Simultaneous Measurement of Total and Nutritional Coronary Blood Flow in Dogs

By ADRIAN D. LEBLANC, PH.D., RICHARD C. RILEY, PH.D., AND RALPH G. ROBINSON, M.D.

SUMMARY
Significant correlation has been shown between the severity of coronary disease and level of coronary blood flow as measured by the rubidium coincidence technique. Contrary to the commonly expressed view, however, the rubidium coincidence method does not evaluate nutritional flow, but estimates total coronary blood flow (CBF_T). An equation is derived that does evaluate nutritional flow (CBF_N), allowing simultaneous measurement of total and nutritional flow.

The extraction ratio of the total body (ER_Tb) relative to the extraction ratio of the heart (ER_C) was measured in a total of 21 dogs by employing the relative uptake of labeled microspheres and diffusible ions, 86Rb and 131Cs.

CBF_T, measured with a bolus injection of 86Rb or 131Cs, was compared with total flow measured by the microsphere technique in dogs, with and without drug administration (norepinephrine, isoproterenol, nitroglycerin). These studies showed that while rubidium gives accurate mean values of total flow (4% mean difference, r = 0.92) individual estimates may be in error by as much as ±42%. It was found, however, that isotopes of cesium are not suitable for measuring either total or nutritional coronary blood flow by the methods described in this paper.

Comparison of CBF_N with CBF_T showed that not only was CBF_N significantly less than CBF_T, but the percentage increase in CBF_N was significantly less than that of CBF_T after norepinephrine or isoproterenol administration.

Additional Indexing Words:
Rubidium-86  Norepinephrine  Cesium-131  Isoproterenol  Nitroglycerin
Radioactive microspheres  Extraction ratios

It is generally believed that the microcirculation of skeletal muscle is composed of both effective (nutritional) and shunt or nonexchanging circuits.1–4 Presumably the coronary vascular bed is also composed of nutritional and nonnutritional circuits. Knowledge of either by itself does not give a complete description of coronary blood flow. Depending on the technique used to measure coronary blood flow, the measured variable may be total flow per unit mass, nutritional flow per unit mass, total organ flow, or total organ nutritional flow.

The rubidium-84 coincidence technique using a bolus injection is the method currently employed by some investigators for measuring coronary blood flow.5–10 Good correlation between coronary blood flow and the severity of coronary artery disease has been demonstrated.11, 12 Contrary to what is generally believed, however, the equation used in the 84Rb coincidence technique employing a bolus injection most likely measures total coronary flow (CBF_T) and not coronary nutritional flow (CBF_N). This will be shown mathematically in the following section and will also be demonstrated experimentally by comparing total coronary blood flow obtained from an intravenous bolus injection of 86Rb with total coronary flow determined by the microsphere technique. The microsphere technique, employing either left atrial or left ventricular injection of radioactive microspheres, has been described.13–15 In addition, an equation will be derived which does estimate nutritional coronary flow (CBF_N) and which employs the same bolus injection used to obtain total coronary flow. Therefore, it was possible to study the effect of drugs on both total and nutritional flow determined simultaneously.

In the derivation of the equation for total coronary blood flow, it is assumed that the extrac-
tion ratio of the total body (ERTB) is equal to that of the heart (ERC). An extraction ratio is the fraction of a diffusible tracer removed in a single passage through an organ. Some estimates using $^{84}$Rb and $^{86}$Rb have been made comparing the total or lower body extraction ratio with that of the left ventricle but not with the entire heart. The above assumption has even greater significance when drugs designed to alter coronary blood flow are administered during coronary blood flow measurement. The main objective of this study was to test the above assumption (i.e., that the extraction ratio of the entire heart is equal to the extraction ratio of the total body) by determining the ratio ERTB/ERC in dogs with and without administration of the coronary vasoactive agents isoproterenol, nitroglycerin, and norepinephrine. The coronary extraction ratio of isotopes of cesium have been shown to be considerably less than that of rubidium. However, since measurement of CBF only requires that ERTB = ERC, the values of ERTB and ERC were determined using $^{131}$Cs as well as $^{86}$Rb.

Methods

1. Theoretical Considerations

By utilizing the Fick principle for measuring organ flow, one can express total coronary blood flow (CBFT) as follows:

$$\text{CBF}_T = \frac{q_t}{\int_0^1 a(t)dt - \int_0^1 v(t)dt} \ (1)$$

where CBF_T = total coronary blood flow (ml/min)
$q_t$ = myocardial uptake of a diffusible tracer at time t ($\mu$Ci)
$\int_0^1 a(t)dt$ = integrated arterial concentration from the time of injection to time t ($\mu$Ci min/ml)
$\int_0^1 v(t)dt$ = integrated myocardial venous concentration from the time of injection to time t ($\mu$Ci min/ml).

The frequency function measured with a nondiffusible tracer is the fraction of indicator entering or leaving a system per unit time at time t. The extraction ratio of the heart (ERC) for any tracer is

$$\text{ERC} = \frac{\int_0^1 a(t)dt - \int_0^1 v(t)dt}{\int_0^1 a(t)dt} \ (2)$$

Substitution of equation 2 into equation 1 gives

$$\text{CBF}_T = \frac{q_t}{\text{ERC} \int_0^1 a(t)dt} \ (3)$$

By using the Fick principle, we can express cardiac output as follows:

$$\text{C.O.} = \frac{Q_t}{\int_0^1 a(t)dt - \int_0^1 v(t)dt} \ (4)$$

where C.O. = cardiac output,
$Q_t$ = total body uptake of a diffusible tracer at time t ($\mu$Ci),
$\int_0^1 v(t)dt$ = integrated total body venous concentration from the time of injection to time t.

The total body extraction ratio (ERTB) is

$$\text{ERTB} = \frac{\int_0^1 a(t)dt - \int_0^1 v(t)dt}{\int_0^1 a(t)dt} \ (5)$$

Substitution of equation 5 into equation 4 gives

$$\text{C.O.} = \frac{Q_t}{\text{ERTB} \int_0^1 a(t)dt} \ (6)$$

Division of equation 3 by equation 6 gives

$$\frac{\text{CBF}_T}{\text{C.O.}} = \frac{q_t}{Q_t} \times \frac{\text{ERTB}}{\text{ERC}} \ (7)$$

This latter equation provides a direct method for calculating the ratio ERTB/ERC and will be referred to when this subject is discussed. Cardiac output can also be expressed by using the Stewart-Hamilton formula:

$$\text{C.O.} = \frac{I}{\int_0^1 a(t)dt} \ (8)$$

where I = amount of tracer injected ($\mu$Ci),
$\int_0^1 a(t)dt$ = integrated first arterial circulation curve ($\mu$Ci min/ml).

Substitution of equation 8 for cardiac output in equation 7 and rearranging gives

$$\text{CBF}_T = \frac{q_t}{Q_t} \times \frac{I}{\int_0^1 a(t)dt} \times \text{ERTB} \ (9)$$

If ERTB = ERC, equation 9 reduces to the equation used in the $^{84}$Rb coincidence method:

$$\text{CBF}_T = \frac{q_t}{K \int_0^1 a(t)dt} \ (10)$$

where K = $\frac{Q_t}{I}$.

Equation 10 is the expression which has been used to estimate nutritional flow.5-10 As shown here, this expression actually estimates total flow.

Nutritional flow can be defined as the product of total flow and the extraction ratio of a flow limited tracer such as rubidium or potassium. For the heart this would be expressed as follows:

$$\text{CBF}_N = \text{CBF}_T \times \text{ERC} \ (11)$$

Substitution of this expression into equation 7 gives

$$\text{CBF}_N = \text{C.O.} \times \text{ERTB} \times \frac{q_t}{Q_t} \ (12)$$
This expression gives the fraction, $\frac{q_t}{Q_t}$, of total body nutrient flow, $C.O. \times ER_{TB}$, which goes to the heart. This equation can be further reduced.

$$ER_{TB} = \frac{a(t) dt - v(t) dt}{a(t) dt}$$

$$ER_{TB} = \frac{a(t) dt + a(t) dt - v(t) dt}{a(t) dt}$$

where $\int a(t) dt = \text{integrated arterial recirculated concentration from the time of injection to infinity}$

$\int v(t) dt = \text{integrated arterial and venous concentration from time of injection to infinity}$

Since the rubidium ion uptake by the lung is very small, $\int a(t) dt \approx \int v(t) dt$ and

$$ER_{TB} = \frac{a(t) dt}{a(t) dt}$$

(13)

Because of the importance of ER$_{TB}$ in the measurement of CBF$_{X}$, the above approximate expression for ER$_{TB}$ was tested in two animals by comparison with ER$_{TB}$ obtained using equation 5. Substitution of equation 13 into equation 12 and using the Stewart-Hamilton formula for cardiac output gives

$$CBF_{X} = \frac{q_t}{K_a \int a(t) dt}$$

(14)

This equation is used to calculate nutritional flow from an intravenous bolus injection of a diffusible tracer.

2. Comparison of CBF$_{X}$ with CBF$_{F}$ and Measurements of ER$_{TB}$/ER$_{F}$

Mongrel dogs weighing 15-22 kg were anesthetized with 30 mg/kg of sodium pentobarbital and respired with compressed air after endotracheal intubation. A left thoracotomy was performed through the fourth and fifth rib. Two multihole catheters were inserted through the left atrial appendage and the combination of catheter and appendage secured. One catheter was inserted through the mitral valve into the left ventricle for obtaining pressures. The other catheter was placed in the left atrium and used for microsphere injection. A Jelco 18 gauge catheter was placed in a brachial vein and used for infusion of drugs. Another Jelco catheter was placed in the contralateral brachial vein in order to inject the bolus of diffusible tracers ($^{89}$Rb$^+$, $^{131}$Cs$^+$). The specific activity of the $^{89}$Rb$^+$ was in the range of 1-10 Ci/g while the $^{131}$Cs$^+$ was carrier free. The activity used per injection was approximately 200-300 $\mu$Ci of $^{89}$Rb$^+$ and 30-40 $\mu$Ci of $^{131}$Cs$^+$.

Just before injection of the isotope, left ventricular pressure and heart rate were obtained. After the pressure and heart rate were measured, the left ventricle catheter, now withdrawn into the left atrium, was connected by means of a four-way stockcock to a 1 cc coil inserted in a 2" $\times$ 2" shielded well detector. The coil in turn was connected to a syringe pump.

The output from the well detector amplifier was split and connected to two single channel analyzers. One analyzer was set to accept $^{89}$Rb$^+$ pulses (650 keV to 1250 keV) while the other was set to accept $^{131}$Cs$^+$ pulses (15 keV to 50 keV). The outputs from the single channel analyzers were fed into two multichannel analyzers (MCA) operating in the multiscale mode. The MCA recording $^{89}$Rb$^+$ activity was set to integrate pulses every 0.5 second while the MCA recording $^{131}$Cs$^+$ activity, because of limited storage capacity, was set to integrate pulses every second. The effect on the time-concentration curve was not significant since both isotopes gave essentially the same value for cardiac output. The average value of cardiac output in four dogs was 2.16 L/min calculated from the $^{89}$Rb$^+$ curve and 2.13 L/min from the $^{131}$Cs$^+$ curve.

After the left ventricle catheter was placed in the left atrium, the multichannel analyzers and the arterial syringe pump (38 ml/min) were started. Without delay, the rubidium or rubidium plus cesium chloride bolus was rapidly injected intravenously followed immediately by a 5 ml saline flush by means of a four-way stopcock. Simultaneously the $^{85}$Sr or $^{141}$Ce microspheres (1 ml) were injected into the left atrium followed by a 5 ml saline flush. The carbonized microspheres which were purchased from 3M Company were 50 ± 10 microns in diameter and were uniformly labeled with either $^{85}$Sr or $^{141}$Ce. The specific activity was approximately 5.7 mCi/g which was equivalent to 2100 particles per $\mu$Ci at the time of assay. The microspheres were suspended in 10% dextrose-saline solution. Prior to injection the syringe containing the microspheres was placed on a mechanical shaker for 15 seconds. The number of microspheres injected was approximately 85,000 and corresponded to 30-40 $\mu$Ci per injection. The manufacturer’s specifications concerning the number and size of the particles were checked using a stage micrometer and hemocytometer.

Arterial blood sampling lasted approximately 1.3 minutes after which the multichannel analyzers were stopped. The animal was sacrificed at 3 minutes after injection of the diffusible tracer by injecting an overdose of pentobarbital into the left atrium. After sacrifice of the animal, a 40 ml blood sample was obtained and the lung, heart, and kidneys removed for radioisotopic analysis. The activity in the arterial blood removed by the syringe pump was also determined. This was usually less than 1% of the injected dose.

The activity in the right ventricle and atria was totaled and reported as right heart activity (R.H.). The activity in the free wall of the left ventricle and that in the interventricular septum was combined and reported as left heart activity (L.H.). From previous studies these anatomical areas were found to have similar flow per unit mass.

When drugs were used, left ventricular pressure and heart rate were obtained before drug infusion and again during infusion after the heart rate and pressure had stabilized. For isoproterenol and norepinephrine, this was approximately 5-10 min after the start of drug infusion. The infusion rate for these two drugs was 0.5-1.0 micrograms per minute per kilogram of body weight. Nitroglycerin was given as a bolus on the basis...
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of 0.04 mg per kilogram of body weight. The pressure and heart rate measurements were taken at 2.5–3.0 minutes after nitroglycerin injection while the flow measurements were begun at approximately 3–4 min after nitroglycerin injection. At this time heart rate and pressure were equal to pre-injection levels.

Two methods were used to calculate total coronary blood flow. To obtain total flow to any area using the microspheres, the following equation was used:

\[
CBF_T(M) = \frac{m}{M} \times C.O. \quad (15)
\]

where \(m\) is the net counts per minute of \(^{85}\text{Sr}\) or \(^{141}\text{Ce}\) microspheres in the tissue and \(M\) is the net counts per minute of microspheres injected corrected for any residual activity in the catheter or syringe and for any activity removed during arterial blood sampling. Cardiac output was calculated by using the \(^{86}\text{Rb}\) or \(^{131}\text{Cs}\) arterial time-concentration curves and the Stewart-Hamilton formula (equation 8).

Total coronary blood flow was also calculated from equation 10 where \(q_t\) and \(Q_t\) are the \(^{86}\text{Rb}\) or \(^{141}\text{Ce}\) concentrations in the heart and total body respectively at the time of sacrifice. The value of \(q_t\) in \(\mu\text{Ci}\) was obtained directly by tissue counting while \(Q_t\) was estimated by determining the activity in the blood at the time of sacrifice and subtracting this from the injected activity. \(\int a(t)\ dt\) was obtained from the arterial well detector described above. The arterial counts were summed to the point of recirculation and the remainder of the curve obtained by plotting the descending portion of the curve on semi-log paper and extrapolating toward infinity. A dilution standard was made and counted in the same geometry as the arterial blood in order to convert \(\int a(t)\ dt\) from counts to \(\mu\text{Ci} \text{ ml/min}\).

Nutritional flow was calculated by using equation 14. The value of \(\int a(t)\ dt\) was obtained by summing the activity in the arterial blood in net counts per minute from time zero up to 1.3 min when the pump was stopped. The remainder of the curve was obtained by plotting the last 40 seconds of the curve on semi-log paper and extrapolating toward infinity. The extrapolated portion of the curve represented 10 to 20% of the total area.

Equation 7 was used to determine the ratio \(\text{ER}_{\text{TR}}/\text{ER}_C\):

\[
\frac{\text{ER}_{\text{TR}}}{\text{ER}_C} = \frac{\text{CBF}_T}{\text{C.O.}} \div \frac{q_t}{Q_t} \quad (7)
\]

The ratio \(\text{CBF}_T/\text{C.O.}\) is simply the fractional uptake of microspheres by the heart while the ratio \(q_t/Q_t\) is the fractional uptake of either \(^{131}\text{Cs}\) or \(^{86}\text{Rb}\). While \(\text{ER}_C\), \(\text{CBF}_T\), and \(q_t\) refer to the entire heart, it is clear that equation 7 could be used for any portion of the heart.

Excluding counting statistics, there are two main sources of error in calculating \(\text{ER}_{\text{TR}}/\text{ER}_C\) from equation 7. In order that the fractional uptake of microspheres by the heart equal the ratio \(\text{CBF}_T/\text{C.O.}\) statistical variation in the number of particles must be small and there must be adequate mixing of the microspheres and blood as the blood is ejected from the left ventricle. In this study the number of particles needed for injection was determined by injecting two dogs with a mixture of \(^{141}\text{Ce}\) and \(^{85}\text{Sr}\) microspheres. Tissue samples were grouped according to the number of microspheres contained in the samples. Variation in the fractional localization of the isotopes gives a measure of the statistical error due to the number of particles since counting error was less than \(\pm 1\%\).

It has been shown that mixing is achieved after left atrial injections of 50 micron microspheres. To determine if the present technique achieved this and also to demonstrate reproducibility, serial injections of \(^{141}\text{Ce}\) and \(^{86}\text{Rb}\) microspheres were made into the left atrium of two dogs. The injections were 5 min apart. The fractional uptake of the two isotopes was determined by tissue counting.

To obtain the coronary extraction ratio of the whole or any portion of the heart, the value of \(\text{ER}_{\text{TR}}/\text{ER}_C\) for this area was divided into \(\text{ER}_{\text{TR}}\). The total body extraction ratio (\(\text{ER}_{\text{TR}}\)) was usually determined by using equation 13. When simultaneous sampling of blood activity was obtained from the right ventricle and aorta, \(\text{ER}_{\text{TR}}\) was calculated by using equation 5.

3. Total Body Extraction Ratio (\(\text{ER}_{\text{TR}}\))

As discussed previously equation 5 (direct) is equivalent to equation 13 (indirect) if the time-concentration curve of recirculated arterial blood is equal to the venous time-concentration curve. As the integration approaches infinity, this will be true if the lung uptake of \(^{86}\text{Rb}\) is small. The average lung uptake of \(^{86}\text{Rb}\) measured in 21 dogs was 2.3%. This is good evidence that equation 13 is a close approximation to equation 5.

In addition, tests were made in order to compare the values of \(\text{ER}_{\text{TR}}\) measured from arterial sampling alone (equation 13) with a direct measurement of the total body extraction ratio (equation 5).

The experimental arrangement was similar to that described in the previous section except for the following changes. A second 3" x 3" detector was added in order to separately sample venous blood. A 5 cc coil was taped to the face of the 3" x 3" shielded detector. The coil in turn was connected to a syringe pump.

The arterial catheter was placed in the descending aorta via the femoral artery while the venous catheter was placed in the right ventricle by catheterizing the femoral vein. A small catheter was inserted and secured in the left atrium for use in injecting \(^{86}\text{Rb}\). The outputs from the arterial and venous detectors were connected to amplifiers, single channel analyzers (250 keV to 1250 keV) and then to the multichannel analyzers (MCA). The MCA receiving pulses from the venous detector integrated the rubidium count every 2.5 sec. The arterial pump speed was 38 ml/min while the venous pump speed was set at 15 ml/min. Venous blood was sampled for approximately 2–2.5 min following injection of isotope. The area under the venous curve was obtained in a manner similar to that which provided the integrated arterial curve. The extrapolated portion of the curve represented 10 to 20% of the total area.
4. Tissue Counting

A major difficulty when assaying solutions of microspheres is the rapid settling rate. For 50 micron particles, this is approximately 4 mm per minute. Unlike other radioactive solutions, doses based on volume are unreliable, therefore, a system must be used for normalizing different doses to be given to an animal and for relating a dose received by an animal to a standard used for determining tissue uptake.

The method employed utilized a hollow plastic tube which would just accommodate a 50 cc syringe and which was centered 23 cm from a shielded 3" x 3" NaI detector. The distance of 23 cm was used in order that error due to the difference in distance between the sample and dose would be less than one-half percent. A paraffin mold was made which fitted into the plastic tube. A center hole was made in the paraffin mold to accommodate a 1 ml syringe containing either the microsphere dose or the diffusible isotope dose. By counting the syringe before and after injection, injected activity in cpm of either the microspheres, $^{86}$Rb$^+$ or $^{131}$Cs$^+$, was determined. Tissue samples were chopped and compacted into 50 ml syringes up to a maximum volume of 40 cc. After counting the 50 cc syringe containing the tissue sample, the tissue sample activity in cpm was compared directly with the injected activity.

Results

1. Microsphere Mixing and Statistical Variation

Table 1 gives the results from the injection of a mixture of $^{111}$Ce and $^{85}$Sr microspheres in two dogs. The data are expressed as a ratio of the percent localization of the two isotopes. Since statistical variation in the number of particles is the principal factor affecting the ratio of the two percentages, reporting the data according to the number of microspheres in any given area should indicate the number of microspheres needed to achieve a given variability. The tissue samples were separated according to the number of microspheres of each isotope contained in the sample. The second line of Table 1 shows that the average value of the %Ce/%Sr ratio remains close to unity, which one would expect if deviation from unity is a result of the number of particles. The third and fourth lines give the ranges and standard deviations of the ratios in each group. The improvement when the number of particles exceeds 1000 can be seen. Above 2000 particles, there is little improvement in the range or standard deviation. These results are very similar to those of Buckberg.28 For this study it was concluded that a minimum of 1000 particles was needed in the area of interest receiving the lowest fraction of the cardiac output, in this case the right heart (approximately 1.5% of cardiac output). This then determines the minimum number of particles required for injection (68,000). Approximately 50,000 microspheres were used in this study.

Table 2 gives the results of serial left atrial injections of $^{85}$Sr and $^{14}$Ce microspheres in two dogs. The values are expressed as a ratio of the fractional localization of injection number 2 to injection number 1. The values near unity for the heart and kidney indicate proper mixing of microspheres and blood.

2. Comparison of a Direct and Indirect Method of Measuring ER$_{TN}$

Table 3 gives the results of three measurements in two dogs of ER$_{TN}$ calculated from equation 13 (indirect) and ER$_{TN}$ calculated using equation 5 (direct). As expected, the results are quite close. In the second dog, two measurements were made. Because of a slowing of heart rate and increasing peripheral resistance, the cardiac output during the second measurement was decreased. As would be expected, ER$_{TN}$ increases. As mentioned earlier, the lung uptake of $^{86}$Rb in 21 dogs was 2.3%. Thus, it was concluded that equation 13 is a good approximation of equation 5.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Microspheres Versus the Ratio %Ce/%Sr</td>
</tr>
<tr>
<td>Number of tissue samples &amp; 7 &amp; 12 &amp; 5 &amp; 4 &amp; 5</td>
</tr>
<tr>
<td>Average %Ce/%Sr &amp; 1.08 &amp; 0.98 &amp; 0.99 &amp; 1.00 &amp; 1.00</td>
</tr>
<tr>
<td>Range of %Ce/%Sr &amp; 0.90-1.24 &amp; 0.82-1.10 &amp; 0.94-1.01 &amp; 0.95-1.06 &amp; 0.97-1.03</td>
</tr>
<tr>
<td>Standard deviation &amp; ±0.11 &amp; ±0.10 &amp; ±0.03 &amp; ±0.04 &amp; ±0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Left Atrial Injection of Microspheres Five Minutes Between Injections</td>
</tr>
<tr>
<td>R = fractional localization of injection #2 &amp; fractional localization of injection #1</td>
</tr>
<tr>
<td>Dog A &amp; Dog B &amp; Mean</td>
</tr>
<tr>
<td>Kidney &amp; 0.97 &amp; 1.02 &amp; 1.00</td>
</tr>
<tr>
<td>Heart &amp; 1.04 &amp; 0.98 &amp; 1.01</td>
</tr>
</tbody>
</table>

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We could expect from these results that administration of isoproterenol will give an accurate estimate of mean changes in total flow while norepinephrine and nitroglycerin will give values which are 10-15% too low. Although the mean values of $ERTB/ERC$ were close to unity, there was considerable variation in all groups tested.

5. Effect of Drugs on CBF_N, CBF_T, and CBF_T(M)

The data in table 5 gives a comparison of total coronary blood flow using the microsphere measurement ($CBF_T(M)$) with CBF_T and CBF_T(M) with CBF_N in control dogs and in dogs during infusion of isoproterenol or norepinephrine and after nitroglycerin administration. The data in this table shows the close mean relationship between total coronary flow determined by using rubidium (equation 10) and total flow determined by using microspheres (equation 15). The linear regression equation for all the data relating total flow in ml/min/100g determined by using microspheres and $^{86}\text{Rb}^+$ is $CBF_T(M) = 7.44 + CBF_T$. It is pointed out that the slope is unity while the Pearson correlation coefficient is 0.92. While the over-all mean difference was only 4%, the data in table 5 indicate that individual estimates of total flow using rubidium may be in error as much as ±42% at the 95% confidence level. They also show that the difference between CBF_N and CBF_T has increased after drug administration. This means that not only is nutritional flow not equal to total flow but upon dilation the two do not necessarily increase proportionately.

6. Comparison of Left and Right Heart Flow with Extraction Ratios

In table 6 the ratio of left heart to right heart flow is compared with the ratio of left heart to right heart extraction ratios. These data show that, despite the flow per 100g of the left heart being nearly twice that of the right, the extraction ratios are nearly identical. This appears to remain true even

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**Table 3**

Comparison of a Direct and Indirect Method of Measuring $ERTB$

<table>
<thead>
<tr>
<th></th>
<th>Animal no. 1</th>
<th>Animal no. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test no. 1</td>
<td>Test no. 2</td>
</tr>
<tr>
<td>C.O. (ml/min/kg)</td>
<td>138</td>
<td>148</td>
</tr>
<tr>
<td>$ERTB$ (indirect)</td>
<td>0.51</td>
<td>0.57</td>
</tr>
<tr>
<td>$ERTB$ (direct)</td>
<td>0.51</td>
<td>0.57</td>
</tr>
</tbody>
</table>

3. $^{131}\text{Cs}^+$ Ratio of $ERTB/ERC$

The ratio of total body extraction of the diffusable ion $^{131}\text{Cs}^+$ divided by the coronary extraction of $^{131}\text{Cs}^+$ was determined in four control dogs. The values were 1.51, 2.39, 1.85, and 1.41 with a mean of 1.79. These results show that the $^{131}\text{Cs}^+$ $ERTB/ERC$ ratio is quite variable from one animal to another and also considerably different from unity. These two facts demonstrate that $^{131}\text{Cs}^+$ should not be used for measuring total coronary flow with the bolus equation. In addition, the mean $^{131}\text{Cs}^+$ extraction ratio for the whole heart was only 0.24 in agreement with published values for the left ventricle. This means that isotopes of cesium in diffusible form are not flow limited and cannot be used for measuring nutritional flow with a bolus technique. Therefore, only $^{86}\text{Rb}^+$ was used as the diffusible ion in the remainder of the study.

4. Effect of Drugs on $ERC$ and $ERTB$ Using $^{86}\text{Rb}^+$

Table 4 gives the mean values of $ERC$ for the whole heart, $ERTB$, and the ratio $ERTB/ERC$ in control dogs and in dogs which received drugs. This table shows that there is not statistically significant change in the ratio $ERTB/ERC$ due to drug infusion ($2\text{-tailed, } P > 0.3$). However, in the case of isoproterenol and norepinephrine, both the $ERTB$ and $ERC$ were significantly decreased from control values ($2\text{-tailed, } P < 0.05$). The values of $ERTB$ and $ERC$ after nitroglycerin are lower than the control values although not significantly.

**Table 4**

Effect of Drugs on Extraction Ratios

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Iso.</th>
<th>Norepi.</th>
<th>NTG</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>$ERC = SD$ (whole heart)</td>
<td>0.57 ± 0.08</td>
<td>0.40 ± 0.10</td>
<td>0.36 ± 0.06</td>
<td>0.47 ± 0.09</td>
<td>0.47 ± 0.12</td>
</tr>
<tr>
<td>$ERTB = SD$</td>
<td>0.56 ± 0.06</td>
<td>0.38 ± 0.06</td>
<td>0.41 ± 0.04</td>
<td>0.53 ± 0.04</td>
<td>0.49 ± 0.09</td>
</tr>
<tr>
<td>$ERTB/ERC = SD$</td>
<td>1.02 ± 0.23</td>
<td>0.98 ± 0.16</td>
<td>1.16 ± 0.08</td>
<td>1.15 ± 0.13</td>
<td>1.07 ± 0.18</td>
</tr>
</tbody>
</table>

Abbreviations: Iso. = isoproterenol; Norepi. = norepinephrine; NTG = nitroglycerin.
Table 5

Comparison of Total Coronary Blood Flow (CBF<sub>T</sub>) Determined by Using Microspheres (CBF<sub>T(M)</sub>) with CBF<sub>T</sub> Determined by Using 86Rb<sup>+</sup> and with Nutritional Coronary Blood Flow (CBF<sub>N</sub>)

<table>
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<tr>
<th></th>
<th>CBF&lt;sub&gt;T(M)&lt;/sub&gt;</th>
<th>CBF&lt;sub&gt;T&lt;/sub&gt;</th>
<th>CBF&lt;sub&gt;T&lt;/sub&gt; - CBF&lt;sub&gt;T(M)&lt;/sub&gt;</th>
<th>CBF&lt;sub&gt;N&lt;/sub&gt;</th>
<th>CBF&lt;sub&gt;N&lt;/sub&gt; - CBF&lt;sub&gt;T(M)&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/min/100 g</td>
<td>ml/min/100 g</td>
<td>Δ</td>
<td>*Δ%</td>
<td>ml/min/100 g</td>
</tr>
<tr>
<td>Mean Controls</td>
<td>117</td>
<td>121</td>
<td>+ 4</td>
<td>23</td>
<td>68</td>
</tr>
<tr>
<td>sp</td>
<td>25</td>
<td>48</td>
<td>+ 3</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td>P &gt; 0.2</td>
<td>NS</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>318</td>
<td>325</td>
<td>+ 7</td>
<td>20</td>
<td>121</td>
</tr>
<tr>
<td>sp</td>
<td>84</td>
<td>70</td>
<td>+ 2</td>
<td>16</td>
<td>82</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td>P &gt; 0.2</td>
<td>NS</td>
<td>P &lt; 0.01</td>
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<tr>
<td>Norepinephrine</td>
<td>280</td>
<td>240</td>
<td>- 40</td>
<td>6</td>
<td>96</td>
</tr>
<tr>
<td>sp</td>
<td>105</td>
<td>88</td>
<td>- 14</td>
<td>24</td>
<td>87</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td>P &gt; 0.2</td>
<td>NS</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Nitroglycerine</td>
<td>195</td>
<td>182</td>
<td>- 13</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>sp</td>
<td>69</td>
<td>49</td>
<td>- 6</td>
<td>16</td>
<td>56</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td>P &gt; 0.2</td>
<td>NS</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

NS = Nonsignificant P > 0.05 (2-tailed t-test); S = Significant P < 0.05 (2-tailed t-test).

*Δ% = (CBF<sub>T</sub> - CBF<sub>T(M)</sub>) / CBF<sub>T</sub>(M)

*Δ% = (CBF<sub>N</sub> - CBF<sub>T(M)</sub>) / CBF<sub>T(M)</sub>

with the extensive hemodynamic changes produced by the drugs being tested.

The fact that the extraction ratios of the left and right heart are essentially the same means that coronary sinus blood is representative of venous 86Rb<sup>+</sup> concentrations from the entire heart. This means that direct Fick methods which employ coronary sinus catheterization and intravenous injections of 86Rb<sup>+</sup> can be used to obtain total heart flow and not just left ventricle flow, i.e., the extraction ratio measured by coronary sinus sampling can be used for the whole heart.

Discussion

Depending on the technique employed to estimate coronary blood flow, one may be measuring total flow per unit mass, nutritional flow per unit mass, total organ flow, or total organ nutritional flow. The present study has demonstrated the importance in recognizing the type of flow being measured. For instance, based on the belief that the 86Rb coincidence technique measures nutritional coronary blood flow, it has been stated that isoproterenol increases nutritional and total coronary flow proportionately.5, 7, 8, 10, 11, 16 It was shown mathematically in Section 1 of Methods that the equation employed to measure nutritional flow actually evaluates total flow. This was further demonstrated by comparisons with total flow determined by the microsphere technique. By employing the definition of CBF<sub>N</sub> as being the product of total flow and the extraction ratio of 86Rb<sup>+</sup>, it was shown that total flow is increased to a much greater degree than is nutritional flow after isoproterenol.

Table 6

Comparison of Mean Left Heart (L.H.) and Right Heart (R.H.) Total Flow with Extraction Ratios in Dogs with and without Drug Administration

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Iso.</th>
<th>Norepi.</th>
<th>NTG</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.H. Flow</td>
<td>2.24 ± 0.28</td>
<td>1.52 ± 0.16</td>
<td>1.93 ± 0.16</td>
<td>1.90 ± 0.15</td>
<td>1.90 ± 0.14</td>
</tr>
<tr>
<td>R.H. Flow</td>
<td>0.86 ± 0.05</td>
<td>1.02 ± 0.06</td>
<td>0.83 ± 0.05</td>
<td>0.92 ± 0.01</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>L.H. ER</td>
<td>2.24 ± 0.28</td>
<td>1.52 ± 0.16</td>
<td>1.93 ± 0.16</td>
<td>1.90 ± 0.15</td>
<td>1.90 ± 0.14</td>
</tr>
<tr>
<td>R.H. ER</td>
<td>0.86 ± 0.05</td>
<td>1.02 ± 0.06</td>
<td>0.83 ± 0.05</td>
<td>0.92 ± 0.01</td>
<td>0.91 ± 0.03</td>
</tr>
</tbody>
</table>

Abbreviations: Iso. = isoproterenol; Norepi. = norepinephrine; NTG = nitroglycerin; SE = standard error.

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administration. This is in agreement with the results found by Love using the vasodilators di-
pyridamole and norepinephrine.21

When discussing total flow using the rubidium bolus equation, the limitation in accuracy due to variation in the ratio ER\textsubscript{BN}/ER\textsubscript{C} noted above should be kept in mind. Because of this variability, it is recommended that at least duplicate determinations be obtained for both control and drug infusion measurements.

In contrast to CBF\textsubscript{T} as determined by using a bolus injection of rubidium, CBF\textsubscript{N} as derived in this study does not require that the ratio ER\textsubscript{BN}/ER\textsubscript{C} be constant. However, for an indicator to qualify as an estimator of capillary flow, it must be totally extracted from the capillaries, it must be non-
extractable from shunt circuits, and back-diffusion must be minimal during the period of measure-
ment. For isotopes of rubidium and potassium, the latter condition is met because of the large intra-
cellular pool into which the ions are diluted. There is evidence that rubidium and potassium ions are totally extracted from the capillaries in a single passage and therefore are flow limited.22-25 Friedman\textsuperscript{4} has presented evidence based on work with the gracilus muscle which indicates that muscle is composed of two parallel circuits, one of which provides a nonlimited permeability exchange for rubidium ions while the other is completely perme-
ability limited. Renkin\textsuperscript{26} theorizes the existence of parallel vascular pathways which differ mainly in the ratio of blood flow to tissue volume and can be characterized as well perfused and poorly per-
fused pathways.

It is a common finding that as blood flow in-
creases, extraction decreases. According to the flow distribution concept suggested by Friedman,\textsuperscript{4} increasing flow leads to reduction of the fractional flow of blood to the nutritional circuit and thereby reduces rubidium ion extraction. Renkin\textsuperscript{26} has attributed this decreasing extraction as flow increases to blood flow redistribution between poorly and well perfused circuits, each being diffusion limited to different degrees. As total flow is reduced, flow is redistributed from the poorly perfused cir-
cuits to the highly perfused circuits. If a substance is diffusion limited, the extraction ratio will depend on capillary permeability, the exchange surface area, and the velocity of flow past the exchange surface. Based on this concept, an extraction ratio less than unity represents the lack of equilibrium between tissue and blood for the tracer.

On the basis of the above definition of isotope extraction, Renkin\textsuperscript{30} derived a widely quoted ex-
pression:

\[
ER = (1 - e^{-PS})
\]

in which \(P\) = permeability of the diffusion barrier (cm/min/100g),

\(S\) = surface area of the diffusion barrier (cm\textsuperscript{2}),

\(F\) = total flow (cc/min/100g),

and \(C\) = \(F (1 - e^{-PS})\).

C, called clearance, is not necessarilry a measure of capillary flow because of the manner in which ex-
traction ratio has been defined. If rubidium extraction by the myocardium is diffusion-limited, then nutritional flow as defined in this work is a measure of clearance. If rubidium extraction is flow limited, nutritional flow is a measure of capillary flow.

The basic principle of microsphere techniques is that radioactive microspheres distribute in the same manner as blood when they are mixed with blood. If the microspheres are larger than the small vessels of an organ, they are totally removed from the blood and the relative distribution of the micro-
spheres is therefore equal to the relative distribution of the blood flow perfusing that organ. Four conditions however must be satisfied for this to be true.

1) The microspheres themselves must not alter blood flow.

2) The microspheres must be removed from the blood stream by becoming impacted in the microcirculation.

3) The microspheres must be uniformly mixed with the blood.

4) Statistical variation due to the number of par-
ticles injected must be small.

Condition 1) has been studied by Kaihara\textsuperscript{13} and others and their results suggest that the number of particles required to alter the circulation is much higher than the doses used in tracer studies, 300,000 or more particles, in dogs. The quantity of micro-
spheres which are not removed by the systemic microcirculation can be estimated by measuring the microsphere content in the lung (Condition 2). However, lung activity is also due to flow through the bronchial arteries, therefore this contribution to lung activity must be considered when estimating systemic shunting. Kaihara\textsuperscript{13} found that 7-10\% of 15\mu microspheres may be shunted whereas no shunting was observed with 50\mu particles. This has
been confirmed by Hoffbrand. Microspheres of \(50\mu m\) in diameter were used in the present study.

The condition of adequate mixing with the blood has been studied by comparing the distribution of microspheres labeled with one isotope to the distribution of microspheres labeled with another isotope injected at different times. Kailhara has shown that adequate mixing was attained when injections are made into the left atrium but not when injections are made into the left ventricle. Others have demonstrated that significant deviations may occur after left ventricular injections.

The number of particles needed for injection is dependent on the number of particles localized in the area of interest. It was determined from the simultaneous injections of two differently labeled microspheres that 1000–2000 microspheres were needed in an area of interest for a 5% or less statistical error. This is in agreement with the results of Buckberg.

These experiments have shown that it is possible to obtain simultaneous estimates of total and nutritional flow by methods which can be used clinically. The addition of nutritional flow requires only slight additional manipulation of the data already obtained for the measurement of total flow. Winbury has shown that when flow was held constant, the \(^{86}\text{Rb}^+\) extraction ratio for the left ventricle went up after nitroglycerin administration. From the present studies, in which flow was permitted to vary, \(E_{RC}\) for \(^{86}\text{Rb}^+\) remained unchanged or decreased. The constant flow studies are analogous to the condition of the patient with coronary artery disease while the present studies might represent the normal individual. Clinically then, we might speculate that the ratio of \(CBF_N/CBF_T\) will increase in patients with coronary artery disease after nitroglycerin administration while in normals we would expect no change or a decrease. Drugs such as isoproterenol and norepinephrine, on the basis of the results presented, also cause a differential effect on \(CBF_N\) and \(CBF_T\) which might prove useful clinically. It is anticipated that the addition of a measurement of nutritional flow to that of total flow will increase the diagnostic usefulness of coronary blood flow measurements.

Conclusions

1) \(^{131}\text{Cs}^+\) has a low coronary extraction ratio measured by the bolus technique in agreement with values obtained by using constant infusion methods. In addition, the cesium ratio \(E_{RB}/E_{RC}\) was substantially greater than unity. These two findings indicate that diffusible isotopes of cesium would not be useful for evaluating either \(CBF_N\) or \(CBF_T\) by using equations described in this paper.

2) Contrary to published reports, this study shows that the equation used with the \(^{86}\text{Rb}\) coincidence technique employing a bolus injection of rubidium estimates total flow and not nutritional flow (equation 10).

3) The main assumption in the derivation of the above equation 10 is that \(E_{RB}/E_{RC}\) is unity. This ratio was found to vary considerably. Mean values, however, are essentially unity and not significantly altered by drugs. This means that this rubidium bolus equation 10 will give reasonably accurate average values of total flow but individual estimates may be in error by as much as ±42%.

4) A rubidium bolus equation for evaluating nutritional flow (\(CBF_N\)) was derived which employs the same bolus injection used for estimating total flow. Comparison of nutritional flow with total flow obtained by using microspheres, \(CBF_T(M)\), showed that not only was \(CBF_N\) significantly less than \(CBF_T(M)\) but \(CBF_N\) increased significantly less than \(CBF_T(M)\) after drugs which increase flow were given.

5) The left heart and right heart extraction ratios were found to be very close even though the flow per unit mass of the left heart was nearly twice that of the right heart. This was true whether or not the animals were given drugs. This means that the coronary sinus is probably representative of venous \(^{86}\text{Rb}^+\) concentrations from the entire heart.

References

TOTAL AND NUTRITIONAL BLOOD FLOW


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Simultaneous Measurement of Total and Nutritional Coronary Blood Flow in Dogs
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