# New Method for Measuring Myocardial Blood Flow by High Resolution Scintigraphy in the Excised Dog Heart

Chia-Yao Hung*, Robert D. Burow, Benjamin J. Scherlag, Garo P. Basmadjian, and Ralph Lazzara

*College of Pharmacy, University of Oklahoma Health Sciences Center and Section of Cardiovascular Disease, VA Medical Center, Oklahoma City, Oklahoma*

The standard method for measuring myocardial blood flow (MBF) with radioactive microspheres requires processing of selected tissue samples usually from the excised heart, and consequent loss of exact relation to myocardial morphology. A computer-based image processing method was developed by using [$^{99m}$Tc]microspheres (mean particle size 20 $\mu$m) for quantitative analysis of MBF in 25 dogs. A computer-controlled gamma camera was used to obtain the images of radioactive microsphere distribution in transaxial slices of the ex vivo heart. Any portion of these slice images could be quantitated by using a computer program based on modification of the formula for determining MBF by the standard microsphere method. Regional myocardial perfusion calculated by this technique correlated well with values obtained with reference microspheres ($r = 0.96$) over a broad range of MBF. The results show that our new method, accurately and with high resolution, delineated zones of differing MBF and confirmed the increase of MBF in surviving myocardium with healing.

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The radioactive microsphere method (*1*) is widely used for the determination of regional blood flow in animals after excision of the heart. This method, however, requires cutting the myocardium into discrete samples which may be a combination of normal tissue, border zone, hyperemic tissue, and severely ischemic tissue. Although the degree of resolution for flow measurement could possibly be improved by cutting myocardium into smaller samples, this becomes tedious and, therefore, impractical for studying flow throughout the heart. Furthermore, the sampling technique requires the preselection of the region of interest. The processing of selected tissue samples can bias the results as well as blur the exact relation to overall myocardial morphology.

We have developed a computer-based image processing method for quantitative analysis of myocardial blood flow in experimental animals. The processing employs a gamma camera to obtain the images of myocardial distribution of technetium-99m ($^{99m}$Tc) microspheres in the ex vivo heart. Scintigraphic quantitation of distribution of the $^{99m}$Tc-labeled microspheres was accomplished by means of a computer program based on modification of the formula for the standard microsphere method.

The purpose of this study was to validate the measurement of regional myocardial blood flow by means of high resolution scintigraphy and to correlate the new methodology with the conventional microsphere method.

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For reprints contact: (*Present address) Chia-Yao Hung, PhD, Div. of Nuclear Medicine, Dept. of Radiology, University of Missouri-Columbia, Columbia, MO 65211-0001.

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## MATERIALS AND METHODS

### Models of Myocardial Infarction

*One-day infarcted dog heart.* Fifteen mongrel dogs (14–22 kg) were anesthetized with intravenously administered sodium pentobarbital (30 mg/kg body weight) and ventilated with room air using a Harvard respirator. Atelectasis was prevented by maintaining an expiratory

pressure of 5–7 cm of water with a trap under controlled ventilation. The heart was then exposed through a thoracotomy at the fourth intercostal space and the pericardium incised. After gentle retraction of the left atrial appendage, the left anterior descending coronary artery (LAD) was isolated from the epicardial surface of the heart. The artery was ligated distal to the anterior septal branch by silk sutures passed around it and subsequently tied in two stages as described by Harris (*2*). The chest was then closed and the animals were allowed to recover for 24 hr.

*Four-day infarcted dog heart.* The experimental preparation of the 4-day infarcted dog heart was the same as described for the 24-hr infarcted dog heart, except the entire operation was performed under sterile conditions. After repair of the thoracotomy, the animal was allowed to recover for 4 days. Experiments were performed in ten adult mongrel dogs of either sex, weighing 15 to 24 kg.

**Regional Myocardial Blood Flow Determination**

Human serum albumin microspheres*, 20 ± 1 μm (mean diameter), were labeled with 10 to 30 mCi (370 to 1,110 MBq) of $Na^{99m}TcO_4$. The labeling kit was vigorously agitated by hand for 5–15 sec and then mixed ultrasonically for 5 min. Microscopic examination assured uniform dispersion of microspheres.

For each blood flow measurement, ~4–6 million microspheres were injected through a polyethylene cannula into the left atrium over a 10-sec period and flushed with 10 ml physiological saline. No significant changes in hemodynamics were observed following injection of this number of microspheres. Starting 5 to 10 sec before each microsphere injection and continuing for a total of 2 min, a reference sample of arterial blood was withdrawn from the right femoral artery with a constant-withdrawal syringe pump (3.88 ml/min). After completion of the experiment, the animal was killed and the heart was excised, and thoroughly rinsed with running water. The atria, epicardial fat, and valves were removed and the remaining ventricles were frozen for 2 hr at −70°C to facilitate sectioning. The ventricles were then cut into "bread-loaf" sections, 3 mm thick (Fig. 1), with a meat slicer†. The uniformity of the thickness was checked to be within ±0.1 mm by use of a thickness gauge‡. The 3-mm sections from apex to base were stained with nitro blue tetrazolium in phosphate buffer (*3*). This allowed visualization of the necrotic tissue, since only viable regions stain dark blue due to the presence of intracellular dehydrogenases (*3*).

By cutting the heart in slices perpendicular to its long axis, i.e., apex-base axis, the determination of regional blood flow could be obtained. Images of the slices and the reference blood sample were obtained using a commercially available gamma camera§, one slice at a time. The camera was equipped with a high resolution, parallel hole collimator (resolving power ~3 mm). A 20% window was centered on the 140-keV photopeak of $^{99m}Tc$. The acquisition time for blood sample and each slice was 5 min. Data were stored in a 128 × 128 byte mode with a zoom of 2.5 ×.

Image processing was accomplished interactively with a computer program. This program, BARFLOW, can be described as follows. Histogram values were converted into corresponding blood flow value (ml/min/100 g) by selecting portions of the image for analysis within a defined rectangular region of interest. The BARFLOW program calculated mean flow within the region of interest (ROI) according to the following formula:

$$MBF = C_I \times RBF \times e^{\lambda t}/C_R \times \text{sample weight}, \quad (1)$$

where MBF = myocardial blood flow in ml/min/100 g,

$C_I$ = activity in the ROI (counts/min);
RBF = withdrawal rate of arterial blood (3.88 ml/min);
$\lambda$ = 0.0019 $min^{-1}$ (decay constant for $^{99m}Tc$);

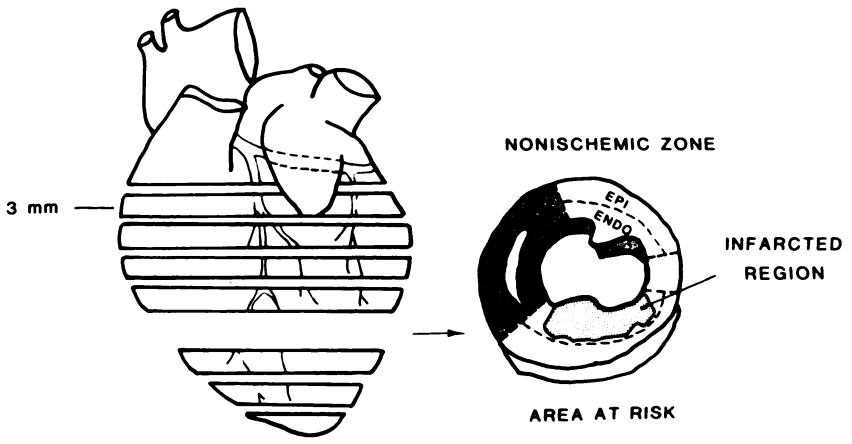


**FIGURE 1**
Tissue sampling for microsphere determination of regional myocardial blood flow. Schematic diagrams indicate site of LAD occlusion, method of slicing heart into transverse sections, and sampling of subepicardial (EPI) and subendocardial (ENDO) tissue in area at risk and nonischemic zone. AO = aorta

t = elapsed time (min) from imaging the reference blood sample to the slice of interest;

$C_R$ = total activity in the reference blood sample (counts/min);

sample weight = tissue sample weight (100 g).

The analog zoom is usually specified as a factor by which each axis of the image is magnified. With our instruments, at a zoom factor of 2.5 a 128 × 128 matrix encompassed the 26.7-cm field-of-view of the camera. The digital resolution was 2.5 × (128 pixels/26.7 cm) = 12 pixels/cm and the dimension of each pixel was 1 cm/12 pixels = 0.083 cm/pixel. Each pixel in this zoomed image represented a mass of $(0.083\ cm)^2$ × thickness of the slice (cm) × specific gravity of the myocardium ($g/cm^3$). According to the CRC Handbook Series in Clinical Laboratory Science (*4*), the specific gravity for myocardium is 1.03 $g/cm^3$. Hence, in a slice 0.3 cm thick, a pixel value represents a mass of 0.0021 g. The sample weight of the ROI could be given by

$$\text{Sample weight (100 g)} = (N \times 0.0021)/100 = N \times 0.000021, \quad (2)$$

where N is the number of pixels in the ROI, we obtain from formulae 1 and 2

$$MBF = C_I \times RBF \times e^{\lambda t}/C_R \times N \times 0.000021. \quad (3)$$

The BARFLOW program allows the selected ROI to be subdivided into several equal sections and calculates the individual blood flow for each section. In this way a section from epicardium to endocardium can be taken and divided into two (epicardium, endocardium) or three (epicardium, mid-myocardium, endocardium) myocardial compartments.

### BARFLOW Versus Standard Method

After the previous experiment was completed, 166 myocardial tissue samples (range 0.05 to 1.2 g) were obtained from different areas of the myocardial slices. The radioactivities of each section of myocardium and the reference blood sample were measured in a well counter. After correcting for background counts and physical decay, blood flow for each tissue sample was calculated by the formula $MBF = C_I \times RBF/C_R \times$ sample weight.

The correlation between values of regional myocardial blood flow for the standard method and BARFLOW was determined with linear regression analysis using a least-squares fitting technique. The coefficient of linear correlation, r, was obtained from the Pearson's product moment.

### Statistical Method

Statistical analysis of the results was made using Student's t-test for independent means. Differences were considered to be significant when $p < 0.05$.

## RESULTS

Figure 2 shows a display of myocardial "bread-loaf" sections (Apices 4 and 8) made through different levels of infarcted heart. Apex 1 represents the initial section 3 mm thick, starting at the apex of the heart. In most cases, 14 or 15 slices are taken until the base of the heart is included. Note the loss of perfused tissue (infarct) in the left anterior subendocardial and intramural

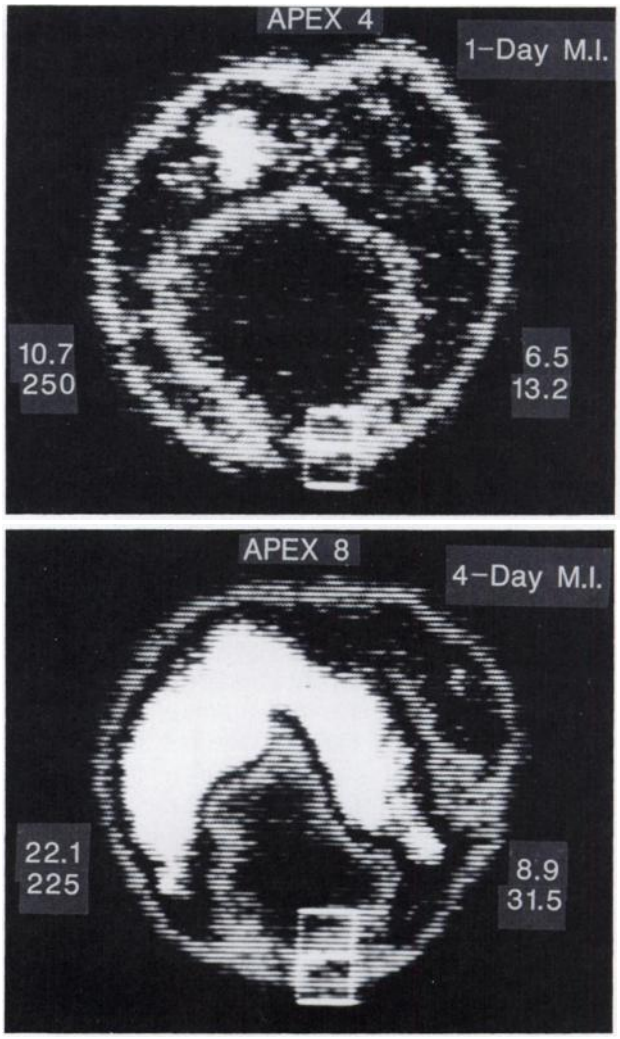


**FIGURE 2**
Flow distribution of myocardial "bread-loaf" section of 24-hr-old infarct (top) and 4-day-old infarct (bottom), caused by LAD occlusion in dog. There was substantial increase of blood flow in surviving subepicardium by 4 days after permanent ligation of LAD. Figure 2 also demonstrates myocardial blood flow distributed to inner (subendocardial) and outer (subepicardial) layers of area at risk. Blood flows in ml/min/100 g are shown on right side of box. Top and bottom numbers represent blood flow of subendocardial and subepicardial portions, respectively. Two rows of numbers are shown on left side of box. Top number indicates average flow in whole box, whereas bottom number is number of pixels in box

areas. Figure 2 also displays a decreased blood flow in the subepicardial layers overlying the infarct.

The resulting images of BARFLOW (Fig. 2) display a box on a pixel by pixel scale that allows the assessment of the regional blood flow in ml/min/100 g. This computer program allows quantitative determinations of flow within any size box superimposed on the image of a given heart slice. The position of the box can be changed, so that one can measure the blood flow in any part of the heart. The BARFLOW program can also be used to show the blood flow in inner (subendocardial) and outer (subepicardial) sections of the normal and infarcted zones, respectively.

Myocardial blood flow is summarized for the normal, ischemic, or infarcted regions of the left ventricular epicardium and endocardium in Figs. 3 and 4, and the ratios of blood flow between various areas in the left ventricle in Table 1. At 24 hr after LAD occlusion,

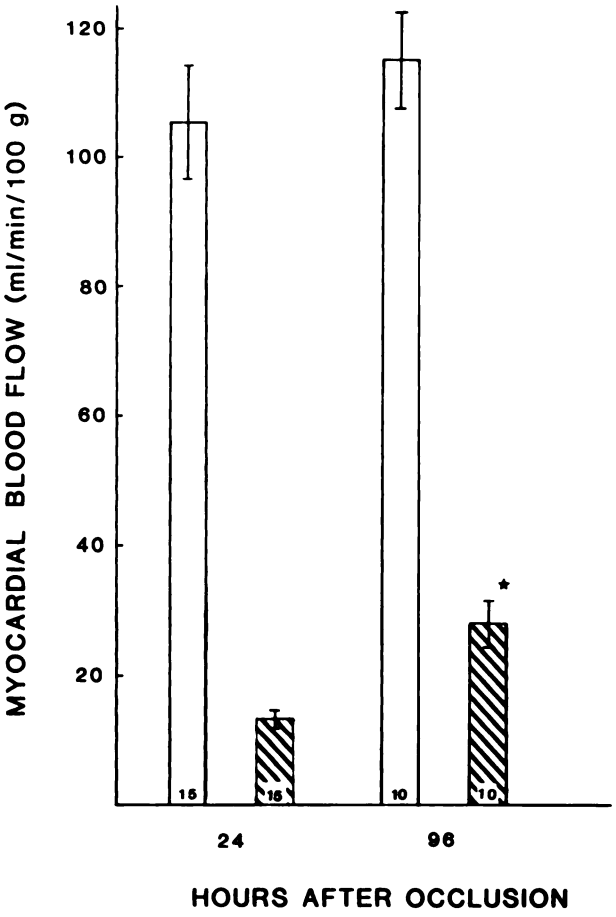


**FIGURE 3**
Blood flow to normal and ischemic zones of epicardial half of left ventricle after LAD occlusion. Numbers at bottom of each bar represent number of dogs studied at each time period. Statistical analysis was by Student's t-test. *p = 0.0002 compared with 24-hr time value. n = As indicated at bottom of bars. (□) Normal; (▨) Ischemic

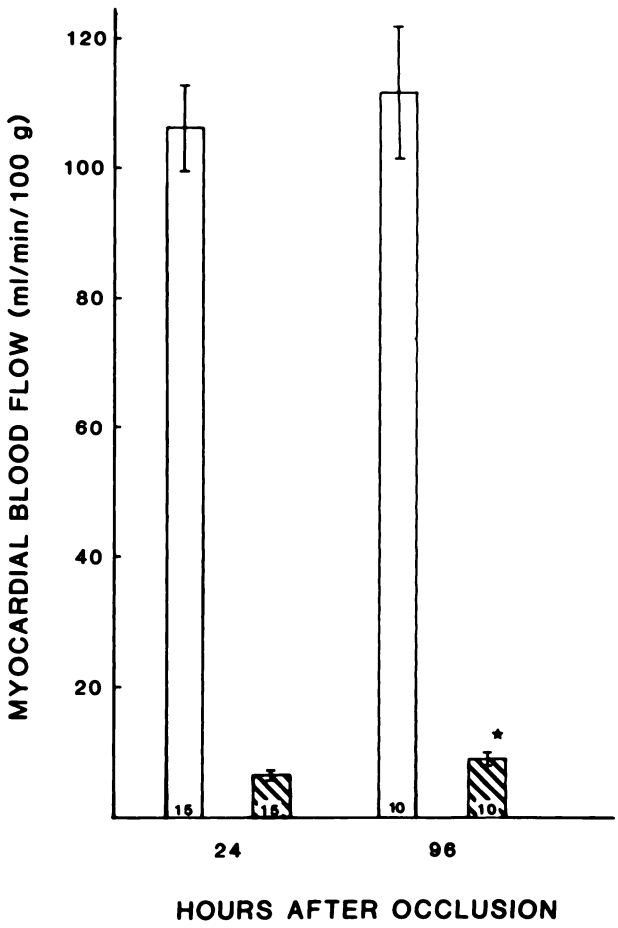


**FIGURE 4**
Blood flow to normal and infarcted zone of endocardial half of left ventricle after LAD ligation. Number at bottom of each bar represents number of dogs studied at each time period. Data analysis was by Student's t-test. *p = 0.04 compared with 24-hr time value. n = As indicated at bottom of bars. (□) Normal; (▨) Ischemic

blood flow was sharply decreased to group means of 6.5 ± 0.6 (mean ± s.e.m.) ml/min/100 g in the endocardium and to 13.1 ± 1.5 ml/min/100 g in the epicardium. By 96 hr, blood flow to the endocardial half of the left ventricular myocardium was slightly increased to 9.1 ± 1.1 ml/min/100 g (p = 0.04) and the increase of collateral blood flow to viable epicardial rim was considerably augmented to 28.1 ± 3.4 ml/min/100 g (p = 0.0002) (Figs. 3 and 4). The increase of blood flow to ischemic endocardium and epicardium was also demonstrated by the ratio of endocardial and epicardial blood flow in ischemic tissues or the ratio of ischemic and nonischemic tissue in endocardial and epicardial tissues (Table 1).

In the normal zone, there was no change in blood flow by 96 hr postocclusion. The subendocardial/subepicardial perfusion ratio in the normal zones is 1.02:1 and 0.96:1 for the 24-hr and 4-day infarcted dog heart, respectively (Table 1).

**TABLE 1**
Microsphere Distribution Ratio in Infarcted Canine Myocardium*

| | Hours after coronary artery occlusion | |
|---|---|---|
| | 24 | 96 |
| Endocardium/Epicardium | | |
| Nonischemic | 1.02 ± 0.03 (n = 15) | 0.96 ± 0.03† (n = 10) |
| Ischemic | 0.56 ± 0.07 | 0.34 ± 0.04 |
| Ischemic/Nonischemic | | |
| Endocardium | 0.06 ± 0.01 (n = 15) | 0.09 ± 0.01 (n = 10) |
| Epicardium | 0.13 ± 0.02 | 0.26 ± 0.04 |

* Values were group means ± s.e.m. for all dogs studied at each time period.
† p = N.S. by Student's t-test vs. 24-hr infarcted dog heart.

Regional myocardial perfusion calculated by the BARFLOW method correlated well with values obtained with the conventional microsphere technique (r = 0.96) over a range of 3.8 to 709.3 ml/min/100 g (Fig. 5).

## DISCUSSION

The excellent correlation between the BARFLOW and standard reference microsphere method over a broad range of blood flow provides evidence that the BARFLOW is a valid method for determining regional myocardial blood flow. The results of this study suggest that BARFLOW can be used to determine myocardial blood flow with better resolution (3.4 mm) than the standard method while maintaining a more intact myocardial morphology. The new technique delineated zones of differing myocardial blood flow and confirmed the increase of blood flow in surviving myocardium with healing at 4 days (*5,6*). It will also allow for the selection of any other areas that one may become interested in since the images are stored for computer retrieval and processing.

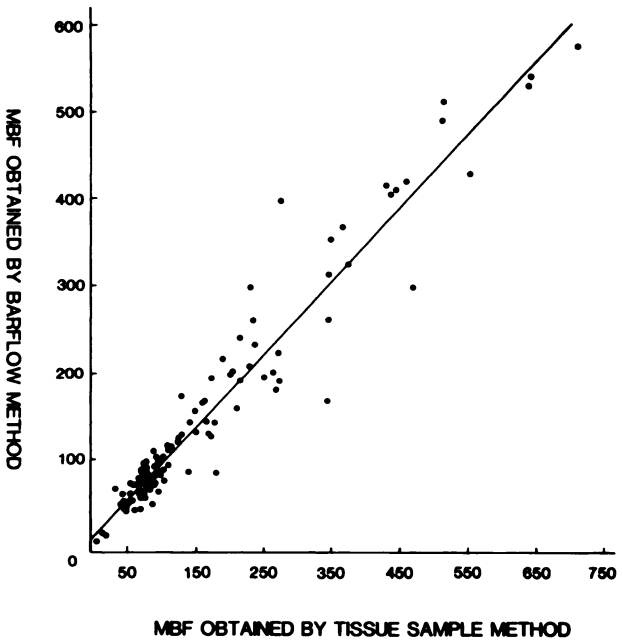


**FIGURE 5**
Correlation between two methods of measuring myocardial blood flow (MBF): BARFLOW and standard microsphere techique. s.e.e. Standard error of estimate. Y = 0.85× + 12.2; r = 0.96; s.e.e. = 3.12; n = 166

The degree of error in the microsphere technique will depend upon the number of microspheres injected and the weight of the tissue sample counted for radioactivity. Buckberg et al. (*7*) have shown that for accurate determination (±10% at the 95% confidence level) of tissue perfusion, the tissue segment analyzed should contain at least 384 microspheres. This criterion is satisfied by injecting 1 million microspheres into the left atrium and analyzing normal tissue samples that weigh more than 0.5 g. To adequately measure blood flow in a low-flow region, the number of injected microspheres must be increased. According to the conclusion from Winkler's experiments (*8*), at least 2.5 times more particles have to be injected, i.e., 2.5 million microspheres, if blood flow of individual tissue samples that weigh ~170 mg taken from freshly infarcted myocardium is to be determined with an acceptable accuracy. In the present study, 4–6 million microspheres were injected into the 24-hr or 4-day infarcted dog heart. Thus, this number of microspheres exceeds the minimal requirement for microsphere content in infarcted tissue and in analyzing sample size. The limits of variability decrease as the number of microspheres present is increased, as predicted from the binomial distribution. In dogs, serial left atrial injection of 15-μm microspheres totaling 48 million caused no significant changes in systemic hemodynamics, regional myocardial flow, or coronary pressure-flow relations (*9*).

The measurement of regional distribution of myocardial blood flow is significantly affected by the size of the microspheres used or the radial distribution of the microspheres within the cross section of the major arteries. Domenech et al. (*10*) reported that large microspheres overestimate subendocardial blood flow in comparison to results obtained with a diffusible indicator (*11,12*). Studies using tracer microspheres 7–10 μm in size in the anesthetized open-chest dog report endocardial to epicardial flow ratios close to unity (*13–15*).

From these data, it is apparent that smaller microspheres, which approximate red cell diameter, should be used to assess the distribution of flow within the myocardium. Because arteriovenous shunting of small

microspheres (average diameter 9 μm) in the myocardium can be a problem (16–19), most investigators who study myocardial perfusion prefer the 15-μm size, even though the ratio of subendocardial to subepicardial left ventricular perfusion may be slightly increased compared to that measured with spheres 9 μm in diameter (*10*). Although the human albumin microspheres used in this study have a mean diameter of ~20 μm, the subendocardial/subepicardial flow ratio in anesthetized dogs was close to unity (0.99:1).

Phibbs et al. (*20,21*) studied the possible mechanisms involved in the uniformity of distribution patterns displayed by the microspheres of different diameter ranges. They found that microspheres of 60–80 μm diameter concentrated centripetally, whereas the microspheres of 7.5–10 μm diameter were fairly evenly distributed throughout the total cross-sectional area of the vessel. Thus, when radial distribution is uneven, as with the larger microspheres, small branch arteries with proportionately low blood flow might receive disproportionately fewer microspheres for that flow. Furthermore, because the particles are labeled with gamma-emitting isotopes and labeling is proportional to the mass of the microspheres, when the particle size is not uniform, the counting rate in various tissue samples is not proportional to the number of particles in a tissue sample.

The diameter range of albumin microspheres used in this study, after labeling with $Na^{99m}TcO_4$, is between 10 and 35 μm and remains essentially unchanged 8 hr after labeling. This uneven distribution of microspheres may account for the high flow value in normal myocardium in two of 25 flow studies. Further studies with more uniform size of albumin microspheres will be required to define a possible effect of radial distribution on estimation of transmural flow.

Autoradiography is primarily a means of determining the location of radioisotopes in a given tissue section, either as gross sample or microscopic section. Digital film-analysis systems using videodensitometry (*22,23*) have been developed to quantitate autoradiograms. Digital film autoradiography has all the advantages and limitations of photographic detection techniques. It has high spatial resolution and is, basically, a simple and inexpensive method. The geometric resolution of digital film autoradiography is impressive (0.05 to 30 μm). This is several orders of magnitude better than the geometric resolution that can be achieved with microspheres. The film sensitivity, however, is relatively low and a long exposure time (days to weeks) is often needed to get a sufficient darkening level. This time depends, of course, on the type of isotope, its specific activity and dose. Moreover, the evaluation of radioactivity from film darkening cannot be considered very reliable because of the intrinsic variability of the photographic process resulting in a difficult system to calibrate.

The ability to measure regional myocardial blood flow with sufficient resolution by this new technique may be useful in experimental research to measure the changes in regional flow that may be related to the genesis of ventricular arrhythmia (*6*), or in whatever area a reference method is required to determine regional myocardial perfusion.

## FOOTNOTES

* 3M Center, St. Paul, MN.

† Model 770, Globe Slicing Machine Co. Inc., Stanford, CT.

‡ Direct Reading Dial Gages, The Dyer Co. Inc., Lancaster, PA.

§ Low Energy Mobile Scintillation Camera, Searle Analytic Inc., Des Plaines, IL.

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