INVESTIGATIVE NUCLEAR MEDICINE

Cardiac Lymphoscintigraphy Following Closed-Chest Catheter Injection of Radiolabeled Colloid into the Myocardium of Dogs: Concise Communication

M. D. Osbakken,* S. Y. Kopiwoda, A. Swan, F. P. Castronovo, and H. W. Strauss

Massachusetts General Hospital, Boston, Massachusetts 02114

A catheter technique for injection of radiolabeled colloids into the myocardium was developed and tested in a series of 15 dogs. A multipurpose angiographic catheter was modified to permit an inner core of PE-50 polyethylene tubing, tipped with a 23-gage needle, to pass through the lumen for intra-myocardial injection of radiocolloids. For injection of the left ventricle, the catheter is introduced through the femoral artery: for the right ventricle, the femoral vein. The catheter advanced under fluoroscopy until the desired surface for injection is reached. The inner core is then extended to lodge the needle in the endocardium. A mixture of Renografin (to confirm the endocardial injection site) and radiolabeled colloid was injected in 13 animals. Ten minutes after injection, scintigraphy was begun and continued for up to 6 hr. In three dogs the procedure was repeated 3 or 4 times. From two to five nodes were visible in all animals, irrespective of whether the right or left ventricular myocardium was injected. In two animals the injection was given intravenously, and no nodes were seen. These data suggest that cardiac lymphatic drainage can be studied with a catheter injection method.

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Previous investigations have described the anatomy, flow patterns, and composition of lymphatic drainage from the heart in experimental animals (*1–18*). The studies describe the normal patterns of drainage and the impact of various disease states and pharmacologic interventions on the flow rate or composition of cardiac lymph. Alterations in cardiac lymphatic drainage have been observed in myocardial disease processes such as fibroelastosis, viral cardiomyopathy, and ischemic heart disease (*19–21*).

Most of the experimental studies of cardiac lymphatic drainage have used an open-chest preparation (*5–13, 15–18*). Although this approach permits precise identification and sampling from lymphatic vessels, the surgical procedure itself may alter lymphatic flow patterns (*12,18*). To overcome this problem, Clark, et al. (*18*) introduced the technique of closed-chest transthoracic injection of Tc-9m-labeled sulfur colloid into the myocardium, with scintigraphic imaging to evaluate cardiac lymphatic drainage. In their study the rate of cardiac loss and the pattern of lymph-node uptake of the tracer were measured over a 24-hr period. Although the transthoracic approach offers an opportunity for a more physiological measurement than the open-chest approach, the technique does not allow precise placement of the colloid deposit at sites in the posterior wall or interventricular septum.

For the study of cardiac lymphatic drainage from multiple sites in the heart, a technique that uses transarterial or transvenous administration of the radiocolloid is desirable. This report describes the preliminary results of such a study in experimental animals.

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For reprints contact: H. William Strauss, MD, Nuclear Medicine Division, Massachusetts General Hospital, Boston, MA 02114.

* Present address: Mary Osbakken, MD, Cardiology Department, Hershey Medical Center, Hershey, PA 17033.

METHODS

Injection catheter. A double-lumen catheter was designed to facilitate percutaneous insertion, advancement, and intramyocardial injection of either radioopaque contrast material or a radionuclide into either the right or left ventricle. The outer catheter was an all-purpose 7 French angiographic catheter; the inner, extendable core was made of PE-50 polyethylene tubing tipped with a 3-mm-long 23-gage needle at the distal end and an injection hub at the proximal end (Fig. 1). The inner catheter was designed to permit the needle to extend 2–3 mm when the connector is pushed home (Fig. 1, B). This needle projection was selected to minimize the likelihood of passing through the myocardium and injecting the pericardial space.

Imaging agents. Two radiocolloids with small particle sizes were selected: A. *Tc-99m antimony sulfide.* This agent has a 5–15-micron particle size, and was recently shown to enter the lymphatic system following depot injection to a greater extent than other technetium-labeled colloids (*22–24*). The radiopharmaceutical was prepared according to the manufacturer's instructions by adding $Na^{99m}TcO_4$ to a solution of antimony sulfide in acid, boiling for 30 min, cooling, and adding buffer. B. *In-111 hydroxide.* A microcolloid with particle size similar to antimony sulfide was produced by the method of Castronovo (*25,26*). Indium-111 chloride was acidified in 0.05*N* HCl, a stabilizer of 10% gelatin solution in saline was added, and the pH was slowly brought to pH 7.4 with NaOH to form the colloid.

To facilitate fluoroscopic monitoring of the injection site, the radiocolloid was mixed with 10% Renografin. Preliminary studies were performed in four rabbits to determine whether mixing the Renografin with Tc-99m antimony sulfide would alter the distribution of the colloid. The rabbits were injected in the footpad: one side with the colloid mixed with Renografin, the other with colloid only. There was no difference in the rate of visualization of the nodes on the two sides. As a result, we felt that injection of the colloid mixed with Renografin was suitable. In studies performed with *In-111 hydroxide* colloid, however, the EDTA in the Renografin was neutralized with calcium chloride before injection.

Animal study. Fifteen adult mongrel dogs of mixed sexes were anesthetized with pentobarbital, placed on a Harvard respirator, and restrained supine on a fluoroscopy table. Thirteen animals had catheter injections into the myocardium; the remaining two had injections into the right or left ventricular cavity to determine whether lymph-node visualization would ocur after intravascular injection of colloid. Of the 13 dogs that had intramyocardial injections, ten had acute studies, whereas three were injected on from three to six separate occasions to determine the reproducibility of the pattern of lymphatic drainage observed from the images.

A baseline 12-lead electrocardiogram was recorded, and Lead 2 was monitored throughout the procedure. A catheter was inserted either into the femoral artery (seven animals, for study of the left ventricle) or the femoral vein (six animals, for the right ventricle). The catheters were filled with Renografin to facilitate advancement, under fluoroscopic guidance, into the cardiac chambers. The catheters were rotated to place the orifice against the free wall of the chamber, and the needle at the tip was then advanced into the myocardium (Fig. 2). Thereafter 0.3–0.5 ml of radiolabeled colloid (containing either 300–500 μCi of Tc-99m antimony sulfide or 70–100 μCi of In-111 colloid) was injected, along with 0.2 ml of contrast, under fluoroscopy (to ensure intramyocardial injection), and the catheter then removed.

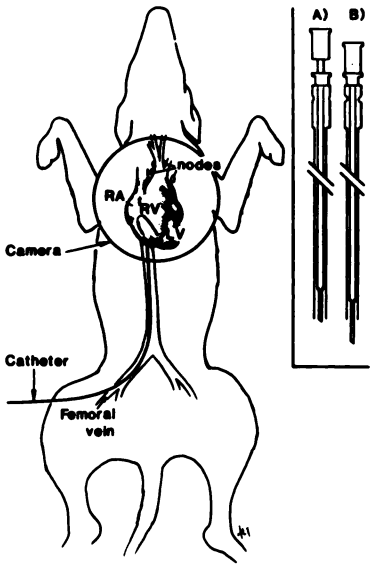

FIG. 1. Catheter positioned in right ventricle by way of femoral vein. Insert: double-lumen catheter used for injection of radioactive colloid into myocardium: A) needle retracted; B) needle advanced.

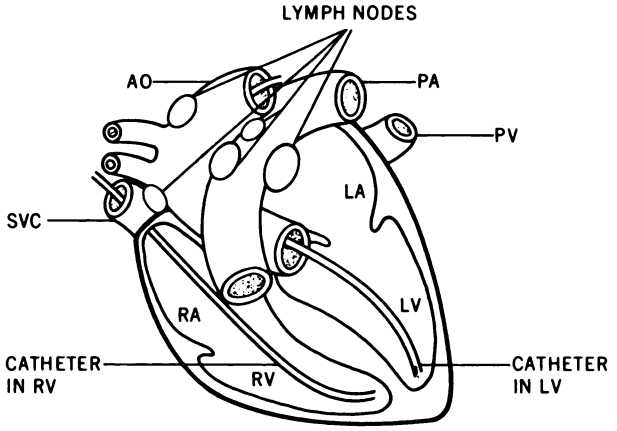

FIG. 2. Heart with right- and left-ventricular (RV and LV) injection catheters in place. Lymph nodes in para-aortic position consistently showed sequestration of radiocolloid.

Two of the ten dogs studied acutely received injections of Tc-99m colloid into the free wall of one ventricle and In-111 colloid in the free wall of the other. The remaining animals had a single injection into the free wall of one ventricle.

Following injection, the animals were placed beneath the detector of a scintillation camera for anterior scintigrams. When Tc-99m colloid was administered alone, a parallel-hole low-energy, all-purpose collimator was used, but for In-111 colloid, either alone or in conjunction with Tc-99m, a parallel-hole medium-energy collimator was used. The pulse-height analyzer was centered at 140-keV with a 20% window to image the Tc-99m, In-111 was imaged at the 247-keV peak with a 20% window. Data were recorded both directly on film from the analog CRT of the scintillation camera and into a dedicated computer interfaced to the camera. Sequential 10-min anterior images of the chest were recorded for 2 to 6 hr, and at 24 hr in six animals. Between 120,000 and 600,000 counts were recorded in each image. After imaging, the ten animals studied acutely were killed and the heart and thoracic nodes excised and imaged in vitro. The three animals studied with multiple injections were brought back to their cages and allowed to recover. The animals were reanesthetized, reinjected, and imaged as described above at intervals of 2–7 days. In one animal three injections were made in the right-ventricular myocardium and one in the left; in a second animal four injections went into the left-ventricular myocardium; while a third animal received three injections in the left-ventricular myocardium.

Data analysis. Each image was analyzed both qualitatively, to identify the number and site of lymph nodes visualized, and quantitatively by placing regions of interest (ROIs) over several zones in the image: (a) the injection site in the myocardium; (b) clearly defined nodes in the thorax; (c) liver; and (d) lungs (to determine background). The activity in each ROI was expressed as counts/pixel, corrected for background and physical decay. The activity in each zone was then related to the activity in the initial image, and plotted as a function of time. In the two animals receiving both radiocolloids simultaneously, corrections were made for indium activity appearing in the technetium window.

RESULTS

All animals survived the injection procedure, but two developed ventricular irritability at the time of injection; it subsided within 3 min of injection without medication. One animal developed transient ST-segment elevation during injection; it resolved within 30 min.

There was some reflux of the injected material in all animals, as observed on fluoroscopy. From two to five nodes were visible in all dogs (Fig. 3–5). The pattern of lymph nodes, seen varied between the animals, but in the

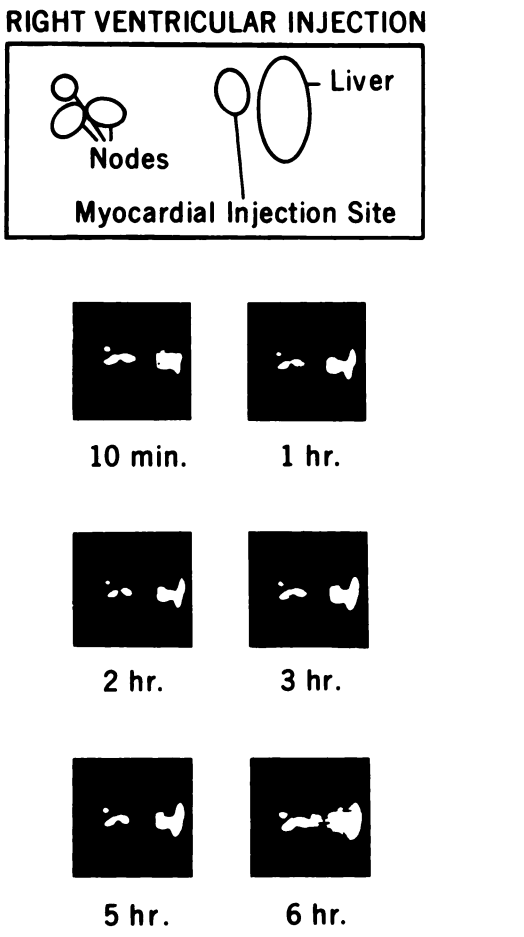

FIG. 3. Sequential images of cardiac injection site and lymph nodes after injection of Tc-99m antimony sulfide ($TcSb_2S_3$) into right ventricle.

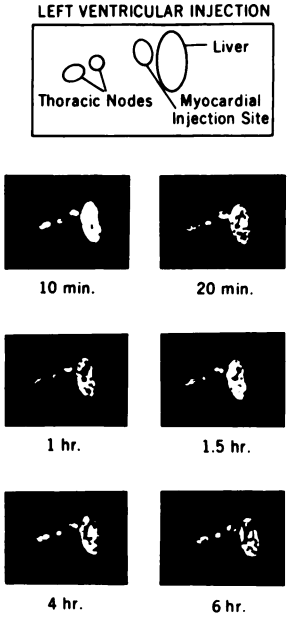

FIG. 4. Sequential images of cardiac injection site and lymph nodes after injection of Tc-99m antimony sulfide ($TcSb_2S_3$) into left ventricle.

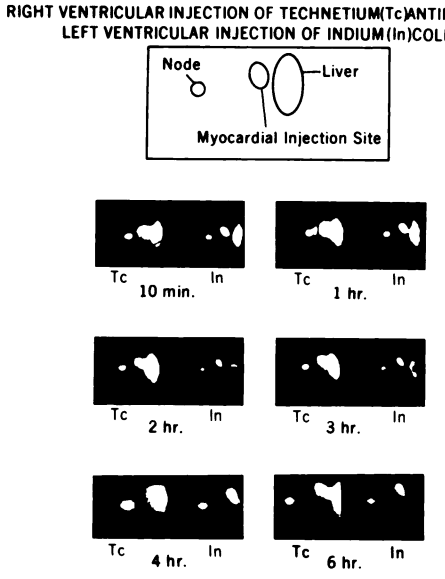

FIG. 5. Sequential images of cardiac injection site and lymph nodes after simultaneous injection of Tc-99m antimony sulfide ($TcSb_2S_3$) into right ventricle and In-111 colloid into left ventricle. Tc = $TcSb_2S_3$; In = indium-111 colloid.

TABLE 1. RATIOS OF NODAL ACTIVITY TO INITIAL ACTIVITY AT INTRAMYOCARDIAL INJECTION SITE, AT TIMES SHOWN, FOR FOUR INDEPENDENT INJECTIONS IN ONE DOG

Time	Node 1	Node 2	Node 3
	Injection 1 (right ventricle)		
1 hr	2%	5%	29%
2 hr	7.6%	11%	44%
3 hr	5%	9.5%	70%
	Injection 2 (left ventricle)		
1 hr	9%	16%	8%
2 hr	23%	14%	8%
3 hr	7%	32%	11%
	Injection 3 (right ventricle)		
1 hr	8%	4%	18%
2 hr	12%	8%	20%
3 hr	5%	2%	21%
	Injection 4 (right ventricle)		
1 hr	25%	25%	26%
2 hr	31%	32%	38%
3 hr	30%	31%	35%

three dogs undergoing multiple injections, the same nodes were generally visualized at each study. In two of the three animals undergoing multiple studies, one of the multiple injections behaved in an unusual manner—the site of deposition in the myocardium appeared far larger than expected, and many more nodes in both the thorax and neck were visualized. Aside from these two unusual results, a similar pattern of nodal visualization was observed after injection in either the right- or left-ventricular myocardium (Figs. 3–5). The ratios (%) of nodal to initial myocardial activity in a typical animal undergoing multiple injections are listed in Table 1. There was wide variation in the radiocolloid concentration in the nodes with each injection.

Verification that the extracardiac activity was actually in the nodes was accomplished by postmortem imaging. Nodes located between the aorta and the pulmonary outflow tract consistently demonstrated concentration of the radiocolloid, as did the injection site in the myocardium. In general, however, there were at least four to seven nonradioactive thoracic nodes harvested from each animal for postmortem imaging. No significant radiocolloid concentration was found in the lungs or the empty thoracic activity.

The hearts were inspected for myocardial damage at the injection site. There were 1- to 10-mm hematomas at the injection sites of six dogs. In the remaining dogs the injection site could not be located with certainty.

The animals injected with radiocolloid intravenously or intra-arterially did not demonstrate any nodal activity either during in vivo or postmortem imaging of the excised nodes. The liver and spleen were visualized in all animals. An incidental finding in these animals was the faint but persistent visualization of the cardiac chambers long after the blood pool had cleared. Postmortem imaging confirmed this observation. Quantitation of this activity as a fraction of the injected dose revealed that in the entire myocardium it represented less than 1% of the injected dose. The significance of this observation is obscure.

Several sets of time-activity curves were generated for each animal: (a) the temporal course of activity at the myocardial injection site; (b) the course of activity in each visualized node; (c) the ratio of myocardial injection to lymph node versus time; and (d) the ratio of lymph node to initial myocardial activity versus time. Representative curves for right- and left-ventricular injections are presented in Figs. 6 and 7. The fall-off in cardiac activity and the changes in nodal activity are similar. The pattern of nodal activity varied from initial concentration with loss over time, through stable concentration over the period of observation, to continued accumulation of activity over time. These data suggest that different flow patterns exist for nodes draining the same area.

Mean curves of cardiac clearance and nodal activity following right- and left-ventricular injection are presented in Figs. 8 and 9. The changes in lymph-node activity are not statistically significant. In 6 hr, approximately 50 ± 10% (decay-corrected) activity had cleared from the site of deposition. Lymph-node uptake is highly variable, as is demonstrated by the large standard deviations.

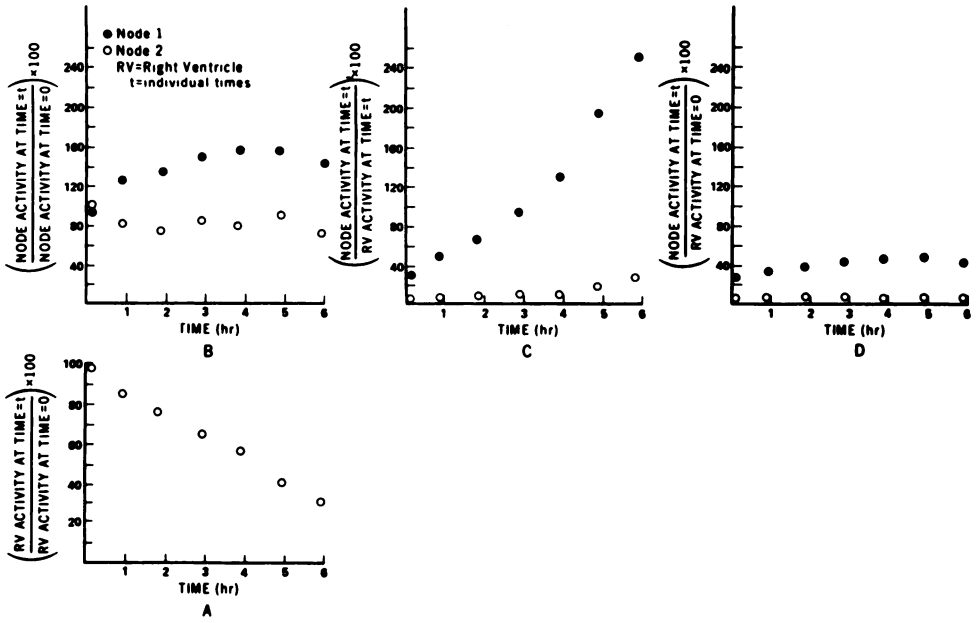

FIG. 6. Typical time-activity curves after right-ventricular injection of Tc-99m antimony sulfide. A) cardiac activity; B) lymph-node activity; C) lymph-node activity related to simultaneous cardiac activity; D) lymph-node activity related to initial cardiac activity.

DISCUSSION

The intramyocardial injection of radiocolloidal materials permits imaging of the distribution of regional lymph nodes draining the heart. Compared with the transthoracic approach of Clark et al. (*18*), the catheter method permits injection into any desired interior surface of the heart. In these studies we injected the free wall of either the right or left ventricle. The drainage pattern from the anterior or posterior surfaces of the left ventricle appears to be similar to that from the right ventricle as shown by concurrent injection studies of two animals. Similar observations about the drainage patterns of the right and left ventricular myocardium have been made by other investigators (*3,5,6*). Repetitive injections into three animals revealed a qualitatively similar pattern of lymph-node visualization when the myocardial injection site was not enlarged.

Measurements of lymph-node concentration, however, demonstrated markedly different patterns of behavior over time in the nodes. Whether this variation is due to

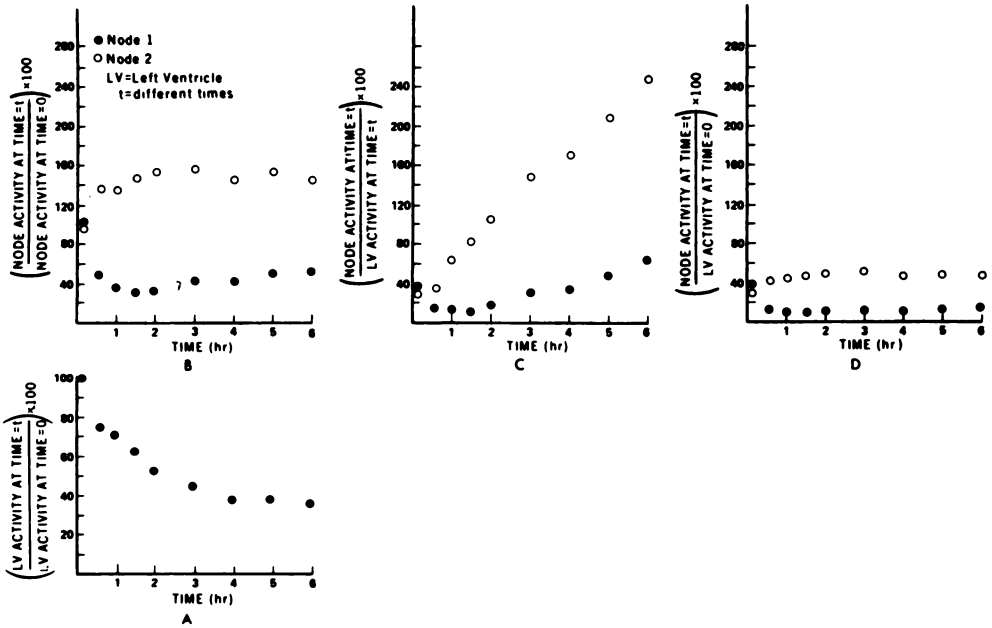

FIG. 7. Typical time-activity curves after left-ventricular injection of Tc-99m antimony sulfide. A) cardiac activity; B) lymph-node activity; C) lymph-node activity related to simultaneous cardiac activity; D) lymph-node activity related to initial cardiac activity.

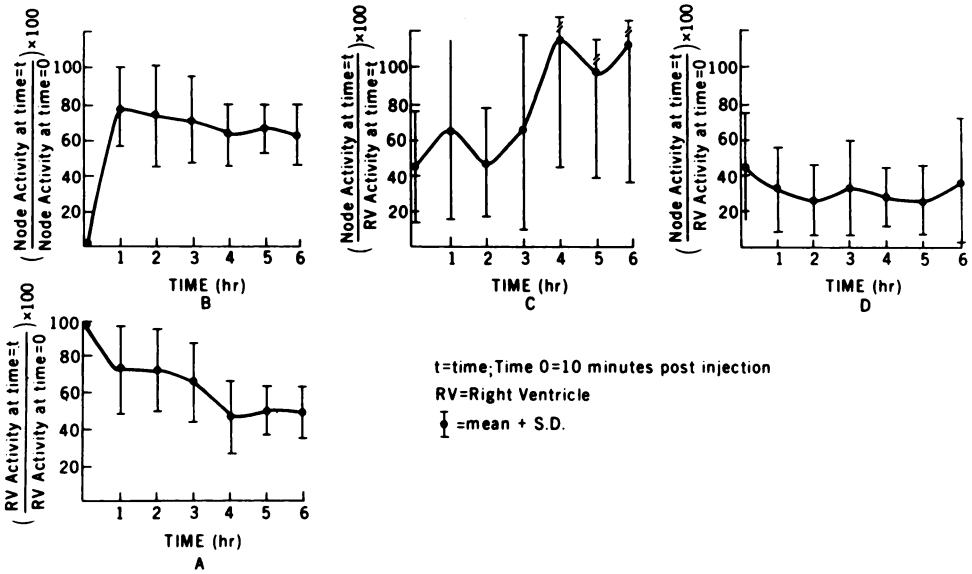

FIG. 8. Mean time-activity curves for six right-ventricular injections of Tc-99m antimony sulfide. These data represent all nodes. Each entry (I) is a mean ± s.d. A) cardiac activity; B) lymph-node activity; C) lymph-node activity related to simultaneous cardiac activity; D) lymph-node activity related to initial cardiac activity.

differences in absorption from each injection site, is not clear.

The catheter injection method was associated with transient arrhythmias in two animals and electrocardiographic changes in one. In addition, the injection site was identifiable at postmortem in six dogs. Although the catheter approach minimizes the possibility of myocardial penetration and laceration of a coronary artery, this may occur. Two of our injections produced unusual results, with visualization of an extensive myocardial stain, possibly secondary to myocardial penetration and injection of at least part of the dose into the pericardial space. However, all the animals survived. Some of the problems associated with the injection may have been secondary to the administration of contrast material. The radiocolloid could have been injected in a very small volume, in physiological saline, but Renografin is hypertonic and may have contributed to the EKG changes.

Although this technique has not been studied for the evaluation of lymphatic drainage before and after an intervention, the catheter-injection approach to such

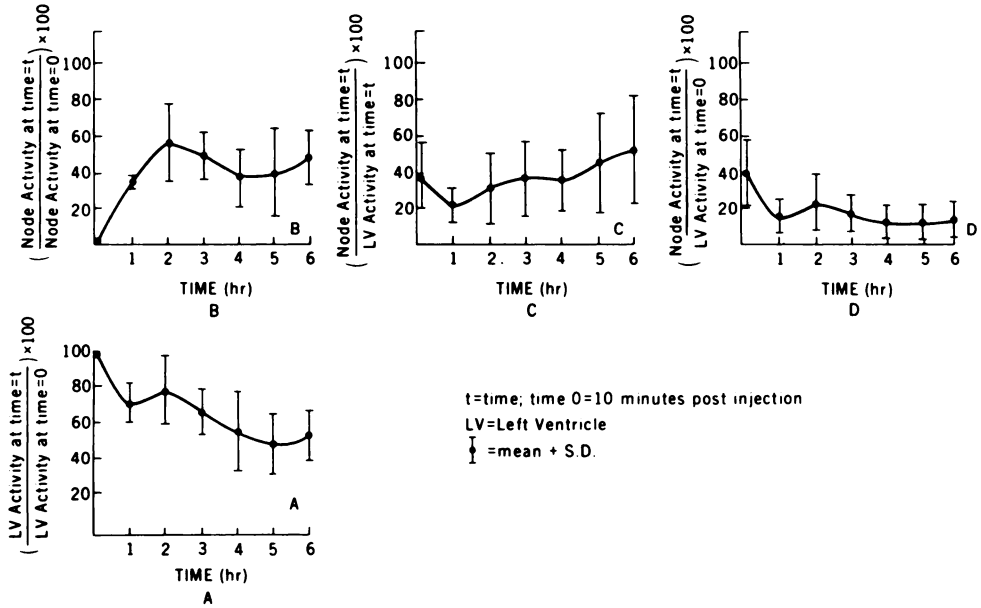

FIG. 9. Mean time-activity curves for five left-ventricular injections of Tc-99m antimony sulfide. These data represent all nodes. Each entry (I) is a mean ± s.d. A) cardiac activity; B) lymph-node activity; C) lymph-node activity related to simultaneous cardiac activity; D) lymph-node activity related to initial cardiac activity.

studies offers several advantages over direct cannulation techniques by permitting: (a) observations in the closed-chest animal; (b) continuous monitoring of several sites; and (c) quantification of the rate of radiocolloid passage through the lymph nodes.

Although no immediate application of the technique in patients is contemplated, we might consider some aspects of cardiac disease where knowledge of the lymphatic drainage may play a role. Acute myocardial ischemia can cause either increased (*2,3,11,14*) or decreased (*8,13*) lymph flow. Feola et al. (*7,12*) and Szabo et al. (*8*) reported that ligation of a coronary artery in the dog causes an increase in lymphatic creatine phosphokinase, lactic dehydrogenase, glutamic-oxaloacetic transaminase, lysoenzymes, lipid concentration, and protein. Araki et al. (*9*) and Uhley et al. (*10*) reported a fall in pH of cardiac lymph and an increase in lactate concentration during ischemia. It has been suggested that lymphatic obstruction augments necrosis after an ischemic episode (*2,3*), and that arrhythmias associated with ischemia may be caused, in part, by toxic materials in lymph bathing vulnerable ischemic cells (*12*). The consequences of obstruction of the lymphatic vessels are equally serious; hemorrhagic necrosis, microinfarction, subendocardial thickening, and large pericardial effusions are some of the findings reported after ligation of lymphatic vessels (*13,15–17*). These investigations suggest that abnormalities in cardiac lymphatics may play an important role in cardiomyopathies (*1–3, 14*). The cited works suggest that additional studies of cardiac lymphatic drainage are in order.

ACKNOWLEDGMENT

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