The Dependence of Accumulation of $^{13}$NH$_3$ by Myocardium on Metabolic Factors and Its Implications for Quantitative Assessment of Perfusion

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SUMMARY  The residual fraction — the fraction of tracer extracted and retained by the myocardium after a bolus injection of $^{13}$N-labeled ammonia (NH$_3$ = NH$_2$) — was studied in isolated perfused rabbit hearts under conditions in which flow and cardiac metabolism could be selectively and independently controlled. Residual fraction and clearance (defined as the half-time [t½] required for elimination of sequestered tracer) of this positron-emitting tracer were monitored and quantified by coincident detection. Hearts were perfused with either modified Krebs-Henseleit buffer alone (KH) or KH enriched with washed sheep erythrocytes (KH-RBC) to augment oxygen-carrying capacity. In 13 hearts perfused with KH, the residual fraction (Res Fx) of $^{13}$N counts was not altered significantly when flow was decreased by 75% from a control rate of 4.2 ml/g/min (Res Fx = 17.9 ± 2.7%; mean ± SEM) to 1.2 ml/g/min (Res Fx = 18.4 ± 1.2%, NS). Clearance of $^{13}$N was faster because t½ decreased from 36 ± 5 minutes to 15 ± 3 minutes (p < 0.01). In 12 hearts perfused with KH-RBC, Res Fx and t½ were not altered despite marked ischemia when flow was diminished by 75% from control flow of 1.4 to 0.3 ml/g/min (control values: Res Fx = 54.6 ± 2.4%, t½ = 41 ± 6 minutes; low flow values: Res Fx = 58.1 ± 4.4%, t½ = 35 ± 10 minutes, NS). In four additional hearts perfused with KH-RBC with 0.02 mg/ml of methionine sulfoximine, a glutamine synthetase inhibitor, myocardial retention of $^{13}$N counts was reduced by > 60% and myocardial clearance was prolonged compared to pre-inhibition values. The results obtained indicate that the retention and clearance of $^{13}$N activity by myocardium are influenced to a considerable extent by the metabolic state of the myocardium. Accordingly, relationships between extraction and retention of tracer and flow per se are complex and preclude direct estimation of perfusion from the amount of tracer sequestered by the myocardium.

BECAUSE of its intrinsic quantitative capabilities, development of positron-emission transaxial tomography has contributed to the noninvasive assessment of myocardial metabolism and perfusion. However, interpretation of results obtained with positron-emitting radionuclides injected intravenously and accumulating in an organ requires elucidation of physiologic, biochemical, and pharmacologic factors that influence the fate of the injected compound. In 1971 Hunter and Monahan noted that ammonia labeled with the positron-emitting radionuclide $^{13}$N-ammonia ($^{13}$NH$_3$)* was extracted by the heart and suggested that this compound might be useful for imaging myocardium. Subsequently, $^{13}$NH$_3$ has been used to detect altered perfusion in ischemic myocardium and brain noninvasively. However, quantitative relationships between regional myocardial blood flow and the extraction, retention and clearance of $^{13}$NH$_3$ detected externally are not well understood.

A tracer whose accumulation in myocardium is dependent exclusively on flow (flow-dependent tracer) should have a constant extraction fraction throughout the range of flow being studied such that the amount extracted is dependent on flow alone, free from influence by opposing effects related to altered residence time. Ideally, a perfusion indicator should have an extraction fraction of 100% by myocardium on a single passage through the coronary circulation, as is the case with microspheres. However, if metabolism of a tracer is altered as a result of changes in the metabolic function of the heart elicited by altered perfusion, the amount taken up by myocardium may not be affected in direct proportion to the change in flow. For example, if a heart metabolizes 20 μM of glucose/100 g/min under conditions of normal flow and flow is reduced by 50%, the extraction fraction of glucose increases by approximately 100% so that the delivered amount remains approximately constant and the tissue continues to metabolize approximately 20 μM/100 g/min despite the reduced flow. For the case of $^{13}$NH$_3$, trapping of the tracer within some tissues depends on the glutamine synthetase reaction. Thus, it is important to determine whether trapping by this mechanism (and its influence on the amount retained after a bolus) is prominent in the heart. If it is, alterations in glutamine synthetase activity induced by transitory or prolonged metabolic consequences of altered flow would be likely to influence extraction and/or retention of $^{13}$NH$_3$ indepen-

*In this study $^{13}$NH$_3$ will be used for convenience even though at physiologic pH, NH$_3$ is, of course, in equilibrium with NH$_2$ — the dominant species. NH$_3$ is readily diffusible into tissue, but the permeability of the ammonium ion is limited.

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dent of the magnitude of flow itself. In general, if a putative perfusion indicator's myocardial activity is a function of both uptake and liberation of tracer already incorporated into the heart, the quantitative relationship between flow and accumulation may be clouded, particularly if efflux from the heart is altered markedly and promptly by metabolic consequences of altered perfusion.

We have found previously that the isolated perfused rabbit heart preparation is particularly useful in characterizing behavior of tracers with potential applicability for imaging in vivo because it permits assessment of residual fraction, the amount of tracer retained after a bolus injection, and the rate of clearance of retained tracer from the heart under conditions in which flow can be controlled precisely and factors that influence cardiac performance and metabolism can be controlled and monitored independently. In addition, such preparations permit evaluation of behavior of the tracer under conditions in which concentrations in the perfusate of substrate, protein, hormones and other moieties can be controlled, and in which recirculation can be excluded, included, or simulated to permit assessment of its possible effects on accumulation of tracer in vivo. By inclusion of washed sheep erythrocytes in the perfusion medium to enhance oxygen carriage, with a preparation recently developed and characterized in our laboratory, perfusion can be performed under conditions in which the absolute magnitude of flow simulates flow in vivo, a particularly important consideration in the present study since extraction of the tracer with buffer-perfused hearts may be influenced by the shorter residence time associated with the high flow conventionally required to provide adequate oxygenation.

Use of the isolated perfused heart preparation provided the basic information needed to interpret results of tomographic studies with 11C-palmitate in dog hearts in vivo, and also in studies of patients with ischemic heart disease. Accordingly, an analogous approach was used in the present study. The behavior of a tracer potentially important in the assessment of perfusion in hearts of experimental animals in vivo and in patients, therefore, was evaluated under more readily controlled conditions with the use of an isolated perfused heart preparation. As shown by the results, analysis of residue detection curves obtained with isolated perfused hearts with erythrocyte-enriched media demonstrate that the fraction of 15NH3 retained by the myocardium after bolus injection is not dependent exclusively on myocardial flow. In fact, residual fraction and clearance of 15N activity are influenced markedly by cardiac metabolism. Thus, the results indicate that though qualitative changes in activity of 15N activity in the myocardium during intervals corresponding to those required for imaging in vivo accompany altered perfusion, retention is not quantitatively or directly related to flow, but depends to a considerable extent on the metabolic status of the heart as well.

Methods

Isolated Heart Preparations

Hearts were isolated from New Zealand white rabbits weighing 2–3 kg and perfused retrogradely through the aorta as described previously. Briefly, hearts were perfused with or without washed sheep erythrocytes at a hematocrit of 40, suspended in modified Krebs-Henseleit solution containing 0.4 mM (27.6 g/l) bovine serum albumin (approximating plasma levels) which contains 0.1 mM fatty acid (Cohn fraction V, Sigma Chemical Co.). The Krebs-Henseleit buffer contained the following ions in mM: Na+143; Cl–128; K+6.1; Ca2+2.5; Mg2+1.2; SO42–1.2; HCO3–30; H3PO41.4. It also contained 5 mM glucose as substrate and 70 μU/ml insulin. All buffer solutions were dialyzed extensively before use and filtered through a 0.45-μm Millipore filter. Sheep erythrocytes were used because of their availability and small size (mean diameter approximately 4.5 μm), and because their affinity for oxygen is independent of the concentration of 2,3-DPG, in contrast to cells from many other species. All hearts were flushed initially with modified Krebs-Henseleit solution (KH) to wash out platelets and other blood elements that might lead to microaggregation and impair perfusion later. Within 2 minutes after excision of the heart, the pulmonary artery was cannulated and the caval and pulmonary vessels ligated. Ninety to 95% of total flow and virtually all nutritional flow (excluding flow through the Thebesian system) was collected via the pulmonary artery. Isovolumically beating hearts were prepared by passing a fluid-filled latex balloon into the left ventricle via the left atrium. With the balloon connected to a Gould P23Db pressure transducer via a short length of polyethylene tubing, left ventricular pressure and dP/dt (obtained by electronic differentiation) were recorded continuously. Perfusion pressure was monitored with the use of a side-arm cannula immediately distal to the aortic valve. Drainage comprising Thebesian flow and retrograde ventricular filling due to occult aortic insufficiency was monitored via a left ventricular stab wound and found to be <10% of pulmonary outflow. Left ventricular end-diastolic pressure was adjusted by changing the volume of the balloon and maintained at 10 mm Hg. The isolated heart was enclosed in a water jacket to maintain myocardial temperature at 37°C (verified by monitoring with a thermistor in some experiments) and paced at a rate of 180 beats/min with pacing electrodes sutured onto the right atrium.

Perfusion was initiated with either KH alone or with KH enhanced with red blood cells (KH-RBC). The perfusion rate in hearts perfused with buffer alone was adjusted to 4.2 ml/g wet weight/min with a constant-flow roller pump (a value obtained in a previous study with hearts perfused with erythrocyte-free buffer at a constant pressure of 60 mm Hg and found to provide adequate oxygenation based on stability of the preparation and ventricular performance). In hearts perfused with KH-RBC the initial flow rate was 1.4
ml/g/min, since under these conditions, ventricular mechanical and metabolic performance was stable, reflecting adequate oxygenation. When hearts were perfused with buffer alone (without RBCs), the perfusate was equilibrated with 95% oxygen and 5% CO₂ through a Silastic membrane oxygenator (fig. 1). When red cells were used in the buffer, equilibration was accomplished with room air and sufficient CO₂ to maintain Pco₂ in the perfusate (monitored with an IL-213 blood gas meter) within the physiologic range. The salutary effects of erythrocyte-enhanced buffer perfusion on physiological and metabolic parameters in this preparation have been documented previously.¹⁷

Residue Detection of ³⁵NH₃

For analysis of cardiac time-activity curves of ³⁵N, 50–100 μCi of ³⁵NH₃ (half-time [t½] = 10.0 min) were injected as a bolus in a volume of 0.1–0.2 ml into the perfusate 1 cm distal to the aortic valve through a side-arm in the aortic cannula providing adequate mixing.¹³ Time-activity curves were monitored with two NaI (TI) detectors placed 180° apart, approximately 3 cm from the surface of the heart at the midventricular level (fig. 1). Simultaneous detection with each of the two detectors of activity within their colinear fields of view permits coincidence detection of pairs of 511-keV photons emitted due to positron annihilation with measured background activity of virtually zero. The detectors were aligned such that the percentage of coincidence counts represented approximately 10% of singles, verified before each experiment with the use of a standard ⁶⁶Germanium source inserted in the field of view. Events were recorded with Ortec model 485 amplifiers, model 488 single-channel analyzers, and model 414A fast-coincidence unit. Output was monitored on-line and data collected with a microcomputer system to correct residue-detection curves for physical decay of the tracer and provide plots such as those shown in figure 2. Curves can be normalized by the computer with peak counts assigned values of 100% and all subsequent counts expressed as a percentage of the peak. Conversely, all curves can be (and were) evaluated also in terms of absolute counts to assure adequacy of the injection and biological integrity of the preparation in each case. All injections were made so that the peak coincidence count rate exceeded 1000 counts/sec.

Residue-detection curves were obtained during a 20-minute interval beginning with the injection. The fraction of ³⁵N counts retained by the heart after a single pass is referred to subsequently as the residual fraction.⁵,⁹,²⁰ Residual fraction was calculated by back extrapolation of the monoexponential tail of the time-activity curve to define the ordinate value of the curve at the time of occurrence of peak count rate. Residual fraction is the ratio of this value to the peak count rate itself (in figure 3, residual fraction = B/C). In this preparation, the fraction of tracer retained is analogous to counts accumulated by regions of interest in the heart during corresponding intervals after injection in imaging studies performed in vivo, as shown in previous studies with ¹¹C-palmitate.¹³,¹⁸,¹⁹ Furthermore, alterations in the residual fraction induced by changes in vitro are paralleled by corresponding changes in behavior of tracer induced by analogous changes in vivo. Clearance of ³⁵N counts from the hearts was expressed in terms of t½

FIGURE 1. Diagram of the perfusion apparatus. IPDAS refers to the laboratory microcomputer with corresponding peripherals for collection of radioactivity data.
KH

Flow = 4.3 ml/g/min
ResFx = 13.8 %
\( t_{1/2} = 36.7 \) min

KH

Flow = 1.6 ml/g/min
ResFx = 12.2 %
\( t_{1/2} = 18.9 \) min

FIGURE 2. Time-activity curves obtained from a representative heart perfused with modified Krebs-Henseleit buffer (KH) at two flows. With low flow, retained \( ^{13}N \) counts (plotted as a percentage of peak counts, Res Fx) do not decrease significantly. However, clearance of tracer is increased (\( t_{1/2} \) declines).

calculated from the best-fit monoeponential function (e\(^{kt}\)) conforming to the terminal portion of the residue-detection curve.\(^*\) Decreases in clearance are reflected by increases in \( t_{1/2} \).

Preparation of \( ^{13}NH_3 \)

Ammonia labeled with \( ^{13}N \) was produced in the Washington University Medical cyclotron by the \( ^{12}C \) (d,n) \( ^{13}N \) reaction in which methane gas is the target gas as described previously.\(^2\) The purity of the \( ^{13}NH_3 \), assessed by gas-liquid chromatography, was consistently > 97% with 0.3% CH\(_3\)\(^{14}NH_3\), and < 2% undefined compounds.

Experimental Protocol

Isolated hearts were randomized after initial equilibration, and perfusion was maintained with either KH alone or initiated with KH-RBC. All hearts were equilibrated for an additional 20 minutes, while metabolic and physiologic performance was assessed. Hearts that did not generate a developed left ventricular systolic pressure greater than 60 mm Hg during this interval were discarded. The first bolus of \( ^{13}NH_3 \) was then injected under conditions of control flow (4.2 ml/g/min in KH hearts and 1.4 ml/g/min in KH-RBC hearts). After a 20-minute interval during which residue-detection curve data were collected and at a time when background activity was as low as it had been initially, the perfusion rate, monitored with timed collection of the pulmonary arterial effluent, was reduced in a stepwise fashion first to 50% and then to 25% of control flow. At each flow, repeat bolus injections of \( ^{13}NH_3 \) were implemented and residue-detection curves obtained again.

In four additional hearts perfused with KH-RBC after the initial acquisition of the \( ^{13}N \) time-activity curve under conditions of control flow (1.4 ml/g/min) the hearts were perfused with 0.02 mg/ml perfusate of 1-methionine-dl-sulfoximine (Sigma Chemical Co.), an irreversible and specific inhibitor of glutamine synthetase.\(^16\), \(^22\) This intervention was used to determine the extent to which the residue-detection curve depended on metabolism of \( ^{13}NH_3 \) via the glutamine synthetase pathway. Flow was maintained at the same rate as that prevailing under the control conditions, and 20 minutes after initiation of infusion of the inhibitor another bolus injection of \( ^{13}NH_3 \) was given and a repeat residue-detection curve obtained.

Statistical Analysis

Between-group data were analyzed with the \( t \) test for small independent samples, and within-group data were analyzed with the \( t \) test for paired samples. Correlation was analyzed with the Pearson product-moment correlation coefficient. Values expressed are means \( \pm \) SEM.

Results

Performance of Isolated Hearts

As demonstrated previously and confirmed in this study, isolated rabbit hearts perfused with modified Krebs-Henseleit buffer containing washed sheep erythrocytes showed better left ventricular performance at comparable levels of end-diastolic pressure and heart rate than the performance of hearts perfused with buffer alone (table 1).\(^17\) Diminished performance was evident in both preparations under conditions in which flow was decreased from control values by 50–75%. However, with a decreased flow, hearts perfused with buffer alone became more hypoxic since the oxygen carrying capacity and hence oxygen content of buffer solutions is markedly limited compared with that of KH-RBC solutions (table 2). Nevertheless, the reduction of flow in the KH-RBC perfused hearts was sufficient to produce severe ischemia, judging from the profound diminution in ventricular function.

Relationships Between Myocardial Flow and \( ^{13}N \) Residual Fraction

The residual fraction in the residue-detection curves obtained represents the amount of \( ^{13}N \) activity retained in the heart during an interval after exposure of the heart to tracer corresponding to the one conventionally used for imaging studies in vivo. Thus, com-
FIGURE 3. Schematic time-activity curve. For convenience and for descriptive purposes only, the curves have been considered in terms of three consistently observed prominent phases. Phase I represents a rapid decline of radioactivity associated with independently measured washout of tracer in the vascular compartment. Phase II reflects some redistribution of tracer between interstitial and intracellular fluid and relatively slow washout of tracer entering the right ventricular cavity. Phase III represents an apparent monoexponential decline of $^{31}$N counts after sequestration in myocardium. Back extrapolation of phase III to the time of peak counts (B/C) defines a residual fraction (i.e., that fraction of peak counts initially extracted and subsequently retained for an arbitrarily defined interval by the myocardium). Clearance ($t_{1/2}$) is calculated as the half-time required for elimination of sequestered tracer, from best-line fit monoexponential conforming to phase III values.

TABLE 1. Performance Characteristics of Langendorff Perfused Hearts

<table>
<thead>
<tr>
<th></th>
<th>Hearts perfused with KH (n = 13)</th>
<th>Hearts perfused with KH-RBC (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coronary flow (ml/g/min)</td>
<td>Peak LVP (mm Hg)</td>
</tr>
<tr>
<td></td>
<td>4.2 ± 0.2</td>
<td>74 ± 5</td>
</tr>
<tr>
<td></td>
<td>2.0 ± 0.1</td>
<td>40 ± 3*</td>
</tr>
<tr>
<td></td>
<td>1.2 ± 0.1</td>
<td>9 ± 2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coronary flow (ml/g/min)</td>
<td>Peak LVP (mm Hg)</td>
</tr>
<tr>
<td></td>
<td>1.4 ± 0.05</td>
<td>91 ± 7</td>
</tr>
<tr>
<td></td>
<td>0.7 ± 0.03</td>
<td>66 ± 6*</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.03</td>
<td>38 ± 7*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
All hearts were paced at 180 beats/min and perfused at 37°C. End-diastolic pressure was maintained at 10 mm Hg. KH refers to hearts perfused with modified Krebs-Henseleit medium; KH-RBC refers to hearts perfused with KH containing washed sheep red blood cells at a hematocrit of 40.

*p < 0.01 compared with control values in the same group.

Abbreviations: LVP = left ventricular systolic pressure; dP/dt = rate of LVP rise.
Table 2. Characteristics of Perfusates For Both Groups of Hearts Perfused at Selected Flow Rates

<table>
<thead>
<tr>
<th>Coronary flow (ml/g/min)</th>
<th>KH</th>
<th>4.2 ± 0.2</th>
<th>Arterial</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arterial</td>
<td>Venous</td>
<td>Arterial</td>
<td>Venous</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.03</td>
<td>7.39 ± 0.02</td>
<td>7.40 ± 0.09</td>
<td>7.24 ± 0.06</td>
</tr>
<tr>
<td>PO2 (mm Hg)</td>
<td>516 ± 22</td>
<td>139 ± 8</td>
<td>526 ± 29</td>
<td>103 ± 9</td>
</tr>
<tr>
<td>PCO2 (mm Hg)</td>
<td>41.6 ± 1.6</td>
<td>42.3 ± 3.9</td>
<td>41.2 ± 1.8</td>
<td>46.9 ± 3.6</td>
</tr>
<tr>
<td>O2 content (ml/dl)</td>
<td>1.6 ± 0.07</td>
<td>0.4 ± 0.03</td>
<td>1.5 ± 0.07</td>
<td>0.3 ± 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coronary flow (ml/g/min)</th>
<th>KH-RBC</th>
<th>1.4 ± 0.05</th>
<th>Arterial</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.7 ± 0.03</td>
<td>0.3 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arterial</td>
<td>Venous</td>
<td>Arterial</td>
<td>Venous</td>
</tr>
<tr>
<td>pH</td>
<td>7.47 ± 0.09</td>
<td>7.39 ± 0.04</td>
<td>7.47 ± 0.03</td>
<td>7.34 ± 0.04</td>
</tr>
<tr>
<td>PO2 (mm Hg)</td>
<td>136 ± 2</td>
<td>45 ± 4</td>
<td>131 ± 4</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>PCO2 (mm Hg)</td>
<td>34.0 ± 1.7</td>
<td>38.2 ± 2.4</td>
<td>34.3 ± 1.6</td>
<td>40.6 ± 3.4</td>
</tr>
<tr>
<td>O2 content (ml/dl)</td>
<td>18.1 ± 0.01</td>
<td>14.3 ± 0.5</td>
<td>18.1 ± 0.03</td>
<td>13.2 ± 0.8</td>
</tr>
</tbody>
</table>

KH and KH-RBC refer to modified Krebs-Henseleit solution without and with enrichment with sheep erythrocytes. Hearts perfused with KH were equilibrated with 95% O2/5% CO2; KH-RBC hearts were equilibrated with room air + CO2.

Values are mean ± SEM.

Comparison of residual fractions under different conditions provides insight into what would be evident if the same conditions prevailed in vivo at the time of imaging. When residual fractions measured in KH or KH-RBC perfused hearts were compared to flow in the same hearts, no correlation was found between measured coronary flow and residual fraction of injected \(^{13}\text{NH}_3\), (table 3, fig. 4). Figures 2 and 5 depict typical time-activity curves from hearts in each group and demonstrate the lack of correlation of the \(^{13}\text{NH}_3\) residual fraction with flow. Although this lack of correlation is evident for both groups of hearts, particularly for the KH-RBC group, it is evident within the range of flow encountered in normal and ischemic myocardium in vivo. Even within zones of frank infarction in dogs, perfusion ranges from 10-40% of values in normal tissue. With analysis with a paired t test, a more sensitive means of detecting significance, no statistically significant relation between residual fraction and flow was evident for either group of hearts (table 3). In fact, rather than paralleling flow, residual fraction in KH-RBC perfused hearts increased from 48 ± 5.3 to 58 ± 4.4% (although values were not statistically different from control values) when flow was reduced from 50 to 25% of control (table 3).

Dependence of Residual Fraction on Metabolism

When the residual fraction of hearts perfused with KH at a flow of 1.2 ml/g/min (conditions in which hearts are hypoxic because of the lack of RBCs) is compared with the residual fraction of hearts perfused with KH-RBC at similar flow (1.4 ml/g/min; conditions in which the hearts are well oxygenated), it is clear that the well-oxygenated hearts retained a much greater fraction of the injected dose of \(^{13}\text{NH}_3\) activity than the poorly oxygenated ones (table 3, fig. 6A). Thus, under conditions of similar flow but disparate oxygenation, residual fraction of \(^{13}\text{NH}_3\) differed. The major contribution of metabolism, as opposed to flow, to retention of \(^{13}\text{NH}_3\) by the heart is evident also based on
results obtained with methionine sulfoximine, an inhibitor of glutamine synthetase. When KH-RBC perfused hearts were maintained in a well-oxygenated state with a flow of 1.4 ml/g/min comparable to flow under control conditions, but infused with 0.02 mg/ml of methionine sulfoximine, the residual fraction of $^{15}$N activity declined by more than 60% from a mean of 54% to 20% ($p < 0.001$) (table 4, fig. 6B). Under these conditions no overall impairment of myocardial metabolism was present since the hearts continued to develop normal ventricular pressure, had normal dP/dt values, and did not become arrhythmic.

### Clearing of $^{15}$N Activity

Clearance of $^{15}$N counts from the myocardium was calculated from residue-detection curves. Clearance of tracer initially extracted and retained in the myocardium appears to be more rapid (t½ decreases) at markedly reduced flow (table 3). Furthermore, clearance was faster in the more poorly oxygenated (KH-perfused) hearts under conditions of comparable flow. In KH-perfused hearts the t½ of the terminal portion of the residue-detection curve (fig. 2) decreased from a mean of 36 ± 5 minutes at a control flow of 4.2 ml/g/min to 21 ± 3 minutes at a flow of 2.0 ml/g/min ($p < 0.01$) and 1½ decreased even further when flow was reduced from 2.0 to 1.2 ml/g/min (t½ = 15 ± 3 minutes). In KH-RBC perfused hearts maintained at a flow of 1.5 ml/g/min, the residual fraction (ResFx) (%) and time to onset of phase III (sec) were: KH-RBC = 38 ± 3; KH-RBC + MS = 38 ± 3.

### Table 3. Effect of Coronary Flow on Retention and Clearance of $^{15}$NH$_3$ from the Myocardium

<table>
<thead>
<tr>
<th>Coronary flow (ml/g/min)</th>
<th>KH (n = 13)</th>
<th>KH-RBC (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary flow (ml/g/min)</td>
<td>4.2 ± 0.1</td>
<td>1.4 ± 0.05</td>
</tr>
<tr>
<td>Residual fraction (%)</td>
<td>17.9 ± 2.7</td>
<td>1.4 ± 0.05</td>
</tr>
<tr>
<td>Myocardial clearance (half-time [min])</td>
<td>36 ± 5</td>
<td>21 ± 3*</td>
</tr>
<tr>
<td>Time to onset of phase III (sec)</td>
<td>208 ± 15</td>
<td>206 ± 11</td>
</tr>
</tbody>
</table>

All hearts were paced at 180 beats/min at 37°C. End-diastolic pressure was maintained at 10 mm Hg. Residual fraction represents the percent of the peak counts retained by backextrapolation of phase III (the monoexponential function best fitting the terminal portion of the residue detection of $^{15}$NH$_3$ time-activity curve) to the time of peak counts. Myocardial clearance (half-time) is the time for the retained counts to decline by 50%. KH and KH-RBC refer to the same solutions as those noted in Table 1.

Values are mean ± SEM.

*p < 0.05 compared with control values in hearts in the same group.

†p < 0.01 compared with control values in hearts in the same group.

### Table 4. Effect of Methionine Sulfoximine (MS) (0.02 mg/ml) on Retention and Clearance of $^{15}$NH$_3$

<table>
<thead>
<tr>
<th></th>
<th>KH-RBC</th>
<th>KH-RBC + MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual fraction (%)</td>
<td>54.6 ± 2.4</td>
<td>20.3 ± 2.0*</td>
</tr>
<tr>
<td>Myocardial clearance (half-time [min])</td>
<td>41 ± 6</td>
<td>60 ± 4*</td>
</tr>
<tr>
<td>Time to onset of phase III (sec)</td>
<td>206 ± 11</td>
<td>444 ± 18*</td>
</tr>
</tbody>
</table>

Hearts (n = 4, each studied with and without inhibitor) were perfused at a constant flow of 1.4 ± 0.05 ml/g/min and paced at 180 beats/min at 37°C with an end-diastolic pressure of 10 mm Hg. KH-RBC refers to erythrocyte-enriched Krebs-Henseleit solution.

Values indicate the mean ± SEM.

*p < 0.01.
fused hearts, t\(\frac{1}{2}\) did not decline at all until flow was reduced by 75% and even then the decline was not statistically significant (table 3). When hearts perfused with the erythrocyte-enhanced media were exposed to

![Figure 6](https://example.com/figure6.png)

**Figure 6.** A) Time-activity curves from representative hearts demonstrating the difference between hearts perfused at comparable flow with Krebs-Henseleit buffer alone (KH), or buffer enriched with erythrocytes (KH-RBC). At this flow, buffer alone does not provide adequate oxygenation but buffer enriched with erythrocytes does. Retention of \(^{13}\)N counts (Res Fx) is substantially less in the hypoxic hearts and clearance is augmented. B) Time-activity curves from the same heart perfused with erythrocyte-enhanced media at the same flow after infusion of 0.02 mg/ml of methionine sulfoximine, a glutamine synthetase inhibitor, in KH-RBC (KH-RBC + MS). Infusion of the inhibitor did not alter myocardial performance. After inhibition of glutamine synthetase, the retention of \(^{13}\)N counts by the myocardium falls markedly and clearance decreases (t\(\frac{1}{2}\) increases). Furthermore, the onset of phase III is prolonged. Thus, both extraction and retention of \(^{13}\)NH\(_3\) are altered metabolically despite no substantial change in flow.

the glutamine synthetase inhibitor methionine sulfoximine, t\(\frac{1}{2}\) increased significantly, from 36 ± 5 minutes before inhibition to 60 ± 5 minutes after inhibition (p < 0.001) (table 4, fig. 6B). These results indicate that even if the amount of \(^{13}\)NH\(_3\) retained initially by the heart were dependent exclusively on flow, the amount eliminated by the myocardium over intervals comparable to those generally required for imaging myocardium with positron-emission detection system in vivo would vary depending on prevailing metabolic conditions of the heart. The faster clearance in KH-compared to KH-RBC-perfused hearts may reflect in part a more general phenomenon — efflux of K\(^+\) and potassium analogs that accompany myocardial hypoxia. To whatever extent \(^{13}\)NH\(_3\) exists or free \(^{13}\)NH\(_3\)\(^+\) (ionic form), its behavior may be influenced in this fashion. The slower clearance after inhibition of glutamine synthetase in the KH-RBC-perfused hearts may reflect differences in the turnover of specific pools of trapped \(^{13}\)NH\(_3\). For example, \(^{13}\)NH\(_3\) incorporated into glutamine may be cleared more rapidly than \(^{13}\)NH\(_3\) incorporated into carbamyl phosphate. Thus, clearance of \(^{13}\)N is likely to be even more susceptible to the influences of altered myocardial metabolism than clearance of tracers such as rubidium that are not actively incorporated into other molecular species.

Since net accumulation measured with an imaging system acquiring data during a selected interval after initial exposure of the heart to tracer is a function of both extraction and clearance, and since clearance of \(^{13}\)N activity is affected markedly by altered metabolism, retention at any given interval after exposure of the heart to tracer (or net accumulation) cannot be expected to depend exclusively on flow even if the initial extraction fraction were entirely independent of flow.

**Discussion**

Noninvasive, quantitative assessment of myocardial perfusion is clinically important because delineation of the distribution and extent of regions of impaired perfusion at rest or with stress is essential to characterize coronary artery disease. Several approaches have been used, including the use of lipophilic gases such as \(^{133}\)Xe and \(^{85}\)Kr, which unfortunately require intracoronary injection. Potassium analogs such as \(^{82}\)Rb and \(^{201}\)Tl are accumulated by normal myocardium, and at least provide semiquantitative information about perfusion defects, even when imaged with single-photon detection systems that suffer from some unavoidable, intrinsic, quantitative limitations.\(^1\) However, the extent to which accumulation and retention during selected imaging intervals of these and related tracers, such as \(^{13}\)NH\(_3\), may vary due to altered metabolism of myocardium under conditions such as ischemia requires elucidation, since it may markedly distort relationships between myocardial uptake and flow.

Although the isolated, perfused heart preparation is a simple preparation, in contrast to the case in intact animals and patients, it permits control of flow and exclusion of recirculation under conditions in which the
metabolic environment and physiologic demands of the heart can be controlled and monitored. Analysis of behavior of tracer in such preparations permits delineation of the relative quantitative importance of specific factors influencing its behavior in both settings.

Results of this investigation indicate that uptake and retention of $^{13}$NH$_3$ do not depend exclusively on flow. The lack of correlation between residual fraction (retention) and flow is apparent under conditions of high flow in the experiments with hearts perfused with buffer alone. Since the oxygen solubility is low in non-hemoglobin-containing solutions, such hearts require high flow rates. The low residual fraction of $^{13}$NH$_3$ in these hearts probably reflects the short residence-time of the tracer within the capillary bed and indicates that extraction of $^{13}$NH$_3$ by the heart is limited severely when the time available for extraction is reduced, as may occur with high flow in vivo in conditions such as those occurring exercise. However, when flow was decreased, retention of $^{13}$NH$_3$ did not increase, probably because the increased residence time was offset by metabolic effects induced by hypoxia and diminishing extraction of the tracer. An additional factor is the increased clearance of tracer that was extracted was augmented by hypoxia, as shown by analysis of the terminal portions of the residence-time curves. Hearts perfused with buffer alone, rendered hypoxic despite flow rates within the physiologic range because of the lack of hemoglobin, retained much less $^{13}$NH$_3$ than hearts perfused at the same levels of flow with erythrocyte-enriched buffer. Thus, flow is clearly not the only factor influencing extraction and retention of ammonia. In fact, the metabolic status of myocardium is an important determinant.

The complex interplay between metabolism and flow and their dual effects on extraction and retention of $^{13}$NH$_3$ are evident also based on the data obtained with KH-RBC perfused hearts. When flow was reduced to levels typical of ischemic zones in vivo, the relationship between perfusion and residual fraction appeared to be biphasic. When flow was reduced by approximately 50% (to a mean of 0.7 ml/g/min), retention decreased slightly (although not statistically significantly), probably because of diminished net transport of NH$_3$ into ischemic cells despite prolonged residence time of the tracer. However, when flow was decreased further, the residual fraction increased beyond values obtained during control conditions, although again not significantly, probably because the effects of increased residence time outweighed the decreased sequestration due to altered myocardial metabolism.

The metabolic dependence of extraction and retention of $^{13}$NH$_3$ by myocardium is indicated also by results obtained with KH-RBC-perfused hearts. When glutamine synthetase (an enzyme mediating incorporation of ammonia into glutamine) was inhibited by methionine sulfoximine (a remarkably specific and irreversible inhibitor of this enzyme), residual fraction decreased by more than 60%.

Correlations between retention of $^{13}$NH$_3$ and 1 based on measurements in tissue samples under specific conditions such as after coronary artery ligation dogs may be due in part to heterogeneity in the sample. Thus, in some regions of myocardium that receive very little flow, virtually no radioactivity may be present. In other regions within a single portion of tissue sampled for analysis in vitro, perfusion may be normal and tracer may accumulate substantially. The amount of radioactivity in the entire tissue sample in vitro will be an algebraic sum of activity in the two (or more) types of tissue. This sum may correlate with fraction of tissue of each type within the sample, hence with overall (average) flow within the sample measured by an independent technique such as the radiolabeled microsphere method. However, conditions do not imply that accumulation of $^{13}$NH$_3$ within a small region of interest perfused homogeneously will correlate with flow within region.

In clinical applications of techniques designed to detect ischemia in vivo, mixtures of cells within single regions of interest of two extreme types (i.e., norm perfused as opposed to virtually nonperfused) much less likely be encountered than a spectrum of regions of interest with relatively homogeneous flow ranging from approximately 10-40% of flow of normal. Coronary artery disease in man is generally analogous to complete ligation of a proximal por of a major coronary artery. In the case of impalpable perfusion sufficient to induce symptoms but not frank, the diminution of flow in regions of interest may be even more modest.

The relative insensitivity of $^{13}$NH$_3$ as a perfusion indicator is emphasized by recent observations in which reconstructive tomographic images were obtained after bolus intravenous injections of $^{13}$NH$_3$ in dogs. The technique permitted detection of ischemic zones only when the proximal coronary stenosis exceeds 47% of the diameter, a value that could result in potential decrease in flow of as much as 95% under conditions of maximum distal vasodilation, based on Poiseuille’s equation.

The clearance of $^{13}$N activity that was extracted hearts perfused with buffer alone was inversely related to flow (table 3). Thus, as myocardium became hypoxic, clearance became more rapid (t½ decrease 1.3). It is possible that the acidosis accompanying hypoxia may have altered the equilibrium between NH$_3$ and NH$_4^+$, thereby altering extraction of tracer. How once inside the cell, the tracer appeared to metabolized in a fashion dependent largely on the thesis of glutamine via the glutamine synthetase pathway, based on previous reports. The altered clearance of intracellular $^{13}$N associated with hypoxia observed in the pre-study may depend on altered Na$^+$-K$^+$-ATPase activity (with $^{13}$NH$_4^+$ functioning as a K$^+$ analog in changes in glutamine synthetase activity; increase
corporation into pools such as carbamyl phosphate or more distal components in the urea cycle; or other factors influencing metabolism of the tracer. Regardless of its basis, it is likely to distort relationships between the net amount of $^{15}$NH$_3$ retained (and hence detectable and quantifiable by positron-emission tomography in vivo) within ischemic myocardium during any selected interval and the absolute level of flow to the region of interest.

Clinical Implications

Despite the potential value of $^{15}$NH$_3$ for detecting regions of compromised myocardium qualitatively, its use for quantification of regional perfusion has serious limitations. Results of this study indicate that even under conditions in which complicating potential effects of recirculation and redistribution of tracer are excluded, the residual fraction of $^{15}$NH$_3$ in myocardium after a bolus injection is not dependent exclusively on myocardial flow but that it is dependent to a considerable extent on the metabolic status of the myocardium. Thus, although qualitative differences in myocardial perfusion may be detectable in vivo with $^{15}$NH$_3$, the nature of the relationships between the amount of $^{15}$N activity in myocardium and perfusion is complex. Accordingly, despite the quantitative attributes of positron-emission tomography, quantitative assessment of perfusion with $^{15}$NH$_3$ must be pursued cautiously with adequate consideration of intrinsic limitations of the approach.

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The dependence of accumulation of $^{13}$NH$_3$ by myocardium on metabolic factors and its implications for quantitative assessment of perfusion.

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