

**RAT MODEL FOR ACUTE MYOCARDIAL INFARCTION:  
APPLICATION TO TECHNETIUM-LABELED  
GLUCOHEPTONATE, TETRACYCLINE,  
AND POLYPHOSPHATE**

Norman Adler, Leopoldo L. Camin, and Peter Shulkin

*New England Nuclear Corporation, North Billerica, Massachusetts*

***Cauterization of rat myocardium serves as a quick (2 min) inexpensive technique to create an animal model of acute myocardial infarction useful in evaluating radiopharmaceuticals as potential clinical imaging agents. Preliminary evidence indicates that it correlates well with behavior in man. Application of the model led to the discovery, confirmed by later investigators, that chelating agents such as glucoheptonate and polyphosphate show significant uptake in recent myocardial lesions.***

The need for a specific diagnostic agent for myocardial infarcts and the potential of radiopharmaceuticals to solve this problem have long been recognized (1-5). Two main lines of inquiry have been explored. One approach is based on the fact that potassium and metabolites of the cardiac muscle are selectively concentrated in healthy heart muscle; radiopharmaceuticals based on these would be expected to show an infarcted or necrotic region as a "cold" (nonradioactive) spot in an otherwise "hot" (radioactive) area. Potassium-43 has, in fact, been used successfully (6), but its limited availability, poor decay characteristics, and high cost have led to a search for alternative radionuclides that would behave in a similar physiologic manner. Monovalent cations close to  $K^+$  in size are the most direct analogues, and variable success has been attained with  $^{129}Cs$ ,  $^{81}Rb$ , and  $^{14}NH_4^+$ . Among this class, thallium, recently proposed by Kawana (7) and Lebowitz (8), has yielded good results in studies to date (9) and  $^{201}Tl$  is now available from a commercial source. Furthermore, long-chain fatty acids are extracted by

the heart muscle. The studies of Bonte (10) and Robinson (11) with radioiodinated oleic acid have shown some promise in this area.

The second line of inquiry is to seek radioactive agents that are preferentially concentrated in the myocardial lesion as opposed to normal myocardium (hot spot in a cold area). Following Malek's original observation that tetracycline localized in infarcted tissue and his attempts to prepare a radioiodinated analogue of tetracycline (12), Holman, Davis, et al have recently reported that  $^{99m}Tc$ -labeled tetracycline permits the detection of experimentally produced myocardial infarcts in dogs (13) and actual infarcts in man (14,15).

Progress in this area has been severely hampered by the difficulty, expense, and time required to induce myocardial infarcts experimentally in animals. Models based on dogs, pigs, and rabbits, as reported in the literature, have indicated that, even with highly specialized techniques and equipment, the mortality rate of the test species has been high and progress painstakingly slow. The need for a suitable low-cost animal model in which myocardial infarction can be rapidly and reliably induced was clearly apparent and was the main initial objective of this study. Subsequent application of the research tool that we developed led to the discovery that  $^{99m}Tc$ -glucoheptonate and other metal complexing agents show promise as agents for the detection of acute myocardial infarcts.

Received April 7, 1975; revision accepted Sept. 25, 1975.

For reprints contact: L. L. Camin, New England Nuclear Corp., Radiopharmaceutical Div., Research Dept., North Billerica, Mass. 01862.

## MATERIALS AND METHODS

**Experimental.** The rat was selected as the species in which to study the experimental induction of myocardial infarcts in view of its ready availability and low cost. Male white rats, CD strain (Charles River Breeding Laboratories), weighing 150–300 gm, were used in our laboratories. The suitability of  $^{99m}\text{Tc}$ -tetracycline as the reference agent is shown by the work of Holman et al (13–15).

**Procedure.** The rats are anesthetized with ether and secured to a dissection board, and a 3-cm longitudinal incision is made in the thorax, 2 cm to the left of the midline. The heart is exposed by subperiosteal resection of the fourth and fifth ribs. Several overlapping burns are made on the left ventricular wall by gently applying a 2-mm-tipped soldering iron (Wahl Iso-Tip, Clipper Co., Sterling, Ill.) to the epicardium. The affected area is approximately 2–4 mm in diameter. The incisions are closed by stapling together the muscle and then the skin. The entire operation (opening, cauterizing, closing) must be completed in under 2 min. The rat is then placed in a cage where it can be observed during recovery. No impairment or change in behavior has been noticed in these rats.

The syringe containing the radiopharmaceutical is counted in an ionization chamber immediately before injection. The animal is anesthetized and secured to the dissection board as before, and 0.25 ml of the radiopharmaceutical is injected into an exposed femoral vein. The thigh incision is then stapled. The syringe is counted again in the ionization chamber and the injected dose (ID) is determined from the difference. After an appropriate interval, the animal is anesthetized and decapitated with a laboratory guillotine, and the blood is collected in a preweighed cup. The heart and other organs of interest are excised. The area of infarction, seen as a reddened area on the surface of the heart, is carefully excised

using microscalpels. A slice of myocardium approximately 1 mm thick is cut around the periphery of the excised section to measure the activity concentration over the infarcted-to-normal transition zone. These samples and the remainder of the heart are then counted in an ionization chamber or scintillation counter and weighed. The average infarct weight in a 200–300-gm animal is 40–60 mg and the weight of the total heart muscle is 600–900 mg. After calculating the concentration of activity in each tissue in units of percent injected dose per gram of tissue, the contrast ratios of infarcted myocardium (MI) to normal myocardium (NM), to blood, to liver, and to total gastrointestinal tract (GI) may be readily calculated.

All the animals treated by the above procedure (well over 100) survived the initial operation; some have been kept alive up to 9 months without pharmacotoxic manifestations. With this procedure, the agent could be injected at any time before or after the infarction. A further variation of this procedure, creation of a zone of damage by a cryothermic process, has been considered and warrants further investigation.

## RESULTS

**Validation of the rat model.** The cauterized rat model was initially assessed by trial with  $^{99m}\text{Tc}$ -tetracycline, an agent known to concentrate in myocardial infarcts in man. The results, listed in Table 1, show that the agent is preferentially concentrated in the experimentally produced lesion. Although the contrast ratios are not exceptionally high, their magnitudes are distinctly above the level of experimental error.

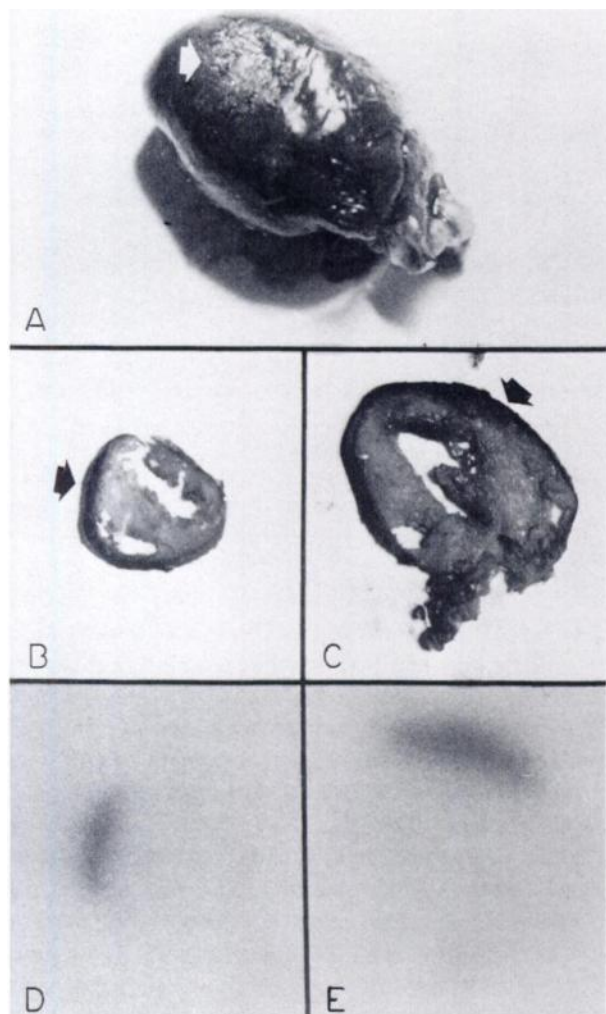
**Autoradiography.** To obtain further validation of the rat model, visualization of localized uptake within the infarcted myocardium was attempted by autoradiography. Infarcted rats were injected with

TABLE 1. COMPARATIVE MYOCARDIAL INFARCT UPTAKE OF  $^{99m}\text{Tc}$ -TETRACYCLINE ANALOGUES\*

	Tetracycline	Demethylchlor-tetracycline	Chlortetracycline	Oxytetracycline	Deoxytetracycline
Percent of Injected Dose (% ID)					
Total blood	2.7 ± 0.3	2.7 ± 0.2	3.2 ± 0.2	3.0 ± 0.3	2.7 ± 0.1
Total heart	0.09 ± 0.02	0.08 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Myocardial infarct (MI)	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Contrast Ratio†					
MI/NM	10.5 ± 2.9	9.2 ± 1.4	9.4 ± 0.8	11.4 ± 3.7	8.6 ± 2.2
MI/blood	4.0 ± 0.7	3.7 ± 0.6	5.0 ± 0.6	4.9 ± 2.0	3.9 ± 1.3
MI/liver	6.2 ± 3.2	4.6 ± 0.6	4.1 ± 0.5	8.1 ± 2.7	5.3 ± 1.6
MI/GI (total)	4.3 ± 2.3	2.8 ± 0.7	4.0 ± 1.0	5.8 ± 1.5	3.5 ± 0.8

\* Mean ± 1 SD of five rats. Animals injected 3 hr after infarction and killed 1 hr after injection.

† Ratio of % ID/gm of infarcted myocardium to % ID/gm of other tissues.



**FIG. 1.** Induced lesions (arrows) are shown on epicardium of left ventricular wall (A). Transverse (B) and longitudinal (C) sections of excised heart are shown with corresponding autoradiographs (D,E). Areas of increased activity clearly correspond to lesion areas.

$^{99m}\text{Tc}$ -glucoheptonate. At the time of death (1 hr after injection), the excised heart typically contained about 40  $\mu\text{Ci}$  (0.07% of the injected dose). The heart was dried for 15 min at 70°C, cooled, and embedded in Tissue Prep (Fisher Scientific Co., No. T565). Sections of the heart, 500  $\mu\text{m}$  thick, were cut by microtome, mounted on white paper, and covered with a strip of film (Kodak x-ray film, RP Royal X-Omat). Exposure times of about 2–10 hr proved suitable.

Figure 1 compares the visually observable myocardial lesion before sectioning with the autoradiographs of selected sections. The transverse autoradiograph (D) shows a transmural region of increased activity corresponding to the experimental lesion. The longitudinal autoradiograph (E) shows a similarly darkened area in the damaged region. These results provide visual evidence that the agent is selectively concentrated in the experimental lesion.

**Nature of lesion.** To derive further insight into the cauterized model, studies were made to assess the blood flow to injured tissue relative to normal tissue. The distribution of  $^{201}\text{Tl}$  10 min after intravenous injection into a rat with a 4-hr-old infarct is shown in Table 2. Significantly reduced accumulation is seen in the damaged zone, consistent with reduced blood flow. The distribution of  $^{99m}\text{Tc}$ -macroaggregated albumin at 10 min after injection in the left ventricle of an infarcted rat is also shown in Table 2. This result confirms that coronary blood flow to the cauterized area is significantly reduced, further suggesting that the behavior of the lesion is consistent with that of an infarct.

**Application to other potential agents.** The rat model permitted rapid assessment of other compounds as potential infarct-imaging agents. As an obvious first step, a number of tetracycline analogues were investigated. All formulations were based on stannous ion and were checked by *in vitro* radiochromatography to ensure less than 5% of free  $^{99m}\text{Tc}$ , and by both *in vitro* radiochromatography and *in vivo* liver uptake to ensure freedom from radiocolloid. The latter may readily form during the process of labeling tetracycline and its analogues unless care is taken. Under the conditions investigated, our results (Table 1) do not indicate any important differences among the analogues, although there is a suggestion that oxytetracycline is marginally preferable. The same conclusion was reached independently after a similar study in dogs (16).

A second study explored the hypothesis that infarcted tissue concentrates calcium at or within the myocardial cell membrane, which may, in turn, interact with a radiolabeled complexing agent present in the vascular pool surrounding the infarct (17,18). As Tables 1 and 3 show, a number of known calcium-complexing agents, particularly polyphosphate

**TABLE 2. COMPARATIVE MYOCARDIAL INFARCT UPTAKE OF  $^{201}\text{Tl}$ \* AND  $^{99m}\text{Tc}$ -MACROAGGREGATED ALBUMIN (PULMOLITE<sup>TM</sup>)\***

	% ID/gm MI	Ratio of Activity Concentration†	
		MI/NM	MI/blood
$^{201}\text{Tl}$ ‡	0.52 ± 0.08	0.22 ± 0.05	14 ± 2
$^{99m}\text{Tc}$ -MAA	3.9 ± 0.8	0.41 ± 0.07	31 ± 4

\* New England Nuclear Corporation.

† % ID/gm for myocardial infarct to % ID/gm for other tissues (ID = injected dose).

‡ Average ± range of two rats. Animals killed 4 hr after induction of infarct and 10 min after intravenous injection.

|| Mean ± 1 SD of three rats. Animals killed 4 hr after induction of infarct and 10 min after injection in the left ventricle.

TABLE 3. COMPARATIVE MYOCARDIAL INFARCT UPTAKE

	% ID/gm MI	Ratio of Activity Concentration*†			
		MI/NM	MI/blood	MI/liver	MI/GI (total)
Polyphosphate‡	2.3 ± 0.2	25.2 ± 8.3	15.9 ± 5.3	4.8 ± 3.4	39.3 ± 12.5
Glucoheptonate	1.0 ± 0.2	15.6 ± 7.2	6.9 ± 4.8	7.3 ± 6.3	2.8 ± 2.1
D-Galacturonic acid	1.2 ± 0.4	9.4 ± 1.7	13.6 ± 3.2	14.6 ± 4.9	5.4 ± 1.2
α-D-Glucoheptonic γ-lactone	0.8 ± 0.2	9.0 ± 2.7	4.9 ± 1.6	6.7 ± 2.7	4.3 ± 1.4
D-(+)-Glucuronic acid	0.1 ± 0.0	5.7 ± 0.4	1.2 ± 0.5	1.5 ± 0.6	0.6 ± 0.3
Erythromycin glucoheptonate	1.2 ± 0.2	5.5 ± 2.1	4.6 ± 2.2	8.4 ± 2.9	3.9 ± 1.4
D-Galactono γ-lactone	1.0 ± 0.1	5.1 ± 0.8	1.6 ± 0.3	3.7 ± 1.2	5.0 ± 1.2
Glycolic acid	1.1 ± 0.7	3.6 ± 2.0	1.3 ± 0.6	1.5 ± 1.2	4.1 ± 3.4
Diethylenetriamine penta-acetic acid (DTPA)	0.06 ± 0.0	3.7 ± 0.5	0.8 ± 0.5	1.4 ± 0.7	1.0 ± 0.5
L-(-)-Malic acid	0.3 ± 0.1	3.5 ± 0.7	0.9 ± 0.2	2.5 ± 0.6	1.3 ± 0.4

\* % ID/gm myocardial infarct to % ID/gm for other tissues (ID = injected dose).  
† Mean ± 1 SD of three rats. Animals injected 3 hr after infarction and killed 1 hr after injection.  
‡ New England Nuclear Kit, consisting of 10 mg pyrophosphate and 30 mg trimetaphosphate.  
|| New England Nuclear Kit.

and glucoheptonate, show significantly better contrast ratios than tetracycline and its analogues at the particular times studied. However, DTPA, also a strong calcium-complexing agent at physiologic pH, shows little uptake in the infarct. Although the rapid blood clearance of DTPA may account for this in part, we note that glucoheptonate is also cleared quite rapidly from blood.

**Comparison in dogs.** The results of our studies using the rat model for screening myocardial infarcts have since been supported by independent studies in dogs. Zweiman et al (20) repeated their work on tetracycline in comparison to glucoheptonate (Table 4). Additional dog studies have confirmed the high uptake for <sup>99m</sup>Tc-glucoheptonate (19–21) and <sup>99m</sup>Tc-pyrophosphate (22–24).

#### DISCUSSION

The guiding premise of this work was that an acceptable animal model for evaluating potential myo-

cardial-infarction agents did not have to rely on specific vascular occlusion to create an infarct (25): the important issue was whether a lesion that would simulate human ischemic-infarction defects could be reproducibly induced sufficiently well to permit its use as a screening tool. Since any animal model invokes the risk of species differences in collateral coronary circulation, thus distorting the results, the need to test the rat model with an agent known to concentrate in myocardial infarcts in man was clearly apparent. The current model met this test for <sup>99m</sup>Tc-tetracycline, the only <sup>99m</sup>Tc agent proved to yield useful images of myocardial infarction in man at the time of this work.

Our model's rating of a number of agents, particularly glucoheptonate and polyphosphate, as superior to tetracycline in terms of their concentration ratios for myocardial infarcts illustrates the method's considerable promise. Both agents, in clinical trial for other indications (glucoheptonate for kidney and brain imaging, polyphosphate for bone imaging), have since been shown to concentrate in fresh myocardial infarcts in man (9,26–28). Although polyphosphate may have an advantage in its higher contrast ratio, it also has a significant disadvantage for this indication in that its high uptake in the ribs may tend to mask the heart unless special instrumentation and viewing angles are employed (28). Glucoheptonate, on the other hand, while having a less advantageous contrast ratio, possesses the advantage of very rapid blood clearance and little uptake in normal organs other than the kidney.

#### ACKNOWLEDGMENTS

The authors wish to express their appreciation to Frank Sheeley and Garth Brown for the preparation and analysis of the tetracycline compounds; to Charles Pfizer Inc. for the samples of oxytetracycline and deoxytetracycline; to

TABLE 4. COMPARATIVE MYOCARDIAL INFARCT UPTAKE OF <sup>99m</sup>Tc-GLUCOHEPTONATE AND <sup>99m</sup>Tc-TETRACYCLINE IN DOGS\* (20)

Agent	% ID/gm MI	Ratio of Activity Concentration†			
		MI/NM	MI/blood	MI/liver	MI/bone
Tetra-cycline	3.3 × 10 <sup>-3</sup>	6.8 ± 3.2	3.2	0.5	8.5
Glucohep-tonate	4.3 × 10 <sup>-3</sup>	11.2 ± 6.0	5.4	0.7	9.5

\* Mean of five dogs. Animals injected 24 hr after infarction and killed 24 hr after injection.

† Ratio in cpm/gm for infarcted myocardium to cpm/gm for other tissues.

|| Mean ± 1 SD of five dogs.

B. Holman and F. G. Zweiman for their data on dogs; and to L. Rosenthal and H. W. Strauss for their comments on glucoheptonate.

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