jnm/ RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

# Radiopharmaceuticals for Acutely Damaged Myocardium II: Synthesis and Evaluation of [$^{203}$Hg] Hydroxymercurifluoresceins

Robert N. Hanson, Michael A. Davis, and B. Leonard Holman

*Harvard Medical School and Northeastern University, Boston, Massachusetts*

***Six [$^{203}$Hg] hydroxymercurifluoresceins were prepared by two methods and compared with [$^{3}$H] fluorescein, [$^{131}$I] rose bengal, and [$^{203}$Hg] mercuric nitrate, in a rat model for myocardial necrosis, to determine their specificities for damaged myocardium (DM). The nonhalogenated [$^{203}$Hg] hydroxymercurifluorescein had the highest ratios of the series for DM/normal heart (51.5 ± 13.5) and DM/blood (22.1 ± 8.1). Halide substituents at the 2′ or 4′ positions of the fluorescein moiety decreased the tissue selectivity, and bis-hydroxymercuration had no significant effect. The six tracers studied had greater absolute uptake and better target-to-nontarget ratios than [$^{3}$H] fluorescein, [$^{131}$I] rose bengal, or [$^{203}$Hg] mercuric nitrate, indicating a cooperative effect between the fluorescein and hydroxymercuri-moieties in the overall sequestration process in damaged tissue.***

**J Nucl Med 18: 803–808, 1977**

The availability of gamma-emitting radiolabeled compounds that localize selectively in ischemic or infarcted tissue and permit the direct visualization of acute infarcts has had a dramatic impact on the practice of both nuclear medicine and cardiology (*1*). Such agents should provide diagnostically superior scintiscans of the diseased area compared with the images produced by the monovalent cations of K-43, Rb-82, Cs-131, and Ti-201 that concentrate in healthy myocardium (*2–4*). The available infarct specific agents, however, have serious drawbacks, particularly for determining the extent of tissue necrosis. Tc-99m tetracycline clears slowly from the blood, precluding external imaging earlier than 24 hr after injection (*5,6*). Technetium-99m pyrophosphate, the most accurate tracer to date for MI detection (*7,8*), is hampered by uptake in overlying ribs and in reversibly ischemic myocardium, which limits its accuracy in assessing infarct size (*9,10*). Other agents such as Tc-99m glucoheptonate give target-to-background ratios too low to be useful clinically (*8*).

In studies using differential fluorescence, Malek (*11*) and Katsuya (*12*) have shown that fluorescein and substituted fluoresceins are sequestered to a greater degree in damaged and tumorous tissue than in normal tissue. In 1966, Malek et al. (*13*) reported the use of [$^{203}$Hg] and [$^{197}$Hg] mercuric acetate to synthesize a series of radiolabeled difluoresceinylmercury, mono- and bis-hydroxymercurifluorescein derivatives. In subsequent papers (*14*) they described alternative syntheses of the compounds to give products of higher specific activity. In animal models employing damaged muscle (rat) and coronary artery ligation (dog), the labeled hydroxymercurifluoresceins showed target-to-nontarget (T/NT) ratios in the 20–70 range (*15*). Attempts by other

Received Jan. 19, 1977; revision accepted March 7, 1977.

For reprints contact: Michael A. Davis, Dept. of Radiology, Harvard Medical School, 50 Binney St., Boston, MA 02115.

investigators to reproduce the syntheses and the biologic data (*16–18*) of the hydroxymercurifluoresceins, however, have produced differing results. It was the purpose of our study to re-evaluate the synthesis and isolation of the radiolabeled hydroxymercurifluoresceins, to examine their tissue selectivity in a model we feel to be more representative of a myocardial infarct than damaged rat thigh muscle, and to try to establish a structure–activity relationship for these compounds as potential imaging agents in myocardial infarction (*19*).

## MATERIALS AND METHODS

**Chemical reagents.** Commercial sources were used for fluorescein, 4′,5′-diiodofluorescein ($I_2F$), and 2′,7′-dibromofluorescein ($Br_2F$), and for the mercuric acetate and mercuric oxide, certified ACS, used in the hydroxymercuration reactions. Our aqueous [$^{203}$Hg] mercuric nitrate had a specific activity 1–5 Ci/mmol, and the [$^{3}$H] fluorescein in absolute methanol, 3.78 Ci/mmol.

**Chemical and radiochemical purity analyses.** The radiochemical purity of [$^{3}$H] fluorescein was ascertained by silica-gel and alumina TLC* and with Whatman No. 3MM paper strips using 6% ammonium hydroxide with methanol (1:1) as the developing solvent. In all three systems greater than 90% of the H-3 activity was associated with the fluorescent component.

Column chromatographic separations of the mercurated fluoresceins employed neutral Woelm Alumina and 0.1*N* $NH_4OH$, 0.1*N* $NaHCO_3$, and 0.1*N* NaOH as the eluents. Chemical purity and radiotracer distribution were determined by ascending paper chromatography with Whatman 3MM paper strips using 28% methanol with ammonia (1:1) as the developing solvent. The nonlabeled components were detected by ultraviolet fluorescence and the mercury-203-labeled materials by gamma-scintillation counting.

Elemental analyses were performed commercially.†

**Chemical syntheses.** The following derivatives were synthesized: hydroxymercurifluorescein (MF); 4′,5′-diiodohydroxymercurifluorescein ($I_2MF$); 2′,7′-dibromohydroxymercurifluorescein ($Br_2MF$); bis(hydroxymercuri)fluorescein ($M_2F$); 4′,5′-diiodo-bis(hydroxymercuri)fluorescein ($I_2M_2F$); and 2′,7′-dibromo-bis(hydroxymercuri)fluorescein ($Br_2M_2F$). For products of low specific activity, the syntheses were carried out using nonradioactive mercuric acetate containing tracer amounts of [$^{203}$Hg] mercuric nitrate (*14*). Isolation by column chromatography with alumina, using a pH-gradient elution, gave unreacted fluorescein with water, the mono(hydroxymercuri) fluoresceins (MF, $I_2MF$, $Br_2MF$) with 0.1*N* $NH_4OH$, and the bis(hydroxymercuri)fluoresceins ($M_2F$, $I_2M_2F$, $Br_2M_2F$) with 0.1*N* NaOH (see Fig. 1). The products were precipitated with glacial acetic acid and collected by centrifugation. After three aqueous washes the residue was dissolved in saturated $NaHCO_3$, filtered through a Millipore filter (0.45 $\mu$) and diluted with distilled water to give an activity of 150–600 $\mu$Ci/ml. The [$^{203}$Hg] hydroxymercurifluoresceins of high specific activity were prepared by mercuric isotope exchange by the procedures described by Ratusky et al. (*14*). The products were precipitated with glacial acetic acid, collected by centrifugation, washed with water, redissolved with saturated $NaHCO_3$, and filtered. The filtrate was diluted with distilled water to give a final activity of 150–600 $\mu$Ci/ml.

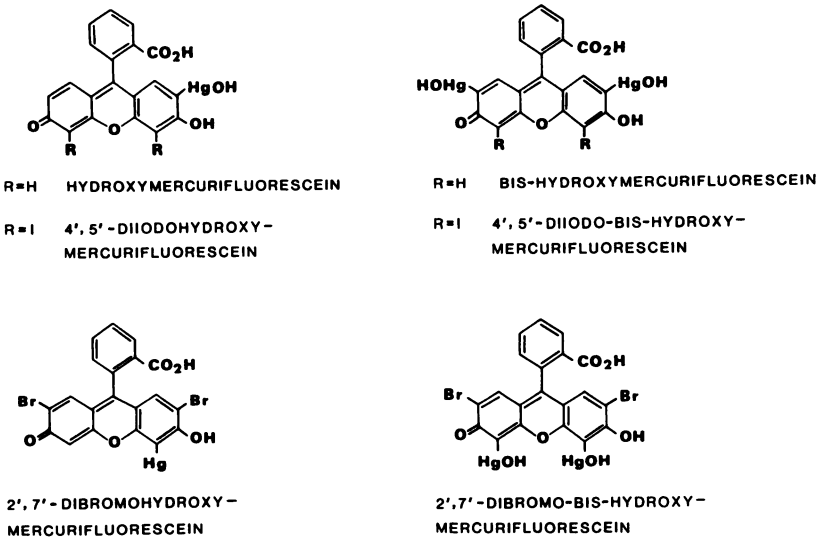


**FIG. 1.** Chemical structures of hydroxymercurifluoresceins and halogenated derivatives.

**TABLE 1. COMPARISON OF ANALYTICAL DATA FOR THE [$^{203}$Hg] HYDROXYMERCURATED FLUORESCEINS PREPARED BY DIRECT RADIOCHEMICAL SYNTHESIS AND BY ISOTOPE EXCHANGE**

| | Direct Synthesis | | | Isotope Exchange | | |
|---|---|---|---|---|---|---|
| | Specific Activity | Chromatographic $R_f$ Values | | Specific Activity | Chromatographic $R_f$ Values | |
| Agent | mCi/mmol | UV monitor | Gamma scintillation | mCi/mmol | UV monitor | Gamma scintillation |
| MF | 20 | 0.69 | 0.26 | 190 | 0.69 | |
| | | 0.26 major | | | 0.26 major | 0.26 |
| | | | | | 0.10 | 0.10 |
| $M_2F$ | 50 | 0.10 | 0.10 | 160 | 0.13 | 0.13 |
| $I_2MF$ | 15 | 0.62 | 0.28 | 200 | 0.62 | |
| | | 0.28 major | | | 0.28 major | 0.28 |
| | | | | | 0.07 | 0.05 |
| $I_2M_2F$ | 30 | 0.07 | 0.05 | 170 | 0.07 | 0.05 |
| $Br_2MF$ | 12 | 0.29 | 0.26 | 130 | 0.29 major | 0.26 |
| | | | | | 0.07 | 0.06 |
| $Br_2M_2F$ | 24 | 0.07 | 0.06 | 230 | 0.07 | 0.07 |

**Animal model.** A myocardium damaged by heat (*19,20*) was produced in albino, outbred Wistar or Sprague–Dawley male rats weighing 150–250 g.

**Biodistribution of radiopharmaceuticals.** Three hours after creation of the lesion, the radiolabeled mercurifluoresceins were injected into the saphenous vein. One hour after administration, the rats were killed and the heart was removed. The severity of the tissue damage was graded on a scale of 1 to 4 (*19*). All of the lesions in this study were graded 3. The damaged area was excised and tissue adjacent to the lesion was separated and discarded. The atria and ventricles were separated. Blood was obtained from a vein in the thoracic cavity. Care was exercised to prevent cross-contamination of samples. Tissue samples were weighed, and counted in an NaI(*Tl*) well scintillation counter.

## RESULTS

**Chemical syntheses.** Table 1 compares the analytical data for the [$^{203}$Hg] hydroxymercurated fluoresceins prepared by synthesis and by isotope exchange. Chromatographic analysis of the products was performed on paper. Ultraviolet fluorescence identified the unlabeled products, and gamma counting the labeled ones. These studies indicated that complete separation of the starting fluoresceins ($R_f$ 0.6–0.7) and the mono(hydroxymercurifluoresceins ($R_f$ 0.2–0.3) was not attained because of the marked tailing of the starting material during column chromatography. For hydroxymercurifluorescein (MF) and diiodohydroxymercurifluorescein ($I_2$MF) the nonmercurated starting material comprised 5–10% of the isolated mono-hydroxymercurated product. A distinct separation between the mono-hydroxymercurated and bis-hydroxymercurated products ($R_f$ 0.05–0.47) was more readily obtained.

Contrary to the report by Malek et al. (*15*) no difluoresceinylmercury ($MF_2$) products from the reactions were observed or isolated. It seems unlikely, although possible, that the higher-$R_f$ material ($R_f$ 0.6) that was present in the mono-hydroxymercurated fraction, and identified by UV fluorescence as the unreacted starting material, was indeed the difluoresceinylmercury ($R_f$ 0.5). When the synthesis was repeated employing mercuric acetate doped with [$^{203}$Hg] mercuric nitrate, the same mixture was obtained. The higher-$R_f$ component was nonradioactive and therefore could not be [$^{203}$Hg] difluoresceinylmercury. This corroborates the findings of others with respect to the absence of difluoresceinylmercury in the synthesis of radiolabeled hydroxymercurated fluoresceins (*22*).

The reaction of [$^{203}$Hg] mercuric nitrate with the nonlabeled hydroxymercurated fluoresceins gave a 60–80% labeling efficiency in which the percent incorporated depended upon the molar ratio of the nonlabeled material to the carrier-free [$^{203}$Hg] mercuric nitrate. The differences in composition of the material obtained from $I_2$F by synthesis and by isotope exchange are presented in Fig. 2. The absence of fluorescein and its halogenated derivatives ($R_f$ 0.6–0.7), and the presence of bis(hydroxymercuri)fluorescein ($R_f$ 0.06) in the hydroxymercurated fluorescein product obtained via exchange, can be ascribed to [$^{203}$Hg] hydroxymercuration, which competes with the desired isotope exchange. This side reaction is not observed during the Hg-203 isotope exchange of the bis(hydroxymercuri)fluoresceins because the compounds no longer have any sites available for hydroxymercuration. The higher specific activities (5–20-fold) that are obtained by isotope exchange result from the greater ease of handling small quantities of the compounds in that technique than in the

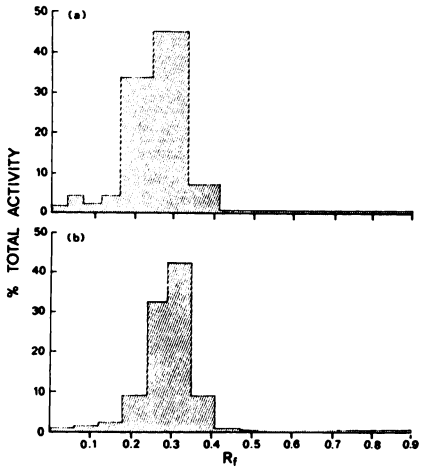


**FIG. 2.** Radiochromatographic comparison of 4',5'-diiodomonohydroxymercuri-fluorescein prepared by isotope exchange (a) and by direct synthesis (b).

total synthesis with [$^{203}$Hg] mercuric acetate and the better subsequent isolation and separation of the mixture of products.

**Biologic evaluation.** Table 2 shows a comparison of the concentration of the Hg-203 radiopharmaceuticals in the damaged myocardium (DM) relative to that in normal myocardium and in blood. In addition to the six [$^{203}$Hg] hydroxymercurated fluoresceins, we included [$^{3}$H] fluorescein, [$^{131}$I] rose bengal, and [$^{203}$Hg] mercuric nitrate to determine the separate contributions of the fluorescein and mercury moieties. The highest absolute concentration in the damaged myocardium (% ID/gDM) was exhibited by the [$^{203}$Hg] mercurated agents (5.0–12.6% ID/gDM) relative to [$^{131}$I] rose bengal (0.7) and [$^{3}$H] fluorescein (0.15). The [$^{203}$Hg] hydroxymercurated fluoresceins tended to give greater uptake than [$^{203}$Hg] mercuric nitrate. Only [$^{203}$Hg] hydroxymercurifluorescein had a lower uptake (5.0 compared with 7.1), whereas the other five had greater uptakes (8.6–12.6 compared with 7.1). The highest concentration ratios for damaged myocardium to normal myocardium (DM/N) and damaged myocardium to blood (DM/blood) were exhibited by [$^{203}$Hg] hydroxymercurifluorescein (51.5 and 22.1) and [$^{203}$Hg] bis(hydroxymercuri)fluorescein (42.8 and 15.6). There was no statistically significant difference between these two agents. The lowest ratios were exhibited by the agents that did not contain both the fluorescein and hydroxymercuri-functional groups—namely [$^{203}$Hg] mercuric nitrate (15.5 and 5.8), [$^{131}$I] rose bengal (7.8 and 3.2), and [$^{3}$H] fluorescein (2.4 and 1.7).

## DISCUSSION

The mechanism that has been proposed for the selective localization of the hydroxymercurifluoresceins in damaged tissue is based on the evidence that lysozymes are released in dying cells (*22*). The lysozymes digest proteins, which exposes sulfhydryl groups capable of forming covalent bonds with divalent mercury. Therefore, relative to normal tissue, the ischemic tissue contains more potential mercury-binding sites. In addition, the membrane properties of ischemic or necrotic cells are altered, permitting the influx of organic molecules and inorganic ions that are normally excluded. The combination of an organic carrier and a sulfhydryl-binding prosthetic group should provide a degree of injured-tissue specificity greater than either can achieve alone. It is assumed that modification in the steric constitution of the agents will be reflected by differences in

**TABLE 2. TISSUE-CONCENTRATION RATIOS OF RADIOLABELED MERCURIFLUORESCEINS IN RATS**

| Compound | No. of animals | % ID/g DM* | DM/normal myocardium† | DM/blood† | Blood/ normal myocardium† | Injured muscle/ normal muscle‡ | Injured muscle/ blood‡ |
|---|---|---|---|---|---|---|---|
| MF | 5 | 5.0 ± 1.5 | 51.5 ± 13.5 | 22.1 ± 8.1 | 2.43 ± 0.35 | 70.3 | 6.7 |
| $M_2F$ | 3 | 9.4 ± 1.5 | 42.8 ± 9.4 | 15.6 ± 2.5 | 2.87 ± 0.12 | 27.6 | 5.2 |
| $I_2MF$ | 6 | 11.0 ± 3.5 | 24.7 ± 6.4 | 10.3 ± 1.5 | 2.42 ± 0.39 | 38.0 | 3.5 |
| $I_2M_2F$ | 3 | 9.9 ± 3.7 | 26.5 ± 8.9 | 12.1 ± 5.4 | 2.28 ± 0.32 | | |
| $Br_2MF$ | 6 | 8.6 ± 2.2 | 25.4 ± 6.8 | 11.3 ± 3.5 | 2.24 ± 0.21 | 18.0 | 5.6 |
| $Br_2M_2F$ | 6 | 12.6 ± 3.3 | 18.4 ± 4.1 | 8.0 ± 1.6 | 2.31 ± 0.31 | | |
| [$^{131}$I] rose bengal | 6 | 0.7 ± 0.2 | 7.8 ± 1.0 | 3.2 ± 1.1 | 2.44 ± 0.37 | | |
| [$^{203}$Hg] mercuric nitrate | 6 | 7.1 ± 3.4 | 15.5 ± 4.6 | 5.8 ± 2.0 | 2.67 ± 0.28 | | |
| [$^{3}$H] fluorescein | 5 | 0.15 ± 0.03 | 2.4 ± 0.5 | 1.7 ± 0.7 | 1.41 ± 0.11 | | |
| Tc-99m $PP_i$ | 6 | 2.2 ± 0.4 | 25.2 ± 9.2 | 12.8 ± 2.1 | 1.97 ± 0.24 | | |

* DM = (heat) damaged myocardium.
† % ID/g tissue.
‡ From Ref. 15.

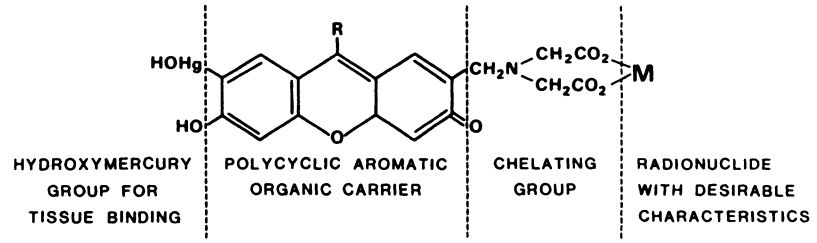


**FIG. 3.** Chemical features of hydroxymercurifluoresceins involved in proposed mechanism of binding.

tissue concentration and selectivity. The position of halide substituents, and the position and degree of hydroxymercuration, are factors considered in assessing the effect of structure upon selectivity for damaged tissue.

As Table 2 indicates, [$^{131}$I] rose bengal (3,4,5,6-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein) — which has no sulfhydryl-binding capability—shows a DM/normal myocardium ratio (selectivity) of 7.8 with only 0.7% ID/gDM (uptake). A similar situation exists with [$^{3}$H] fluorescein. They are capable of penetrating damaged tissue, but because they do not bind to the tissue, efflux also occurs, lowering the amount retained. [$^{203}$Hg] mercuric nitrate also shows a good DM/normal myocardium ratio of 15.5, but since it can bind covalently to exposed sulfhydryl groups, its % ID/gDM (7.1%) is ten times that achieved by rose bengal. All of the [$^{203}$Hg] hydroxymercurifluoresceins—whether halogenated or nonhalogenated, mono- or bis-mercurated—possess greater tissue selectivity than [$^{131}$I] rose bengal, [$^{203}$Hg] mercuric nitrate, or [$^{3}$H] fluorescein. There is an apparent cooperative effect whereby the organic moiety provides DM/normal myocardium selectivity at the cell-membrane level, and the mercuric prosthetic group makes irreversible binding possible once the agent is within the cell (see Fig. 3).

If one assumes that the % ID/gDM at 1 hr is a measure of tissue uptake, there appears to be no significant difference among the hydroxymercurifluoresceins relating to molecular structure. Uptake in the lesion is comparable to mercuric ion alone and is greater by a factor of ten than for the nonmercurated fluorescein rose bengal. All eight of the agents examined had similar blood/normal myocardium ratios (2.24–2.87), indicating that normal myocardium was incapable of discriminating between these exogenous compounds. The DM/normal myocardium values, therefore, reflect true selectivity toward damaged tissue and show that the nonhalogenated hydroxymercurifluoresceins MF and $M_2F$ are most likely to penetrate and bind to ischemic cells. Halogenation at either the 2′ or 4′ positions results in comparable decreases in tissue selectivity. Binding is not affected by either position or degree of mercuration, since compounds $I_2MF$ and $Br_2MF$, $I_2M_2F$, and $Br_2M_2F$ have statistically identical biologic profiles and the bis(hydroxymercuri)fluoresceins behave like the mono-hydroxymercurated analogs. Only one hydroxymercuri-group is necessary for, or involved in, binding to the cellular protein. There appears to be little advantage to bis-hydroxymercuration except that an agent of higher specific activity can be obtained.

A comparison of our results with those reported by Malek et al. (*15*) shows general agreement with regard to the order of selectivity of the agents. Our necrotic-tissue model, however, provides greater reproducibility, so that the data can generate meaningful correlations between the structures of the agents and their effectiveness. It is apparent that both the fluorescein structure and the hydroxymercuri-prosthetic group are necessary, since the six radiotracers that were examined were superior to [$^{131}$I] rose bengal, [$^{203}$Hg] mercuric nitrate, and [$^{3}$H] fluorescein as DM-seeking agents. Halogenation of the hydroxymercurifluorescein at either the 2′,7′ or 4′,5′ positions results in a decreased selectivity, whereas the position or degree of hydroxymercuration is not a significant factor. The mono(hydroxymercuri) fluoresceins have potential as MI-seeking agents and warrant further study in more realistic models. Because the radioisotopes of mercury have poor physical properties for gamma imaging, and mercuric compounds have an undesirable in vivo distribution, mercury-203-labeled agents are unlikely candidates for clinical use. This study suggests, nevertheless, that the hydroxymercurifluoresceins could provide a suitable carrier for nuclides that have better imaging characteristics and would deliver lower absorbed radiation doses (Fig. 3). Work in this direction is currently in progress.

ACKNOWLEDGMENTS

The authors wish to thank Alice Carmel for expert technical assistance and Rebekah A. Taube for her editorial work on the numerous drafts and the final manuscript.

This investigation was supported in part by USPHS, DHEW (NIH) Grants GM 18674, HL 17739, HL 11668, and by grants from the American Heart Association (75-796) and its Northeast Massachusetts Chapter Affiliate (1279).

FOOTNOTES

* Eastman Chromagram Sheets, Eastman Kodak Co., Rochester, N.Y.

† Schwartzkopf Microanalytical Lab., Woodside, N.Y.

REFERENCES


1. HOLMAN BL: Radionuclide methods in the evaluation of myocardial ischemia and infarction. *Circulation* 53: Suppl I:112–I:119, 1976

2. LOVE WD, ROMNEY RB, BURCH GE: A comparison of the distribution of potassium and exchangeable rubidium in the organs of the dog, using rubidium-86. *Circ Res* 2: 112–122, 1954

3. CARR EA, BEIERWALTES WH, WEGST AV, et al.: Myocardial scanning with rubidium-86. *J Nucl Med* 3: 76–82, 1962

4. CARR EA, GLEASON G, SHAW J, et al.: The direct diagnosis of myocardial infarction by photoscanning after administration of cesium-131. *Am Heart J* 68: 627–636, 1964

5. HOLMAN BL, DEWANJEE MK, IDOINE J, et al.: Detection and localization of experimental myocardial infarction with $^{99m}$Tc-tetracycline. *J Nucl Med* 14: 595–599, 1973

6. HOLMAN BL, LESCH M, ZWEIMAN FG, et al.: Detection and sizing of acute myocardial infarcts with $^{99m}$Tc(Sn) tetracycline. *N Engl J Med* 291: 159–163, 1974

7. PARKEY RW, BONTE FJ, MEYER SL, et al.: A new method for radionuclide imaging of acute myocardial infarction in humans. *Circulation* 50: 540–546, 1974

8. HOLMAN BL, TANAKA T, LESCH M: Evaluation of radiopharmaceuticals for the detection of acute myocardial infarction in man. *Radiology* 121: 427–430, 1976

9. ZWEIMAN FG, HOLMAN BL, O'KEEFE A, et al.: Selective uptake of $^{99m}$Tc complexes and $^{67}$Ga in acutely infarcted myocardium. *J Nucl Med* 16: 975–979, 1975

10. ABDULLA AM, CANEDO MI, CORTEZ BC, et al.: Detection of unstable angina by $^{99m}$Technetium-pyrophosphate myocardial scintigraphy. *Chest* 69: 168–173, 1976

11. MALEK P, ZASTAVA V, KOCANDRLE V, et al.: Aims and perspectives of topical diagnosis of tissue damage. *Cas Lek Cesk* 105: 273–279, 1966

12. KATSUYA H, KINOSHITA T, DOI Y, et al.: Mercurochrome fluorescence in tumor tissue. *Nature* 198: 497–498, 1963

13. MALEK P, VAVREJN B, RATUSKY J, et al.: Further advances in topical diagnosis of ischemic lesions of the myocardium *in vivo*. *Cas Lek Cesk* 105: 1273–1278, 1966

14. RATUSKY J, KRONRAD L, MALEK P, et al.: Preparation of radioactive hydroxy-mercury derivatives of the fluorescein series applicable as IDRS. *Radiochem Radioanal Lett* 6: 63–71, 1971

15. MALEK P, RATUSKY J, VAVREJN B, et al.: Ischaemia detecting radioactive substances for scanning cardiac and skeletal muscle. *Nature* 214: 1130–1131, 1967

16. HUBNER PJB: Radioisotopic detection of experimental myocardial infarction using mercury derivatives of fluorescein. *Cardiovasc Res* 4: 509–514, 1970

17. SPAR IL, MICHAELSON SM, GREENHOUSE VY, et al.: Use of $^{203}$Hg-fluorescein (Mercurascan) in the detection of myocardial infarction (abstract). *J Nucl Med* 11: 362–363, 1970

18. RAMANATHAN P, GANATRA RP, DAULATRAM K, et al.: Uptake of $^{203}$Hg-hydroxymercury-fluorescein in myocardial infarcts. *J Nucl Med* 12: 641–643, 1971

19. DAVIS MA, HOLMAN BL, CARMEL AN: Evaluation of radiopharmaceuticals sequestered by acutely damaged myocardium. *J Nucl Med* 17: 911–917, 1976

20. ADLER N, CAMIN L, SHULKIN P: Rat model for acute myocardial infarction: Application to technetium-labeled glucoheptonate, tetracycline, polyphosphate. *J Nucl Med* 17: 203–207, 1976

21. KRONRAD L, HLADIK V: Preparation of some mercuri-derivatives of iodinated fluorescein labelled with iodine-131. *J Labelled Compd* 8: 293–304, 1972

22. BEAUFAY H, DEDUVE C: Tissue fractionation studies. 9. Enzymic release of bound hydrolases. *Biochem J* 73: 604–609, 1959