

Some sources of error in measuring regional blood flow with radioactive microspheres

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BUCKBERG, GERALD D., JOHN C. LUCK, D. BRUCE PAYNE, JULIEN I. E. HOFFMAN, JOSEPH P. ARCHIE, AND DAVID E. FIXLER. *Some sources of error in measuring regional blood flow with radioactive microspheres.* J. Appl. Physiol. 31(4): 598-604. 1971.—Radioactive microspheres (8–50 μ diameter) distribute in proportion to regional blood flow so that their concentrations in all arteries should be similar but for random variation. Varying numbers of microspheres were injected into the left atrium and ventricles of lambs, sheep, and dogs, and microsphere concentrations were measured in samples drawn simultaneously from two or more arteries (femoral, carotid, or aorta), in the free walls of the left and right ventricles and both kidneys. Coronary and renal blood flows were measured with microspheres (using peripheral reference samples) and by independent methods. Despite some nonrandomness (poor mixing) errors were usually under 20% for all injection and sampling sites in both species as long as each sample had over 400 microspheres; lesser numbers caused errors up to 113%. Variability was less in dogs than sheep, less for left atrial injections, but equivalent in all arteries. Coronary and renal flows by microspheres were usually within 20% and 6%, respectively, of measured flow. The greatest precision occurs on injection of the largest number of microspheres consistent with no physiological changes.

binomial distribution; Poisson variate; coronary blood flow; renal blood flow; left atrial injection; left ventricular injection

RECENTLY RUDOLPH AND HEYMANN (8) introduced a method of measuring regional blood flow with radioactive microspheres 50 μ in diameter. These were injected into the circulation and traveled to the small peripheral vessels where they were trapped; the organs were then removed and their radioactivity measured. Repeated measurements could be made by using different nuclides and separating them by gamma spectrometry. In several studies they and other workers (2, 3, 5, 8) showed that the fraction of total radioactivity in various organs was similar to the fraction of cardiac output reaching those organs during the transit of the spheres, but the variability of the method and the factors responsible for that variability have not yet been fully defined.

For this method to be able to measure flow to any organ the microspheres must be well mixed at the site of injection, should be distributed after injection in proportion to regional blood flow, should all be trapped in small vessels in

the first circulation and should not disturb the circulation. Then, if these criteria are met, flow to any organ can be calculated in two main ways. 1) The cardiac output is measured and multiplied by the proportion of microspheres found in an organ to the total number of microspheres injected to give the flow to that organ. Measuring cardiac output and the proportion of organ microspheres are both subject to error. 2) The flow to (F_k) and radioactivity (C_k) in one organ are measured and, since the method implies that the ratio of flow and radioactivity will be the same in all organs, we can determine the flow (F_u) to any other organ by measuring its radioactivity (C_u) and calculating $F_u = F_k \cdot C_u / C_k$. To avoid the problems of measuring a known organ flow Neutze and Wyler (personal communication), Makowski et al. (5) and Domenech et al. (2) withdrew a known quantity of arterial blood (reference sample), measured its radioactivity and that of the organ in which the flow was required, and then calculated the flow to that organ.

The reference sample method is the simplest of all but its own errors need to be assessed. We have, therefore, investigated three major aspects of the method. First, we studied the criteria for an adequate reference sample—the minimum number of microspheres it must contain, the ideal volume of blood collected and the optimal duration and method of collecting the sample. In addition, we tested the implied assumption that all arteries are equally good for supplying the reference sample. Second, we determined if the microspheres were well mixed when injected into the left atrium or left ventricle and if there was any species variability between dogs and sheep. To do this one or more batches of microspheres were injected into the left atrium or ventricle and their distribution in right and left ventricles, right and left kidneys, and in two or more simultaneously obtained reference samples was examined. Third, we determined the accuracy and limitations of the method in measuring organ flow by making independent measurements of renal and coronary blood flow and comparing these with flows calculated by the reference sample method.

MATERIAL AND METHODS

We anesthetized 14 lambs, 13 sheep, and 30 dogs with sodium pentobarbital 30 mg/kg intravenously, intubated

the trachea, and ventilated them with room air with a Harvard respirator. Polyethylene catheters (PE-240) were inserted into one or both femoral and carotid arteries to collect the reference samples. To avoid trapping microspheres between the arterial wall and the catheter we tied a ligature around the artery about 1 mm from its tip; these peripheral arterial collections were compared with those obtained by advancing one of the femoral arterial catheters into the abdominal aorta (central sample).

Arterial blood pressure was recorded from a Statham P23Dc transducer and a catheter placed in the internal mammary artery through a left lateral thoracotomy. We placed a small polyethylene catheter into the left atrium through a branch of a pulmonary vein. When injecting microspheres into the left ventricle we catheterized the left ventricle retrograde and placed the tip well below the aortic valve. The position of the catheter was not confirmed by fluoroscopy. All injecting and sampling catheters had single end holes. In eight studies we monitored mean and phasic flow in branches of the left and right coronary arteries with 2-mm and 2.5-mm Biotronics electromagnetic flowmeter transducers. We did not use the few studies (under 2% of the total) in which microsphere injection changed arterial systolic, diastolic, or mean pressure or cardiac rate or rhythm, or mean or phasic coronary blood flow.

Right heart bypass studies were done in 15 dogs and 6 sheep as previously described (2). Coronary venous return was continuously monitored with a Statham 4001 cannulating electromagnetic flowmeter transducer and measured by direct collection into a graduated cylinder for timed intervals. In sheep, the left hemiazygous vein was ligated at its entry into the coronary sinus before beginning the bypass.

To measure renal blood flow we tied a polyethylene tube (PE-240) into the right renal artery in three dogs and perfused the kidney by connecting the other end of the tube to the right femoral artery. Flow to the right kidney was measured by placing in the line a calibrated 5-mm-diameter Statham 4001 electromagnetic flow transducer.

The microspheres were suspended in 10% dextran and 0.5% Tween 80, placed in an injection chamber (8) and injected into the left atrium or ventricle over 10–20 sec as previously described (2). We used 50- μ -diameter microspheres labeled with ^{125}I , ^{145}Ce , ^{51}Cr , ^{85}Sr , and ^{95}Nb ; 15- μ -diameter microspheres labeled with ^{125}I ; 12- μ -diameter microspheres labeled with ^{95}Nb ; and microspheres of 8–10 μ diameter labeled with ^{141}Ce or ^{85}Sr . When two differently labeled batches of microspheres were injected simultaneously they were mixed in the same injection chamber. We obtained reference samples in most studies by adjusting the stopcock on the arterial catheter to establish the desired flow rate (usually 15–25 ml/min, but sometimes as low as 3 ml/min or as high as 60 ml/min) and collected the blood into weighed plastic vials, changing the vials every 30 sec for 1–2 min. In other studies, reference flows were withdrawn by a Harvard withdrawal pump for 60 sec. The connecting tubing and stopcocks were examined for radioactivity after completing both types of collections. The heart and kidneys were cut up and placed in vials for counting by the method of Rudolph and Heymann (8).

STATISTICAL ANALYSIS

Even if the spheres are well mixed and distributed in proportion to regional flow, the number of spheres per unit volume per unit time would not be identical in two reference samples drawn simultaneously because of random variation. This variability would be equally true in blood flowing to organs so that analysis of paired reference samples gives information about the precision of measuring any flow by this method.

If two simultaneously collected reference samples have volumes per unit time of V_1 and V_2 (total volume V_t) and the number of spheres in each sample respectively X_1 and X_2 (total X_t) we can test the differences in concentrations X_1/V_1 and X_2/V_2 and determine if they are greater than expected by chance. To do this we put forward the hypothesis that the total number of spheres X_t should be divided between the two samples in proportion to their volumes. Therefore, the expected number of spheres in sample 1 would be fX_t , where f is V_1/V_t . The difference between the observed and expected numbers ($X_1 - fX_t$) should be a random variate with standard deviation $\sqrt{X_t f(1-f)}$ according to the binomial distribution (9). The ratio of these is z , a standard deviate with a mean equal to 0 and a standard deviation of 1.

The z ratio can be used to determine if the difference between the observed and expected numbers of spheres in the smaller of the two samples exceeds what would be expected from random variability; a z value of 1.96 or more would occur in only 5% of samples chosen at random. Furthermore, by setting $z = 1.96$, the 95% confidence limits of X_1 can be determined for specific values of X_t and f . These limits are in numbers of spheres and can be transformed into relative concentrations of spheres per unit time by dividing the number of spheres by the proportion of total volume in that sample. Finally, the difference between the 95% limiting concentrations in the two samples can be expressed as a percentage error by dividing that difference by the weighted mean of the two concentrations and multiplying by 100. Thus the percentage error at the 95% limit (we are concerned only with the upper limit) is

$$100[X_1/f - X_2/(1-f)]/(X_1 + X_2)[f + (1-f)] \\ = 100[X_1(1-f) - X_2f]/f(1-f)X_t$$

Another way of examining the theoretical distribution of the spheres is to consider the proportion of them reaching any organ or part of an organ as binomially distributed but with f being a very small fraction of the total; for most organs this will be true, since the proportion of cardiac output going to any one organ is usually much less than 10% (3). The variability of distribution of spheres to any one organ if the experiment could be done many times under identical conditions should then approximate a Poisson distribution with X being the mean number of spheres reaching that organ and \sqrt{X} being the standard deviation. It is then possible to calculate what value of X is required so that the 95% limits or 99% limits of repeated measurements of X will be within a given percentage of X , e.g., 10%, 5%, or 1%. For the 95% limits to be within 10% of X , $1.96\sqrt{X} =$

TABLE 1. Required numbers of microspheres

Confidence Level	Precision		
	10%	5%	1%
95%	384	1,536	38,400
99%	685	2,742	68,564

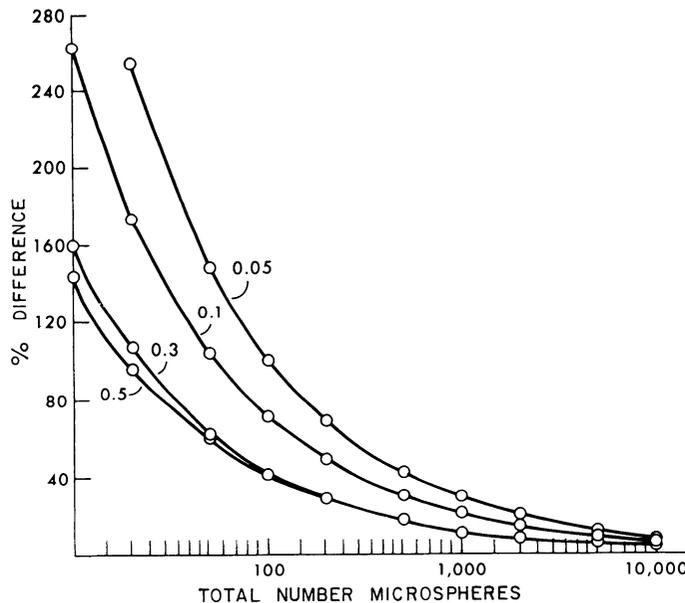


FIG. 1. Vertical axis: percentage difference between microsphere concentrations of two simultaneously drawn samples. Horizontal axis: combined number of microspheres in the two samples plotted on a logarithmic scale. Each line represents the upper 95% confidence limit for a different value of $f = V_1/V_t$, that is, the relative volume of blood in the smaller sample (see text) and, therefore, the relative numbers of total microspheres expected in that sample. Note that the smallest expected differences occur when $f = 0.5$. All values have been corrected for continuity.

0.1X and X should be 384. Table 1 shows the required number of microspheres for different levels of precision and confidence. For practical purposes the number of 400, close to 384, will be chosen as a limiting number which allows enough precision without requiring an unphysiologic excess of spheres to be given to the animal.

RESULTS

Figure 1 gives the theoretical upper 95% limit of the percentage error between microsphere concentrations per unit time for different values of f and X_t , assuming that only random factors affect the distribution. The main determinant of the percentage error is the total number of spheres in the two samples, and the error decreases as the total number of microspheres increases. Both of these conclusions are predictable from any statistical analysis of variability. This figure also shows that variability is determined by sphere numbers and not absolute sample volumes.

If spheres are reasonably well mixed the ratio of the number of spheres in two samples will resemble the ratio of the volumes of those samples. For any total number of microspheres, variability is least when the volumes of the two

samples are equal ($f = 0.5$). If the two sample volumes are slightly unequal ($f = 0.3$) variability is only slightly increased for any number of microspheres. If, however, the volumes are markedly different ($f = 0.1$ or less) then the variability for any total number of microspheres is much greater for small numbers of microspheres but the discrepancy diminishes rapidly as numbers increase.

In analyzing the results of the experiments, no differences could be attributed to the diameter of the microspheres so that their size has been ignored in what follows. We collected 50 right and left femoral arterial samples in 4 sheep and 10 dogs in consecutive 30-sec samples for 1.5–2 min and found that at least 98% of the collected microspheres appeared in the 1st min. The percentage of these appearing within the first 30 sec varied from about 40% to 98% and was largest when the duration of the injection was shortest.

When blood dripped freely from the stopcocks the volumes collected in the first two 30-sec periods differed by less than 15% in 166 of 210 samples in both sheep and dogs. Heart rate and rhythm and systolic and diastolic blood pressures did not change when the collected volumes differed. We have not included any results obtained when these volumes differed by more than 15%. This variability was avoided when a constant flow rate was achieved by drawing samples into a syringe with a withdrawal pump. With both collection methods no radioactivity was found in the stopcocks or the tubing connecting the catheter and the syringe after completing a 1-min collection.

With left atrial injections of microspheres in dogs there was no difference in the relation of variability to the number of spheres collected for different pairs of sampling sites: carotid-carotid, carotid-femoral, femoral-femoral, or peripheral arterial-abdominal aorta (Fig. 2A). There was no systematic difference between left and right carotid or femoral arteries or between peripheral and central samples. Since 27/99 (27%) points fell above the upper 95% confidence limit, there was some nonrandomness. However, with few exceptions, the deviations were quite close to the upper 95% limit, and even these deviations became smaller as the total number of spheres increased. The biggest discrepancies were always with fewer than 800 total spheres or less than 400 spheres in any one sample when 17/38 (45%) of the differences exceeded 20%. Above a total of 800 spheres the maximum difference was 34%, only 1/61 (1.6%) exceeded 20% and only 6/61 (10%) exceeded 10%. The mean difference was 4.5%. In the only experiment in which the difference between two samples exceeded 20% with a total of more than 800 microspheres, there were only 230 microspheres in one sample.

We pooled the data for left atrial injections in lambs and sheep since there was no difference between them (Fig. 2B). When the sphere concentrations per unit time were compared in two different reference samples drawn at the same time, 44/132 (33%) points fell above the upper 95% limit, but the difference between sheep and dogs was not significant ($0.5 > P > 0.25$). Below 800 total microspheres, 13/35 (24%) were above the upper 95% limit, whereas above this total 31/77 (40%) exceeded this limit; the difference is not significant, ($0.10 > P > 0.05$). However, below the total of 800 spheres the differences between the two samples ranged from 0 to 113%, with a mean of 26%, and 14/55 (25%)

exceeded 20%, and above 800 the differences ranged from 0 to 73% with a mean of 11%, and only 7/77 (9%) of the differences exceeded 20%; the difference between the numbers exceeding 20% in these two groups is significant ($0.05 > P > 0.025$).

For comparison of left atrial and ventricular injections we analyzed only samples containing more than 400 spheres. The simultaneously collected femoral and carotid samples agreed within $10 \pm 8\%$ (mean and standard deviation) after injecting 23 different batches of microspheres into the left ventricle in six dogs. This variability is more than that seen after left atrial injection in the dog ($P < 0.05$) and occurred because four reference samples disagreed by 13–34% in one dog who had six successive left ventricular injections; the other two injections showed the reference sample concentrations agreeing within 2%. If we exclude these four discrepant results, there was no difference in variability resulting from left atrial or ventricular injections.

There was no significant difference between carotid-carotid or femoral-femoral comparisons when comparing the results from 14 different left ventricular injections ($9 \pm 4\%$) in 4 sheep to those after left atrial injections in 23 different sheep ($10 \pm 5\%$). There was considerably more variability ($20 \pm 13\%$) when we compared carotid-femoral samples after injecting spheres into the left ventricle, 20 of 39 of these paired samples disagreeing by more than 20%. This is a significant difference from the femoral-femoral and carotid-carotid comparisons ($0.025 > P > 0.01$) and the discrepancy between sampling sites was not reduced with increased numbers of spheres. Unlike dogs, in sheep the mean concentration of microspheres was 6% lower in the carotid than in the simultaneously collected femoral arterial samples ($0.025 > P > 0.01$). However, there was no significant difference between left and right femoral or left and right carotid arteries.

We compared sphere concentrations in different volumes by separating results when flow rates were either more or less than 20 ml/min. Although there was slightly better agreement between collection sites when more than 20 ml/min was collected, this was principally related to having more spheres in larger volumes. When samples containing 400–1,000 spheres were compared there was no significant difference related to volume. In fact, only $7 \pm 4\%$ (SD) difference between 23 paired samples was seen in dogs when more than 400 spheres were collected in each sample and flow rates were below 3 ml/min.

Coronary blood flow. We made 21 measurements of coronary blood flow in 6 sheep and 70 measurements in 15 dogs (Fig. 3). Coronary flows ranged from 65 to 550 ml/min in these studies and the reference samples all had over 400 microspheres. In sheep and lambs, calculated coronary blood flow after left atrial injection averaged 1.4% less than measured flow, with a range of difference from 38% to –26%. Only 3 of 21 calculated flows differed by more than 20% from measured flows. Because of the variability of the measurements the slope of the regression line (0.71) was not significantly different from a slope of 1.

In dogs after left atrial injection the differences between calculated and mean flows averaged –3.6%, with extremes of 25% and –35% and with 10 of 50 differences exceeding 20%. After left ventricular injection the mean difference

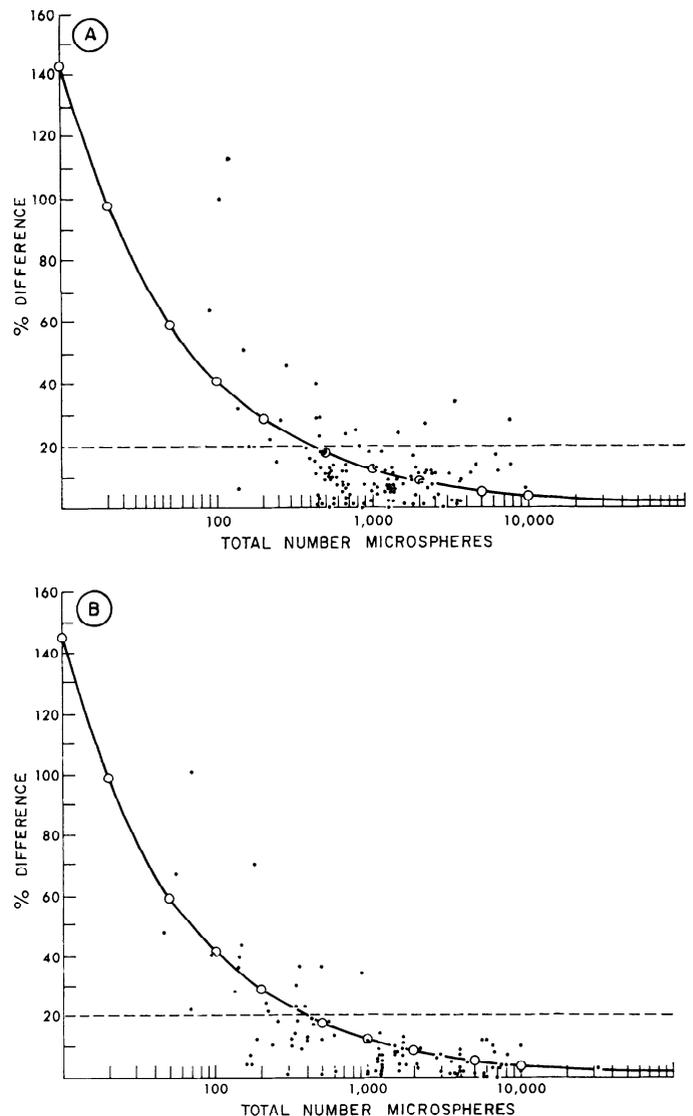


FIG. 2. Vertical axis: percentage difference between microsphere concentrations of two simultaneously drawn samples. Horizontal axis: total number of microspheres in both samples irrespective of site plotted on a logarithmic scale. Curved solid line shows the upper 95% confidence limit for this difference for a range of f values from 0.3–0.5. Dashed horizontal line indicates a difference of 20% between sample concentrations. A: left atrial injection in dogs. B: left atrial injection in sheep and lambs.

was 4.7%, the range was from 21% to –20% and only 2 of 20 differences were over 20%. The slopes of the regression lines after left atrial and ventricular injections did not differ significantly and averaged 0.89; the intercept was not significantly different from a slope of 1 ($0.05 > P > 0.025$). There was no difference in results when either femoral or carotid reference flows were used in either species. Less than 1% of the total spheres contained within the heart appeared in the coronary sinus drainage in all studies, including those with 8- to 10- μ -diameter microspheres.

We also compared the ratios of microspheres per 100 g of left (LV) and right (RV) ventricles in 23 dog experiments when we injected two or more different batches of microspheres simultaneously. Two of these studies were done in

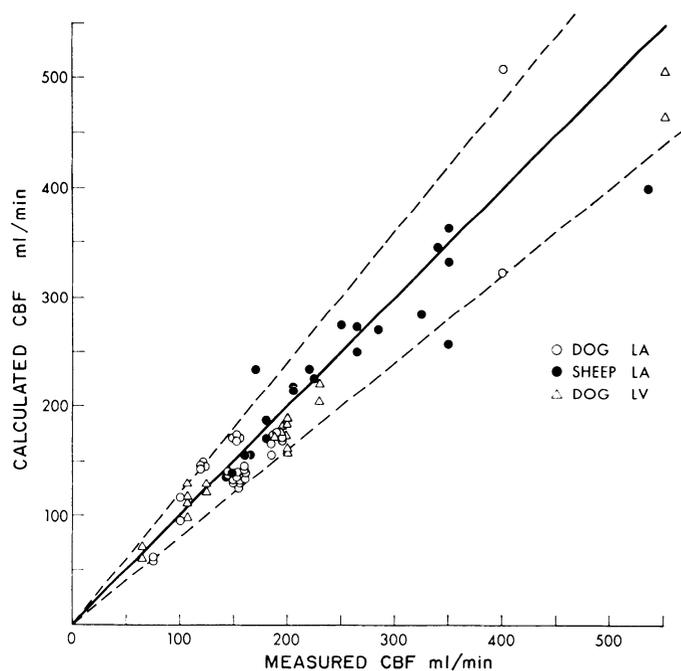


FIG. 3. Horizontal axis: measured coronary venous drainage during right heart bypass in dogs and sheep. Vertical axis: coronary blood flow calculated by microsphere method using reference samples as the known flow. Solid line is the line of identity, and broken lines indicate 20% variation from this. CBF = coronary blood flow.

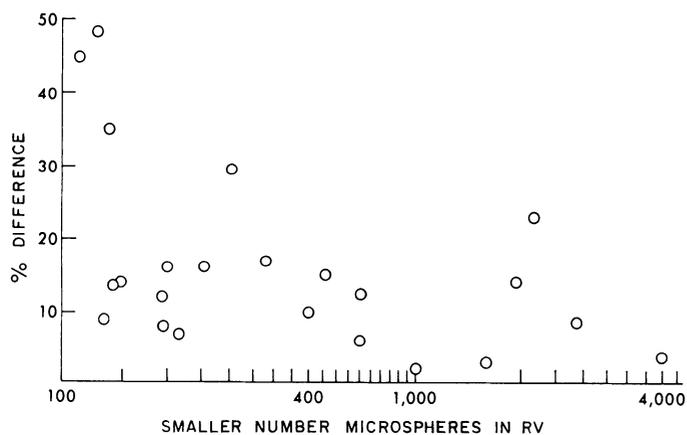


FIG. 4. Vertical axis: ratio of flow per 100 g of left and right ventricular free walls in dogs after simultaneous injection of two different batches of microspheres. Horizontal axis: smaller number of microspheres in right ventricular free wall plotted on a logarithmic scale. LV = left ventricle; RV = right ventricle.

dogs undergoing right heart bypass. When we plotted the percent difference between the LV/RV ratios against the number of spheres contained within the right ventricle, most of the variability occurred within less than 400 spheres in the right ventricle (Fig. 4). The ratios were more than 15% different in 7 of 13 studies when the right ventricle contained less than 400 spheres and only 1 of 10 were more than 15% different when over 400 spheres were present within the right ventricle; the difference is not quite significant ($0.10 > P > 0.05$). There was under 10% difference in LV/RV ratios in two sheep with simultaneous injections of isotope and with more than 400 microspheres in the right ventricle.

Renal blood flow. When we compared 12 renal blood flow measurements made with cannulating electromagnetic flowmeter transducers in three dogs with flows calculated by the reference method we found that these agreed within $6 \pm 2\%$. Renal blood flow was not changed by the microsphere injections and remained stable between injections (3-hr observation period). The ratio of microsphere concentrations per gram of left and right kidneys was 1.0 ± 0.07 sd in 19 studies in six sheep with intact renal vessels who received one batch of microspheres at a time. Each kidney contained at least 4,000 microspheres.

DISCUSSION

Our results show that differences between microsphere concentrations in two or more samples collected simultaneously can largely be explained by random variation. In general, the limits of variability decrease as sphere numbers increase and do so almost as predicted from the binomial distribution. However, the excess number of differences above the upper 95% confidence limit indicates that some nonrandomness exists; it is presumably related to inadequate mixing which can occur with any number of injected microspheres. This nonrandomness does not lead to serious practical difficulties as long as there are at least 400 microspheres in each sample, since Fig. 2, A and B, shows that differences above 20% are rare when these numbers are used. The same freedom from serious error should hold if instead of two reference samples we consider one reference sample and one organ (Fig. 3).

The theoretical and experimental results suggest that the usefulness of the reference sample in predicting regional flow is determined by the number of microspheres contained in both the reference sample and the organ of interest. This can be achieved by injecting sufficient microspheres to be sure that the organ receives enough microspheres and collecting enough blood so that an adequate number of microspheres are present in the reference sample. We used this approach to study coronary blood flow and chose to accept an error of up to 10% with 95% confidence because of possible practical limitations of the number of spheres that could safely be injected. Since we were interested in studying the distribution of flow to each ventricle, and the right ventricle usually receives about 20% of coronary blood flow (1% of cardiac output), we injected 40,000 microspheres in order to have about 400 in the right ventricle. Figure 4 shows the variability in RV/LV ratios during simultaneous injection of more than one microsphere and illustrates how the variability increased when the right ventricle did not contain enough spheres. The variability in this graph is similar to that seen in Fig. 2, A and B, where we compared two simultaneously collected reference samples. If we were interested in studying the blood flow to the papillary muscle of the right ventricle (assuming it receives 10% of right ventricular blood flow), we would need to inject 400,000 spheres.

This analysis also points out one of the most important aspects of the microsphere method, namely, that its precision depends mainly on the numbers of microspheres in the organs and reference samples and not on the amount of radioactivity. Counting accuracy is of secondary importance

and can be improved by longer counting times if the count rate is very low. If, however, small numbers of microspheres lead to marked random variability of their distribution to different organs, no degree of counting accuracy will correct the error. For example, if an organ containing two differently labeled batches of microspheres had count rates of 5,000 counts/min from each nuclide and if the activities per microsphere were 5,000 and 1 count/min, then that organ would contain respectively 1 and 5,000 of each type of microsphere. Although the efficiency of counting each nuclide would be similar, the errors in measuring flow would be vastly different for the two types of microspheres.

The volume of blood that must be collected at the reference sites can be estimated from predicting the cardiac output and blood volume and by judging what volume of blood could be removed without changing blood pressure and cardiac output significantly. For example, if cardiac output were estimated at 1 liter/min and blood volume at 1 liter, withdrawal of 10 ml of blood (1% of cardiac output) in 1 min would probably be well tolerated. Thus to get 400 microspheres in the reference sample, 40,000 microspheres would need to be injected. This would assure a measurement of flow correct to within 10% to any organ with a blood flow of about 10 ml/min, but an organ with a blood flow of about 1 ml/min would receive only about 40 microspheres so that in about 5% of the injections errors of over 37% would be expected in calculating flow to that organ (Fig. 1). If the reference sample were restricted to 1 ml of blood, then 400,000 microspheres should be injected and this total number would allow flows as low as 1 ml/min to be estimated with no more than about 12% error. (Clearly introduction of large numbers of microspheres to a small unit of tissue must be shown not to interfere with organ function or flow if subsequent studies are to be done.) Use of small numbers of microspheres will be associated with large errors and low precision of estimate, but should not lead to a systematic over- or underestimate of mean flow to any organ. With small numbers and large errors, however, more experiments will be needed to demonstrate the significance of any given difference.

The volume of blood collected in the reference sample did not directly influence the results, as predicted from theory, and when small volumes were associated with large numbers of microspheres the errors were low. This confirms the findings of Makowski et al. (5) who collected reference samples at 1.25 ml/min and obtained agreement within 10% between paired samples which contained 400–2000 microspheres each.

The cause of the increased variability seen after left atrial injection of microspheres in sheep compared to dogs is not clear. It did not seem to be related to cardiac output or heart rate. The significantly 6% lower mean values in carotid as compared with simultaneous femoral samples might be related to the different anatomy of the aortic arch in these species. In sheep, the brachiocephalic trunk arises immediately above the aortic valve, so that any tendency for axial concentration of microspheres in the aortic root might lead to lower microsphere concentrations in the brachiocephalic trunk and its branches. More distally mixing would be better, thus explaining the equality of microsphere concentrations in left and right carotid or

femoral arteries in both species. This discrepancy between carotid and femoral microsphere concentration in sheep suggests that in them femoral reference samples should be used to calculate flow from abdominal organs, whereas carotid reference samples should be used for upper body flow.

Inadequate mixing may be a major problem after left ventricular injection since in both species variability was greater after left ventricular than atrial injection. This increased variability might have been due to the position of the catheter, for if it was in the outflow tract there might well have been inadequate mixing of injected microspheres by the time they reached the root of the aorta; the variability might be exaggerated in sheep because of their aortic root anatomy. Poor mixing certainly accounted for the four very marked discrepancies found in left ventricular injection in one dog in our series. Unless a particular catheter position in the left ventricle has been shown to give good mixing under all circumstances, the investigator is advised to collect simultaneous femoral and either brachial or carotid reference samples and regard major discrepancies between their microsphere concentrations as evidence of poor mixing.

Although accuracy increases as more microspheres are present within the reference flow and the organ of interest, the maximal number of microspheres that may be injected without causing physiological changes is not known for any organ. It will vary with the size of the microspheres and of the animal and probably with organ flow. In our studies here and previously (2), injection of up to 1 million 50- μ or 10- μ spheres 5 or 6 times has not caused any detectable change in heart rate, left ventricular or aortic blood pressure, the maximal rate of rise of left ventricular pressure, aortic or coronary blood flow, or postischemic hyperemia in a branch of the left coronary artery. Others have found no changes in the distribution of flow to many organs after several injections of microspheres. We, therefore, do not believe that in the numbers used here any significant changes in organ flow were caused during or after the injections.

The smaller spheres have several advantages over larger ones in that they are distributed more like red blood cells (7), occlude less of the vascular bed, are less variable in size, and more of them can probably be safely given, thus allowing more reliable measurements of flow to smaller regions. Another advantage of the 8- μ microspheres that we have found is their equal concentration per gram of subendocardial and subepicardial muscle. This is similar to the distribution of diffusible indicators but differs markedly from the distribution found with 14- to 50- μ microspheres (2). These different distributions relative to microsphere size are probably related to the greater axial concentration of the larger microspheres (7) and would not be affected (other than randomly) by the numbers of microspheres injected.

Use of the 8- to 10- μ -diameter microspheres, however, depends on all of them being trapped in the vascular bed under examination. We have shown that usually under 1% of small microspheres entering the myocardial vascular bed pass into coronary venous drainage, but this percentage would need to be determined for any other organ studied.

We collected the reference flows initially in plastic vials

rather than using a constant withdrawal syringe so that we could see if changes in flow rate occurred during these injections and determine if a significant number of spheres were present in the last 30-sec sample. Unfortunately almost 25% of samples collected in this way had to be discarded because the collection volumes differed by more than 15%. We used a withdrawal pump in recent studies to avoid this problem, but could only assume a steady state by monitoring arterial blood pressure and heart rate. We assume the variations in flow rate at the reference site were caused by obstruction in the withdrawal line since they occurred despite a steady state of arterial, systolic, and diastolic pressures, heart rate and rhythm, and mean and phasic coronary blood flow. It is important to show that microspheres are not contained within the last small quantity of collected blood (any collection method) because regional flow would be overestimated if some microspheres were not accounted for in the reference sample. This would be particularly important if injections are made when cardiac output is low (shock) and reference samples should probably be collected for more than 1 min at these times so that microspheres with transit times above 1 min will be captured.

Our findings of good correlation between measured and calculated coronary blood flow in dogs confirms the studies of Domenech et al. (2). Although the slope of the regression line relating calculated and measured flows was significantly different from 1, the actual difference was small and due to three unusually discrepant observations. The results in sheep show that this method can also be used to study total coronary blood flow in that species. The accuracy of coronary flow predictions after both left ventricular and left atrial injections in dogs indicates that coronary flow can be studied without opening the chest, since the left ventricle can be catheterized retrograde from a peripheral artery. These data differ from the results of Kaihara et al. (4) who reported variable percentages of total injected microspheres within the heart after left ventricular injections. Our re-

sults are not really comparable to theirs since their conclusions were based on only two microsphere injections made by percutaneous left ventricular puncture techniques and they did not measure coronary blood flow. It is, however, important to realize that at times left ventricular injections can be associated with more variable results. We have not yet measured coronary blood flow after injecting microspheres into the left ventricle of sheep, but we would expect more variable results than in dogs because of the increasing variability between reference sites seen after left ventricular injections in sheep.

The variability in calculated coronary flows in dogs was greater than that in the reference flows. The difference might be related to slightly uneven mixing of spheres as they leave the heart. This variability would probably be more pronounced were it not for the vortices produced in the aortic sinuses; these vortices probably cause more homogeneous distribution of particles (1). It is possible that additional variability is introduced with separating the different components of radioactivity when analyzing superimposed compound energy spectra.

The excellent correlation between measured and calculated total renal flow might be expected since the kidneys receive a large proportion of cardiac output and contain a large percentage of the total injected microspheres. Our results are similar to those reported by Neutze, Wyler, and Rudolph (6). This correlation further demonstrates the importance of the numbers of microspheres in an organ in determining the accuracy of measurement of its flow.

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