# COMPARISON OF $^{86}$Rb AND MICROSPHERE ESTIMATES OF LEFT VENTRICULAR BLOODFLOW DISTRIBUTION

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***Diffusible radioactive tracers and nondiffusible microspheres have both been used to estimate the distribution of left ventricular (LV) blood flow. These methods were directly compared in five open-chested dogs 1½ hr after ligation of branches of the anterior descending coronary artery. Strontium-85-labeled 15 micron-diam microspheres (RM) were injected into the left atrium and $^{86}$RbCl was given intravenously; ninety seconds later the LV was excised and divided into multiple samples encompassing a wide range of flows. Tissue contents of RM and Rb, expressed relative to nonischemic posterior wall myocardium, were not significantly different at normal flows. However, in the peri-ischemic region where RM content exceeded posterior wall, there was relatively less Rb than RM, and where RM content fell below 20% in the ischemic region, there was more Rb than RM. At normal or hyperemic flows the ratio of tracer content in the inner half to that in the outer half of the LV (I/O) was 1.04 for RM and 1.02 for Rb, not significantly different. As flow fell below 0.60 cc/min/gm, I/O declined similarly for RM and Rb, except that below 0.10 cc/min/gm, I/O was significantly higher for Rb (0.30 versus 0.16, $p < 0.005$). The results suggest that the two methods are equally suitable for measuring bloodflow distribution except at high or very low flow levels where microspheres may reflect blood flow more accurately.***

Measurement of total myocardial or left ventricular blood flow may give an incomplete or misleading picture in the setting of myocardial ischemia. Since ischemia is regional rather than total, knowledge of bloodflow distribution is also essential. Two types of radioactive tracers have been used to determine flow distribution: diffusible indicators, such as $^{86}$Rb or $^{131}$I-iodoantipyrine, and nondiffusible microspheres. Both have been validated for measuring total left ventricular flow by comparing tracer flow estimates with simultaneous determinations by electromagnetic flowmeters, rotameters, or timed venous collections (*1–4*). Validation of either tracer for estimation of blood flow distribution has proved difficult because of the lack of a suitable standard. Direct comparisons of the two techniques are therefore necessary but have been scanty (*5–9*).

In this study, $^{86}$RbCl and 15 micron-diam $^{85}$Sr-labeled microspheres were injected in a series of dogs 90 min after coronary ligation. The relative amounts of each tracer were directly compared in multiple tissue samples from areas of low and high flow. In addition, the distribution of each tracer between inner and outer portions of the left ventricular wall was examined.

## METHODS

Five adult male greyhound dogs weighing 20–28 kg were anesthetized with intravenous thiopental and kept unconscious with intermittent injections of pentobarbital. Respiration was maintained by Harvard pump and cuffed endotracheal tube. The heart was exposed through a left thoracotomy, the pericardium incised, and the edges sutured to the chest wall. With limited dissection silk ligatures were passed around all of the major free wall branches of the left anterior descending coronary artery and the arteries were acutely ligated. Epicardial electrocardiograms were taken to verify the presence of myocardial ischemia.

Ninety minutes after ligation, 1–2 million 15 ± 5 micron (mean ± s.d.)-diam carbonized radioactive

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microspheres were injected into the left atrium in a volume of 5 ml over 5–10 sec following several minutes of vigorous mechanical agitation to disperse clumps. The spheres, labeled with the gamma-emitting nuclide $^{85}Sr$, were obtained from the 3M Company (Minneapolis, Minn.) as 1 mCi of nuclide suspended in 10 ml of 10% dextran with 1 drop of Tween 80 added (specific activity 10.5 mCi/gm). Starting 10 sec before injection and continuing for 2–3 min afterwards, blood was withdrawn simultaneously from brachial and femoral arteries using a Harvard pump (approximately 1.5 cc/min). These collections served as a reference for calculating myocardial blood flow as described below.

Immediately after the blood withdrawals were completed, 100–200 $\mu$Ci of $^{86}RbCl$ were injected intravenously as a bolus. Exactly 90 sec later the heart was rapidly excised and washed free of blood. The left ventricular free wall was removed and 25–50 full-thickness pieces weighing 1–4 gm were taken. Each piece was divided into inner and outer halves. After discarding gross epicardial fat and blood vessels, samples were weighed, placed in glass tubes, and counted for 5 min in a 2-in. well scintillation counter (Wallac) at two energy windows (corresponding to the 513-keV and 780-keV photoelectric peaks of $^{85}Sr$ and $^{86}Rb$, respectively). The reference arterial blood samples taken during microsphere injection were counted in aliquots in the same way as the myocardial samples. Geometric differferences between blood and tissue samples were found to be unimportant. For each sample the activities of the two isotopes were separated by gamma spectrometry (*10*) and the counts per gram of myocardium computed. The relative amount of each tracer in each sample was expressed as the ratio of radioactivity per gram of the sample to that of the nonischemic posterior left ventricular free wall, calculated separately for $^{86}Rb$ and microspheres. Each sample thereby had a corresponding measurement of $^{86}Rb$ and microsphere content. In addition, microsphere flow was calculated in absolute terms (cubic centimeters per minute per gram) by the formula: $MBF = Cm \times RBF/Cr$, where MBF is myocardial blood flow, Cm is counts per gram in myocardial sample, RBF is reference blood flow (Harvard pump withdrawal rate), and Cr is total counts in reference blood sample (obtained by averaging the total counts of the simultaneous brachial and femoral samples). This relation follows from the fact that if microspheres are adequately mixed in the left atrium, their distribution to myocardium and peripheral artery is in proportion to the blood flow to each (*11*).

To compare the microsphere and $^{86}RbCl$ methods, samples were pooled from the five dogs and grouped according to their relative microsphere content. Mean $^{86}Rb$ content was then compared to mean microsphere content in each group. Distribution of the two tracers between inner and outer halves of the left ventricular wall was compared by grouping full-thickness samples according to their absolute microsphere flow values. Mean inner/outer wall tracer contents were compared in each group. Statistical significance was determined by Student's t-test adapted for paired comparisons.

The precision of the microsphere method has previously been shown to depend on the number of particles present in myocardial and blood samples and the number of counts achieved (*11*). In this study nonischemic muscle contained over 1,000 spheres/gm and reference bloods over 2,000 spheres, yielding an accuracy of 10–20% in estimating flow (*11*). However, some samples with very low flow contained small numbers of spheres (less than 100) and low counts (less than 1,000); in these samples, $^{86}Rb$ counts were also low. To achieve acceptable accuracy, results were expressed in terms of pooled rather than individual samples. All groups including the one with lowest flow had over 7,000 spheres, resulting in an error of less than 10%. It should be emphasized, however, that low counts and small numbers of spheres increase data scatter but do not bias the results in either direction.

## RESULTS

Acute myocardial ischemia was present in all of the animals, evidenced by a cyanotic poorly contracting region on the anterior left ventricular surface and ST segment elevation in epicardial electrocardiograms taken from this area. Within the territory of ligated arteries, tissue contents of microspheres and $^{86}Rb$ were low, more so in the center than at the edge. In peri-ischemic muscle, tracer content was high, exceeding that of the posterior free left ventricular wall. These observations agree with previous reports in this animal model (*12*).

Microsphere and $^{86}Rb$ distributions are compared in Fig. 1. In the middle and low flow ranges with microsphere content 20–80% of the nonischemic posterior wall, $^{86}Rb$ and microsphere distribution did not differ significantly. However, in high flow areas, where microsphere content exceeded posterior wall, there was relatively less $^{86}Rb$ than microspheres. In contrast, in very ischemic regions where microsphere content was less than 20% of posterior wall, there was relatively more $^{86}Rb$ than microspheres. When microsphere content was 80–100%, there was more Rb present than microspheres although the difference for the 80–90% range was of

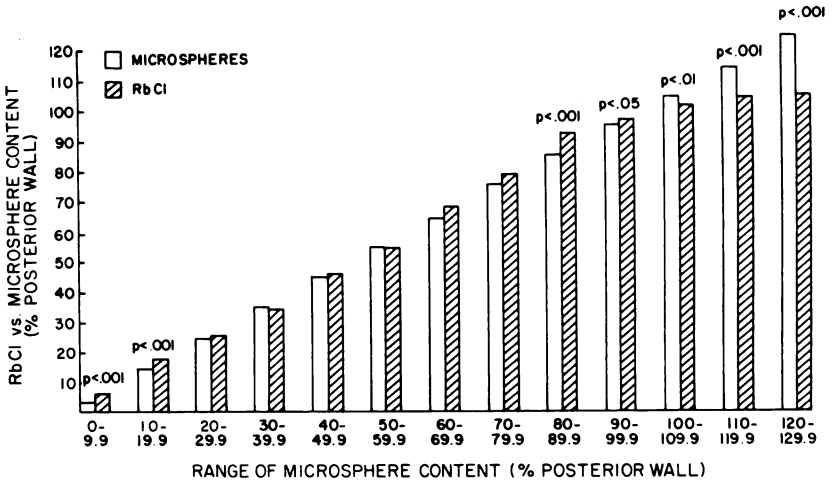


**FIG. 1.** Samples are grouped along horizontal axis by their microsphere content, expressed as percentage of nonischemic posterior left ventricular wall. Relative content of microspheres and RbCl are compared by vertical bars. Note that when microsphere content exceeds 100% there is relatively less RbCl than microspheres, and when microsphere content is below 20%, there is relatively more RbCl.

borderline significance. These findings were similar for inner wall and outer wall samples of the same flow (i.e., with the same microsphere content) as shown in Table 1. Both types of samples were therefore included in the analysis in Fig. 1. The findings were also similar when Rb content was compared directly to microsphere content in each sample without reference to nonischemic myocardium. When microsphere content became very low, the ratio of Rb to microsphere radioactivity increased whereas the opposite was true when sphere content became high.

The distribution of each tracer between inner and outer halves of the left ventricular wall are compared in Figs. 2 and 3. When full wall flow, measured with micropheres, exceed 0.60 cc/min/gm, inner/outer (I/O) wall ratios by both methods were close to unity and did not differ significantly from each other. Pooling all samples with flow between 0.60 and 1.29 cc/min/gm, I/O was 1.04 ± 0.03 for microspheres (mean ± s.e.m.) and 1.02 ± 0.03 for $^{86}$Rb. The difference between these values was not significant ($t = 1.107$, $n = 104$) and neither value was significantly different from unity. As full wall flow declined below 0.60 cc/min/gm, I/O decreased similarly for microspheres and $^{86}$Rb except that when flow was less than 0.10 cc/min/gm, I/O was significantly higher for $^{86}$Rb. Apparently this was related to the relatively greater amounts of $^{86}$Rb present in the very low flow inner half of such areas (see Fig. 1).

## DISCUSSION

We have shown that the distribution of microspheres and $^{86}$Rb in left ventricular myocardium is similar over middle flow ranges but at high flow there is disproportionately less $^{86}$Rb than microspheres and at very low flow, disproportionately more. The excess of Rb in samples having microsphere content 80–100% of posterior wall provides an irregularity in the data and is difficult to explain. Recently Yipintsoi, et al (*5*) have compared the distributions of

**TABLE 1. RELATIVE TISSUE CONTENTS OF MICROSPHERES (M) AND Rb IN INNER AND OUTER WALL SAMPLES***

| Inner wall samples | | | | | Outer wall samples | | | | |
|---|---|---|---|---|---|---|---|---|---|
| M | Rb | n | t | P | M | Rb | n | t | P |
| 2.4 | 5.1 | 36 | 13.00 | 0.001 | 8.0 | 11.3 | 6 | 2.16 | NS |
| 14.7 | 16.6 | 16 | 2.28 | 0.05 | 14.8 | 18.7 | 19 | 6.47 | 0.001 |
| 25.4 | 27.0 | 12 | 1.06 | NS | 22.9 | 23.9 | 7 | 0.70 | NS |
| 35.0 | 33.3 | 4 | 0.83 | NS | 34.9 | 35.1 | 14 | 0.16 | NS |
| 43.5 | 49.5 | 2 | 1.50 | NS | 44.5 | 45.4 | 14 | 0.42 | NS |
| 54.6 | 54.2 | 9 | 0.22 | NS | 54.9 | 54.4 | 9 | 0.17 | NS |
| 65.3 | 70.1 | 7 | 1.50 | NS | 63.4 | 66.6 | 10 | 0.78 | NS |
| 75.8 | 83.0 | 8 | 1.76 | NS | 75.0 | 75.3 | 12 | 0.08 | NS |
| 85.9 | 92.5 | 8 | 2.30 | NS | 84.4 | 91.8 | 21 | 4.61 | 0.001 |
| 95.0 | 97.8 | 25 | 1.52 | NS | 94.6 | 96.1 | 40 | 1.48 | NS |
| 104.3 | 102.9 | 35 | 1.15 | NS | 104.8 | 99.1 | 21 | 3.08 | 0.01 |
| 115.3 | 106.6 | 16 | 6.01 | 0.001 | 112.3 | 100.5 | 11 | 5.21 | 0.001 |
| 123.7 | 105.3 | 7 | 4.10 | 0.01 | 126.5 | 102.0 | 2 | 3.77 | NS |

* Values for M and Rb expressed as percentage of nonischemic posterior wall and grouped according to content of M. n, Number of samples in each group. t and p, Statistical significance of M = Rb difference by paired Student's t-test.

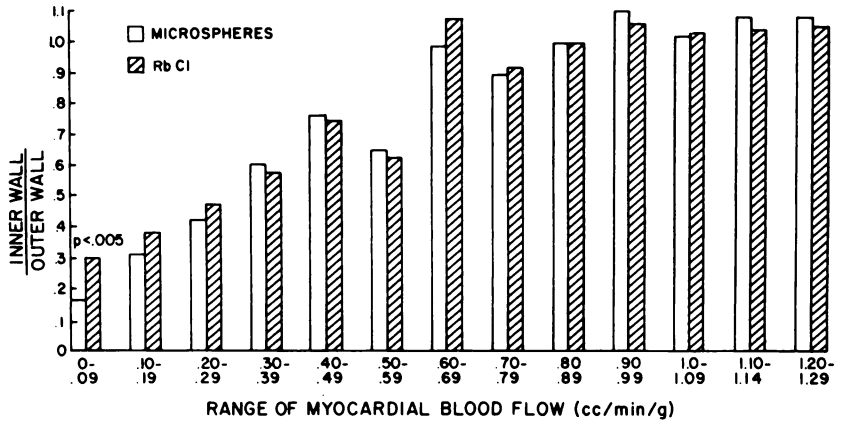


**FIG. 2.** Samples are grouped along horizontal axis by their full wall blood flow. Relative distributions of RbCl and microspheres between inner and outer halves of wall are shown as vertical bars. Note that there is no significant difference between RbCl and microspheres except at very low flows.

several sizes of microspheres and several diffusible indicators in isolated perfused hearts. They also found a relative excess of $^{86}$Rb in low flow areas and a relative lack in high flow regions compared with microspheres although they interpreted the results in the opposite way, i.e., as a relative lack and excess of microspheres, respectively.

Previous studies have shown that $^{86}$Rb uptake systematically underestimates coronary blood flow, particularly at high flows (*3*). In contrast, microspheres measure bloodflow accurately if given in adequate amounts and do not cause a systematic under- or overestimation (*4,6,9,11*). The falsely low flows with $^{86}$Rb appear to be related to the fact that myocardial extraction of $^{86}$Rb (i.e., percentage uptake during each passage through the coronary circulation) is not constant but decreases as flow rate increases (*1–3,13*). This has been thought to result from the shorter time available for $^{86}$Rb absorption in the capillary bed (*3,13*). As a result, the myocardial content of $^{86}$Rb does not increase in direct proportion to blood flow (*1*). These considerations also hold for isotopes of potassium, which distribute similarly to Rb (*14,15*) and are being presently used to estimate myocardial blood flow in man.

The disproportionately high Rb content we found at very low flows is also most likely due to the inverse relation between flow and extraction. As flow decreases, Rb extraction rises relative to nonischemic muscle and Rb content becomes disproportionately high (*1*). Although we cannot dismiss an underestimation of flow by microspheres in this setting, the recent data of Utley, et al (*9*) showing close agreement between microspheres and antipyrine, a diffusible tracer the extraction ratio of which is independent of flow, supports our hypothesis. By using the logarithm of Rb uptake rather than the uptake itself to estimate blood flow, it is possible to at least partially correct for the flow-dependence of Rb extraction (*1*). After this correction, it is unclear whether microspheres or diffusible indicators give a "better" estimate of flow distribution. Lacking an absolute standard of comparison, the two methods can only be compared against each other. As pointed out by Yipintsoi, et al (*5*), both methods may give "correct" distribution data in the sense that microspheres may tend to reflect red cell distribution whereas $^{86}$Rb may better reflect plasma flow distribution.

Early measurements of flow distribution within the left ventricular wall using intramyocardial injections of diffusible radioactive tracer suggested that the subendocardium was relatively underperfused at rest (*16*). However, more recent studies have found subendocardial flow to be equal to or slightly greater than subepicardial flow based on the distributions of both diffusible tracers (*17–20*) and microspheres (*6,7,12,21–23*). When myocardial blood flow is reduced, subendocardial perfusion has been found to

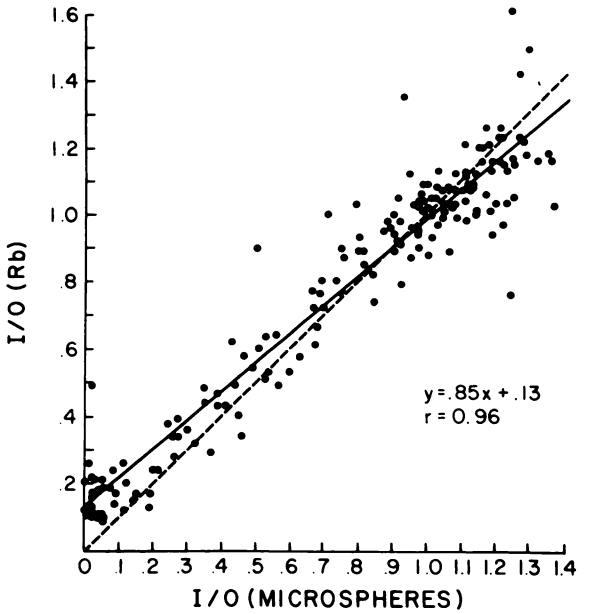


**FIG. 3.** Inner/outer wall tracer contents (I/O) compared for microspheres and Rb. Each point represents single myocardial sample. Solid line represents regression equation and broken line is line of identity. Standard error of estimate of slope is 0.019. Significant deviation of slope from unity is most likely due to dependency of myocardial Rb extraction on flow rate although disproportionate relation between microsphere distribution and flow is also possible.

fall disproportionately (*6,7,12,19,20,22*). The possible reasons for this maldistribution have been reviewed (*24*).

In the present study we have verified these observations and shown the distribution of $^{86}Rb$ and 15 micron-diam microspheres between subendocardium and subepicardium to be similar except in areas of very low flow. The higher I/O ratios found in these areas with $^{86}Rb$ may in part be caused by isotope uptake from the left ventricular cavity (*25*). However, the relative excess of $^{86}Rb$ in subepicardial as well as subendocardial regions of low flow (Table 1), suggests that more complete extraction related to lower subendocardial flow was also responsible.

Large microspheres (50 micron-diam) have been shown to give falsely high values for subendocardial flow and I/O ratios of 1.5 or greater, apparently due to either particle streaming or entrapment in the subendocardial vascular plexus (*4,24*). Although I/O ratios with $^{86}Rb$ and 15 micron spheres were not significantly different in this study, others have shown 5–10% lower ratios with diffusible indicators compared with small microspheres (*5,7,9*) and slightly smaller ratios with 7–10 microns than 15 micron spheres (*5,9,23*). These discrepancies have been attributed to anatomic differences between subendocardial and subepicardial vessels (*5*) or possible differences in flow rate (*5*) or velocity (*9*) in the two layers. Since there is no accepted standard, the question of which tracer if any reflects true transmural bloodflow distribution remains unsettled.

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