Considerations of Carrier (continued)

The recent efforts by A. P. Wolf and others (*1–3*) to bring rigor and clarity to the discussion of the carrier question in radiochemistry are both admirable and overdue. The proposed radioactivity categories of carrier-free (CF), nearly carrier-free (NCF), no carrier added (NCA), and carrier added (CA) are unambiguous and, for most applications, comprehensive. However, experimental conditions are possible in which the assignment of an activity to the NCA classification would imply a chemical flexibility that may not be obtained without further sample manipulation. For example, a procedure has lately been developed (*4*) in which the resultant activity is formally categorized as NCA in accordance with the recommended definitions, but it belongs more logically in a gray area between NCA and CA. At this point it is not clear how prevalent such examples are likely to become in nuclear medicine or in other disciplines. It would perhaps be wise to consider other possible carrier designations at this time, however, with an aim toward generating a more inclusive set of guidelines.

The analytical separations required for the isolation of Br-77 from mixed spallation products and residual target material necessitate the addition of approximately a milligram of chloride pseudo-carrier (*4*). The chloride stabilizes and "carries" the radiobromine throughout the procedure, just as would the addition of stable bromide. Moreover, the chloride can subsequently compete and interfere in syntheses of Br-77-labeled pharmaceuticals, and the chlorinated analogs so obtained can saturate receptor binding sites. Thus, although the chloride gives rise to the same problems that would occur with the use of bromide carrier, the Br-77 could nevertheless be categorized as NCA under the current definitions. In the absence of further processing, such a classification would misrepresent the *effective* specific activity of the radionuclide and could hinder the assessment of efficacy for particular experimental applications.

This illustration is, of course, not a novel situation, but is a special case of the use of what Friedlander, Kennedy, and Miller term a "nonisotopic" carrier (*5*). Such pseudocarriers can usually be separated from the radionuclide(s) of interest by chemical methods, and high specific activities can be obtained without resorting to electromagnetic mass separation. When the nonisotopic carrier is homologous with the activity, however, as chloride is with bromide, the necessary chemistry may no longer be trivial, and sophisticated chromatographic techniques may be required. Not all institutions are equipped for such separations, particularly when they also involve the presence of high radiation levels.

We wonder whether Dr. Wolf would think it worthwhile to incorporate these considerations in his carrier classifications, perhaps by including "homologous carrier added" or by slightly modifying the definition of NCA?

PATRICK M. GRANT
Chevron Research Company
Richmond, California
DAVID A. MILLER
California State University
Northridge, California

REFERENCES

1. WOLF AP, FOWLER JS: Organic radiopharmaceuticals. Recent advances. In *Radiopharmaceuticals II.* New York, The Society of Nuclear Medicine, 1979, pp 73–92
2. TEWSON TJ, WELCH MJ: Re: terminology concerning specific activity of radiopharmaceuticals. *J Nucl Med* 22:392, 1981
3. WOLF AP: Reply. *J Nucl Med* 22:392–393, 1981
4. GRANT PM, WHIPPLE RE, BARNES JW, et al: The production and recovery of ^{77}Br at Los Alamos for nuclear medicine studies. *J Inorg Nucl Chem* (in press)
5. FRIEDLANDER G, KENNEDY JW, MILLER JM: *Nuclear and Radiochemistry.* 2nd edition. New York, Wiley & Sons, 1964, p 405

Tc-99m PPi Localization in Acute Experimental Myocardial Infarction: Application of Macro- and Microautoradiography

Recently Tc-99m pyrophosphate (PPi) has been used routinely in clinical cardiology to determine the size of acute myocardial infarcts (*1–3*). The determinants of Tc-99m PPi uptake have been extensively studied and its preferential localization in the infarcted myocardium is well accepted (*3–5*).

To document directly the relationship between the site of Tc-99m PPi uptake and the pathological features of myocardial infarction, we present an application of macro- and microautoradiograms combined with conventional histochemical methods.

Twenty mongrel dogs were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and underwent thoracotomy with sterile surgery. The left anterior descending coronary artery was ligated just distal to the first diagonal branch, and the thoracotomy was closed. Forty-eight hours after coronary ligation, 30 mCi of Tc-99m complexed with 5 mg of stannous pyrophosphate was injected intravenously, and the dogs were killed 90 min later by lethal doses of sodium pentobarbital followed by KCl. The heart was immediately removed and transmural blocks (2 by 2 cm) including both normal and infarcted regions were cut out, coated with embedding material, and frozen with dry ice in hexane for 30 min. The frozen sample was then sliced with a cryomicrotome into 8- and 20-μm sections for micro- and macroautoradiography, respectively. Rapid freezing and tissue slicing in a cryostat is necessary to minimize loss of Tc-99m radiation due to decay, and to preserve tissue enzyme activities.

The thick sections were placed on glass slides and immediately dried by cool air. For the Tc-99m macroautoradiograms, a slide was covered with Saran Wrap 1.27 μm in thickness, and placed in contact with x-ray film.* The films were exposed for 24 hr, then developed, fixed, rinsed, and dried using an automatic developer. The other was fixed with cold acetone and stained by incubating it in the buffered nitroblue tetrazolium (NBT) solution at 37 °C to demarcate a necrotic area.

For the first 15 dogs we used a dipping method for microautoradiography, and this failed because of the water solubility of Tc-99m PPi. From then on, the glass slides were previously coated with nuclear tracking emulsion.† An 8-μm section was cut with the cryomicrotome and placed on the precoated glass slide in the darkroom. The exposure time for the microautoradiogram was 12 hr. After photographic processing with Copinal‡ and Pan fix,‡ the section was stained with hematoxylin and eosin HE. This technique, used with another five dogs, in which the exposure of the emulsion to Tc-99m was performed under dry conditions, provided the first successful microautoradiographic location of Tc-99m PPi in infarcted myocardium.

Figure 1 shows an HE stain (a), an adjoining NBT section (b), and the macroautoradiogram (c), from a two-day-old myocardial infarct. The infarcted area is clearly demarcated in both HE and NBT sections, and the Tc-99m PPi is seen to be deposited predominantly around the boundary of the infarcted region. Figure 2 shows photomicrograms from 8-μm sections stained with HE;

FIG. 1. Low-power photomicrograms of transmural sections stained with hematoxylin and eosin (a), NBT (b), and a Tc-99m autoradiogram (c). The sample includes lateral border of myocardial infarct from a dog's heart at 2 days after LAD occlusion. Upper and lower edges correspond, respectively, to endocardial and epicardial surfaces. Uptake of Tc-99m PPi is restricted to infarcted area, which is well outlined in the HE and NBT sections as the weakly stained area. Magnification 2X. Note that center of myocardial necrosis contains less radioactivity than border area, in spite of the similar staining characteristics with NBT and HE.

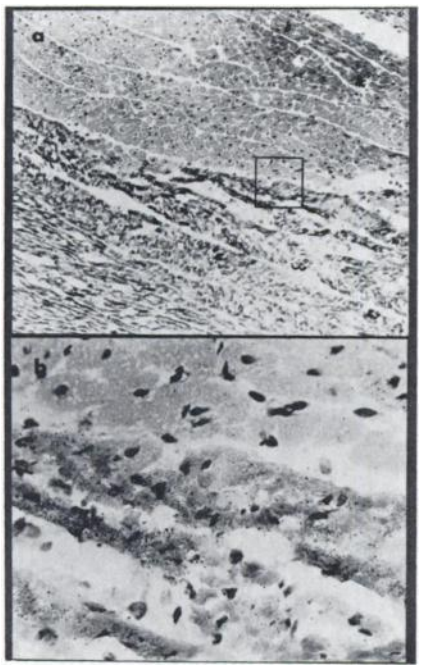

FIG. 2. Low-power (a, 108X) and high-power (b, 820X) photomicrographs of boundary between normal and infarcted myocardium. In this HE section the silver grains, reflecting presence of radioactivity, locate predominantly at necrotic cells with pyknotic nuclei and altered cytoplasmic staining.

(a) at low power and (b) a high-power view of the square area in (a). The Tc-99m PPi was deposited predominantly in the necrotic cells.

The particulate radiation from Tc-99m is due to Auger electrons, whose low penetrating power is an asset in high-resolution autoradiography. The reported resolution of Tc-99m autoradiograms is less than 0.4 μm (7). Thus, Tc-99m is a favorable tracer for use in microscopic studies. In comparison with tritium-labeled phosphonate (5), Tc-99m PPi offers the advantages of much shorter processing time and the direct visualization of the location of clinically available radiopharmaceuticals.

HITONOBU TOMOIKE
MOTOOMI NAKAMURA
KUNIHIRO WATANABE
TETSUJI INOU
TAKESHI KUROZUMI
KENZO TANAKA
Kyushu University
Fukuoka, Japan

FOOTNOTES

* Sakura® X-ray films QS: Konishiroku Photo Co., Ltd., Tokyo, Japan.
† NR M_2: Konishiroku Photo Co., Ltd., Tokyo, Japan.
‡ Fuji Photofilm Co., Ltd., Tokyo, Japan.

REFERENCES

1. WYNNE J, HOLMAN BL, LESCHE M: Myocardial scintigraphy by infarct avid radiotracers. *Prog Cardiovasc Dis* 20: 243–266, 1978
2. COWLEY MJ, MANTLE JA, ROGERS WJ, et al: Technetium-99m stannous pyrophosphate myocardial scintigraphy. *Circulation* 56:192–198, 1977
3. WILLERSON JT, PARKEY RW, BONTE FJ, et al: Pathophysiologic considerations and clinicopathological correlates of technetium-99m stannous pyrophosphate myocardial scintigraphy. *Semin Nucl Med* 10:54–69, 1980
4. MARCUS ML, TOMANEK RJ, EHRHARDT JC, et al: Relationships between myocardial perfusion, myocardial necrosis, and technetium-99m pyrophosphate uptake in dogs subjected to sudden coronary occlusion. *Circulation* 54:647–653, 1976
5. BUJA LM, TOFE AJ, KULKARNI PV, et al: Sites and mechanisms of localization of technetium-99m phosphorus radiopharmaceuticals in acute myocardial infarcts and other tissues. *J Clin Invest* 60:724–740, 1977
6. LEDERER CM, HOLLANDER JM, PERLMAN I: *Table of Isotopes.* New York, John Wiley & Sons, Inc., 1967, p 47, 236
7. TILDEN RL, JACKSON J JR, ENNEKING WF, et al: ^{99m}Tc-polyphosphate: Histological localization in human femurs by autoradiography. *J Nucl Med* 14:576–578, 1973