NEW BONE SCANNING AGENT: ^{99m}Tc-LABELED 1-HYDROXY-ETHYLIDENE-1, 1-DISODIUM PHOSPHONATE

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The radionuclide ^{99m}Tc has nearly ideal physical characteristics for imaging and is presently being used as a label for numerous radiopharmaceuticals. Consequently, most of the major organ systems have been studied with the aid of this radionuclide. One of the most recent to be considered is the skeletal system as reported by Subramanian and coworkers who labeled inorganic polyphosphates with ^{99m}Tc and observed satisfactory bone images (*1,2*).

Our laboratory is presently investigating several organo-phosphorus compounds which also show promise as satisfactory bone imaging agents after tagging with ^{99m}Tc. One such compound which currently shows the most promise is a phosphonate derivative with the chemical name 1-hydroxy-ethylidene-1, 1-disodium phosphonate (HEDSPA). Phosphonates are compounds containing a carbon-to-phosphate bond, and diphosphonates are compounds containing a carbon-to-disphosphate bond (*3*). The structures of several inorganic and organic phosphorus compounds are shown in Table 1.

Phosphonates have been used both in industry and in medicine: for example, as additives to detergent tablets (*4*), as scale and corrosion inhibitors (*5*), and as antioxidants (*6*). In 1966 several phosphonates were found to significantly inhibit bone resorption in vivo (*7*), and three years later Fleisch and coworkers reported diphosphonates to inhibit the dissolution and crystal growth of hydroxyapatite (*8*). More recently, diphosphonates have shown promise in the clinic for the treatment of myositis ossificans progressiva (*9,10*), Paget's disease (*11*), and calcinosis universalis (*12*).

This paper describes the synthesis and quality control of 1-hydroxy-ethylidene-1, 1-diphosphonic acid (HEDPHA), and the formulation and quality control of ^{99m}Tc-HEDSPA.

MATERIALS AND METHODS

The HEDPHA was synthesized by treating acetic acid with PCl_3 according to the simplified procedure of Castronovo (*13*). The quality and quantity of the product were determined by titrating 1 ml of the HEDPHA with 1 *N* NaOH to pH 12. A linear plot of the titration curve (Fig. 1) was then constructed, and simple stoichiometric relationships were employed for characterizing the product (*14*). In addition, 1 ml of HEDPHA was analyzed for carbon, oxygen, phosphorus, and hydrogen content*.

The disodium salt HEDSPA was prepared by ad-

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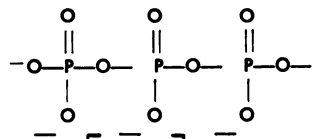
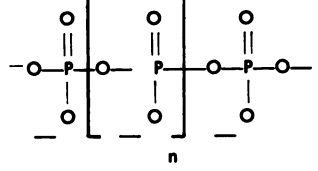
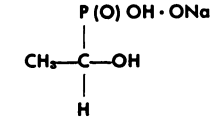
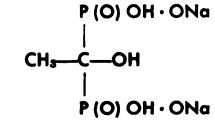
TABLE 1. STRUCTURE OF SEVERAL INORGANIC AND ORGANIC PHOSPHORUS COMPOUNDS

Compound	Structure
Inorganic phosphorus tripolyphosphate	
Inorganic polyphosphate; n = No. of phosphates	
Organic phosphorus phosphonate (1-hydroxy-ethylidene-1-mono-sodium phosphonate)	
Organic phosphorus diphosphonate (1,hydroxy-ethylidene-1, 1-disodium phosphonate) HEDSPA	

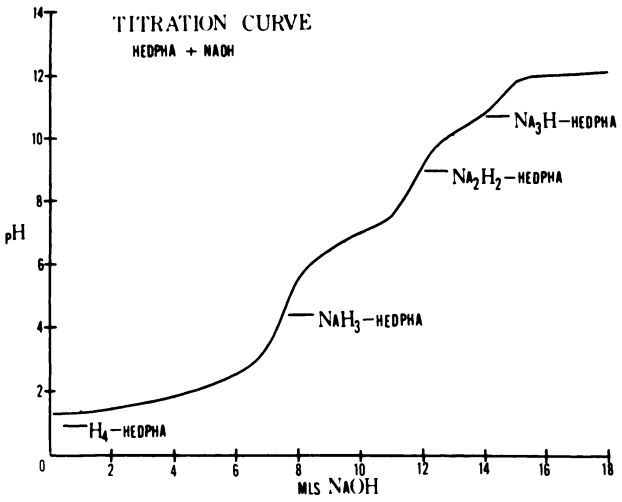


FIG. 1. Qualitative analysis of HEDPHA. Titration with NaOH resulted in three isoelectric points at pH 4.4, 8.5, and 10.7.

justing a known amount of the HEDPHA to pH 8.5. Lethality studies of intravenously administered HEDSPA were carried out in adult male (20–25 gm) Swiss-Webster mice*. The levels administered were expressed as the number of milligrams HEDSPA per kilogram body weight. Control mice were injected with the vehicle used in the formulation and no harmful effects were noted. Each group of ten mice received a single dose of HEDSPA (tail vein, pH 7.0) and were observed for 7 days after injection. If death did not occur by 7 days, the animals survived at least 60 days when the observations were terminated. The dosage levels administered during the studies aimed at determining the MLD ranged from 0.1 to 0.5 gm/kg of body weight.

The ^{99m}Tc-HEDSPA was prepared by using sterile and pyrogen-free reagents, and all glassware and other materials used were sterile and apyrogenic as outlined by current USP procedures (*15*). The ^{99m}Tc-HEDSPA was formulated by adding 0.1 mg of freshly prepared stannous chloride in 1 ml of 0.02 *N* HCl into 5 mg of HEDSPA and the solution was mixed for 4 min. Two to six ml of freshly eluted ^{99m}Tc were then added and the solution mixed for 2 min. The ^{99m}Tc-HEDSPA was then adjusted to pH 7.2–7.4 with NaOH and passed through a 0.22-micron Millipore filters into a 30-ml evacuated sterile vial.

The quality control procedures for ^{99m}Tc-HEDSPA involved the following: descending paper chromatography with 85% methanol and Sephadex Gel column chromatography.

In vivo tissue distribution of the ^{99m}Tc-HEDSPA was carried out in adult male (20–25 gm) Swiss Webster mice. One hundred microcuries of ^{99m}Tc-HEDSPA (0.1 mg) were injected via the tail vein in a total of 15 mice. At 1,3, and 6 hr after administration five mice were sacrificed by decapitation and the following organs were isolated and their content of radioactivity measured: liver, spleen, lung, kidneys, blood (7% BW), femur (total skeleton = 10% BW), skeletal muscle (43% BW), bone marrow, and thyroid. All samples were counted in a Nuclear-Chicago well scintillation counter with a 5-in. NaI(Tl) crystal. Sufficient counting time was used to keep statistical errors below 5%.

The pharmacodynamics of ^{99m}Tc-HEDSPA was followed in young (2-kg) and adult (4-kg) white New Zealand rabbits. The formulation [300 μCi ^{99m}Tc-HEDSPA (1 mg)] was injected through the marginal ear vein in three young and three adult rabbits sedated with sodium pentobarbital. Each rabbit was scanned in the lateral and posterior positions at various time periods up to 6 hr after injection with a Nuclear-Chicago Pho/Gamma III camera with a 4,000-hole parallel collimator.

RESULTS

HEDPHA is an organic acid which when titrated to pH 11.0 with NaOH shows a tribasic tritation curve with three equivalence points at pH values 4.4, 8.5, and 10.7 (Fig. 1). The curve serves to measure the quality of the reaction product and represents a simple and accurate way of determining whether HEDPHA is present in the reaction mixture. An inability to illustrate a tribasic titration curve means a poor product yield or no yield at all, and the synthetic procedure should be repeated. The results from the elemental analysis are shown in Table 2. The reported percentages of carbon, hydrogen, oxygen, and phosphorus agree closely with the theoretical values for four waters of hydration.

The quantitative analysis of HEDPHA is a straightforward stoichiometric relationship, and a typical run resulted in 1.8 gm/ml of yield or a total of 63 gm of HEDPHA. The amount of the disodium

TABLE 2. HEDPHA-(HOH)$_n$ ELEMENTAL ANALYSIS

Theoretical	n (No. of H_2O's)	C (7.77%)	H (5.28%)	O (62.99%)	P (22.41%)
$C_2H_8O_7P_2$	0	11.66	3.91	54.36	30.06
$C_2H_{10}O_8P_2$	1	10.72	4.50	57.13	27.65
$C_2H_{12}O_9P_2$	2	9.92	5.00	59.49	25.59
$C_2H_{14}O_{10}P_2$	3	9.24	5.43	61.52	23.82
$C_2H_{16}O_{11}P_2$	4	8.64	5.80	63.29	22.27

* Goffman Farms, Westboro, Mass.

salt (HEDSPA) produced from 1.8 gm of HEDPHA was 2.07 gm. This solution was brought to 207 ml with distilled water yielding a stock solution of 10 mg HEDSPA/ml. Microscopic analysis of the precipitated salt resulted in needlelike crystals (Fig. 2). The HEDSPA has proven to be extremely stable both in solution and in crystal form. This represents a definite advantage over polyphosphate which only remains chemically stable over a few months as reported by Subramanian, et al (*2*). The formulation of ^{99m}Tc-labeled HEDSPA necessitates the use of tin-reduced pertechnetate since the compound will not complex with technetium as pertechnetate. Chemical quality control of the formulation resulted in a chromatographic pattern as illustrated in Fig. 3. Reduced pertechnetate is known to have an R_f of zero in 85% methanol system (*16*), and the ^{99m}Tc-HEDSPA complex has an R_f of zero as determined by our studies. Because the two substances did not separate well, we investigated another method, Sephadex-gel chromatography. The ^{99m}Tc-HEDSPA was eluted in the 10–16-ml range (void volume) and the reduced pertechnetate was adsorbed on the column (Table 3). The percentage labeling for the formulation illustrated in Table 3 was calculated to be approximately 92%. Typical formulations currently provide percentage yields of 80–95%. In several instances the label has broken down on the column, resulting in yields of approximately 60%.

The blood clearance of 0.1 mg of ^{99m}Tc-HEDSPA in mice was quite rapid as illustrated in Table 4. By 3 hr after injection approximately 1% of the dose remained in the blood, and by 6 hr this value dropped to well below 1%. At 3 hr the cumulative urinary excretion was approximately 40% and the cumu-

TABLE 3. SEPHADEX-GEL CHROMATOGRAPHY ELUTION CHARACTERISTICS OF ^{99m}Tc-HEDSPA

Fraction	ml	Dose (%)
1	2	0
2	4	0
3	6	0
4	8	0
5	10	2
6	12	47
7	14	48
8	16	8
9	18	0
10	20	0
.	.	.
.	.	.
.	.	.
20	40	0
Total	40	92

TABLE 4. DISTRIBUTION OF ^{99m}Tc-HEDSPA IN ADULT MALE MICE

Organ	Dose per organ* (%)		
	1 hr	3 hr	6 hr
Blood	4.35	1.02	0.08
Femur	1.21	1.40	1.45
Bone (average)	49.6	55.3	54.7
Marrow	0.83	0.52	0.21
Muscle	3.14	1.54	0.45
Liver	1.24	0.87	0.63
Spleen	0.08	0.02	—
Kidneys	3.63	3.21	0.91
Lung	—	—	—
Thyroid	—	—	—
Urine (bladder)	38.7	39.2	—

* Average value for five animals each.
0.1 mg HEDSPA/animal, 5 mice/group.

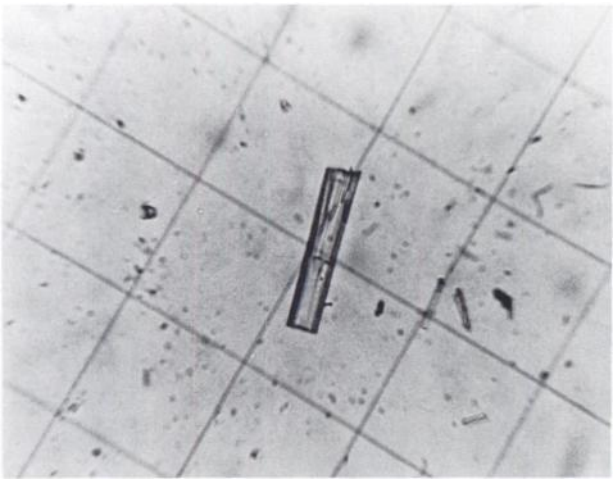


FIG. 2. Photomicrograph of HEDSPA showing needlelike crystals 430×.

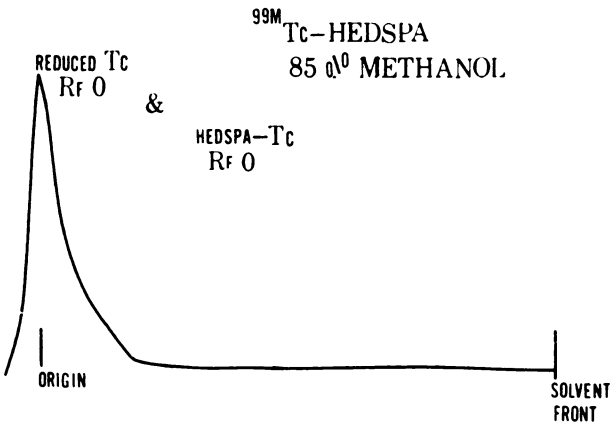


FIG. 3. Radiochromatogram scan of ^{99m}Tc-HEDSPA in 85% methanol. Both labeled HEDSPA and reduced pertechnetate remained at origin (R_f = 0).

lative skeletal uptake was 55.3%. Little activity was detected in the remaining organs with the liver concentrating less than 1% after 1 hr, the muscle less than 1%, and the bone marrow 0.52% at 3 hr. These data suggested rapid blood and soft tissue clearance with over 95% of the dose being concentrated by the skeletal system or excreted into the urine. Because of its chelate properties, a portion of the ^{99m}Tc-HEDSPA was rapidly excreted by the kidneys into the urine. This pharmacological property serves as a natural "outlet" for that fraction of the dose which fails to adsorb onto bone.

Dosimetry calculations, assuming 50% of the administered dose of 1 mCi ^{99m}Tc-HEDSPA to concentrate in bone, are outlined in Table 5. The rad doses are considerably lower for the ^{99m}Tc agent than those of ^{87m}Sr, ^{85}Sr, and ^{18}F.

The rabbit assay showed the rate of clearance from the blood of the ^{99m}Tc-labeled HEDSPA to be a function of age—the younger the animal, the faster the rate of clearance. For an adult rabbit the earliest time for obtaining a skeletal image with good resolution was 2.5–3 hr after injection (Fig. 4). However, young rabbits cleared the HEDSPA much faster, and the earliest time for good skeletal imaging was 1.5–2 hr after injection (Fig. 5). The bladder and kidneys are also evident in Figs. 4 and 5.

The lethality studies in mice resulted in a minimum lethal dose of 0.2 gm/kg body weight. No deaths occurred at the 0.1 gm/kg dose level.

TABLE 5. DOSE CALCULATIONS OF ^{99m}Tc-HEDSPA*

Organ	HEDSPA (rads/ mCi)	^{87m}Sr rads (1 mCi)	^{18}F (rads/ mCi)	^{85}Sr rads (0.1 mCi)
Whole body	0.011	0.02	0.05	1.3
Whole body + sk†	0.098	—	—	—
Whole body + sk + Bl‡	0.114	—	—	—
Skeletal	0.045	0.16	0.29	5.5
Bone marrow	0.01	0.02	0.04	—
Kidneys	0.006	—	—	—
Kidneys + Bl‖	0.012	—	—	—
Bladder§	0.49	—	—	—
Bladder¶	0.07	—	—	—
Male gonads	0.114	—	—	—
Female gonads	0.134	—	—	—

* Assume 50% of administered dose to be concentrated in skeletal system.
† Whole body dose + skeletal contribution.
‡ Whole body dose + skeletal contribution + bladder contribution.
‖ Kidney + bladder contribution.
§ 50% of dose, $T_{1/2}$ eff-6 hr—worst possible case.
¶ Bladder dose, patient voids 1 hr after administration.

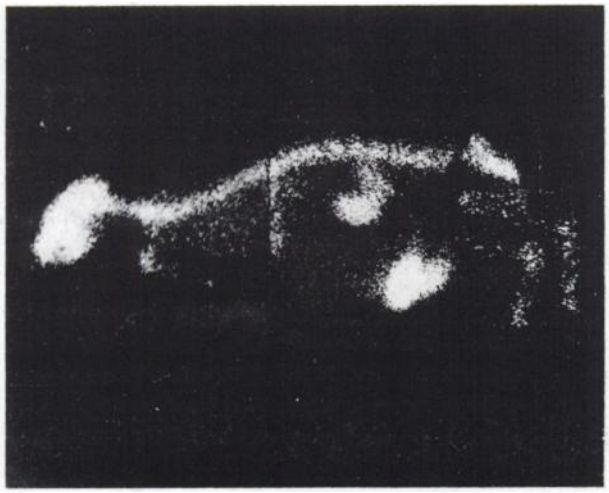


FIG. 4. Composite left lateral scintigraph of adult rabbit 2.5 hr after intravenous injection of 1 mg ^{99m}Tc-HEDSPA. Pho/Gamma III camera with 4,000-hole technetium collimator. Uptake in bone, kidneys, and bladder.

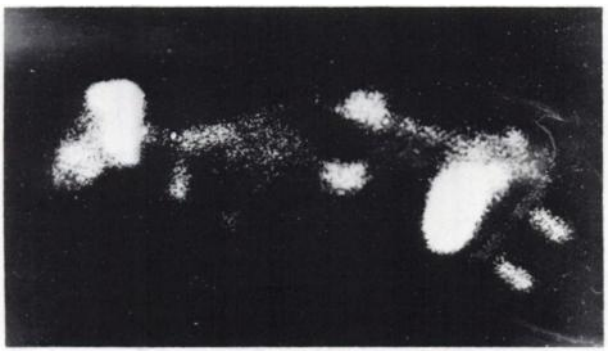


FIG. 5. Composite left lateral scintigraph of young rabbit 1.5 hr after intravenous injection of 1 mg ^{99m}Tc-HEDSPA. Pho/Gamma III camera with 4,000-hole technetium collimator. Uptake in bone, kidneys, and bladder regions. Increased uptake in rapidly growing areas of bone, skull, and epiphysial regions.

DISCUSSION

The development of ^{99m}Tc-HEDSPA is a simple "in house" procedure for providing a chemically stable skeletal localizing agent in gram amounts at a minimal cost. In addition, the ability to label HEDSPA with ^{99m}Tc provides the nuclear medicine physician with a totally new radiopharmaceutical for investigating various bone pathologies, especially in the area of pediatrics where a low rad dose is important (Table 5).

After injection, diphosphonates are "chemisorbed" onto the bone, which leads to, at most, a monomolecular layer (*17,18*). Chemisorption can be regarded as a zero-order reaction and subsequently, it is not surprising that diphosphonates have been shown to inhibit either dissolution or crystal growth of hydroxyapatite, both being a function of the amount administered (*8,19*). In 1971, King and associates investigated the dose-related effects of diphospho-

nates on bone formation in animals (*20*). They concluded that at systemic doses of 5 mg/kg/day × 28 days in dogs there resulted no toxic effects.

Based on the minimum lethal dose of 0.2 gm/kg of body weight in mice, the safety factor for a 1.0-mg dose of HEDSPA in a 70-kg human would be 1.4×10^4.

SUMMARY

This paper outlines the development of a new bone scanning agent from a combination of raw materials (PCl_3 and HOAc) to successful skeletal imaging by ^{99m}Tc-HEDSPA. Simple and accurate quality control steps are outlined for both the HEDPHA and the ^{99m}Tc-HEDSPA. A biological assay is introduced based on the earliest time for obtaining a quality bone image in young and adult rabbits after administration of 1 mg of ^{99m}Tc-HEDSPA.

The ease of synthesis of the compound, its chemical stability, and its ability to complex with ^{99m}Tc will greatly increase HEDSPA's usefulness to the nuclear medicine physician as a skeletal imaging agent.

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