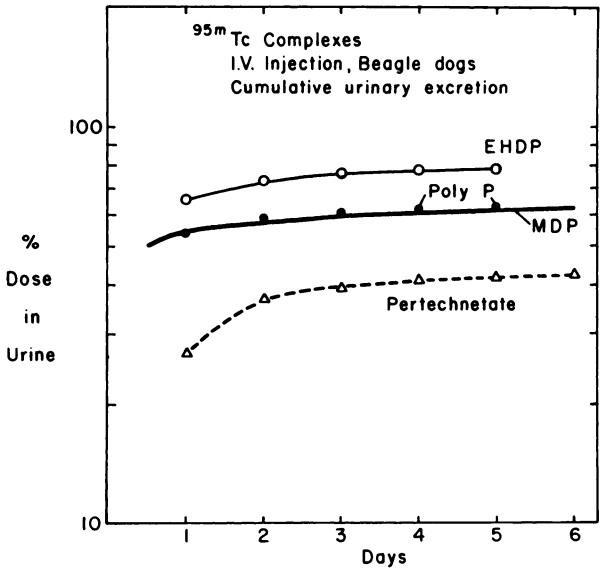

FIG. 3. Urinary excretion of MDP in dogs compared with two other skeletal agents and pertechnetate (corrected for physical decay).

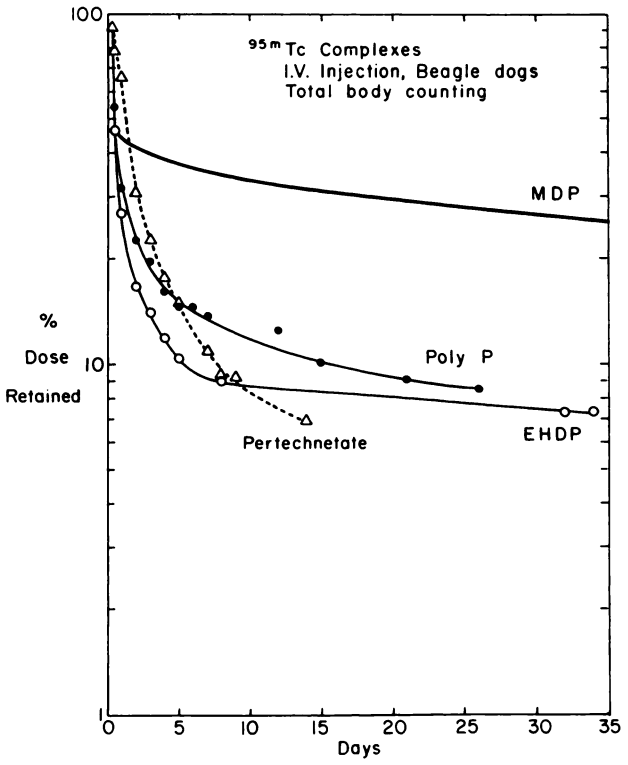

FIG. 4. Total-body retention of MDP in dogs compared with two other skeletal agents and pertechnetate (corrected for physical decay).

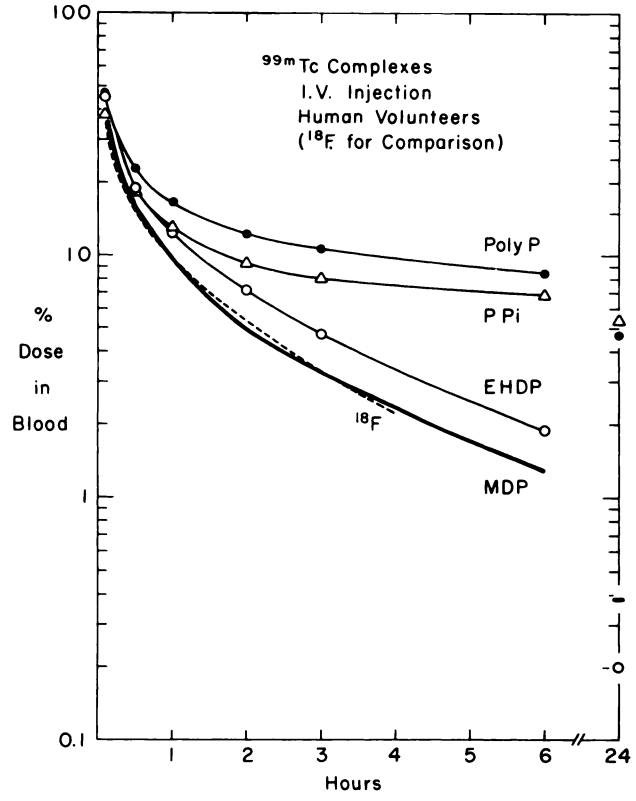

FIG. 5. Blood clearance of MDP in humans compared with three other ^{99m}Tc complexes and ^{18}F (corrected for physical decay), assuming blood volume was 7% of body weight. PPi indicates pyrophosphate and PolyP denotes polyphosphate.

radioactivity. Both diphosphonates showed negligible diffusion of the radioactivity into the circulating red cell fraction within the first 48 hr after injection. For polyphosphate, on the other hand, diffusion of radioactivity from plasma into the red cell fraction increased with time. At 2 hr, about 30% of the remaining polyphosphate blood activity was located in the red cell fraction, increasing to 90% of the remaining blood activity by 48 hr. Hence, red cell diffusion appeared to be one factor explaining the slowed blood clearance of polyphosphate.

In the dog the urinary excretion of all three skeletal complexes was considerably faster than that of pertechnetate (Fig. 3). Urinary clearance rates of MDP and polyphosphate were almost identical during this 6-day study and considerably slower than for EHDP. During the first 6 days, the fecal excretion of MDP was much less than for either EHDP or polyphosphate.

The total-body retention of these skeletal complexes in the dog, as assessed by urine and fecal excretion measurements, was similar to the results obtained by total-body counting. Beyond 5 days excretion measurements became technically difficult and were therefore discontinued. The total-body counting was carried up to 35 days after injection for the diphosphonates and for 26 days for polyphosphate. The MDP had a large slow component of retention representing about one-third of the injected radioactivity; this component was about three times larger than for EHDP and had a longer biologic half-life. This larger component was assumed to be due to a greater skeletal retention of MDP. The biologic half-time of the slow component of retention of polyphosphate was much shorter than that of either diphosphonate, probably due to metabolic breakdown of this compound at bone crystal surfaces.

Biologic distribution in man. Blood clearance data in normal adult male volunteers up to 24 hr following intravenous injection are shown in Fig. 5 and Tables 3 and 4. The same six subjects were injected with three of the ^{99m}Tc agents at different times, permitting a statistical comparison of blood levels by Student's paired t-tests. The polyphosphate data were obtained in ten volunteers, six of whom were the same subjects injected with the other agents. The most rapid blood clearance during the first 6 hr was obtained with MDP and its blood clearance curve (Fig. 5) was virtually identical with that of ^{18}F previously published (*11*). The blood clearance of EHDP was somewhat slower than that of MDP for the first 6 hr ($P = 0.01$) but at 24 hr the blood level of EHDP was somewhat lower than that of MDP. The blood clearance for pyrophosphate was

TABLE 3. DISTRIBUTION OF ^{99m}Tc SKELETAL AGENTS IN BLOOD IN HUMAN VOLUNTEERS*

	MDP			EHDP			Pyrophosphate			Polyphosphate		
	6†			6†			6†			10†		
Time	Whole blood	Plasma	Plasma protein	Whole blood	Plasma	Plasma protein	Whole blood	Plasma	Plasma protein	Whole blood	Plasma	Plasma protein
3 min	50.5 ±10.6	49.8 ±9.8	15.7 ±5.9	55.0 ±10.0	53.5 ±10.0	12.8 ±2.7	48.4 ±8.9	48.0 ±8.9	23.6 ±6.3	— —	— —	— —
5 min	39.2 ±3.9	38.8 ±4.6	10.6 ±3.5	45.4 ±10.4	43.7 ±10.4	9.82 ±2.4	37.9 ±4.6	37.7 ±4.5	19.2 ±4.8	46.1 ±7.2	43.0 ±8.2	16.3 ±5.6
30 min	15.8 ±0.83	15.2 ±0.84	4.74 ±1.5	18.9 ±2.7	17.7 ±2.3	4.11 ±0.96	18.1 ±3.0	17.2 ±3.2	8.86 ±2.2	22.7 ±2.2	21.0 ±2.6	9.08 ±3.1
1 hr	9.74 ±0.78	9.52 ±0.79	3.02 ±0.98	12.2 ±1.8	11.9 ±1.8	3.25 ±0.88	13.0 ±2.0	11.7 ±2.0	5.94 ±0.97	16.6 ±1.4	14.8 ±1.9	7.20 ±2.3
2 hr	4.82 ±0.58	4.91 ±0.61	1.85 ±0.35	7.11 ±1.0	7.12 ±1.0	2.02 ±0.47	9.35 ±1.7	7.35 ±1.7	4.21 ±0.83	12.1 ±1.0	10.6 ±1.5	5.52 ±1.9
3 hr	3.22 ±0.87	3.15 ±0.34	1.42 ±0.35	4.68 ±0.87	4.68 ±0.84	1.37 ±0.30	7.95 ±1.7	5.57 ±1.2	3.42 ±0.67	10.6 ±0.80	8.92 ±1.1	4.86 ±1.6
6 hr	1.26 ±0.37	1.27 ±0.28	0.52 ±0.27	1.89 ±0.45	1.87 ±0.44	0.61 ±0.09	6.83 ±1.4	3.93 ±1.5	3.42 ±0.67	8.39 ±0.79	6.84 ±1.0	4.08 ±1.3
9 hr	0.74 ±0.39	0.77 ±0.17	0.56 ±0.10	0.84 ±0.43	0.91 ±0.40	0.31 ±0.30	6.41 ±1.3	3.40 ±0.82	2.65 ±0.70	6.58 ±1.0	5.83 ±1.1	1.55 ±0.32
24 hr	0.38 ±0.21	0.40 ±0.12	0.40 ±0.08	0.20 ±0.16	0.19 ±0.13	0.09 ±0.07	5.44 ±1.0	2.19 ±0.43	1.85 ±0.38	4.70 ±0.89	3.65 ±1.0	— —

* Percent administered radioactivity in total circulating blood fractions; mean values and standard deviations.
† Number of subjects.

TABLE 4. COMPARISON OF BIOLOGIC FATE OF ^{99m}Tc SKELETAL COMPLEXES IN HUMANS

	MDP		EHDP		Pyrophosphate		Polyphosphate	
Component	% Dose	$T_{1/2}$ (hr)	% Dose	$T_{1/2}$ (hr)	% Dose	$T_{1/2}$ (hr)	% Dose	$T_{1/2}$ (hr)
				Blood clearance				
1st	77.6	0.04	74.2	0.05	74.0	0.03	71.3	0.04
2nd	20.1	0.85	21.5	1.04	18.3	0.64	19.3	0.82
3rd	1.5	14.5	2.5	7.62	7.4	53.7	9.4	23.5
				Whole-body retention				
1st	28.8	0.27	30.8	0.55	27.6	0.36	14.5	0.35
2nd	41.3	1.7	43.1	2.50	27.8	2.78	28.9	1.12
3rd	30.0	68.6	26.1	52.9	44.6	199.1	58.6	43.3

much slower than that of either diphosphonate, particularly at 3 hr and beyond and the clearance of polyphosphate was the slowest of the four complexes. As in the dog experiments, the diffusion of the labeled diphosphonates into the circulating blood cell fraction was negligible. For polyphosphate, from 10–15% of the remaining blood activity was localized in the red cell fraction between 1 and 3 hr, increasing to 22% at 24 hr. Pyrophosphate exhibited a greater degree of red cell diffusion; from 10–30% of the remaining blood activity was contained within the red cell fraction between 1 and 3 hr, increasing to 60% at 24 hr. For all of these agents, however, the total fraction of administered activity remaining in the blood stream at 24 hr is very small.

The plasma protein binding, as assessed by trichloroacetic acid precipitation, was considerably greater for the two phosphate complexes than for the diphosphonates within the first few hours. About 30% of the MDP plasma activity was protein-bound in the first hour, increasing to 45% by 3 hr and 100% by 24 hr. For EHDP about 25% of the plasma activity was protein-bound within the first hour, 30% by 3 hr, and 45% by 24 hr. For both pyrophosphate and polyphosphate, from 40–50% of the plasma activity was protein-bound within the

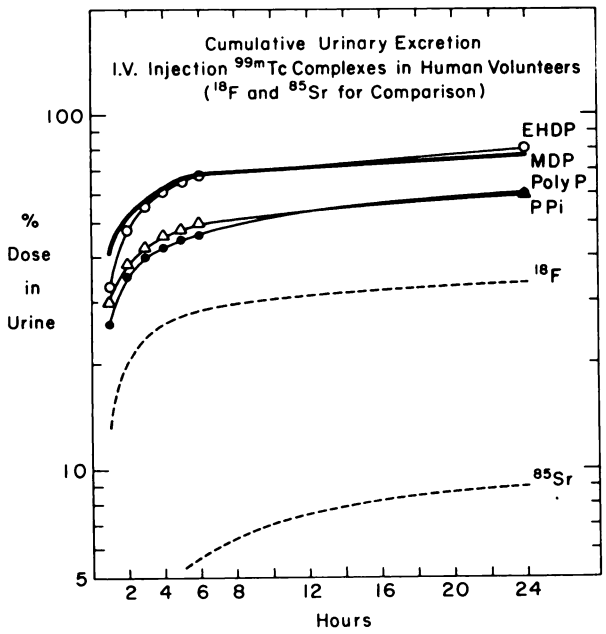

FIG. 6. Urinary excretion of MDP in humans compared with three other ^{99m}Tc complexes, ^{18}F and ^{85}Sr (corrected for physical decay). PPi indicates pyrophosphate and PolyP denotes polyphosphate.

first hour, and 55–60% by 3 hr and beyond. No reliable whole-blood clearance data could be found in the literature for ^{85}Sr for comparison with the ^{99m}Tc skeletal agents. However, in comparison with numerous ^{85}Sr plasma clearance curves (*12,13*), it is apparent that the disappearance of this older agent from the blood stream is even slower than that of ^{99m}Tc-polyphosphate.

In the present study the dog and human blood clearance curves for polyphosphate are essentially the same during the first 6 hr after injection and thereafter the levels are somewhat lower in the dog. The blood clearance of MDP is considerably faster in the dog than in man. The EHDP blood levels are slightly lower in the dog than in the human for the first 3 hr but somewhat higher in the dog at 6 and 24 hr.

The urinary clearance of the four ^{99m}Tc skeletal agents in the six male volunteers is shown in Fig. 6 and Table 5. The urinary excretion at 24 hr of EHDP and MDP is similar; however, excretion is slightly greater for MDP in the first 2 hr after injection ($P = 0.01$). Pyrophosphate and polyphosphate have a similar urinary excretion rate throughout the first 24-hr period, lower than that of the diphosphonates. Urinary clearance of all four ^{99m}Tc agents is greater than ^{18}F. The urinary excretion of ^{85}Sr is very low and a major fraction of this radionuclide is eventually excreted in the feces. The 24-hr urinary excretion for EHDP and polyphosphate is similar between dog and man but somewhat greater in man for MDP. Exponential analysis of the total-body retention curves in man, obtained from the urinary excretion data, is summarized in Table 4. The fast component of retention of these agents is not as large a fraction of the administered activity as in the dog (Table 2).

Clinical trials of all four ^{99m}Tc agents showed that each was capable of producing excellent images of the skeleton. Figure 7 illustrates comparative images performed with all four agents in the same normal individual. As a rule images of high quality could be obtained with the MDP complex 2 hr after intravenous injection. A longer interval of 3–4 hr was often required for the EHDP complex. With the polyphosphate and pyrophosphate complexes, a 4-hr interval usually was required to allow the soft-tissue radioactivity to clear to obtain similar satisfactory visualization of the skeleton. From the overall subjective evaluation of the clinical studies, MDP seemed to produce the best clinical results consistently, somewhat better than EHDP and pyrophosphate. Polyphosphate ranked below the other three agents. A wide variation was observed in the quality of images produced from the same preparation in different individuals on the same day. Clinical studies in elderly patients tended to be poorer in quality than those in young individuals. Images of excellent qual-

TABLE 5. CUMULATIVE URINARY EXCRETION OF ^{99m}Tc BONE AGENTS IN HUMAN VOLUNTEERS*

	MDP	EHDP	Pyrophosphate	Polyphosphate
Time (hr)	6†	6†	6†	10†
0–1	40.7 ± 3.47	32.9 ± 5.89	29.9 ± 3.9	25.8 ± 4.8
0–2	52.2 ± 4.00	47.3 ± 6.05	38.0 ± 4.3	35.1 ± 4.5
0–3	58.9 ± 4.69	55.5 ± 6.71	42.7 ± 4.1	40.0 ± 4.6
0–4	61.8 ± 4.36	60.8 ± 6.99	45.9 ± 4.3	42.5 ± 5.1
0–5	66.3 ± 5.08	64.7 ± 6.95	48.2 ± 4.5	44.9 ± 5.2
0–6	68.2 ± 5.20	67.7 ± 7.02	49.8 ± 4.5	46.0 ± 5.7
0–24	76.5 ± 5.59	79.2 ± 7.24	58.9 ± 5.2	60.1 ± 5.2

* Percent administered radioactivity in urine; mean values and standard deviations.
† Number of subjects.

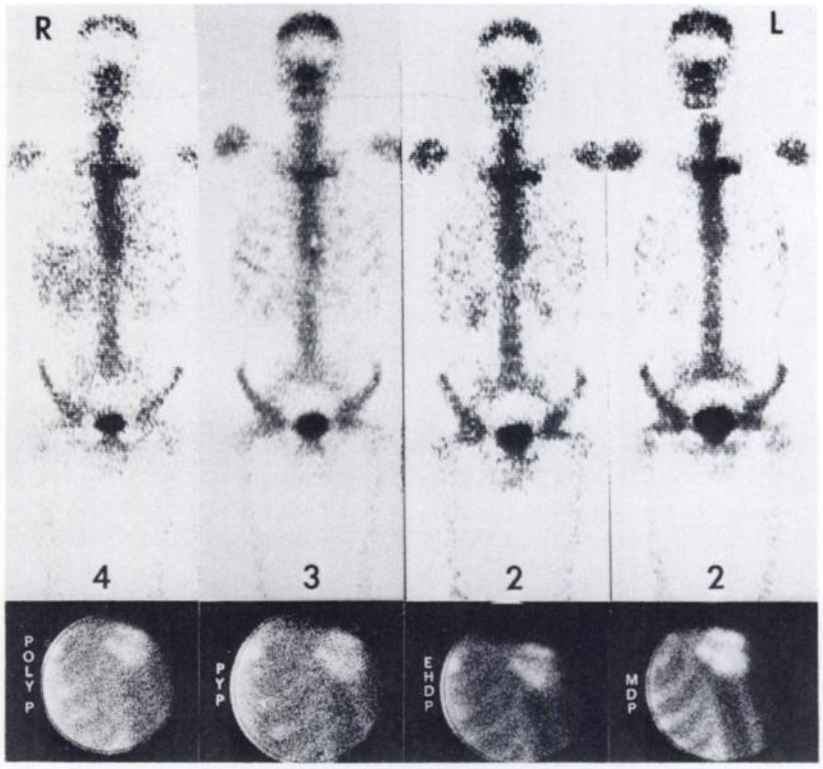

FIG. 7. Comparative anterior scans and camera images of left upper chest and shoulder after 15 mCi of each of four ^{99m}Tc skeletal complexes in same normal individual. Numbers indicate number of hours between injection and scan. Soft-tissue activity appears lowest with MDP and highest with polyphosphate.

TABLE 6. RADIATION DOSE ESTIMATES (RADS/mCi)

Organ	^{99m}Tc-EHDP or ^{99m}Tc-MDP	^{99m}Tc-pyrophosphate or ^{99m}Tc-polyphosphate
Skeleton	0.038	0.054
Red marrow	0.025	0.038
Total body (conventional)	0.007	0.010
"Average soft tissue"	0.009	0.013
Kidneys	0.031	0.047
Bladder wall	0.44	0.32
Liver	0.008	0.014
Ovaries	0.017	0.020
Testes	0.012	0.014

ity were almost invariably obtained in children and young adults with any of these agents.

Toxicity. The acute toxicity of the two diphosphonates, MDP and EHDP, was similar in rodents. In both the mouse and rabbit the acute LD_{50} after intravenous injection was 45–50 mg/kg (compounds considered in acid form). Death was due to tetany. No adverse reactions of any kind were encountered in any patient following the administration of MDP or the other three agents.

Radiation dose estimates. Using the preceding data on the biologic distribution and excretion, estimates were made of the radiation dose delivered to various organs after the intravenous administration of the four technetium-labeled skeletal agents, summarized in Table 6. The MIRD Committee "S" tables (*14*) (absorbed dose per unit cumulated activity) were employed for these calculations. The radiation doses for ^{99m}Tc-MDP were not significantly different from those of ^{99m}Tc-EHDP. Similarly, the values obtained for ^{99m}Tc-pyrophosphate were similar to those of ^{99m}Tc-polyphosphate. For most organs except the bladder wall, the radiation levels were slightly higher for the phosphates than the diphosphonates, chiefly due to the slower renal clearance of the former.

For the estimates of the skeletal radiation doses, actual bone concentration data from humans were not available. For a conservative estimate, therefore, it was assumed that 50% of the administered dose of each of the agents concentrated in the skeleton instantaneously and did not undergo any biologic elimination for several hours until the skeletal retention coincided with the measured total-body retention curve. It was further assumed that no further biologic excretion occurred after 24 hr. These appear reasonable assumptions on the basis of available data. If the skeleton represents 10% of the total-body weight, a 50% concentration is equivalent to about 7×10^{-3}% of the administered activity per gram of bone. In biopsy specimens of human cortical bone obtained 2 or 3 days after the administration of ^{85}Sr (*15*), the concentration was 6×10^{-3}%/gm. The current tissue radioassay studies in rabbits showed that the ratio of technetium–strontium concentrations for these skeletal complexes approached but did not exceed unity. In another study (*16*), the retention of

^{85}Sr in the human skeleton was 47% 2 days after administration as estimated by assay of specimens obtained at autopsy. In direct assays of ^{99m}Tc-pyrophosphate (*17*), the concentration in the femur was 8×10^{-3}%/gm in miniature swine and 6×10^{-3}%/gm in the dog.

The estimated skeletal radiation levels are "average skeletal doses" that assume uniform distribution. However, numerous radioassays of a variety of bone-seeking agents have invariably shown a striking nonuniformity of distribution. For example, ^{85}Sr has had a higher concentration in the vertebrae and ribs than in long bones of humans; in different parts of the same rib, the concentration has varied by a factor of 3 (*16*). In long bones the highest concentration of ^{85}Sr was immediately adjacent to the marrow cavity (1.1×10^{-2}% dose/gm). The next highest concentration was adjacent to the periosteum and the content was relatively low in the interior of the cortex (by a factor of 10) and in trabecular bone (by a factor of 2). In all likelihood the biologic retention in each of these areas is different, being longest in the layer adjacent to the periosteum.

The radiation dose estimates for a "standard man" as listed in Table 6 are not applicable to children. The localization of other bone-seeking agents in the growing skeleton suggests that their concentration is higher and their retention longer than in adults. From radiostrontium assays in young rabbits (*18*), the concentration in the metaphyses of growing long bones immediately adjacent to the epiphyseal cartilage is two and one-half to three times higher than the average concentration of the diaphysis. Moreover, in biopsy specimens of bones of children (*15*), the metaphyseal ends contain about three times the average concentration of the cortex (16.7×10^{-3}% dose/gm). The total-body retention of ^{85}Sr in the beagle at 24 hr is 90% in the newborn animal compared with only 70% in the full-grown dog (*19*). Unfortunately, the methodology has not yet been developed to permit valid radiation dose calculations for the pediatric age groups for these skeletal-localizing agents.

The red marrow receives most of its radiation dose from the skeletal radioactivity but also receives contributions from other soft tissues and from the intrinsic radioactive content of the marrow itself. The intrinsic marrow concentration was assumed to be 1% of the administered radioactivity for the diphosphonates and 2% for polyphosphate and pyrophosphate. In using the "S" tables for calculation of the skeletal contribution to the marrow radiation dose, the cumulative activity in cancellous bone and cortical bone was not known. Consequently, it was assumed that the total cumulative activity for the skeleton was equally divided between cortical and cancellous bone since the surface areas of these two bone types are approximately equal (*14*).

Table 6 provides an estimate of the total-body radiation dose, as conventionally calculated, using the current human total-body retention data. However, this conventional method assumes uniform distribution of the radioactivity which is not true for these bone-localizing agents. Therefore, an additional calculation was made for the "rest of the body", excluding the skeleton (the "average soft-tissue radiation dose"). It was assumed that 50% of the administered radioactivity instantly localized within the skeleton, leaving 50% distributed in the soft tissues. The average soft-tissue radiation level proved to be slightly greater than that of the conventional total-body radiation dose. The radiation dose received by the blood was not significantly different from these values.

The "target" tissue which receives the largest radiation dose from these agents is the bladder wall, chiefly because of the radioactive urine within the bladder. This dose level is somewhat higher for the diphosphonates due to their more rapid urinary clearance. It was assumed for these calculations that the radioactivity was administered when the bladder was empty, and that there were three daytime voidings every 4 hr followed by an overnight voiding. These radiation levels are reduced significantly under other physiologic conditions, particularly if the radioactivity is administered with the bladder partially filled and the patient well hydrated.

The radiation dose received by the ovaries was significantly greater than that of the "average soft tissue" since they receive radiation from the bladder contents in addition to contributions from the skeleton and other soft tissues. The calculated radiation dose received by the testes was lower than that of the ovary.

The radiation dose estimates for the kidneys were significantly higher than those of the average soft tissue whereas those of the liver were not. For these organs, a biologic half-time of 24 hr was assumed. Based on the current rabbit assay data, and also on the data of Eckelman, et al in miniature swine (*17*), it was assumed that the two kidneys contained a maximum concentration of 3% and the liver, 2% for pyrophosphate and polyphosphate. Based on the rabbit data alone, it was assumed for the diphosphonates that 2% of the administered radioactivity was the maximum concentration in the two kidneys and 1% in the liver.

DISCUSSION

More than a decade ago, the chemist Van Wasser pointed out that "boiler scale" due to the precipita-

tion of calcium salts from water could be prevented by low concentration of linear open-chain polymers of phosphate. In 1961, Fleish and Neuman (*20*) reported that polyphosphates were potent inhibitors of in vitro crystallization of calcium phosphate from solution in the presence of collagen, effective in concentrations as low as 10^{-7} *M*. They prevented also the dissolution of hydroxyapatite crystals in vitro. However, they proved ineffective in vivo because of their rapid enzymatic destruction by polyphosphatases found in bone, kidney and gut, and other mammalian tissues (*21*). Divalent cations, probably magnesium and possibly zinc or cobalt, were coenzyme factors in this destruction. The alkaline phosphatases also could function as polyphosphatases. Pyrophosphate (the smallest polyphosphate, with only two phosphate groups) was found to be a ubiquitous product of cell metabolism normally present in the plasma (0.16–3.4 μmole/liter) and sometimes present in abnormally high concentrations in pseudogout, osteoarthritis, and acromegaly, and low in osteogenesis imperfecta. Tripolyphosphate also was found in some mammalian tissues. Pyrophosphate was found in urine, but longer chain polyphosphates were excreted primarily as orthophosphate.

Subsequently, diphosphonate analogs were synthesized with a P-C-P bonding sequence instead of the P-O-P sequence of pyrophosphate. Although these two chemical structures were similar, calculations for P-C-P bond angles were smaller (117 deg) than the P-O-P bond angles (128.7 deg) and the P-C interatomic distance (1.79 Å) longer than that of P-O (1.63 Å) (*22*). The diphosphonate bonding proved very stable chemically and not subject to enzymatic hydrolysis in vivo. Russell, et al (*23*) compared ten different diphosphonates with pyrophosphate and condensed phosphates using hydroxyapatite crystals in vitro and in mouse calvaria in tissue culture. The three compounds most effective in inhibiting renal and aortic calcifications in rats treated with large doses of vitamin D_3 were ethane-1-hydroxy-1: 1-diphosphonate (EHDP), methylene diphosphonate (MDP), and dichloromethylenediphosphonate (Cl_2MDP). EHDP later was used extensively in studies of bone metabolism (*24–27*). It prevented immobilization osteoporosis in young rats and parathyroid hormone-induced bone resorption. However, it did not prevent osteoporosis induced by cortisone in rats or by low-calcium, high phosphate diet in cats or soft-tissue calcification in rabbits on a high calcium diet. In dogs 5 mg/kg daily inhibited the mineralization of bone after 6 days, produced morphologic osteomalacia, and after several weeks, the formation of osteoid decreased also. The accretion of calcium in bone was slowed and the plasma clearance of calcium delayed. Autoradiographs of ^{32}P-EHDP showed localization on the mineral surfaces of bone (*25*).

Therapeutically, this drug has been used in Paget's disease (*28*) in daily doses of 20 mg/kg for about 6 months, reducing the bone pain, serum alkaline phosphatase, and hydroxyproline excretion and also reducing the increased uptake of bone-seekers. In addition, it retards the formation of dental calculi and ectopic calcification in myositis ossificans and calcinosis universalis; however, it apparently has no effect on postmenopausal osteoporosis. Dichloromethylene diphosphonate does not impair bone mineralization in equivalent doses like EHDP, and it is especially potent in decreasing bone resorption (*23*); nevertheless, this agent has not been used therapeutically as yet. In essence, the diphosphonates have no effect on the first step in bone mineralization—the nucleation of amorphous calcium phosphate. However, they markedly inhibit the second step—the conversion of this amorphous form to hydroxyapatite crystals—by surface absorption. Similarly, they inhibit the dissolution of the crystals by "coating the surface." They may have an additional cellular effect (*24*) and inhibit the action of pyrophosphatases by complexing divalent cations in bone (*29*).

Technetium-99m-complexes are now the most widely used agents for skeletal imaging; hence, a detailed knowledge of their biologic distribution is important. Although the mechanism of skeletal localization is not completely understood, the available evidence suggests that they undergo chemisorption at the surface of bone mineral. One autoradiographic study of ^{99m}Tc-polyphosphate of a human femoral head showed radioactivity deposited along the surfaces of small vascular channels and marrow spaces in less mineralized surface layers just beneath the osteoid tissue of viable bone (*30*).

This current comparison of four ^{99m}Tc complexes suggests that MDP is the most satisfactory agent for skeletal imaging whereas linear "long-chain" polyphosphate is the least satisfactory. A fifth agent, ^{99m}Tc-monofluorophosphate, was not evaluated. However, another comparative study in humans (*31*) indicates that its urinary excretion is even slower than that of polyphosphate.

In the organ radioassay experiments in rabbits, the skeletal concentration of the diphosphonates was greater than that of pyrophosphate or polyphosphate; however, no significant difference was found between EHDP and MDP. Simultaneous ^{99m}Tc to ^{85}Sr concentration ratios showed that these technetium complexes achieve a concentration in axial skeleton of about 90% of the strontium concentration whereas in the extremities their concentration is considerably

lower than that of strontium. In previous assays in animals (*32*), the clearance rate and tissue distribution of polyphosphate and pyrophosphate were similar.

In the dog and human, the four ^{99m}Tc skeletal complexes have the same order of blood clearance rates. In both species polyphosphate has the slowest and MDP the fastest clearance. The EHDP does not clear as rapidly as MDP and this difference is statistically significant in the human volunteers. Pyrophosphate in the human is not cleared quite as rapidly as EHDP but not as slowly as polyphosphate. Although pyrophosphate was not studied in dogs in the present work, the blood clearance obtained by Hosain (*33*) for pyrophosphate was similar to our values for EHDP. We believe that our comparison of the blood clearance rates in humans was more reliable than in the dog study because each agent was tested in the same group of individuals whereas, in the latter, each agent was evaluated in a different group.

The faster blood clearance observed with the diphosphonates is compatible with the clinical observation that in skeletal images there is less activity in the liver and other soft tissues than is observed with pyrophosphate and particularly with polyphosphate.

Recker, et al (*34*) measured the plasma clearance and urinary excretion following intravenous injection of ^{14}C-labeled EHDP in normal male volunteers. In comparing their data with the current human ^{99m}Tc-EHDP data, the ^{14}C-labeled compound has a somewhat faster plasma clearance. By 24 hr, only 52% of the ^{14}C-labeled compound is recovered in the urine compared with 79% of ^{99m}Tc activity injected as the EHDP complex. This marked discrepancy indicates that a sizable fraction of the ^{99m}Tc activity administered as EHDP is excreted in some form other than the diphosphonate complex.

In the human volunteers the urinary excretion of ^{99m}Tc administered as a diphosphonate is significantly greater than ^{99m}Tc administered as polyphosphate or pyrophosphate. This agreed with a previous report that the urinary clearance of EHDP is greater than that of either polyphosphate or pyrophosphate (70% compared with 55%).

The total-body retention of none of the technetium-complexes was as great in the adult beagle as that previously reported for ^{85}Sr (*19*). The metabolic studies in dogs, however, suggest that the skeletal retention of MDP may be greater than EHDP; however, it is possible that this apparent difference could have been due merely to the biologic variation between the two groups of animals. A small fraction of the technetium administered to dogs as either the EHDP or polyphosphate complex was eventually excreted in the feces. On the other hand, the gastrointestinal excretion of MDP was negligible.

In clinical practice considerable variation in the quality of skeletal images has been observed with different commercial preparations of the same compound and in different lots from the same manufacturer. This variability is most marked with polyphosphate (*32*) and appears to be less so with pyrophosphate. The variability in preparations of EHDP, we suspect, is largely due to the presence of acetate, which is an end product of the synthetic process. The batch-to-batch variability with MDP appears to be less than that with EHDP.

MDP like the other agents evaluated had a low order of toxicity, as evidenced by acute toxicity experiments in rodents and complete absence of adverse reactions clinically. Dunson, et al (*32*) found that a low degree of toxicity was common to EHDP, polyphosphate, and pyrophosphate. The LD_{50} in rabbits and rats was 40–70 mg/kg for rapid injection and 70–100 mg/kg for a slower injection. When death occurred from acute toxicity, lung hemorrhages and tetany were observed. On repeated injection of 20 mg/kg, no adverse effects were noted in dogs, pigs, rabbits, or rats. Daily oral administration of EHDP (10 mg/kg) over many months, however, will produce osteomalacia as determined by microradiography (*25,28*).

According to Gosselin, et al (*35*), sodium phosphate polymers are somewhat more toxic than orthophosphate, and the degree of toxicity is directly related to the stability of the calcium complex. With pyrophosphate, tetany in rats is produced by about 22 mg/kg and EKG changes of hypocalcemia at 12 mg/kg intravenously (*36*). If one may extrapolate these data to humans, the "safety factor" between a diagnostic injection and the lowest dose for minimally detectable hypocalcemia is 55 or higher.

Our knowledge of the biologic distribution of these ^{99m}Tc skeletal-localizing agents is still incomplete. Most important, no radioassay data are available for the skeleton itself at various intervals after administration in humans. Significant differences in the distribution of the four agents evaluated, however, are apparent. Differences in soft-tissue activity (highest with polyphosphate and lowest with MDP) are discernible in clinical images. It is suspected that the actual differences in skeletal concentration between these skeletal agents may be relatively minor. The soft-tissue radioactivity probably depends primarily on the plasma clearance, which in turn depends chiefly on the rate of renal clearance.

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