Ischemia and Infarction in the Isolated Rabbit Heart: A Model for the Evaluation of Myocardial Imaging Agents

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An isolated-heart preparation has been adapted to permit rapid evaluation of, and imaging by, myocardial tracers. The rabbit heart provided a mass large enough for imaging and serial biopsies. Coronary arteries could be selectively ligated and provided landmarks for epicardial ST segment mapping. Uptake ratios between a tissue sample and normal myocardium (T/N), obtained using Tc-99m glucoheptonate as an infarct-seeker, increased with duration of the ischemic period, which was followed by reflow. After 25 min of occlusion the T/N was 4.5; after 40 min T/N = 6; after 60 min T/N = 8; and after 120 min T/N = 19.

This well-controlled adjunct to in vivo studies allows evaluation of myocardial imaging agents without interfering with systemic effects or blood and tissue background. Functional and structural changes can be sequentially measured and correlated with the localization of various radiopharmaceuticals.

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The introduction of myocardial imaging by Carr and colleagues in 1962 (*1,2*) has stimulated a vigorous research effort due to the potential of this technique as an essentially noninvasive method for the diagnosis, location, and quantification of ischemic and infarcted myocardium. Much effort has been directed toward evaluating the usefulness of various radionuclides and radiopharmaceuticals in imaging either healthy myocardium or damaged myocardium. Concomitantly, attempts have been made to define the mechanisms by which these materials are selectively concentrated, using a number of in vivo and in vitro models. These range from chronic studies of myocardial infarction in dogs (*3*) to tissue cultures subjected to thermal and/or anoxic insults (*4*).

The work to be described demonstrates the suitability of an isolated rabbit heart model for the investigation of myocardial infarct-imaging agents. The use of this model circumvents many of the problems of such research. The model allows measurement and control of perfusate temperature, pH, substrate, gas and pharmacologic content, as well as heart rate, left ventricular (LV) end-diastolic pressure, and coronary artery flow or perfusion pressure. It permits the creation of either prolonged or transient global or regional ischemia, continuous monitoring of hemodynamic and metabolic functions, and serial electrocardiographic mapping. The rabbit heart has multiple, readily accessible and well-defined epicardial coronary arteries, and its left-ventricular mass is sufficient to allow scintillation imaging with standard equipment and to permit serial LV biopsies. Although the heart in our model contracts isovolumetrically, the system may readily be adapted for study of the ejecting heart.

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METHODS

The apparatus used (Fig. 1) was essentially that described by Kligfield et al. (*5*), scaled to the rabbit heart. The major addition was the use of a mobile gamma scintillation camera for imaging.

The perfusate was a modified Krebs–Ringer bicar-

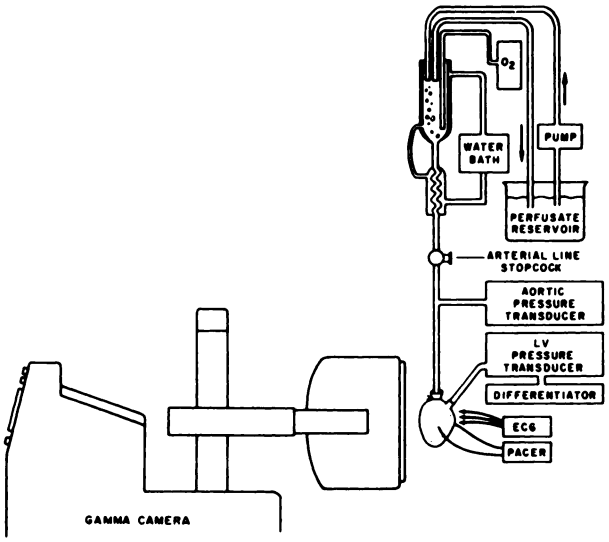

FIG. 1. Apparatus for the perfusion of isolated rabbit heart, with gamma scintillation camera in position for imaging.

bonate solution (KRB), similar to that suggested by Apstein et al. (*6*) modified by using 0.6 m*M* $MgSO_4 \cdot 7H_2O$ and omitting the lactate.

Male, New Zealand white rabbits, weighing between 3 and 4 pounds, were given 1000 units of heparin intravenously 5–15 minutes before sacrifice. The animal was then fastened to a dissection board, killed by a blow to the head, and the heart and aortic root were rapidly excised and placed in ice-cold KRB. The aorta was secured to the perfusion cannula, and within 3 min of opening the thorax, perfusion of the coronary arteries was begun at an average perfusion pressure of 100 cm H_2O.

After several ventricular beats, the left atrial appendage was removed and a deflated latex balloon, produced from an LV mold and tied to the end of a polyethylene catheter, was inserted through the mitral valve into the left ventricle. Once in place the balloon was expanded with water until the LV end-diastolic pressure was between 3 and 5 mm Hg. Pressure-volume curves were obtained for each balloon before insertion to ensure that at operating volume the balloon would be flaccid, with no pressure differential across its wall. A polyethylene catheter, tied into the pulmonary artery, was either connected to a micro-oximeter for continuous measurement of venous oxygen tension or was allowed to drain for venous effluent collections and coronary flow measurements. Arterial perfusate samples were taken from, and all injections made into, the arterial-line stopcock approximately 10 cm above the heart (Fig. 1).

The procedures described above required 5–10 min. Perfusion of all hearts was continued for the remainder of a 20-min *control period.* During this time, each heart was evaluated for rate and regularity of contraction and LV pressure development. Preparations that did not demonstrate regular sinus rhythm and an LV peak systolic pressure of more than 80 mm Hg during the control period were considered inadequate and discontinued. Regularly beating hearts with intrinsic rates below 150 beats per min (BPM) were demand-paced, from the right ventricle, at 150 BPM during both control and ischemic periods.

Following the control period, hearts were subjected to either further *control perfusion* or *regional ischemia* and *reflow.* The effect of these maneuvers on the myocardial uptake of Tc-99m glucoheptonate (TcGH)—a radiopharmaceutical with established affinity for damaged myocardium soon after the onset of ischemia (*7*)—was then studied by scintillation imaging and well counting of tissue samples. Histochemical changes were demonstrated grossly by staining heart slices with nitro blue tetrazolium (NBT), a formazan dye that forms a deep-purple precipitate when reduced by the dehydrogenases active in normal myocardium, whereas infarcted areas remain unstained (*8*). The sharp delineation between viable and necrotic myocardium that this method provides has recently been confirmed by electron microscopy (*9*). Hearts were fixed for light or electron-microscopic study by substituting McDowell-Trump fixative (1% glutaraldehyde and 10% formalin in sodium phosphate buffer, osmolarity = 200 mOsm) for the KRB perfusate at the end of the experiment and perfusing the vascular system for approximately 5 min at 100 cm H_2O pressure. Following this, small LV samples were taken and kept overnight in the same fixative. The following day, tissues to be processed for electron microscopy were washed in 4% sucrose in 0.1 *M* cacodylate buffer, post-fixed with cold 1% osmium tetroxide in 0.1 *M* cacodylate buffer, dehydrated, and embedded in Lufts Epon. Ultrathin sections were stained with aqueous uranyl acetate and lead citrate and examined with an electron microscope. Sections to be examined by light microscopy were routinely processed for paraffin embedding and were stained with hematoxylin and eosin.

Control perfusions. In order to document the stability of this preparation, as a function of perfusion time, 15 hearts were perfused for 1(2),* 2(5), 2.5(3), 3(3), 3.5(1), or 4.5(1) hr following the initial 20-min control period. In each of these hearts LVP was monitored continuously to assess functional integrity, and serial scintigrams were taken following injections of 1–4 mCi of TcGH. At the end of each experiment, hearts were cut into 2–3 mm slices, in a plane parallel to the atrioventricular groove, for histochemical (NBT) and additional scintigraphic study. Transmural sections from the septal, anterior, lateral, and posterior LV walls of these slices were then taken for scintillation well counting to measure the normal variations in LV TcGH activity.

Perfusate flow studies, examining the distributions of Sr-85-labeled microspheres (15 ± 5 μ) and Tl-201, were done in two hearts in order to assess regional myocardial perfusion. One heart, perfused for a total of 60 min, was

given a 5-μCi bolus injection of Tl-201 after 28 min of perfusion, and a 1.5 nCi bolus injection of ~16,000 Sr-85 microspheres after a total of 58 min. Two minutes later the experiment was terminated by a 5-min perfusion with McDowell-Trump fixative. The second heart was given the same Tl-201 treatment, but the microspheres were injected at 178 min. This experiment was also terminated 2 min later with McDowell-Trump fixative. Samples from each heart were taken for well counting by differential spectrometry. The integrity of the myocardium following these periods of perfusion was compared, at the electron-microscopic level, with sections from hearts fixed in situ by aortic root perfusion with McDowell-Trump fixative.

Regional ischemia and restored flow. In a group of ten hearts we studied the effect of varying durations of coronary artery occlusion, followed by reflow, on TcGH accumulation. After the standard control period, each heart underwent a 25-, 40-, 60-, or 120-min period of ligation of either the anterior descending (LAD) or diagonal branch (LDB) of the left coronary artery, and 120 min of reflow. Reversible coronary artery ligation was achieved by passing a 5-0 silk suture under the vessel and tying it over a piece of PE-50 polyethylene tubing placed longitudinally over the vessel. The tubing served to protect the vessel and facilitate removal of the ligature. Immediately after removal of the ligature to restore flow, a 4 liter volume of KRB perfusate containing 11 m*M* dextrose was substituted for the usual KRB solution containing 5.5 m*M* dextrose, and a 4-mCi bolus of TcGH was injected into the arterial line. Venous effluent immediately preceding the injection, and all of that following it, was then added, with constant mixing, to the 4-liter perfusate and recirculated. This allowed prolonged perfusion of the heart with KRB containing ~1 mCi of TcGH per liter of perfusate. The dextrose content of the KRB was doubled to assure adequate substrate levels during recirculation. The pH and pO_2 of the recirculating perfusate were identical to values measured initially (pH 7.3-7.4, pO_2 550-600 mm Hg).

Scintigrams of the beating heart and heart slices were obtained at the end of each study. Slices were then stained with NBT and photographed, and well counting was done on transmural samples from stained slices designated as "infarct," "adjacent-to-infarct," or "distant-from-infarct" according to NBT staining criteria. Tissue stained by NBT and containing the lowest cpm/g (wet) was designated as normal. Tissue sample-to-normal (T/N) ratios were then calculated by dividing cpm/g of the various transmural samples by the normal cpm/g.

Epicardial ECG mapping and LV biopsies. Two studies were done to determine the feasibility of documenting local myocardial damage by epicardial electrocardiographic mapping in the isolated rabbit heart. Up to 12 small platinum electrodes were fastened to the LV epicardium (Fig. 6). A reference electrode was fastened to the epicardium of the right ventricle, and the stainless steel perfusion cannula, tied into the aortic root, served as ground. Hearts used in these studies were not paced. Control electrocardiographic maps were obtained at the end of the control period and these tracings were compared with those made after a 15-min occlusion of the LDB.

Serial transmural LV biopsies were taken during perfusion of four hearts, to study their effects on LV rhythm and performance. After the initial control period, the LV balloon catheter was temporarily deflated and the myocardial wall punctured with a 2-mm trephine. This was done every 30 min for 2 hr over both the anterior and lateral LV walls. Care was taken to avoid epicardial arteries, and the biopsies were taken sequentially from the apex toward the base to avoid interrupting the arterial supply to subsequent biospy sites. In two hearts, obvious perfusate leakage required ligation of a cut artery with 5-0 silk.

RESULTS

Control perfusion. Functional, scintigraphic, histochemical, and electron-microscopic data from control hearts all indicated that there was minimal loss of integrity in the model over the range of perfusion times studied. Function, as measured by heart rate and LVP, did not vary more than 20% from initial control values in any of the hearts studied. The maximum change observed in LV end-diastolic pressure was an increase of 5 mm Hg after 4.5 hr of perfusion.

Scintigrams of beating control hearts (Fig. 2A) and heart slices (Fig. 2B) showed homogeneous uptake of TcGH throughout the LV. Well counting of transmural LV samples showed a maximum cpm/g variation of 60% around the circumference of the LV, i.e., the greatest ratio of high cpm/g to low cpm/g found in any of these hearts was 1.6.

In agreement with these functional, electron-micro-

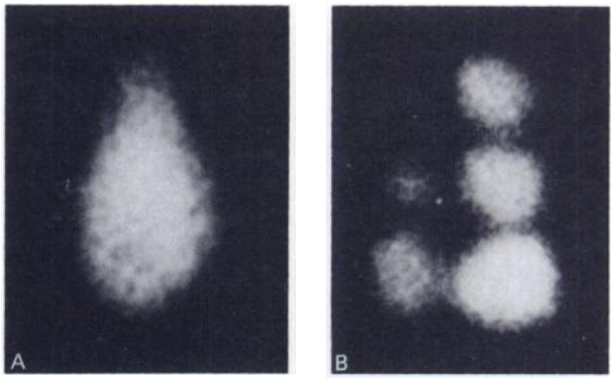

FIG. 2. Control anterolateral TcGH scintigrams: (A) beating isolated rabbit heart, and (B) heart slices made in a plane parallel to atrioventricular groove at end of study.

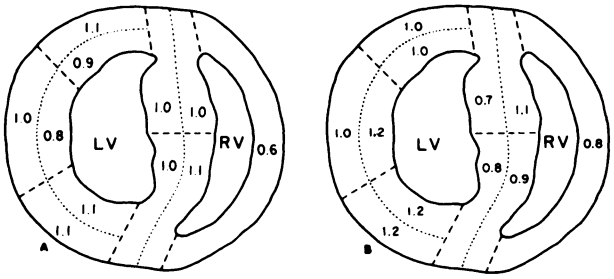

FIG. 3. Regional myocardial perfusate flow in isolated rabbit heart following 3 hr of perfusion, as indicated by distributions of (A) thallium-201, and (B) strontium-85 microspheres. Values shown represent ratio of cpm/g (wet weight) of myocardium in each area to average activity over left ventricle and interventricular septum.

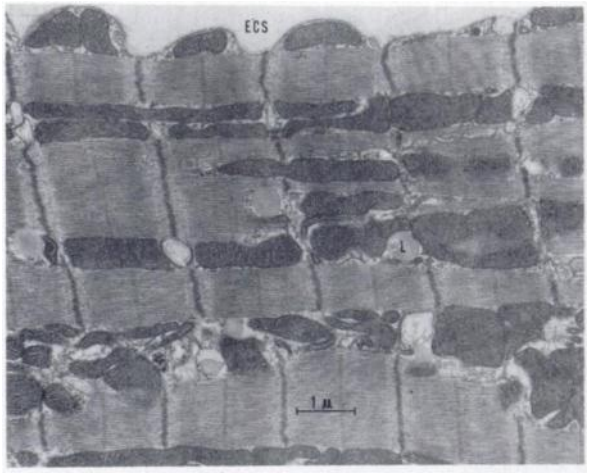

FIG. 4. Electron micrograph of rabbit myocardium (× 23,419) perfused for 3 hr at 37°C with modified, oxygenated, Krebs–Ringer bicarbonate solution. There is moderate expansion of extracellular space (ECS) and occasional lipid droplets (L).

scopic, and scintigraphic findings, all control slices stained homogeneously blue with NBT, implying viable myocardium.

Figure 3 shows the distributions of Sr-85 microspheres and Tl-201 in a heart perfused for 180 min. Both of these blood-flow markers were homogeneously distributed throughout the myocardium after 1 (not shown) and 3 hr of perfusion. The ultrastructural appearance of myocardium fixed after both 1 (not shown) and 3 hr of perfusion (Fig. 4) was essentially that of myocardium fixed in vivo, except for moderate expansion of the extracellular space and the presence of occasional lipid droplets.

Regional ischemia and reflow. None of the hearts subjected to 25 min of coronary artery occlusion, followed by reflow, showed damage detectable by NBT staining or uptake of TcGH by scintigraphy. Light and electron-microscopic comparison of normal myocardium from the ventricular septum and myocardium supplied by the occluded vessel also showed minimal structural change. Well counting done on transmural LV samples, however, showed T/N ratios of up to 4.5 in the myocardium supplied by the occluded vessel. This is well outside of the normal T/N range.

A single heart subjected to 40 min of coronary artery occlusion and 120 min of reflow did not show areas of increased TcGH uptake in scintigrams of the beating heart, or in LV cross sections. There were, however, small patches of LV myocardium where NBT stained less intensely than in surrounding tissue. Well-counting data from such areas showed a T/N ratio of 6.0. Samples of surrounding myocardium had T/N ratios of 1.5–3.0. Light microscopic examination of whole cross sections showed cytoplasmic hypereosinophilia and pyknotic nuclei consistent with coagulative necrosis in the areas lightly stained by NBT.

Ligation of either the LAD or LDB for 60 min, followed by 120 min of reflow, produced easily discernible areas of increased activity on scintigrams of the beating heart and LV cross sections (Figs. 5A and B). This myocardial uptake of TcGH corresponded grossly to areas of the anterolateral wall of the LV unstained by NBT (Fig. 5C). Well-counting data on tissue from the damaged area showed a T/N ratio of 8.0 whereas the adjacent myocardium had a T/N ratio of 5.4.

Results after 120 min of coronary occlusion, followed by reflow, were similar to those obtained after 60 min of

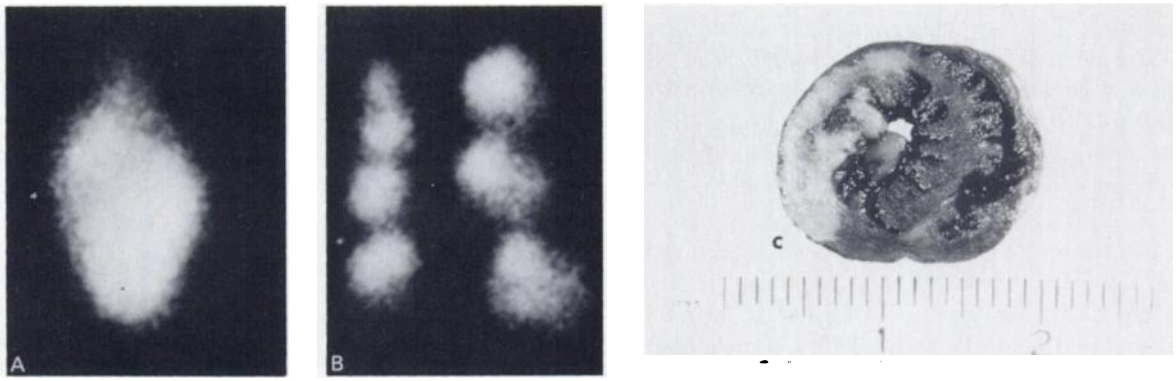

FIG. 5. Scintigrams of (A) beating heart, and (B) heart slices, following 60 min of coronary ligation and 120 min of reflow. (C) Slice as in (B) stained with NBT, which stains normal myocardium purple (black in photograph) and leaves infarcted tissue unstained (*10*).

occlusion with the important exception that in the former the myocardium from the area unstained by NBT had a T/N ratio of 19.0

The T/N ratios for all of these hearts are summarized in Table 1.

Epicardial ECG mapping. Control and postligation epicardial ECG maps are shown in Fig. 6. Inverted T waves and elevation of ST segments were observed over the LV area supplied by the occluded vessel after 15 min of occlusion (Fig. 6B). No dysrhythmias or injury currents were observed in control tracings (Fig. 6A) indicating that electrode placement did not introduce artifact.

LV biopsies. The biopsy procedure did not cause dysrhythmias in any of the hearts studied. There was an average decrease of 19% in LV peak systolic pressure (LVPSP) after 2 hr of perfusion and four biopsies in the four hearts studied (mean control LVPSP = 94 mm Hg; mean final = 76 mm Hg). Six control hearts, without biopsy, showed an average decrease of 14% in LVPSP (mean control = 86 mm Hg; mean final = 74 mm Hg) over this same period. This indicates that transmural samples weighing approximately 10 mg may be obtained sequentially during perfusion, with minimal disruption of function.

DISCUSSION

Previous work by Weiss et al. (*10*) demonstrated the use of an isolated rabbit-heart preparation in imaging areas of decreased activity with C-11-labeled palmitate. Our data further demonstrate the utility of this model. It permits measurement of functional and metabolic changes at the organ level as well as at the cellular, which tissue cultures do not do. Unlike many in vivo ischemic models, our model uses a common and inexpensive animal of specified age, sex, size, and strain. The instrumentation and technical skills required are minimal. These factors not only reduce the time and expense of investigation, they also increase the reproducibility of experimental conditions in different laboratories. The perfusate and ischemic injury can be varied to degrees that may not be compatible with life in the whole animal. The model can be made more physiologic by conversion to an ejecting heart preparation and by using plasma or whole blood for perfusion. Comparisons of uptake in the presence and absence of plasma protein may well be helpful in understanding the binding mechanisms of these blood-borne substances (*11*). Unlike other in vitro techniques, this model permits the creation of *ischemia*, avoiding the necessity for making inferences about ischemic damage from thermal or anoxic insult alone.

Interstitial edema, ascribable to the salt-solution perfusate (*12*), can be overcome by the addition of dextran or protein colloid to the perfusate, or by the use of whole blood. However, perfusate flow studies with Tl-201 and Sr-85 microspheres indicated that the edema observed in this preparation does not interfere with regional perfusate flow.

Although identical to the in vivo ischemic change described in Ref. *9*, the lipid droplets seen after 3 hr of control perfusion in our model probably result from slight tissue hypoxia due to poor oxygen transport by the perfusate. This change, by itself, does not indicate significant structural damage.

Coronary artery ligation for periods of 40 min or more, followed by release, consistently produced infarcts in the isolated rabbit heart, in agreement with previous in vivo canine studies (*13*). Reflow also optimized one of the two prerequisites for the uptake of a flow-dependent agent, namely, access to the target tissue. This would help to account for the consistent and graded nature of the T/N ratios in Table 1.

The disparity between the well-counting data and the

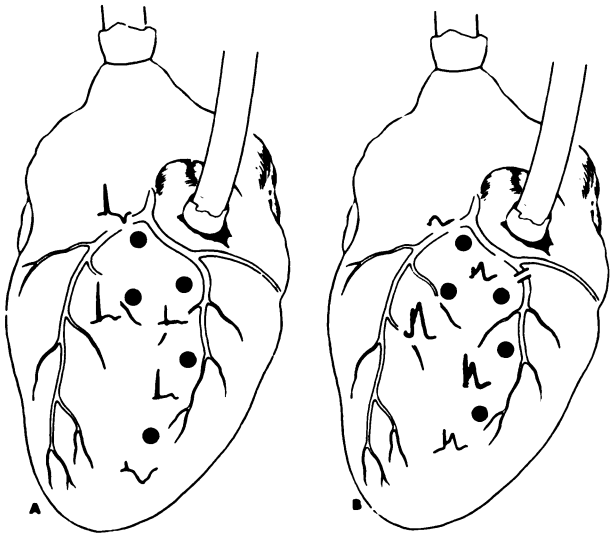

FIG. 6. Epicardial ECG recordings (A) at end of control period and (B) after 15 min of coronary occlusion (‖) in the same heart.

TABLE 1. TcGH RATIOS BETWEEN EXPERIMENTAL MYOCARDIUM AND NORMAL*

Treatment		Tissue-to-normal ratio		
Minutes of coronary occlusion	Minutes of reflow	Distance from infarct	Adjacent to infarct	Infarct center
25	120	1.2†	—	4.5†
40	120	1.5	3.0	6.0
60	120	1.4	5.4	8.0
120	120	1.4	3.3	19.0

* Each in cpm/g (wet weight): "normal" is lowest cpm/g found in area stained with nitro blue tetrazolium.

† "Central" and "distant" areas as judged by epicardial distribution of occluded vessel.

results from TcGH imaging can be attributed, in part, to poor collimation. Although the beating of the heart works against resolution, a higher-resolution collimator could offer some improvement.

The limitation most peculiar to this model is its relatively short "life span." Because it is practical to continue perfusion for only a few hours (up to 4 hr in our experiments) the preparation may be unsuitable for agents that are best administered several hours or days after in vivo infarction. It will probably be most useful for the preliminary screening of tracers expected to localize in damaged myocardium soon after the onset of ischemia. Myocardial cells die progressively as a function of the duration of the insult (*13,14*), and surrounding the developing infarct, for several hours, there is a population of damaged cells that can still recover (*15*). Agents sensitive to early changes in size and/or viability of this cell population may offer a means of evaluating the effects of therapy designed to limit infarct size, as well as diagnosing and quantifying myocardial damage.

In addition to permitting evaluation of agents showing areas of increased activity early in the course of myocardial infarction, the isolated-rabbit-heart model also provides an opportunity to study the localization and kinetics of uptake and release of perfusion indicators, such as Tl-201, and to correlate infarct-avid radiopharmaceuticals with such indicators.

The above results demonstrate that the perfused isolated rabbit heart is a stable, well-controlled model whose mechanical, electrophysiologic, and histologic properties may be readily evaluated and correlated with regional tracer uptake. The method has potential both as a rapid, inexpensive screening procedure and as a means for investigating the subcellular localization and binding mechanisms of myocardial imaging agents.

FOOTNOTE

* Numbers in parentheses indicate number of hearts studied at each perfusion time.

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