High Myocardial Accumulation of Radioiodinated Digoxin Derivative: A Possible Na,K-ATPase Imaging Agent

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In view of the high binding ability of cardiac glycosides to the myocardial Na,K-ATPase, radioiodinated digoxin derivatives were surveyed as candidates for myocardial imaging, with particular emphasis on the noninvasive monitoring of cardiac glycoside therapy. Among the radioiodinated digoxin derivatives surveyed, ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) showed the highest accumulation in the myocardium and similar binding ability to Na,K-ATPase as digoxin itself against ouabain displacement, as indicated by in vivo and in vitro studies. Based on these results, ^{123}I labeling of digoxin-histamine(bis(O-carboxymethyloxime)) and imaging in a dog demonstrated uptake in the myocardium.

J Nucl Med 1992; 33:545–549

Na,K-ATPase spans the plasma membrane (*1*) and is of major importance in myocardial function (*2*). Its content is known to be changed by some physiological or pathophysiological states such as aging (*3*), cardiomyopathy, diabetes mellitus, hypothyroidism, hyperthyroidism (*4*) and ischemia (*5*).

Digoxin is a cardiac glycoside that has a high binding ability to Na,K-ATPase and plays an essential role in the medical treatment of heart failure (*6*). Basic tracer studies using tritiated digoxin show its selective accumulation in the myocardium, with left ventricular myocardium to serum level ratios being in the range of 20 to 60 in human subjects (*7*). Thus, if digoxin could be labeled with a radionuclide suitable for in vivo use, it may be useful for myocardial Na,K-ATPase imaging.

Recently, ^{123}I has become commercially available and its superior characteristics (half-life = 13 hr, pure gamma = 150 keV and ease of radiolabeling) have encouraged investigation into uses of radioiodinated ligands. Digoxin itself has no radioiodination site but some of its derivatives with histamine or tyrosine residues have been used for radioiodination (*8*). These compounds, labeled with ^{125}I, are routinely used in digoxin radioimmunoassay so that the immunoreactivity of these radioiodinated digoxin derivatives to anti-digoxin antibodies has already been clarified. However, their myocardial accumulation has not been evaluated.

In this study, the myocardial accumulation of these ^{125}I-labeled digoxin derivatives was investigated. Based on those results, ^{123}I labeling of a digoxin derivative and a canine heart imaging study was performed.

MATERIALS AND METHODS

Iodine-125-digoxin-iodohistamine(bis(O-carboxymethyloxime)), ^{125}I-digoxigenin-iodohistamine(3-oxime), and ^{125}I-digoxigenin-iodohistamine(3-ester) prepared as for radioimmunoassay tracers, were obtained from Amersham Japan Co. Ltd., Shionogi Co. Ltd. and Dainabbot Radioisotopes Lab. Ltd. (Japan), respectively (their structures are shown in Fig. 1). The specific radioactivities of these compounds were similar to that of commercially available ^{125}I-NaI (no-carrier added, 629 GBq/mg of I). Tritium-3-digoxin was purchased from New England Nuclear Co. Ltd. (555–925 GBq/mmol).

Preparation of ^{123}I-digoxin-iodohistamine-(bis(O-carboxymethyloxime))

A solution of digoxin-histamine(bis(O-carboxymethyloxime)) [a gift from Amersham International, 33.3 μg in 33.3 μl of phosphate buffer (0.01 *M*, pH 8, containing 50% ethanol], ^{123}I-NaI (100 μl, 74 MBq, a gift from Nihon Medi-Physics Co. Ltd., Japan) and chloramine-T (2 μmol in 40 μl of phosphate buffer) was mixed and the reaction was allowed to proceed for 30 min at room temperature. The reaction was stopped by the addition of sodium metabisulfite (3.68 mg/0.1 ml phosphate buffer), following which ^{123}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) was purified with reversed-phase high-performance liquid chromatography. Elution conditions were as follows:

Column: Ultrosphere ODS, 4.6 × 250 mm column
Solvent A: 20 ml of 0.5 *M* phosphate buffer (pH 8.0) in 1,000 ml of methanol
Solvent B: Methanol
Gradient: solvent B = 50 % to 100 % over 30 min.
Flow rate: 1 ml/min

Received Mar. 26, 1991; revision accepted Nov. 20, 1991.
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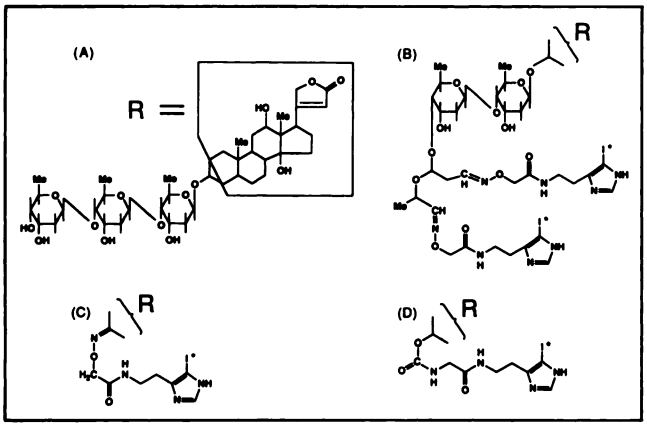

FIGURE 1. Structures of the radioiodinated digoxin derivatives used in this study. (A) digoxin, (B) ^{125}I-digoxin-iodohistamine-(bis(O-carboxymethyloxime)), (C) ^{125}I-digoxigenin-iodohistamine (3-oxime) and (D) ^{125}I-digoxigenin-iodohistamine (3-ester).

Biodistribution Studies

Male guinea pigs weighing 250 g were injected with radioactive tracers (2.5 ml, 16.3–43 KBq) via the femoral vein and then killed by cervical decapitation at 15 and 60 min after injection. The radioactivity of ^{125}I was measured with an autogamma counter (ARC300, Aloka, Japan). Samples containing ^{3}H were solubilized with 1 ml of NCS tissue solubilizer (Amersham Canada Ltd, Canada) mixed with 8 ml of toluene scintillator (containing 18 m*M* of DPO and 0.137 m*M* of POPOP) and radioactivity measured with a liquid scintillation counter (TRI-CARB 1900CA, Packard, USA). For ^{3}H-digoxin, plasma radioactivity was measured instead of the blood. Tissue accumulation was calculated as the percent injected dose/g tissue.

For the in vivo inhibition studies, 0.25 mg/kg of ouabain was injected together with the radioactive tracer, following which the radioactivity distribution was studied at 60 min after injection. As a control, animals were injected with a similar volume of saline plus the radioactive tracer.

In Vitro Na,K-ATPase Binding Studies

With the crude Na,K-ATPase fraction separated from guinea pig kidney cortex (*9*), the effect of ouabain on the binding of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) was studied according to the method described previously (*10*). In brief, the kidneys of four guinea pigs were sliced in half lengthwise and the cortex was dissected off. The combined cortices were divided into three equal portions of about 6 g each. Each portion was homogenized in a homogenizer with a Teflon pestle. Homogenization was at 1000–1500 rpm with 34 ml of a solution of 0.25 *M* sucrose, 0.02 *M* NaCl, 5 m*M* H_2Na_2EDTA, 1 m*M* $MgCl_2$, and 10 m*M* imidazole. The homogenate was centrifuged at 300 × g for 10 min and the supernatant was saved. The same homogenization procedure was repeated on the precipitate twice. The three supernatants were combined and diluted to 320 ml with the homogenizing solution, mixed and centrifuged at 35000 × g for 30 min. All subsequent centrifugations were at this volume, force, and duration also. The sediment was homogenized again in the same volume of a solution of 0.25 *M* sucrose, 2 m*M* H_2Na_2EDTA, 0.1 m*M* $MgCl_2$, 4 m*M* imidazole and 0.02% (w/v) sodium heparin. After centrifugation the sediment consisted of a translucent light yellow upper layer of membranes and an opaque brown lower layer of mitochondria. The supernatant was discarded by aspiration to avoid losing any of the loose upper layer. About 2 ml of a solution of 2 *M* urea, 6 m*M* $(NH_4)_2SO_4$, 2 m*M* H_2Na_2EDTA, 0.1 m*M* $MgCl_2$ and 12 m*M* imidazole was added to each precipitate. This supernatant was decanted and saved. The supernatant was diluted to 60 ml with more of the urea solution, mixed and allowed to stand overnight. The next day 100 ml of a solution of 15 m*M* NaCl, 1 m*M* H_2Na_2EDTA, and 3 m*M* imidazole was added and the suspension was centrifuged. The upper half of each precipitate was suspended, combined, and diluted to 10 ml in a solution of 10 m*M* imidazole, 0.1 m*M* H_4EDTA, and 5 m*M* HCl. The standard reaction mixture consisted of the enzyme solution, ouabain solution (0–10^{-5} *M*), 2 m*M* ATP (Tris salt), 5 m*M* $MgCl_2$, 1 m*M* EDTA-Tris, 100 m*M* NaCl, 50 m*M* Tris-HCl (pH 7.4) and ^{3}H-digoxin (925 Bq) or solution in a total volume of 2 ml. The reaction was started by the addition of the labeled compound and incubated for 1.5 min at 37 °C. The enzyme then was added to the reaction mixture and incubated for 3 min at 37°C. The reaction mixture was filtered with glass fiber filter (Toyo Roshi, Ltd, Japan). The filter was placed into a vial and then 1 ml of NCS tissue solubilizer and 8 ml of toluene scintillator (18 m*M* DPO and 0.137 m*M* POPOP) were added to the vial. The radioactivity bound to the filter was measured with a liquid scintillation counter (TRI-CARB 1900CA, Packard). Similarly, ^{125}I-digoxin derivative (1700 Bq) was used following the above procedure instead of ^{3}H-digoxin. The filter was placed into a plastic tube and the radioactivity bound to the filter was measured with a gamma counter (ARC300, Aloka Co Ltd., Japan).

Canine Scintigraphic Study

A male mongrel dog weighing 20 kg was anesthetized with pentobarbital and placed in the lateral decubitus position. Saline infusion was performed via a foreleg vein to keep the administration route open. Through the infusion tube, 23.5 MBq of ^{123}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) was injected and the radioactivity distribution was detected with a scintillation camera having an ultra-high resolution collimator (LFOV, Shimadzu Co. Ltd., Japan). The dog was then killed by the bolus injection of a lethal dose (100 mg/kg) of pentobarbital at 70 min after injection of the tracer. After the collection of blood, samples of heart, lung and liver tissues (each weighing several grams) were removed and weighed, and their radioactivity levels were measured to calculate the relative myocardial accumulation. The radioactivity of ^{123}I was measured with an autogamma counter (ARC-300, Aloka, Japan).

RESULTS

Figure 2A shows the biodistribution of ^{3}H-digoxin in guinea pigs. Tritium-labeled digoxin showed a high level of accumulation in the myocardium (the heart-to-blood ratio was 2.92 ± 0.24, at 60 min after injection). Figures 2B–D show the biodistribution of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)), ^{125}I-digoxin-iodohistamine(3-oxime), and ^{125}I-digoxigenin-iodohistamine(3-ester), respectively. Among the tested digoxin derivatives, ^{125}I-digoxin-iodohistamine (bis(O-carboxymethyloxime)) with a sugar residue, showed the highest myocardial accumulation to that of ^{3}H-digoxin. Moreover, the in vivo myocardial accumulation of ^{125}I-digoxin-iodohistamine (bis(O-carboxymethyloxime)) was inhibited by the injec-

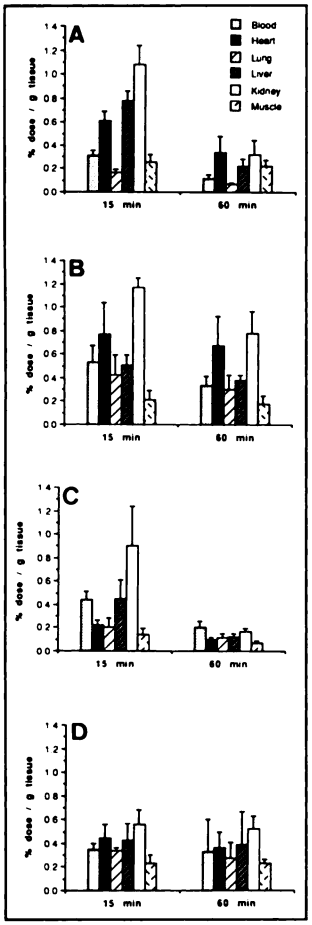

FIGURE 2. Biodistribution of digoxin and its radioiodinated derivatives in guinea pigs. Average of four animals (1 s.d.) (A) ^{3}H-digoxin, (B) ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)), (C) ^{125}I-digoxigenin-iodohistamine(3-oxime), and (D) ^{125}I-digoxigenin-iodohistamine(3-ester).

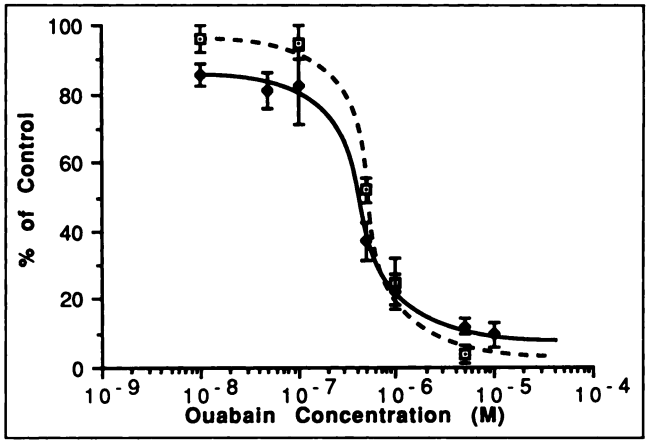

FIGURE 4. Inhibition of ^{3}H-digoxin or ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) binding to guinea pig kidney Na,K-ATPase by ouabain in vitro (average and 1 s.d. of four experiments). (□: ^{3}H-digoxin, ◆: ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)).

tion of ouabain (Fig. 3), and this inhibition was specific to the myocardium with no decrease being found in other tissues.

Based on the accumulation of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) in the myocardium, its in vitro binding to Na,K-ATPase was studied in comparison with that of ^{3}H-digoxin (Fig. 4). Studies with ouabain showed a similar IC_{50} value for the binding of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) and ^{3}H-digoxin to Na,K-ATPase.

Based on these plausible results, ^{123}I labeling studies of digoxin-iodohistamine(bis(O-carboxymethyloxime)) was performed; labeling was easily completed by the conventional chloramine-T method with a labeling efficiency of 39.0 % (36%–44%, average of three experiments). Iodine-123-digoxin-iodohistamine(bis(O-carboxymethyloxime)) was then purified by reversed-phase HPLC (Fig. 5). The fraction showing the same retention time as the ^{125}I-labeled compound (retention time = 17 min) was collected, the solvent was evaporated, and the residue was dissolved in saline. The solution was filtered through a 0.22 μm pore filter to make an injectate.

In one dog study, ^{123}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) showed high accumulation in the myocardium from 10–60 min after injection (Fig. 6). The accumulation rate relative to the surrounding organs at 70 min after injection is shown in Table 1. Very high heart-to-blood and heart-to-lung ratios were obtained.

DISCUSSION

There are species differences in both the ventricular Na,K-ATPase content (*11*) and the stability of the digoxin-ATPase complex (*12*). In addition, it is well known that there are species differences in the pharmacological activity of digoxin, since it is very low in mice or rats, moderate in guinea pigs, and very high in dogs or human beings

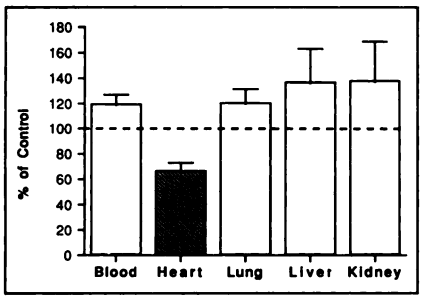

FIGURE 3. In vivo inhibition of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) accumulation in guinea pigs by ouabain. (Average and 1 s.d.).

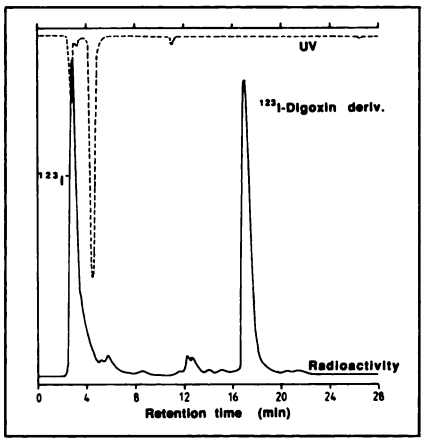

FIGURE 5. A typical elution profile of the labeling solution of ^{123}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)). Elution conditions are described in the text.

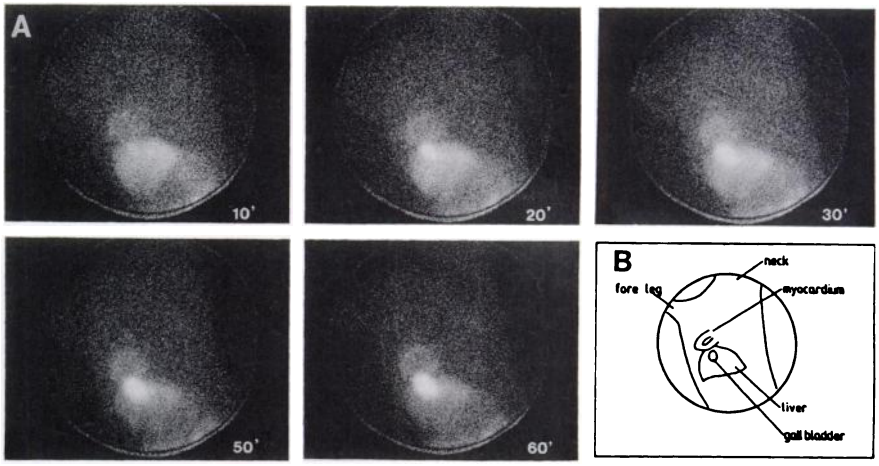

FIGURE 6. (A) Scintigraphy with ^{123}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) in a mongrel dog. Images obtained 10, 20, 30, 50 and 60 min after intravenous injection of the labeled compound. (B) Schematic explanation of the scintigram.

(*13*). These facts indicate that experimental animal models for the survey of Na,K-ATPase-seeking radioligands should be chosen carefully, and that the choice should probably be based on their sensitivity to cardiac glycosides.

In the present biodistribution studies of ^{3}H-digoxin, heart to blood ratio of 2.92 at 60 min after injection was obtained in guinea pigs. In our preliminary studies, no remarkable myocardial accumulation was found in mice (data not shown). It was interesting that this difference corresponded to the difference in drug effect of digoxin between guinea pigs and mice.

Also with the radioiodinated digoxin derivative possessing a sugar residue, ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime), a selective myocardial accumulation was found. On the other hand, the iodinated digoxin derivatives with no sugar residues (^{125}I-digoixigenin-iodohistamine(3-oxime) and ^{125}I-digoxigenin-iodohistamine(3-ester)) showed low myocardial selectivity, that is, low heart-to-blood ratios. It has been reported that the association rate constant of the enzyme-cardiac glycoside complex is dependent on the nature of the steroid moiety (*14*), while the dissociation rate is dependent on the nature of the sugar residues (*15*); the binding of cardenolide aglycones to Na,K-ATPase is reversible, but the binding of their glycosides is irreversible (*16*). Thus, steroid as well as sugar residues might play an important role in the myocardial accumulation of radioiodinated digoxin derivatives.

The high myocardial accumulation shown by ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) led us to study its binding characteristics to Na,K-ATPase. With respect to guinea pig kidney Na,K-ATPase, the IC_{50} of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) in ouabain displacement studies was similar to that of ^{3}H-digoxin. Also the in vivo myocardial accumulation of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) in guinea pigs was inhibited by ouabain, but it was not found in other tissues. Thus, the high myocardial accumulation of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) is considered to be dependent on Na,K-ATPase binding.

Based on these results, ^{123}I-labeling of digoxin-iodohistamine(bis(O-carboxymethyloxime)) was performed. Labeling was successfully achieved under no-carrier added conditions and the labeled compound was easily purified by HPLC. Thus, a preliminary canine imaging study was performed; the heart was clearly visualized and was labeled at a far higher ratio than in guinea pigs. The pharmaceutical effect of digoxin is known to be higher in dogs than in guinea pigs. This might also be an indication of a positive correlation between the myocardial accumulation of ^{125}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) and its binding to myocardial Na,K-ATPase.

From the first description in 1785 of the use of foxglove leaf in treating the symptoms of chronic congestive heart failure (*17*), digitalis has become the most important drug used in treating congestive heart failure, atrial flutter, and atrial fibrillation (*18*). However, the therapeutic index of digitalis is very narrow and digitalis toxicity is a common problem in current practice (*18*). In some circumstances, such as myocardial ischemia (*19*), it can even be harmful. Radioimmunoassays for the measurement of plasma digoxin and digitoxin levels may be important in the prevention of some cases of digitalis toxicity. However, such assays should not replace good clinical judgment (*18*), because they are only an indirect evaluation of drug activity and various factors modify the significance of a given digitalis concentration. The use of ^{123}I-labeled digoxin radiopharmaceutical might provide direct information about the drug-Na,K-ATPase interactions in the human

TABLE 1
Biodistribution of ^{123}I-digoxin-iodohistamine(bis(O-carboxymethyloxime)) in a Mongrel Dog*

Ratio/	Blood	Lung	Muscle	Liver
Left ventricular muscle	31.2	3.55	7.67	1.25
Right ventricular muscle	24.8	2.83	6.10	1.00
Septum	32.0	3.64	7.85	1.28

* Ratio of cpm/g tissue. The dog was killed at 70 min postinjection.

myocardium, which might also be useful for improving the control of digitalis therapy.

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EDITORIAL

How Magic Is the Bullet, and What Will It Do?

In their article on the selection and testing of ouabain analogs for cardiac imaging, Fujibayashi and his colleagues have re-opened for us a chapter in one of the earlier books in nuclear medicine: radiopharmacy. First, their exploration of analogs had embedded two challenges: which bullet is most magic in seeking out the myocardium in preference to other sites, with special attention to other **nearby** sites; which could interfere with image interpretation? And which bullet will carry a strange—a biologically foreign—gamma-emitting element (preferably iodine or technetium) to the target? The chemistry imposes two demands: to find the magic carrier and to devise a bond sufficiently attractive to remain attached to the carrier for several hours, while under the influence of the body's fluids, without so altering the handling of the compound that it might lose some of its magic.

Phase one of the search used the biologically perfect master spy, ^3H, which allows the candidate bullet to be tested for its magical qualities: How well will it seek the target when it has to carry no (biologically) sinister gamma-emitting baggage? Compounds A and B passed the test. (Perhaps the radiopharmacists in Dr. Fujibayashi's group added to their store of knowledge about the nature of the interaction between ouabain, its biochemical close cousins, and the myocardial membrane in the process of looking at these data. Why A and B, and not C and D?)

In phase two, selection of the gamma-carrying element, has meant "try iodine first" in the "before technetium" decades, and then "we should consider technetium" in the subsequent history of radiopharmacy. The radiochemical triumph of taming ^{123}I among its lesser imaging-friendly cousins is a legacy to subsequent generations. Tracer iodine is more expensive and less available than tracer technetium, but the art of modern radiopharmacy delivery services, at least in big cities, addresses this issue.

Phase three, the first to engage the nuclear medicine department with its imaging equipment, requires the choice of a surrogate for man, the animal model. Some care is required: a small kinship of myocardial tracers

Received Jan. 7, 1992; accepted Jan. 7, 1992.
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