## RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

# Tc-99m-Labeled Polystyrene and Cellulose Macromolecules: Agents for Gastrointestinal Scintigraphy

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**Several polystyrene resin and cellulose derivatives were evaluated for potential use as Tc-99m-labeled particulate markers for studies of gastric emptying and intestinal transit time, and for imaging segments of the gastrointestinal tract. The polyamine and quaternary ammonium polystyrene resins bound pertechnetate (Tc-99m) anions effectively; the labeling efficiency was over 95% at physiological pH values. In-vitro stability studies of Tc-99m-labeled resins in simulated gastric and intestinal fluid showed that less than 8% of the label was released after 24 hr. The commercial resins Dowex 2-X8, AG 1-X2, and Bio-Rex 9, labeled with Tc-99m, may be used as particulate markers of solid digesta in external scintigraphic studies of the gastrointestinal tract. Dowex 2-X8 showed relatively more extensive uptake of pertechnetate and greater stability in simulated gastric and intestinal fluids.**

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Preparations such as Tc-99m-tagged chicken liver, Co-57cyanocobalamine-tagged liver, and I-131$\alpha$-cellulose have been used to measure the rate of gastric emptying of solid and semisolid digesta in man and animals (*2,4*). Although these radioagents are suitable for gastric emptying studies, they have certain obvious disadvantages. For instance, the preparation of the Tc-99m-tagged chicken-liver test meal takes up to 2 hr and requires the housing of live chickens. Moreover, we believe that Tc-99m-tagged chicken liver breaks down in the intestine, making it unsuitable as a marker for intestinal transit studies. Finally, the radiation characteristics of Co-57 and I-131 are undesirable compared with those of Tc-99m.

Another radiodiagnostic agent for measuring the gastric emptying of solid digesta is free from the previously mentioned disadvantages. It was first introduced in 1975 and since then it has been tested in humans (*1,3,8–10*). This is a particulate marker (40–100 mesh) consisting of triethylenetetramine polystyrene (P-TETA) resin labeled with Tc-99m. The Tc-99mP-TETA is an easily prepared radioagent requiring less than 10 min of handling once the resin is available (*1,3,6,9*). The labeled resin then can easily be mixed with a test meal such as oatmeal and administered to the patient. Since P-TETA resin is not commercially available, we decided to investigate commercially available resins for potential use as Tc-99m-labeled particulate markers for studies of gastric emptying and intestinal transit time, as well as for imaging various segments of the GI tract (*11*). Some commercially unavailable resins and cellulose derivatives were also investigated for the same purpose.

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### MATERIAL AND METHODS

**Synthesis of polyamine-polystyrene resins.** This has been described previously (*7*). Briefly, chloromethylated "popcorn" polystyrene (*7*) (Cl 13%, 10 g) was added to 30 ml of dry pyridine. After 30 min, a tenfold molar excess of the polyamine was added slowly under constant stirring. Three hours later the temperature of the mixture was raised to 95°C and maintained there at that temperature for 5 hr. The resin was recovered and washed with pyridine, methanol, and water, then dried in a vacuum oven at 60°C for 24 hr. The following polyamines were used: ethylenediamine (EDA), diethylenetriamine (DTA), and triethylenetetramine (TETA).

**Synthesis of cellulose acetyl iminodiacetic acid.** Bromoacetylcellulose was synthesized according to a pre-

viously described procedure (*6*) (Anal: Br 19.01%). Three grams of bromoacetylcellulose and 26.53 g iminodiacetic acid, disodium salt, were mixed in dry dimethyl formamide (DMF, 400 ml). The mixture was heated to 75°C in an oil bath for 16 hr with continuous stirring. It was then poured into water and the precipitated solid was collected and washed repeatedly with water. The cellulose was then lyophilized for 24 hr to remove the water. Infrared spectrum (KBr) 1640–1600 ($\upsilon$ $COO^-$) and 1400 $cm^{-1}$. Anal: N = 1.6%, C = 37.83%, H = 5.68%, Br = 0.64%.

**Uptake of sodium pertechnetate (Tc-99m) by the polystyrene resins.** Polystyrene resin (*7*) (0.5 g) was placed in a 150-ml beaker with buffer (75 ml). Then an aliquot of sodium pertechnetate* in saline (3–10 mCi) was added. The mixture was stirred at room temperature and at predetermined time intervals 1-ml samples were withdrawn using a pipet with a glass-wool filter on its tip. The samples were counted in a gamma well counter with multichannel analyzer† to determine the remaining, unbound pertechnetate. Three different buffers were used: 0.2 *M* potassium chloride, pH 1.5; 0.2 *M* sodium acetate, pH 5.0; and 0.2 *M* sodium borate, pH 8.0.

**Uptake of sodium pertechnetate (Tc-99m) by cellulose derivatives.** Ten milligrams of the cellulose derivatives were mixed with buffer (0.2 ml) containing sodium pertechnetate (0.8–1 mCi). Aliquots (10 $\mu$l) were withdrawn at predetermined time intervals. The percent uptake of the sodium pertechnetate by the cellulose derivatives was determined by instant thin-layer chromatography (ITLC-SG) using 85% v/v methanol. The same three buffers were used.

The same procedure was used to test the uptake of pertechnetate by the stannous pyrophosphate complex of cellulose acetyl iminodiacetic acid, and of polystyrene iminodiacetic acid.

**Preparation of Tc-99m-labeled resins.** Polystyrene resin beads (1 g) were placed in a 20-ml beaker with distilled water (5 ml). Sodium pertechnetate in saline (3–30 mCi) was added. The mixture was stirred for 15 min and the labeled resin recovered by filtration. The resin was washed with two 50-ml aliquots of distilled water, then counted in an ionization chamber‡ to determine the bound activity. A similar procedure was used for the preparation of certain Tc-99m-labeled cellulose derivatives.

Note that some of the resins have the capacity of binding more than 30 mCi of $Na^{99m}TcO_4$ per gram of resin: so by using an excess of pertechnetate specific activities of 30 mCi/g or higher can be achieved.

The following resins were used: P-TETA, Dowex 2-X8, AGl-X2, Bio-Rex 9.

**Preparation of polystyrene-iminodiacetic acid/$SnCl_2$ complexes.** One gram of polystyrene resin (Chelex-100,§ 70–78% moisture by weight) was brought to a beaker with 10 ml of 0.1% w/v $SnCl_2 \cdot 2H_2O$ in 0.025% w/v acetic acid solution. The mixture was lyophilized for 24 hr to remove the water. Approximately 0.25 g of the polystyrene resin/$SnCl_2 \cdot 2H_2O$ complex was recovered (0.012 mmol/g of Chelex-100). The Dowex 50-X8/Sn complex was prepared using the same method (0.012 mmoles per gram of Dowex 50-X8).

**Preparation of the polymer stannous pyrophosphate complexes.** Cellulose acetyl iminodiacetic acid (10 mg) was mixed with 1/5 of contents of a stannous pyrophosphate kit* (2.4 mg of sodium pyrophosphate and 0.68 mg of stannous chloride) reconstituted with saline. The mixture was lyophilized for 5 hr. The polystyrene iminodiacetic acid (Chelex-100) complex was prepared in the same manner.¶

**Stability of Tc-99m-labeled resins in simulated gastric juice and intestinal fluid.** Simulated gastric juice contained 2 g sodium chloride, 3.2 g of pepsin, 7 ml of concentrated hydrochloric acid, all dissolved in sufficient distilled water to make 1000 ml. The pH was about 1.2. Simulated intestinal fluid contained 6.8 g of monobasic potassium phosphate, 190 ml of 0.2 *N* sodium hydroxide and 10.0 g of pancreatin, all dissolved in sufficient distilled water to make 1000 ml. The pH of the solution was adjusted to 7.5.

The Tc-99m-labeled polystyrene resin (1 g, 30 mCi) was placed in a beaker containing 50 ml of simulated gastric juice (pH 1.2) or intestinal fluid (pH 7.5). The mixture was stirred and 1-ml samples were taken from the solution at predetermined time intervals using a pipette with a glass-wool filter tip. The samples were counted in a well counter with multichannel analyzer.†

## RESULTS AND DISCUSSION

The three polyamines, ethylenediamine (EDA), diethylenetriamine (DTA) and triethylenetetramine (TETA) were attached to chloromethylated "popcorn" polystyrene (13% Cl). The microanalysis results indicated that the chlorine was displaced completely by the polyamine. The latter was attached at more than one point on the polymer matrix; this was deduced from the microanalysis results for chlorine and nitrogen.

The attachment of the iminodiacetic moiety to the cellulose was achieved by activating the cellulose with bromoacetylbromide, ($CH_2BrCOBr$). The bromacetylated cellulose was then reacted with iminodiacetic acid, $HN(CH_2CO\ OH)_2$. The microanalysis results indicated that approximately one iminodiacetic acid moiety was attached to the cellulose for every four glucose units.

The uptake of pertechnetate anions from the crosslinked polystyrene and its chloromethylated derivative was less than 10% (Table 1). This was attributed to the fact that both polymers were hydrophobic and lacked any hydrophilic groups. The introduction of a hydroxyl group onto the polymeric matrix** improved the hy-

**TABLE 1. PERCENT BINDING AND RELEASE OF $^{99m}TcO_4^-$ ANIONS BY POLYSTYRENE AND CELLULOSE MACROMOLECULES**

| Polymer | Particle size (mesh) | 3 mCi 60 min pH 1.5 | 5.0 | 8.0 | In vitro % release gastric fl. | In vitro % release intest. fl. |
|---|---|---|---|---|---|---|
| P-Ø* | | | | | | |
| Polystyrene | 40–100 | <10 | <10 | <10 | — | — |
| P-Ø-$CH_2Cl$ | | | | | | |
| Chloromethylated polystyrene | 40–100 | <10 | <10 | <10 | — | — |
| P-Ø-$CH_2OH$ | | | | | | |
| Hydroxymethylated polystyrene | 40–100 | 50 | 57 | 72 | — | — |
| P-Ø-EDA | | | | | | |
| P-EDA | 40–100 | 94.5–99.8† $\bar{x}$ = 97.1 | 99.6–99.9 $\bar{x}$ = 99.8 | 97.8–99.2 $\bar{x}$ = 98.5 | — | — |
| P-Ø-DTA | | | | | | |
| P-DTA | 40–100 | 96.3–99.6 $\bar{x}$ = 97.9 | 97.3–99.6 $\bar{x}$ = 98.7 | 97.3–99.4 $\bar{x}$ = 98.2 | — | — |
| P-Ø-TETA | | | | | | |
| P-TETA | 40–100 | 92.4–98.4 $\bar{x}$ = 95.2 | 98.8–99.0 $\bar{x}$ = 98.9 | 96.0–99.7 $\bar{x}$ = 98.1 | 2.9–3.1 $\bar{x}$ = 3.0 | 6.3–8.1 $\bar{x}$ = 7.2 |
| P-Ø-$CH_2N(CH_2CH_2OH)(CH_3)_2$ | | | | | | |
| Dowex 2-X8 | 50–100 | 97.1–99.5 $\bar{x}$ = 98.5 | 99.3–99.9 $\bar{x}$ = 99.7 | 99.8–99.9 $\bar{x}$ = 99.9 | 0.7–1.3 $\bar{x}$ = 1.1 | 0.6–1.1 $\bar{x}$ = 0.8 |
| P-Ø-$CH_2{}^+N(CH_3)_3$ | | | | | | |
| AGI-X2 | 200–400 | 96.0–96.4 $\bar{x}$ = 96.3 | 98.0–99.1 $\bar{x}$ = 98.6 | 99.6–99.7 $\bar{x}$ = 99.7 | 3.9–6.7 $\bar{x}$ = 4.6 | 1.4–3.7 $\bar{x}$ = 2.6 |
| P-Ø-$CH_2$-CH($CH_2$-$N^+(CH_3)_2$)-$CH_2$-$CH_2$ | | | | | | |
| Bio-Rex 9 | 200–400 | 94.8–95.9 $\bar{x}$ = 95.3 | 99.3–99.4 $\bar{x}$ = 99.3 | 99.1–99.8 $\bar{x}$ = 99.5 | 3.3–5.0 $\bar{x}$ = 4.4 | 3.5–4.2 $\bar{x}$ = 3.8 |
| P-Ø-$CH_2NH(CH_2COOH)_2$ | | | | | | |
| Chelex-100 | 100–200 | 72.6–88.3 $\bar{x}$ = 78.6 | 21.1–24.8 $\bar{x}$ = 23.3 | 5.6–6.9 $\bar{x}$ = 6.4 | — | — |
| Chelex-100 + $SnCl_2$ $2H_2O$ | 100–200 | 85.7–96.7 $\bar{x}$ = 92.2 | 86.2–92.5 $\bar{x}$ = 88.5 | 87.0–93.6 $\bar{x}$ = 90.1 | — | — |
| Chelex-100 Sn-pyrophosphate | 100–200 | 94.4–95.7 $\bar{x}$ = 95.3 | 98.0–98.2 $\bar{x}$ = 98.1 | 97.4–98.8 $\bar{x}$ = 98.3 | | |
| P-Ø-$SO_3H$ | | | | | | |
| Dowex 50W-X8 | 100–200 | <5 | <5 | <5 | — | — |
| Dowex 50W-X8 $SnCl_2$ $2H_2O$ | 100–200 | 91.0–95.1 $\bar{x}$ = 93.1 | 65.3–71.5 $\bar{x}$ = 68.0 | 65.7–76.1 $\bar{x}$ = 71.1 | — | — |
| Cellulose-AIDA | 20 | 0.9–1.2 $\bar{x}$ = 1.0 | 0.9–2.4 $\bar{x}$ = 1.4 | 0.5–1.0 $\bar{x}$ = 0.7 | — | — |
| Cellulose-AIDA Sn-pyrophosphate | 20 | 95.6–97.0 $\bar{x}$ = 96.2 | 92.3–96.3 $\bar{x}$ = 94.9 | 92.9–96.6 $\bar{x}$ = 95.3 | — | — |
| Cellulose-DEAE | 20 | 0.8–3.3 $\bar{x}$ = 2.1 | 5.6–12.4 $\bar{x}$ = 9.4 | 3.7–9.2 $\bar{x}$ = 6.2 | — | — |

* Ø stands for the aromatic ring of the polystyrene resin.

† Existence of the mean and range denotes the result of three replicates.

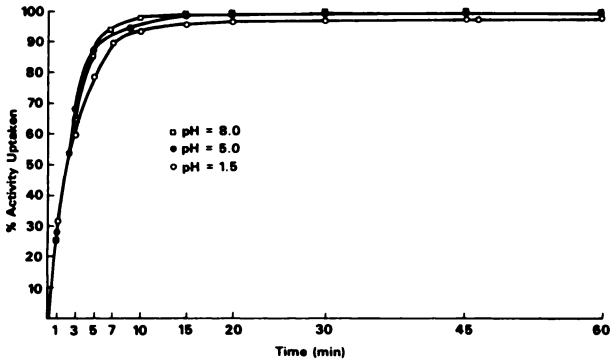


**FIG. 1.** Uptake of pertechnetate (Tc-99m) anion from aqueous solutions by polystyrene resin, Dowex 2-X8, at various pH values.

drophilic character of the polymer and increased uptake of pertechnetate anions, probably due to adsorption. Attachment of the polyamines (EDA, DTA, TETA) to chloromethylated polystyrene increased the hydrophilicity of the polymer and converted it into a weak anion-exchange and chelating resin (*7*). The introduction of the polyamine on the resin dramatically increased the rate of uptake of pertechnetate anions, so that in the first 10 min over 95% of the free pertechnetate was bound to the polyamine resins. As is shown in Table 1, the pH of the solution had a slight effect on the uptake (*9*). Incubation of the polystyrene polyamine resins P-EDA, P-DTA, and P-TETA with pertechnetate anions (10 and 30 mCi) at pH 5.0 resulted in extensive uptake of the anions by the resins: over 95% of the anions were bound. Similarly, the commercial resin, Dowex 2-X8,§ which is a strong anion exchanger bearing quaternary ammonium moieties, ($-N^+(CH_3)_2CH_2CH_2OH$), bound pertechnetate extensively (Fig. 1). Half a gram of the resin, incubated with 3 and 10 mCi of $TcO_4^-$ at pH 5, bound approximately 99% of the activity in 10 to 15 minutes. Other commercial resins bearing quaternary ammonium moieties, AGl-X2§ and Bio-Rex 9,§ also bound $TcO_4^-$ extensively at pH 5.0 (Table 1). Similarly, the binding at pH 8.0 for Dowex 2-X8, AGl-X2, and Bio-Rex 9 was over 99%, while the binding at pH 1.5 was 97.5, 96.2, and 95.3%, respectively.

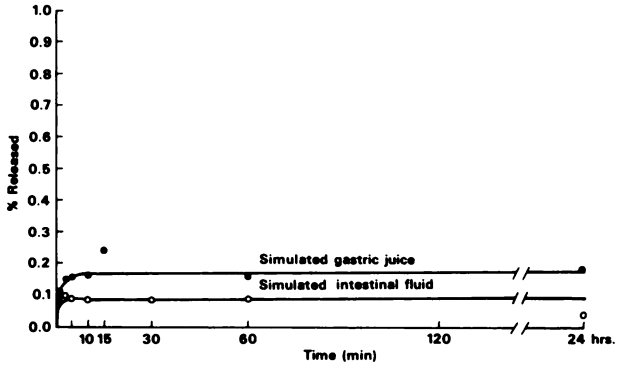


**FIG. 2.** Percent of radioactivity lost from Tc-99m-labeled Dowex 2-X8 in simulated gastric juice, pH 1.2 (-●-●-), and in simulated intestinal fluid, pH 7.5 (-O-O-).

Chelex-100,§ which bears iminodiacetic acid moieties, bound $TcO_4^-$ less efficiently (Table 1). However, in the presence of stannous chloride the uptake of $TcO_4^-$ increased considerably. The stannous pyrophosphate complex of Chelex-100 bound over 95% of the activity at pH 1.5, 5, and 8.

Polystyrene resins bearing sulfonic acid ($-SO_3H$) moieties (Dowex 50-X8) did not bind $TcO_4^-$, but the Dowex 50-X8/$Sn^{++}$ complex showed increased uptake of $TcO_4^-$ at all pH values tested.

Diethyl amino ethyl cellulose (cellulose-DEAE) at pH 1.5 bound 2.1%, while at pH 8.0 it bound 6.2%. Cellulose-acetyl-iminodiacetic acid (cellulose AIDA) bound 1.0% at pH 1.5 and 0.74% at pH 8 (Table 1). The cellulose-AIDA/Sn pyrophosphate bound 96.2% at pH 1.5, while at pH 5 and 8 it bound 94.9 and 95.3%, respectively. Since cellulose-AIDA was available in milligram quantities, a different procedure was used to test the uptake of $TcO_4^-$ by the cellulose derivatives.

The stability of Tc-99m-labeled P-TETA, Dowex 2-X8, AGl-X2, and Bio-Rex 9 were tested in vitro in simulated gastric juice and intestinal fluid USP. Tc-99m-labeled P-TETA (3 mCi/g) lost ~3% of the label in simulated gastric juice in 24 hr and 7% in simulated intestinal fluid (Table 1). After 24 hr the rest of the resin carriers released less than 5% of the label in both simulated gastric and intestinal fluid (Table 1). Figure 2 shows the release of the label from the Tc-99m-labeled Dowex 2-X8 in simulated gastrointestinal fluids.

In conclusion, commercial resins bearing strong anion-exchange functions such as $-N^+(CH_3)_2$-$CH_2CH_2OH$ and $-N^+(CH_3)_3$ (Dowex 2-X8, AGl-X2) can easily be labeled with Tc-99m and may be used as particulate markers in studies for determination of gastric emptying and intestinal transit times, as well as for imaging segments of the gastrointestinal tract. Dowex 2-X8 was superior to AGl-X2 and Bio-Rex 9. Tc-99m-labeled Dowex 2-X8 was more stable in simulated gastrointestinal fluids, and the unlabeled resin showed more extensive uptake of pertechnetate than AGl-X2 and Bio-Rex-9.

## FOOTNOTES

* Mallinckrodt Diagnostics, Mallinckrodt, Inc., St. Louis, MO.

† Canberra MCA, Canberra Industries, Inc., Model Omega-1 MCA, Meriden, CT.

‡ Radx, Assayer 1, Houston, TX.

§ Bio Rad Laboratories, Richmond, CA.

¶ Personal communication with Dennis P. Swanson, University of Michigan.

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REFERENCES

1. DOMSTAD PA, KIM EE, COUPAL JJ, et al: Biologic gastric emptying time in diabetic patients using Tc-99m-labeled resin oatmeal with and without metoclopramide. *J Nucl Med* 21: 1098-1100, 1980
2. HINDER RA, KELLY KA: Canine gastric emptying of solid and liquids. *Am J Physiol* 233 (Endocrinol. Metab. Gastrointest. Physiol. 2):E335-E340, 1977
3. KIM EE, CHOY YC, DOMSTAD PA, BEIHN RM, et al: Biologic gastric emptying time using Tc-99m TETA polystyrene resin in various clinical conditions. *Eur J Nucl Med* 6:155-158, 1981
4. MALAGELADA JR, CARTER SE, BROWN ML, et al: Radiolabeled fiber. A physiologic marker for gastric emptying and intestinal transit of solids. *Dig Dis Sci* 25:81-87, 1980
5. MEYER JH, MACGREGOR MB, GUELLER R, et al: $^{99m}$Tc-tagged chicken liver as a marker of solid food in the human stomach. *Dig Dis* 21:296-304, 1976
6. STO T, MORI T, TOSA T, et al: Studies on immobilized enzymes. IX. Preparation and properties of aminocylase covalently attached to halogenoacetylcelluloses. *Arch Biochem Biophys* 147:788-796, 1971
7. SHAMBHU MB, THEODORAKIS MC, DIGENIS GA: Polystyrene resins with immobilized polyamines: Preparation, characterization and ability to bind Cu (II) ions. *J Polym Sci Polym Chem Ed* 15:525-531, 1977
8. THEODORAKIS MC, BEIHN RM, DIGENIS GA, et al: A new radiodiagnostic agent to evaluate gastric emptying time. *J Nucl Med* 16:575, 1975 (abst)
9. THEODORAKIS MC, DIGENIS GA, BEIHN RM, et al: Rate and pattern of gastric emptying in humans using $^{99m}$Tc-labeled triethylenetetraamine-polystyrene resin. *J Pharm Sci* 69:568-571, 1980
10. THEODORAKIS MC, BEIHN RM, DIGENIS GA: $^{99m}$Tc-labeled triethylenetetraamine-polystyrene: An agent for imaging segments of the gastrointestinal tract in humans. *Int J Biol Nucl Med* 7:374-377, 1980
11. THEODORAKIS MC, WHITLOCK TW, BUSS SL: $^{99m}$Tc-labeled resins for gastrointestinal scintigraphy. Second International Symposium on Radiopharmacology, Chicago, Illinois, 1981, p 85 (abst)

## Announcement of Berson-Yalow Award

The Society of Nuclear Medicine invites manuscripts for consideration for the Fifth Annual Berson-Yalow Award. Work will be judged on originality and contribution to the fields of basic or clinical radioassay. The manuscript will be presented at the 30th Annual Meeting of the Society of Nuclear Medicine in St. Louis, MO, June 7-10, 1983, and a suitably engraved plaque will be awarded to the authors by the Education and Research Foundation of the Society of Nuclear Medicine.

The manuscript should be approximately ten pages in length (typed, double-spaced). A letter requesting consideration for the award, including the author's full mailing address and telephone number, should accompany the manuscript. Original manuscript and eight copies must be received by January 14, 1983 at the Society of Nuclear Medicine office, 475 Park Ave. So., New York, NY 10016, Attn: Mr. Dennis L. Park.

**Deadline for receipt of manuscripts: January 14, 1983.**

## Announcement of the Paul C. Aebersold Award for Outstanding Achievement in Basic Science Applied to Nuclear Medicine—1983

Nominations are invited for this award, which commemorates the contributions of Dr. Paul Clarence Aebersold to the applications of nuclear physics to nuclear medicine and radiation biology, and his contributions to the Society of Nuclear Medicine. Dr. Aebersold contributed greatly to the emergence of nuclear medicine as a discipline by his energetic leadership in the provision of cyclotron-generated and reactor-produced radionuclides, and by his numerous publications and lectures.

In giving this award, the Society thus symbolically signifies its appreciation of the warm and vital person who became our first Honorary Member and whose enthusiastic encouragement and support contributed importantly to the formation and success of the Society of Nuclear Medicine.

Nominations should be supported by the curriculum vitae of the nominee and at least two letters supporting the nomination. These letters should describe briefly the contributions in basic science for which the nominee is proposed. The nominee need not be a member of the Society of Nuclear Medicine.

Please submit nominations and supporting documents to:

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**Deadline for nominations: December 31, 1982.**