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^{99m}Tc-PYROPHOSPHATE FOR BONE IMAGING

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Of the various ^{99m}Tc-phosphates suggested for bone imaging, ^{99m}Tc-pyrophosphate appears to have the desirable characteristics of commercial availability of sodium pyrophosphate and analytical controls available to assure the quality of the final product. Systematic variations of the parameters of the ^{99m}Tc-tin-pyrophosphate preparations were evaluated by gamma camera images in swine. These studies indicate that the best distribution is obtained by adding 1.75 ml of 0.1 M sodium pyrophosphate at pH 10 to 0.25 ml of 1 mg/ml stannous chloride followed by 1.50 ml $^{99m}TcO_4^-$. In vivo organ distribution indicates the importance of normalizing doses on a per weight basis for comparative animal studies. Data reported for ^{99m}Tc-phosphates without consideration of the dose on a weight basis can be misleading. If the optimal dose of pyrophosphate is used, ^{99m}Tc-pyrophosphate results in high-quality bone images in animals and humans and can be easily prepared and assayed.

Technetium-99m-phosphates are now considered to be the agents of choice for bone imaging. We have chosen to study ^{99m}Tc-pyrophosphate because of the commercial availability of sodium pyrophosphate and because of the analytical controls available to assure the quality of the product.

MATERIALS AND METHODS

Reagents. Sodium pyrophosphate $Na_4P_2O_7$ 10 H_2O Baker Analyzed Reagent and stannous chloride $SnCl_2 2H_2O$ Baker Analyzed Reagent were used.

Method of preparation. To a 6-ml evacuated vial was added 0.25 ml tin chloride (1 mg/ml as $SnCl_2 2H_2O$), then 1.75 ml of 0.1 *M* pyrophosphate at pH 10.0 followed by 1.50 ml of pertechnetate. The tin chloride solution is prepared by dissolving 25 mg $SnCl_2 2H_2O$ in 2 ml concentrated HCl with heat and diluting to 25 ml with N_2-purged sterile water. All solutions except the eluted $^{99m}TcO_4^-$ are prepared in a nitrogen-purged glove box. Preprations were carried out with kits up to 4 weeks old.

Variables. The following variables were studied in miniature swine (Pitman-Moore strain, females 8–15 months) varying in weight from 7 to 50 kg. All studies were done in duplicate.

1. Original pH of pyrophosphate solution.
2. Final pH of mixture.
3. Pyrophosphate concentration.
4. Tin concentration.
5. Reproducibility.

After supra vena cava injection gamma camera image quality was recorded using Polaroid and 35-mm film after 300 K collection on a Nuclear-Chicago HP gamma camera. Scans were taken at 3–5 hr. There was little change in distribution over this time period.

Tissue distribution. Tissue distribution of the optimal radiopharmaceutical was obtained in miniature swine, mongrel dogs, and rabbits. In the first series (Table 1) the animals were injected with amounts of pyrophosphate which are 5–15 times greater than that received by humans on a weight basis. The swine were injected with 4 ml of the standard preparation (see the "Methods" section) whereas the dogs and rabbits were injected with 1 ml. In the second series with rabbits (Table 2) the effect of the amount of pyrophosphate injected per kilogram was compared with a similar variation with ^{99m}Tc-ethane-1-hydroxyl-1, 1-diphosphonic acid (^{99m}Tc-EHDP from Medi+Physics). The initial dose (Columns 1 and 4) was calculated on a weight basis using the amounts suggested by the manufacturer based on toxicity studies for human use. This dose should indicate the distribution to be expected in humans assuming no species difference. The high-

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TABLE 1. DISTRIBUTION OF ^{99m}Tc-PYROPHOSPHATE IN SWINE, DOGS, AND RABBITS*

Species	Pyrophosphate (mg salt/kg†)	% dose blood	% dose $gm^{-1} \times 10^3$				
			Kidney	Liver	Muscle	Red marrow	Femur
Swine (1)‡	1.8	3.2	8.6	0.60	0.18	7.1	8.3
Swine (1)	1.5	2.8	11.3	0.40	0.08	11.2	5.6
Dog (2)	4.8	2.5	8.0	1.40	0.27	0.68	6.2
Rabbit (3)	5.8	4.2	73.7	24.6	4.2	34.0	91.4

* All animals were sacrificed at 2 hr except swine No. 2 sacrificed at 3¼ hr.
† Suggested human dose is 0.32 mg salt/kg.
‡ The number in parentheses denotes the number of animals in each group.

TABLE 2. DISTRIBUTION OF ^{99m}Tc BONE AGENTS IN RABBITS

Organ	% dose per organ					
	^{99m}Tc-pyrophosphate			^{99m}Tc-EHDP		
	Phosphate salt conc. (mg/kg)					
	0.32	1.60	8.92	0.014	0.100	0.400
Liver	0.99	1.58	20.99	0.69	2.40	1.36
	3.07	1.39	15.88	0.74	1.10	1.75
	0.94	—	—	1.18	0.97	4.46
	—	—	—	—	2.77	3.38
Avg.	1.67	1.49	18.44	0.87	1.81	2.74
Kidney	1.14	1.43	1.41	3.42	1.97	1.96
	1.56	1.69	1.43	2.87	1.35	1.59
	1.34	—	—	4.57	1.67	2.09
	—	—	—	—	1.28	3.42
Avg.	1.35	1.56	1.42	3.62	1.32	2.27
Muscle	2.56	0.84	1.47	1.60	1.52	1.30
	2.38	0.81	0.96	1.84	1.64	1.29
	1.60	—	—	1.93	1.89	1.35
	—	—	—	—	1.61	3.16
Avg.	2.18	0.83	1.22	1.79	1.67	1.70
Femur	58.34	42.75	32.47	41.64	49.04	45.19
	57.84	50.36	35.75	41.57	49.36	44.96
	59.64	—	—	39.00	50.88	40.25
	—	—	—	—	50.43	43.40
Avg.	58.61	46.56	34.11	40.74	49.93	43.45
Red marrow	0.76	1.26	13.17	0.36	0.79	0.49
	1.05	0.85	4.79	0.32	0.58	0.48
	0.34	—	—	0.63	0.89	0.94
	—	—	—	—	0.84	1.12
Avg.	0.72	1.06	8.98	0.44	0.78	0.76
Blood	3.71	2.46	4.65	2.97	2.45	3.15
	3.78	2.77	3.26	2.98	3.57	2.78
	2.91	—	—	5.66	2.86	2.93
	—	—	—	—	2.12	5.05
Avg.	3.47	2.62	3.51	3.87	2.75	3.48
Urine	38.77	50.31	25.84	34.55	48.28	41.05
	35.51	42.63	30.63	37.70	48.38	47.14
	37.84	—	—	42.03	48.21	47.97
	—	—	—	—	48.98	47.53
Avg.	37.37	46.47	28.24	38.09	48.25	44.17

est dose (Columns 3 and 6) represents the total human dose injected in an animal (which had a smaller blood volume). The intermediate dose was included to substantiate any trends in distribution. The ^{99m}Tc-pyrophosphate was prepared similarly to the standard preparation and varying volumes were injected to give the desired phosphate weight per kilogram, i.e., 0.36 ml of a 1:10 dilution with nitrogen-purged saline diluted immediately before injection was used for the lowest value, 0.36 ml of a 1:2 dilution was used for the intermediate value, and 1 ml of a 1:2 dilution was used for the highest value. For the ^{99m}Tc-EHDP the preparation was made with varying amounts of pertechnetate solution as advised by the manufacturer so that for the lowest value, 0.9 ml of 0.4 mg EHDP in 3.6 ml was injected, for the intermediate dose 0.6 ml of 1.0 mg EHDP in 2.25 ml was injected, and finally 2.1 ml of 3 mg EHDP in 6.5 ml was injected. In rabbits the percent body weight values quoted by Subramanian, et al (*1*) are used. Ten to 15 μCi of ^{85}Sr were also injected in all rabbits to correct for biological differences in metabolism.

Quality assurance. The stannous ion content of the tin chloride solution of the pyrophosphate kit was determined by a potentiometric titration using iodine solution and a combination platinum electrode (*2*). Titrations were carried out on the stannous chloride stock solution and on the final product. The pyrophosphate content was determined using lanthanum precipitation and titration of excess fluoride using a fluoride electrode (*2*).

Paper. Paper chromatography (Whatman No. 1) in saline and 0.05 *M* pyrophosphate indicated the presence of a weak ^{99m}Tc-chelate. In the diluting media, saline, all ^{99m}Tc activity was found at the origin indicating nonchelated ^{99m}Tc. However, in 0.05 *M* pyrophosphate eluant, ^{99m}Tc activity was present at the solvent front. Eluting the weak chelate with an equimolar solution of pyrophosphate maintains equilibrium during analysis whereas the saline eluant dilutes the effective pyrophosphate concentration and causes dissociation of the weak chelate.

Toxicity. The amount of tin used is well below the toxic level (*3*). The pyrophosphate is reported to have a LD_{50} of 243 mg salt/kg by Gosselin, et al (*4*) by intraperitoneal injection in rats. However, a

TABLE 3. SCALE FOR EVALUATION OF GAMMA CAMERA IMAGES

4—Individual vertebras, no interference from other organs including muscle.
3—Vertebras with some interference from blood and muscle background.
2—Vertebras but interference from liver radioactivity.
1—No vertebras, activity concentrated in liver, blood, and/or muscle.

recent study shows that the LD_{50} for intravenous bolus injection in rats and rabbits is 38 mg salt/kg and, hypocalcemia and EKG changes are noted at lower levels (*5*). Injection of 1 ml of final product (22 mg salt/ml) gives a safety factor of 125 for a 70-kg man based on the LD_{50}.

Clinical studies. Patients are injected with 7–15 mCi of the standard preparation (see "Methods") ^{99m}Tc-pyrophosphate in 1-ml solution. Gamma camera and rectilinear images were obtained 3 hr after injection.

RESULTS

The optimal formulation for ^{99m}Tc-pyrophosphate was determined by comparison of gamma camera images in swine. Scan quality was based on a scale of 1 to 4 with 4 indicating high bone concentration and low interference from concentration of ^{99m}Tc in other organs (Table 3).

Table 4 lists the factors studied and the scan quality obtained.

From these data the optimal compound is prepared as follows:

0.25 ml of 1 mg/ml tin chloride
1.75 ml of 0.1 *M* sodium pyrophosphate at pH 10.0
1.50 ml of $^{99m}TcO_4^-$.

Titration of the tin chloride solution indicated that greater than 75% of the tin remained as stannous ion after 1 month. The stannous ion content in the final product decreased 16.4% 4 hr after preparation. If 0.5 ml of air is injected into the reaction vial, 34% of the stannous ion is oxidized after 4 hr. The pyrophosphate solution is stable for at least 3 months at pH 10.0. Paper chromatography in 0.05 *M* pyrophosphate indicated greater than 95% ^{99m}Tc-pyrophosphate. Kits were used for 4 weeks in both the human and animal study.

A gamma camera image of the optimal preparation in swine (Fig. 1A) shows good delineation of the vertebras with little ^{99m}Tc activity concentrated in other organs.

The distribution of the ^{99m}Tc-pyrophosphate was determined in swine, dogs, and rabbits using pyrophosphate doses in excess of that used in humans (Table 1).

To assess the effect of dilution on the organ distribution of ^{99m}Tc-pyrophosphate and ^{99m}Tc-EHDP, distribution studies were carried out with various phosphate concentrations (Table 2). As increasing pyrophosphate or diphosphonate was injected, the activity in liver increased and, for pyrophosphate, the bone concentration decreased while the red marrow increased. Strontium-85 distribution did not indicate biological differences in the rabbits.

DISCUSSION

The short chain sodium phosphates are by far the most readily available and easily controlled substrates suggested for bone imaging with ^{99m}Tc. The simplest phosphate, orthophosphate, does not localize primarily in bone but rather results in high blood and bone marrow concentration (*6*). The dimer pyrophosphate has also been suggested (*7*). A recent report by Perez, et al (*8*) using ^{99m}Tc-pyrophosphate was not encouraging. A more detailed report (*9*) shows that various ratios of pyrophos-

TABLE 4. COMPARISON OF GAMMA CAMERA IMAGES IN SWINE FOR ^{99m}Tc-PYROPHOSPHATE

pH of pyrophosphate solution before addition to tin chloride solution		Molar concentration of pyrophosphate solution before addition to tin chloride		Final pH of solution after mix of all components		Concentration of chloride before mixing with pyrophosphate		Reproducibility of optimal product	
Values	Scan quality	Values	Scan quality	Values	Scan quality	Values	Scan quality	Values	Scan quality
pH = 3.5	2	0.20 M	3	pH = 5.9	3	1,000 μg/ml	4	Scan #1	4
5.5	2	0.10 M	4	6.3	4	1,500 μg/ml	4	Scan #2	4
10.0	4	0.05 M	1	6.6	4	2,000 μg/ml	3	Scan #3	4
		0.02 M	1	7.7	3				
				11.8	1				
				12.6	1				

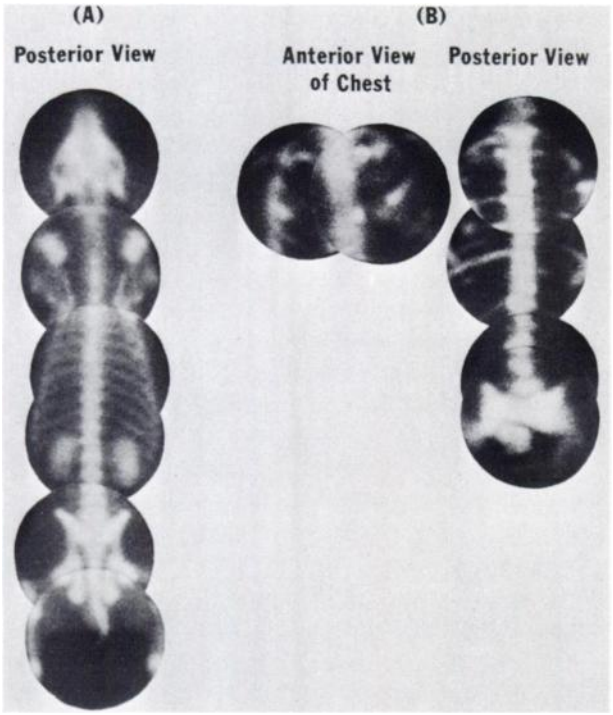

FIG. 1. (A) Scan of miniature swine 2 hr after injection of optimum ^{99m}Tc pyrophosphate preparation (Table 4). Each image was recorded to collect 300K counts. (B) Human subject using optimum ^{99m}Tc pyrophosphate compound demonstrating multiple skeletal metastases. Each image was recorded to collect 300K counts.

phate to tin were not tested and when compared with our data their preparation does not give the maximum bone-to-muscle or bone-to-blood ratio. A qualitative report by Kaplan, et al (*10*) shows a difference in distribution between ^{32}P-orthophosphate and ^{32}P-phosphate polymers in that the bone-to-muscle ratio is greater with the polymers. A quantitative comparison describing the same findings has been published by Anghileri (*11*). Compartmental analysis of the disappearance of ^{32}P-pyrophosphate in dogs shows that the majority of the pyrophosphate leaves the first compartment (plasma) intact and that which is converted to orthophosphate is done so at the bone surface (*12*). This type of behavior would not be detrimental to bone uptake of the ^{99m}Tc because it is unlikely that the ^{99m}Tc would leave with the weak complexing agent orthophosphate to be redistributed in the soft-tissue phosphate pool. This is shown by the low muscle uptake for the standard ^{99m}Tc-pyrophosphate preparation.

Since the tin pyrophosphate chelate has an equilibrium constant of approximately 10^7 (*13,14*) when compared with the stable chelate of tin diethylenetriamine-pentaacetic acid (DTPA) (*15*), it is important to study the product in as large an animal model as practical because of dilution effects. The miniature swine grows to a full weight of 60 kg and is ideal for such a study. The cranial bones of this animal do not contain marrow and therefore skull images are a good indication of bone mineral uptake. The results in swine (Table 4) show that the pyrophosphate stock solution must be kept at high pH; this prohibits premixing the tin and phosphate without using lyophilization techniques. The stability of pyrophosphate to hydrolysis at high pH has been reported (*16*) and confirmation of its integrity for at least 3 months has been obtained by the methods described. In addition, it seems that the insolubility of sodium pyrophosphate (6.23 mg/100 ml at 20°C) or the insolubility of tin pyrophosphate (*17*) was the operational factor in the production of a poor scan when 0.2 *M* solutions were used. With the low concentrations, 0.002–0.05 *M*, it appears that dissociation to tin oxide causes a high blood background. Furthermore, large amounts of tin and high pH also favor formation of tin oxide rather than tin pyrophosphate. As judged by liver uptake in these experiments, ^{99m}Tc follows a similar chemistry and probably is absorbed to any tin oxide formed.

The distribution data for ^{99m}Tc-pyrophosphate (Table 1) reveal bone concentration with low amounts in blood, liver, kidney, muscle, and red marrow. The bone-to-marrow ratios are lower than others have reported for ^{99m}Tc-phosphates. We think this is in part because red marrow was carefully dissected from the fat and periosteum, and the bone fragments were scraped clean before being weighed and counted. The results listed in Table 2 (Columns 3 and 6) show that the total amount of injected phosphate does affect tissue distribution. If the human dose is injected into the rabbit with its smaller blood volume, the data would suggest that EHDP is a superior agent to pyrophosphate. However, if pyrophosphate or diphosphonate concentrations are injected on a per kilogram basis (Table 2, Columns 1 and 4), then the agents appear to be comparable, and there is increased bone concentration for the ^{99m}Tc-pyrophosphate. The amounts present in liver after the higher doses are probably due to colloid formation since tin pyrophosphate and tin orthophosphate are only slightly soluble and may be precipitated in the high ionic strength of the plasma. Comparison of our data with that for other ^{99m}Tc-phosphates in rabbits (*18,19*) is not possible because the injected amount is not given in the studies. Without normalization of the dose to a per weight basis, comparative animal data for the ^{99m}Tc-phosphates frequently will be misleading; however, the ratios of bone-to-nontarget areas for pyrophosphate are comparable to those that have been reported for EHDP (*19*).

To date about 150 scans have been done in patients and no adverse reactions have been noted.

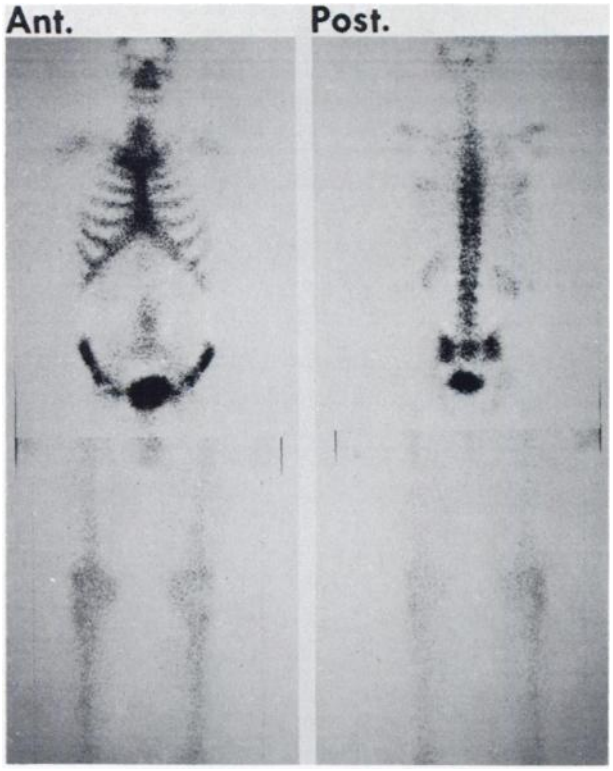

FIG. 2. Normal anterior and posterior bone scan in patient scanned 2 hr after injection of 15 mCi of optimum ^{99m}Tc pyrophosphate compound. Scan image is 5:1 minification. Maximum count rate was 125,000 cpm; scan speed was 610 cm/min.

Scan quality has been comparable to that obtained with the other ^{99m}Tc-phosphate agents in our clinic (Figs. 1B and 2). If the proper dose is used, ^{99m}Tc-pyrophosphate will result in good skeletal images and has the distinct advantage of simple quality control by standard analytical techniques and starting materials which are commercially available.

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