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# Myocardial Infarct Imaging Agents. III. Synthesis and Evaluation of [$^{203}$Hg] Hydroxymercuriphthaleins

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***Four Hg-203-mercurated phthaleins were prepared, purified, and compared with [$^{203}$Hg] mercuric nitrate, [$^{3}$H] phenolphthalein, [$^{203}$Hg] hydroxymercurifluorescein and Tc-99m-pyrophosphate in a rat model of myocardial necrosis to determine their specificities for damaged myocardium. The ratios of damaged myocardium to normal myocardium, and to blood, for the [$^{203}$Hg] hydroxymercuriphthaleins (20.7–34.1 and 12.1–20.1, respectively) were somewhat lower than those reported previously for [$^{203}$Hg] hydroxymercurifluorescein, but were higher than those found with [$^{203}$Hg] mercuric nitrate, [$^{3}$H] phenolphthalein, and Tc-99m pyrophosphate. Both the hydroxymercuri- functional group and the phthalein moiety are required for selectivity. The removal of the oxygen bridge present in fluorescein, and the replacement of carboxylic acid by sulfonic acid, had no significant effects on the sequestration process in damaged tissue.***

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The drawbacks of the currently available radiopharmaceuticals for the imaging of infarcted myocardium have led us to search for agents with superior selectivity for, and uptake in, damaged myocardial tissues. Our original study surveyed several classes of radiotracers, including the Tc-99m bone seekers, Tc-99m-labeled sulfur-containing chelates, and Hg-203 organomercurials (*1*). We found that [$^{203}$Hg] diiodohydroxymercurifluorescein—originally found valuable in this service by Malik et al. (*2*)—gave, in our model, a selectivity ratio of 31.7 for damaged to normal myocardium, compared with the 25.2 ratio obtainable with Tc-99m pyrophosphate. We have since examined a series of six [$^{203}$Hg] hydroxymercurifluoresceins (*3*) and evaluated the following structural parameters: (a) the contribution of the fluorescein and mercuric groups, (b) mono vs. bis hydroxymercuration, and (c) the effect of halogen substituents upon the uptake and selectivity of the tracers. The results indicated that both the polycyclic organic moiety and the hydroxymercuri- group are required for uptake and selectivity, with the hydroxymercuri- function being somewhat more important.

In the present study we have continued our investigation of the effects of structural modification of the organomercurials upon their uptake and sensitivity for damaged myocardial tissue. By preparing and evaluating the [$^{203}$Hg] mono- and bis(hydroxymercuri)phenolphthaleins, the corresponding phenolsulfonophthaleins, and the nonmercurated [$^{3}$H]phenolphthalein, one can determine the structure-avidity relationships resulting from the deletion of the oxygen bridge, carboxylic acid replacement by sulfonic acid, and the presence or absence of covalently bound mercury (Fig. 1). The interpretation of the data has delineated the critical sites of modification of the radiopharmaceutical and has indicated rational approaches to the development of superior agents.

## MATERIALS AND METHODS

**Chemical reagents.** Phenolphthalein (PhPh) and phenolsulfonophthalein (PhSPh) were purchased

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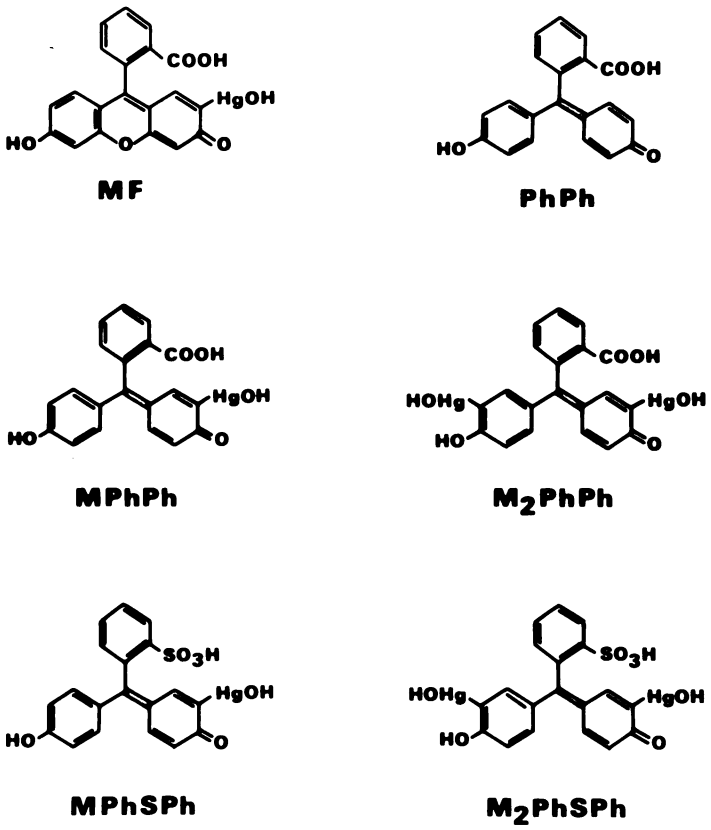


**FIG. 1.** Chemical structures of hydroxymercurifluorescein (MF), phenolphthalein (PhPh), mono- and bis(hydroxymercuri)phenolphthalein (MPhPh, $M_2$PhPh), and mono- and bis(hydroxymercuri)phenolsulfonophthalein (MPhSPh, $M_2$PhSPh).

commercially* and used without further purification. Mercuric oxide, certified ACS, was used in the hydroxymercuration reactions. Aqueous [$^{203}$Hg] mercuric nitrate, specific activity 1–5 Ci/mmol, and [$^{3}$H] phenolphthalein in methanol, 480 mCi/mmol, were obtained from a commercial supplier†.

**Chemical and radiochemical purity analysis.** The radiochemical purity of [$^{3}$H] phenolphthalein was ascertained by silica gel and alumina TLC (Eastman Chromogram sheets 6060 and 6062 respectively) and by paper chromatography (Whatman No. 3 paper) using methanol:water:ammonia (5:4:1) as the developing solvent. The strips were cut into 1-cm segments and counted by liquid scintillation. In all three systems, greater than 90% of the radioactivity was associated with the fluorescent component.

Column chromatographic separations of the mercurated phthaleins employed alumina as the adsorbent and 0.1 N $NH_4OH$, 0.1 N $NaHCO_3$ and 0.1 N NaOH as the eluents. Chemical purity and radionuclide distribution were determined by ascending paper chromatography (Whatman No. 3) using methanol:water:ammonia (5:4:1) as the developing solvent. The non-labeled components were detected by ultraviolet fluorescence, and the Hg-203-labeled components were located by ultraviolet fluorescence and gamma scintillation counting.

Elemental analyses of the nonradioactive hydroxymercuriphthaleins were performed commercially‡.

**Chemical syntheses.** Hydroxymercuriphenolphthalein (MPhPh), bis(hydroxymercuri)phenolphthalein ($M_2$PhPh), hydroxymercuriphenolsulfonophthalein (MPhSPh), and bis(hydroxymercuri)phenolsulfonophthalein ($M_2$PhSPh) (Fig. 1) were prepared by hydroxymercuration of phenolphthalein and phenolsulfonophthalein following the procedure of White (*4*). The Hg-203-labeled phthaleins were obtained via mercuric isotope exchange using the technique described by Ratusky et al. (*5*). The products were precipitated with acetic acid, collected by centrifugation, washed three times with distilled water, redissolved in a small volume of saturated sodium bicarbonate and filtered. The filtrate was diluted with distilled water and adjusted to pH 7–8, giving a final activity of 150–600 $\mu$Ci/ml.

**Damaged-myocardium model.** Albino outbred Wistar or Sprague-Dawley male rates weighing 150–250 g were used. The heart was damaged by the discrete application of heat (*6*).

**Biodistribution of radiopharmaceuticals.** Three hours after the creation of the lesion, the radiolabeled phthaleins were injected into the saphenous vein. Approximately 10 $\mu$Ci (0.5–1.0 mg/kg) were given, and the rats were killed 1 and 3 hr later. The heart was removed and the tissue damage graded on a scale of 1 to 4 as described previously (*1*),

depending upon the severity of the lesion. All of the lesions in this study were grade 3. The damaged area was dissected from the normal myocardial tissue and the tissue immediately adjacent to the lesion was separated and discarded. Blood was obtained from a vein in the thoracic cavity. Tissue samples were weighed and counted in a NaI(Tl) well counter.

## RESULTS

**Chemical syntheses.** The exchange reaction of [$^{203}$Hg] mercuric nitrate with the non-labeled hydroxymercurated phthaleins gave a 60–80% incorporation of label with resultant specific activities of 30–150 mCi/mmol. As previously described with the hydroxymercurifluoresceins, [$^{203}$Hg] mercuric isotope exchange of the mono-hydroxymercurated phthaleins produced 10–15% of the [$^{203}$Hg] bis(hydroxymercuri)phthaleins in addition to the desired [$^{203}$Hg] mono(hydroxymercuri)phthaleins. No attempt was made to remove this minor component from the mixture. Because of the increased aqueous solubility conferred by the sulfonic acid moiety, the purification of the labeled hydroxymercurated phenolsulfonophthaleins usually gave only a 30–50% yield of final products. Losses in the purifications of the hydroxymercurated phenolphthaleins were significantly less.

**Biologic evaluation.** Table 1 shows the 1-hr values for the concentration of Hg-203 radiopharmaceuticals in the damaged myocardium (DM) relative to those found in the normal myocardium (N) and in blood (B). The values for [$^{3}$H] phenolphthalein and [$^{203}$Hg] mercuric nitrate are included to indicate the contributions of the individual phthalein and mercury components, and data previously obtained for [$^{203}$Hg] hydroxymercurifluorescein and Tc-99m pyrophosphate ($PP_i$) are also shown for comparison. The highest absolute concentration in the damaged myocardium was exhibited by the Hg-203-mercurated agents (3.5–15.8% ID/g DM) compared to [$^{3}$H] phenolphthalein (0.15%) or Tc-99m pyrophosphate (2.2%). The uptake of the [$^{203}$Hg] hydroxymercuriphthaleins—with the exception of bis(hydroxymercuri)phenolsulfonophthalein, ($M_2$PhSPh)—tended to be lower than that of [$^{203}$Hg] hydroxymercurifluorescein (MF) (5.0%) and [$^{203}$Hg] mercuric nitrate (7.1%). The ratios for damaged myocardium to normal myocardium (DM/N) and to blood (DM/B) for the [$^{203}$Hg] hydroxymercuriphthaleins (20.7–34.1, 12.1–20.1) were somewhat lower than those reported for [$^{203}$Hg] hydroxymercurifluorescein (51.5, 22.1), although higher than the ratios found with [$^{203}$Hg] mercuric nitrate (15.5, 5.8) or [$^{3}$H] phenolphthalein (3.2, 4.0). None of the Hg-203 organomercurials tested at 3 hr showed a significant improvement in any of the parameters measured compared to the 1 hr values.

**TABLE 1. TISSUE CONCENTRATION RATIOS OF RADIOLABELED MERCURIPHTHALEINS IN RATS 1 HR AFTER ADMINISTRATION***

| | %ID/g DM† | DM/N | DM/B |
|---|---|---|---|
| MPhPh | 3.5 ± 1.0 | 34.1 ± 6.9 | 17.7 ± 5.1 |
| $M_2$PhPh | 5.1 ± 2.2 | 27.3 ± 5.9 | 20.1 ± 7.6 |
| MPhSPh | 3.7 ± 1.2 | 23.9 ± 6.9 | 12.1 ± 4.6 |
| $M_2$PhSPh | 15.8 ± 6.6 | 20.7 ± 9.5 | 19.9 ± 8.4 |
| $Hg(NO_3)_2$ | 7.1 ± 3.4 | 15.5 ± 4.6 | 5.8 ± 2.0 |
| $^{3}$H-PhPh | 0.15 ± 0.06 | 3.2 ± 0.8 | 4.0 ± 1.4 |
| MF | 5.0 ± 1.5 | 51.5 ± 13.5 | 22.1 ± 8.1 |
| $^{99m}TcPP_i$ | 2.2 ± 0.4 | 25.2 ± 9.2 | 12.8 ± 2.1 |

* All values represent the mean ± 1 S.D. of six rats.
† DM = heat damaged myocardium; N = normal myocardium; B = blood.

## DISCUSSION

The mechanism that has been proposed for the selective localization of organomercurials in damaged tissue is based upon evidence that enzymes are released from the lysosomes of dying cells as a result of decreased oxygen uptake and lowered intracellular pH (7). The action of the lysozymal hydrolases and proteases upon the intracellular constituents exposes sulfhydryl groups and other moieties that are capable of forming covalent bonds with divalent mercury. Moreover, ischemia alters the membrane permeability of myocardial cells, thereby permitting the influx of organic molecules and inorganic ions that are normally excluded. The uptake and selectivity of infarct-avid organomercurials represent the combined ability of the agent to cross the ischemic cell membrane and to bind to the intracellular components made available from the release of the lysozymal enzymes. In previous studies we observed that the uptake and selectivity of these agents are greatly enhanced if the hydroxymercuri- group is present in combination with a polycyclic aromatic moiety, as opposed to some small aliphatic or aromatic molecule (*1,3*).

The structural modification made by preparing the [$^{203}$Hg] hydroxymercuriphthaleins has not greatly altered the uptake and selectivity of these compounds compared with [$^{203}$Hg] hydroxymercurifluorescein. As had been observed with the MF series, both the hydroxymercuri- functional group and the phthalein ring system are required for selectivity. Neither [$^{203}$Hg] mercuric nitrate nor [$^{3}$H] phenolphthalein delivers the DM/N ratios shown by the organomercurials. The removal of the oxygen bridge that is present in the fluorescein molecule appears to result

**TABLE 2. COMPARISON OF FIGURE OF MERIT (FOM) VALUES FOR FIVE MERCURY-LABELED COMPOUNDS AND FOR Tc-99m PYROPHOSPHATE**

| | MPhPh | $M_2$PhPh | MPhSPh | $M_2$PhSPh | MF | Tc-$PP_i$ |
|---|---|---|---|---|---|---|
| % ID/g DM | 3.51 | 5.11 | 3.66 | 15.8 | 5.13 | 2.25 |
| % ID/g N | 0.10 | 0.19 | 0.15 | 0.76 | 0.10 | 0.09 |
| % ID/g B | 0.20 | 0.25 | 0.30 | 0.77 | 0.22 | 0.17 |
| FOM DM:N* | 3.22 | 4.57 | 3.23 | 13.55 | 4.71 | 1.92 |
| FOM DM:B* | 2.95 | 4.41 | 2.85 | 13.63 | 4.51 | 1.79 |

* FOM DM:N $= \frac{(\%ID/g\ DM - \%ID/g\ N)^2}{\%ID/g\ DM + \%ID/g\ N}$; FOM DM:B $= \frac{(\%ID/g\ DM - \%ID/g\ B)^2}{\%ID/g\ DM + \%ID/g\ B}$.

in a slight decrease in selectivity for both MPhPh and MPhSPh relative to MF. Uptake of these agents is not significantly affected. The other structural modifications—such as mono in contrast to bis hydroxymercuration and carboxylic in contrast to sulfonic acid—had only minor effects upon selectivity.

The uptake and binding characteristics of these compounds are consistent with the proposed model for infarct-seeking agents. The uptake is facilitated by the phthalein moiety and the binding is effected by the presence of the hydroxymercuri- group. Although not statistically significant, the increased uptake and localization of $M_2$PhSPh relative to the other organomercurials may indicate either a somewhat different mode of uptake or a site of binding that requires the presence of both hydroxymercuri- groups and the sulfonate moiety. There is insufficient data at present, however, to substantiate this speculation. In the damaged myocardium model localization of these agents is essentially complete within 1 hr of administration, since neither the uptake nor the DM/N ratio is significantly altered when evaluated at 3 hr. The DM/B values are somewhat improved when examined at a later time, but the clearance of the majority of the unbound agents from the blood also appears to occur within the first hour. The rapid localization and blood clearance of these compounds are significant factors in their potential development as myocardial infarct-imaging agents.

These Hg-203-labeled compounds provide a basis for subsequent rational development of imaging agents. The studies have demonstrated which types of structural modification are permitted without loss of selectivity. The rapid and significantly large absolute uptake of the agents in the damaged tissue, coupled with their fast blood clearance would provide greater photon flux from the infarct for a suitably labeled organomercurial than could be obtained with Tc-99m pyrophosphate. A figure of merit (Table 2) that is a criterion of the diagnostic image quality would predict that radiopharmaceuticals with localizing properties similar to those of the hydroxymercuri-phthaleins or fluoresceins would provide images superior to those currently available from Tc-99m pyrophosphate. Further work on the development of such organomercurials labeled with I-123 or Tc-99m is currently in progress.

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FOOTNOTES

* Eastman Organic Chemicals, Rochester, N.Y.

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