Measurement of Regional Myocardial Blood Flow With N-13 Ammonia and Positron-Emission Tomography in Intact Dogs

ANIL SHAH, MD, HEINRICH R. SCHELBERT, MD, FACC, MARKUS SCHWAIGER, MD, EBERHARD HENZE, MD, HERBERT HANSEN, BA, CARL SELIN, MS, SUNG-CHENG HUANG, DSc
Los Angeles, California

N-13 ammonia mimics certain properties of microspheres. It rapidly clears from blood into myocardium where it becomes fixed in proportion to myocardial blood flow. Used with positron emission tomography as a means for quantifying in vivo myocardial indicator concentrations, N-13 ammonia may be useful for noninvasive determination of myocardial blood flow with the arterial reference sampling technique. This possibility was examined in 27 experiments in 10 chronically instrumented dogs at control, high and low blood flows. Myocardial blood flow was calculated in vivo from the myocardial N-13 tissue activity concentrations derived from serial cross-sectional images of the heart, the 2 minute arterial input function and the withdrawal rate of arterial blood.

Regional myocardial blood flow can be evaluated both invasively and noninvasively. It is most accurately determined with radioactive microspheres and the arterial reference sampling technique (1,2). The procedure entails administration of radioactive microspheres into the left atrium or ventricle, withdrawal of arterial blood and measurement of regional microsphere tissue concentrations in myocardium by in vitro counting of tissue samples. The latter requirement precludes use of this technique in human beings. However, regional myocardial indicator tissue concentrations can now be measured in vivo with positron emission computed tomography (3-7). Moreover, the positron-emitting blood flow tracer, N-13 ammonia, exhibits properties that to some extent resemble those of radioactive microspheres (8,9). N-13 ammonia is extracted and fixed in myocardium in proportion to blood flow. However, its first capillary transit extraction fraction is less than 100% and decreases further with higher flows. The tracer clears rapidly from blood so that the arterial input function is nearly complete within minutes. Unlike radioactive microspheres, N-13 ammonia is administered intravenously, so that use of this tracer in combination with positron emission tomography would allow noninvasive measurements of regional myocardial blood flow in human beings. It was, therefore, the purpose of our study to examine in closed chest dogs the possibility of employing the arterial reference sampling technique with N-13 ammonia and positron emission tomography for measuring regional myocardial blood flow in vivo.

Methods

Animal preparation. Ten mongrel dogs, weighing 20 to 30 kg (mean 25.2), were anesthetized with sodium pentobarbital (25 mg/kg body weight), intubated and ventilated with room air. Catheters were inserted into the carotid artery and through the femoral artery into the abdominal aorta for withdrawing arterial blood and monitoring systemic blood...
After a left thoracotomy, an electromagnetic flow probe (series 500, Biotronics) was placed around the proximal left circumflex coronary artery. Mechanical zero flow was established during 10 second coronary occlusions with a snare placed distally to the flow probe. A fine polyethylene cannula was advanced through a puncture wound into the left atrium. The arterial input function for N-13 ammonia was accomplished by drawing a line on the dog's chest along the demarcation line through the chest wall in the direction of the imaging plane into the thorax and myocardium to identify the anatomic cross-section of the heart that had been imaged by positron emission tomography. The dog was then killed with concentrated potassium chloride solution, the chest cavity opened and the heart removed. A 1 cm thick cross-sectional slice of the left ventricle along the needle insertion sites (that is, corresponding to the imaged cross section) was obtained and photographed, and the wall thickness determined at six equally spaced sites. The left ventricular cross section was then divided into approximately 1 g samples. Each tissue sample was weighed and transferred into counting tubes, and the tissue microsphere activity concentrations determined by well counting.

Measurement of regional myocardial blood flow with radioactive microspheres. Regional myocardial blood flow at the time of each N-13 ammonia injection was determined with the microsphere technique (1). Carbonized polystyrene microspheres (15 ± 5 µ in diameter, suspended in 10% dextran with 1 drop of Tween 80; 3M Company) were resuspended with a vortex shaker and then by ultrasonification. Approximately $2 \times 10^6$ microspheres labeled with cobalt-57, tin-113, niobium-95, scandium-46 or ruthenium-103 were injected. The activity in the blood and myocardial tissue samples was measured in a sodium iodide (thallium) well counter.

Regional myocardial blood flow (RMBF) was calculated using equation 1:

$$RMBF = \frac{F_a \cdot C_m}{C_b} \cdot 100 \text{ (ml/min per 100 g)},$$

where $F_a$ is the withdrawal rate of arterial blood (ml/min), $C_m$ is the activity in the myocardial tissue sample (counts/min per g) and $C_b$ is the total activity in the arterial blood sample (counts/min).

Positron emission tomographic imaging, data analysis and calculation of blood flow with N-13 ammonia. N-13 ammonia was produced at the University of California at Los Angeles Medical Cyclotron as described previously (8,9). Serial positron tomographic imaging in the ungated, fixed time mode was begun at the time of tracer injection and continued until five 1 minute images were recorded. The spatial resolution was 17 mm at full width-half maximum with a slice thickness of 19 mm. Because of a 20 second interval between the acquisition of each image, the total imaging time amounted to 6.3 minutes. To evaluate possible changes in tissue N-13 ammonia concentrations over time, additional images were obtained in five studies 20 minutes after tracer injection.

Six regions of interest were assigned to the left ventricular myocardium in each image. Their location corresponded to those on the anatomic cross sections where re-
Regional wall thickness was determined. Each region of interest was 0.84 cm² in area (21 pixels). The counts per pixel were then determined, averaged for the entire region of interest and expressed as mean counts/pixel per min. Regional myocardial blood flow was calculated by the arterial reference sampling technique using equation 1. N-13 ammonia activity was measured by well counting.

The activity of the myocardial tissue sample (Cₘ) was determined from the cross-sectional positron emission tomographic images from myocardial regions of interest as described earlier (7,12,13) using equation 2:

\[ C_m = \frac{C_{mo}}{k \times RC \times g} \]

(2)

where Cₘ₀ is the observed myocardial N-13 tissue activity concentration (determined from the region of interest on the tomographic image) and k is the calibration factor that normalizes tissue activity concentrations measured by positron emission tomography to tissue activity concentrations measured by well counting; k was determined as described previously (7). The recovery coefficient, RC, corrects for the underestimation of myocardial indicator tissue concentrations by positron emission tomography, which results from the partial volume effect (7,14), and g is the specific gravity of myocardium. The activity of the myocardial tissue sample (Cₘ₀) was derived from the regions of interest assigned to the left ventricular myocardium and was expressed as average counts/pixel per min. Corrections for physical decay of N-13 activity, for cross-contamination of N-13 activity between blood pools and myocardium as well as for partial volume effect were performed as described previously (12–14). All correction techniques for deriving true tracer concentrations from the tomographic images are described in detail in previous reports (7,12–14).

Statistical analysis. All values are expressed as mean values ± standard deviation (SD). Measurements of regional myocardial blood flow by the two independent techniques were compared and the curve fitted (least square fitting) with polynomials. Statistically significant differences were determined by analysis of variance or, when appropriate, by Student’s t test for paired and unpaired data.

Results

Hemodynamic findings. In the 10 control experiments, myocardial blood flow measured by microspheres averaged 99 ± 41 ml/min per 100 g. Mean arterial blood pressure was 110 ± 18 mm Hg and heart rate was 108 ± 24 beats/min. In the eight hyperemic experiments, myocardial blood flow increased to 169 ± 66 ml/min per 100 g, while arterial blood pressure averaged 90 ± 20 mm Hg and heart rate was 142 ± 20 beats/min. In the nine partial coronary occlusion experiments, blood flow in the underperfused segment averaged 58 ± 28 ml/min per 100 g, while mean arterial pressure and heart rate averaged 106 ± 16 mm Hg and 118 ± 28 beats/min, respectively.

Positron emission tomographic imaging and blood flow measurements. Positron emission tomography yielded good quality images of the myocardial distribution of N-13 ammonia. Examples of serial images recorded at control, during coronary hyperemia and during circumflex coronary artery constriction are shown in Figure 1. The changes in myocardial and blood N-13 activity concentrations after intravenous administration of N-13 ammonia in a control experiment are shown in Figure 2. N-13 activity in the blood is highest in the third sample (withdrawn 30 to 60 seconds after injection). It rapidly declines thereafter and remains relatively constant after 2.5 minutes.

Myocardial N-13 activity concentrations increase most early after tracer injection and then stabilize (Table 1). These changes were similar for all experiments and are summarized for all studies in Figure 3. These time-dependent N-13 activity concentrations were similar for the control, hyperemic and partial occlusion experiments. Myocardial N-13 activity concentrations increased most dramatically (by 13 to 29%) from the first (at 0.5 minute) to the second measurement (at 1.83 minutes). For the next 4 minutes, myocardial N-13 activity concentrations increased less rapidly. Interimage changes in myocardial N-13 activity concentrations were only small. In five studies with additional images at 20 minutes after N-13 ammonia administration, regional N-13 activity concentrations were only 13 to 15% higher than those at 6.3 minutes.

Figure 1. Serial 1 minute cross-sectional images recorded at the same level through the left ventricle at the control study (A), during dipyridamole-induced hyperemia (B) and after constriction of the left circumflex coronary artery (C). Homogeneous uptake of N-13 ammonia throughout the left ventricular myocardium is evident in the control and hyperemic studies. The increase in myocardial blood flow from control to hyperemia is reflected by higher N-13 activity concentrations. The images in C, obtained after partial left circumflex coronary occlusion, show a marked reduction in tracer concentration in the posterolateral wall of the myocardium (arrow).
Table 1. Mean Myocardial N-13 Activity Concentrations ± 1 Standard Deviation and Their Changes Over Time in Each Experimental Protocol

<table>
<thead>
<tr>
<th>Scan</th>
<th>Time (min)</th>
<th>Control Studies (n = 10)</th>
<th>Partial Coronary Occlusion Studies</th>
<th>Dipyridamole Hyperemia Studies (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ischemic Zone (n = 9)</td>
<td>Nonischemic Zone (n = 9)</td>
</tr>
<tr>
<td>1</td>
<td>0.50</td>
<td>11,886 ± 2,944 (82%)</td>
<td>6,541 ± 1,854 (86%)</td>
<td>11,945 ± 6,896 (87%)</td>
</tr>
<tr>
<td>2</td>
<td>1.83</td>
<td>14,461 ± 3,679 (100%)</td>
<td>7,574 ± 3,321 (100%)</td>
<td>13,772 ± 7,062 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>3.17</td>
<td>15,425 ± 4,122 (106%)</td>
<td>8,253 ± 2,643 (108%)</td>
<td>15,021 ± 8,329 (109%)</td>
</tr>
<tr>
<td>4</td>
<td>4.50</td>
<td>16,123 ± 4,487 (111%)</td>
<td>8,453 ± 2,491 (111%)</td>
<td>15,888 ± 8,312 (115%)</td>
</tr>
<tr>
<td>5</td>
<td>5.83</td>
<td>16,680 ± 4,523 (115%)</td>
<td>8,678 ± 2,344 (114%)</td>
<td>16,357 ± 8,297 (115%)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>17,542 ± 4,728 (121%)</td>
<td></td>
<td>20,540 ± 3,708 (116%)</td>
</tr>
</tbody>
</table>

*Time indicates the mid-point of each scan acquisition period for each consecutive scan. The numbers in parentheses indicate the percent changes from scan 2, designated as 100%. Intergroup comparisons reveal only insignificant differences between the progressive increases of myocardial N-13 activity concentrations with the exception of scan 1 of the dipyridamole hyperemia studies (*). Tissue concentration differences between each consecutive scan were insignificant by analysis of variance but significant by Student’s paired t test (except for difference between scans 4 and 5).
Figure 3. Mean myocardial (MYOC) N-13 tissue concentrations as a function of time in all 27 experiments. The rather large standard deviation is due to the fact that tissue concentrations were averaged from all (control, hyperemic and low flow) experiments. The myocardial N-13 tissue concentrations were obtained from regions of interest assigned to the left ventricular myocardium and are given for the time of the mid-point of each scan.

Blood flows were also calculated from the myocardial N-13 activity concentrations at 3.17, 4.50 and 5.83 minutes after tracer injection and the arterial input function integrated only to 2.0 minutes. The rationale for the latter approach was that the arterial input function was nearly complete and residual activity in the blood represented primarily N-13-labeled amino acids. Blood flows calculated by the first approach decreased progressively and significantly with time (Table 2, Fig. 4). Flows calculated from the 5.83 minutes scan and the arterial input function to that time were 24.3 ± 8% lower than those calculated at 1.83 minutes after tracer injection. Conversely, flows calculated from the same scan but using the integral of the arterial input function to 2.0 minutes were 13.3 ± 6.5% higher than those calculated at 1.83 minutes. Comparisons by Student’s t test for paired data disclosed the moderate changes in calculated flows to be statistically significant from 1.83 to 4.5 minutes but not from 4.5 to 5.83 minutes.

Discussion

Current scintigraphic techniques such as thallium-201 imaging are useful for evaluating the relative distribution...
of blood flow in myocardium, but fail to provide quantitative estimates in ml/min per 100 g. As this study indicates, such measurements may be possible with N-13 ammonia and positron emission tomography. Previous studies with planar imaging (15) or positron emission tomography (16) demonstrated the use of N-13 ammonia in human beings as a tracer of blood flow for detecting coronary artery disease and for assessing its extent. These earlier investigations were largely qualitative. A more recent clinical study (16) allowed estimates of absolute increases of blood flow from control to dipyridamole-induced hyperemia, estimates that were based on changes in N-13 ammonia tissue concentrations in human myocardium and approximations of the arterial input function. The estimated increases were comparable with those measured with coronary sinus thermistors by Brown et al. (17).

Relation between myocardial blood flow and N-13 ammonia tissue concentration. The rationale for use of N-13 ammonia for determining in vivo regional myocardial blood flow is based on the properties of this tracer which, to some extent, resemble those of radioactive microspheres. Myocardium accumulates and retains the tracer in proportion to blood flow. However, the extraction fraction is less than 100% and declines with higher flows. Accordingly, the overall relation between blood flow and myocardial N-13 ammonia tissue concentration is nonlinear. N-13 ammonia becomes metabolically fixed in myocardium (9,18–20), yet the trapping mechanism appears to be relatively insensitive to changes in the hemodynamic and metabolic state of the heart (9). Clearance of N-13 activity from myocardium is slow. At the time of positron emission tomographic imaging, the tracer distribution in myocardium reflects, therefore, the distribution of myocardial blood flow at the time of the injection (9). Finally, N-13 ammonia clears rapidly from the blood. After intravenous injection, N-13 activity in blood declines within 2 minutes to approximately 5% of its peak value and in 5 minutes to about 1 to 2% (8,21). Consequently, tracer recirculation and its effects on tracer tissue concentrations should be small after the initial 2 minutes after tracer injection. The residual blood activity thereafter represents N-13 bound primarily to amino acids rather than N-13 ammonia (22). Their extraction by dog myocardium is low (23,24).

Regional myocardial blood flow was calculated from the arterial input function of N-13 ammonia from the time of injection to 2 minutes and from the second of the serial tomographic images acquired at 1.83 minutes (mid-point) after tracer injection. The second scan was chosen because myocardial N-13 ammonia uptake was still incomplete at

Figure 5. Relation between regional myocardial blood flow (MBF) determined with the microsphere technique and with N-13 ammonia and positron emission tomography for all 27 experiments.

<table>
<thead>
<tr>
<th>Scan no.</th>
<th>Time (min)</th>
<th>Input function</th>
<th>Input function</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.17</td>
<td>+6.7 ± 6.6%†</td>
<td>-6.0 ± 5.7%†</td>
</tr>
<tr>
<td>4</td>
<td>4.50</td>
<td>+10.6 ± 4.4%*</td>
<td>-17.4 ± 7.3%†</td>
</tr>
<tr>
<td>5</td>
<td>5.83</td>
<td>+13.3 ± 6.5% NS</td>
<td>-24.3 ± 8.0†</td>
</tr>
</tbody>
</table>

The table lists the average percent changes in blood flow calculated from scan 2 and the integral of arterial N-13 activity concentrations to that time. Statistically significant differences between interscan changes are indicated by † = p < 0.05 and * = p < 0.001. NS = not significant.
of myocardial blood flow. Each of the bars in A show the mean and 1 standard deviation for blood flow calculated from each of the second to the fifth of the serial images and the arterial input function to the time of each scan (B). Note the significant decline in calculated values as compared with the values calculated from each of the serial images and the arterial input function to only 2 minutes (A).

Figure 6. Effects of the time interval between N-13 ammonia administration and myocardial N-13 tissue concentration measurements and arterial blood sampling on the in vivo calculation of myocardial blood flow. Each of the bars in A show the mean and 1 standard deviation for blood flow calculated from each of the second to the fifth of the serial images and the arterial input function to the time of each scan (B). Note the significant decline in calculated values as compared with the values calculated from each of the serial images and the arterial input function to only 2 minutes (A).

The observed data scatter may be attributed to several factors. Flow determined with particulate tracers such as microspheres is known to differ from flow measured with diffusible indicators (25). Also, the cross-sectional images demonstrated previously that indicator tissue concentrations were recorded in an ungated mode. Wisenberg et al. (7), demonstrated previously that indicator tissue concentrations are determined more accurately from electrocardiographic gated rather than from ungated images. Because of the longer acquisition times required for gated image acquisition, the use of gated image acquisition in this study was limited by the time of the first scan. It increased by about 18% from the first to the second scan, while subsequent increases were only small and ranged from 2.3 to 7.3% between scans acquired over the next 4 minutes. These increases were similar in the hyperemic, control and low flow experiments. The step-up in tissue concentrations from the first to the second image with only minor subsequent increases implies that the arterial input function for N-13 ammonia was nearly complete at the time of the second image acquired 1.83 minutes after tracer injection.

Methodologic considerations. In vivo measurements of myocardial blood flow systematically underestimated blood flow determined by the microsphere technique. This is because N-13 ammonia is extracted less than 100% during a single capillary transit. Over the flow range from 40 to about 200 ml/min per 100 g, this relation is almost linear as indicated by the linear regression equation for flows ranging from 44 to 180 ml/min per 100 g of $y = -7.4 + 0.94x$, a correlation coefficient of 0.92 and a standard error of the estimate of 16 ml/min per 100 g. At higher blood flows, the relation stabilizes. This plateau is most likely the result of a declining "extraction fraction" for N-13 ammonia rather than an artifactual decrease in count recovery. Altered wall motion can cause artifactual changes in count recovery, but in this experiment cannot account for the leveling off of measured values at high flow rates. In the hyperemic experiments, dipyridamole lowered peripheral resistance with a compensatory increase in heart rate. Higher heart rates result in an increase in mean myocardial wall thickness and, hence, improve rather than worsen the retrieval of myocardial activity from the cross-sectional images. The stabilizing of in vivo measurements at higher levels is, as is the case for most diffusible indicators, a function of the flow-dependent decrease in the extraction fraction.

In vitro versus in vivo calculated flows. Using the regression equation describing the relation between in vitro and in vivo calculated flows, the in vivo calculated flows can be corrected. For example, applying this correction to our data, in vivo calculated data correlated linearly with the in vitro microsphere measurements (Fig. 7). The slope of the regression line approaches unity, indicating that the correction yields values that are comparable with those calculated by the in vitro technique. Although encouraging, there remains considerable scatter of the data points about the regression line, with an average standard error of 24 ml/min per 100 g. Moreover, the validity of this correction requires further validation in a prospective manner.

The observed data scatter may be attributed to several factors. Flow determined with particulate tracers such as microspheres is known to differ from flow measured with diffusible indicators (25). Also, the cross-sectional images were recorded in an ungated mode. Wisenberg et al. (7), demonstrated previously that indicator tissue concentrations are determined more accurately from electrocardiographic gated rather than from ungated images. Because of the longer acquisition times required for gated image acquisition, the use of gated image acquisition in this study was limited by

Figure 7. Relation between in vivo and in vitro calculated myocardial blood flows (MBF) after correcting each individual data point by the polynomial regression equation shown in Figure 5.
the relatively short physical half-life of N-13 ammonia. Last, because of variations in the arterial input function, the cut-off time at 2 minutes may not always accurately reflect the true arterial input function and introduces additional errors.

Low spatial resolution of the imaging device used in this study. This is another potential source of error. It results in substantial loss in recovered counts (approximately 30 to 50%) as a consequence of the partial volume effect. Accordingly, the factor required for correction of regional wall thickness is large and likely to affect the accuracy of the measurements. Discrepancies between average wall thickness of the live beating heart and the wall thickness measured postmortem further reduce the accuracy. Inconsistencies between the sites of in vivo tissue concentration and of postmortem wall thickness measurements may further contribute to the data scatter. These factors seem largely responsible for variations in segmental tracer concentrations between the various regions of interest along myocardial cross-sectional images that averaged 9 ± 3% in the control experiments. These variations prompted us to use the average instead of the regional flow values. Nevertheless, regional measurements of blood flow are possible as indicated in Figure 4. Many of these limitations can be overcome with newer, high temporal and spatial resolution tomographs that will reduce the magnitude of correction factors for partial volume effect, permit measurements of segmental wall thickness directly from the images and allow electrocardiographic synchronized image acquisition.

Effects of time on measurements of blood flow. In vivo measurements of blood flow with N-13 ammonia and positron emission tomography are affected by the time of measurement after tracer injection. Calculation of blood flow from later scans (3.17 to 5.83 minutes) and the integral of the arterial concentration to the time of image acquisition yielded progressively lower values. Calculated flows at 5.83 minutes were 24.3 ± 8.0% lower than those calculated from the second scan. Use of the arterial input function to only 2 minutes for deriving blood flow from consecutive scans reduced the magnitude of time-dependent changes. For example, the calculated values from the third, fourth and fifth scan, respectively, were only 6.7, 10.6 and 13.3% higher than those calculated from the 2 minute scan. Thus, the latter approach reduces the effect of time dependency on calculated flows.

Possible solutions to minimize the effects of time on calculated blood flow are correction of values for the time between tracer injection and imaging. For example, blood flows calculated from the fifth scan could be corrected for the 13.3% overestimation. This will be important when myocardial blood flow is determined with this approach with a single slice tomograph and the entire heart imaged by recording serially contiguous cross-sectional images. With the multislice capability of newer tomographs, several cross sections could be acquired simultaneously, which would obviate the need for serial imaging.

Clinical applications. In comparison with other more complex techniques currently developed for measuring myocardial blood flow like the rubidium-82 technique (26,27) or the oxygen-15 water technique (28,29), the simplicity of the current approach offers several advantages. The technique requires only one measurement of the myocardial N-13 tissue concentration between 2 and 6 minutes after intravenous tracer injection, withdrawal of arterial blood for 2 minutes, well counter measurements of the arterial input function and calibration of the positron emission tomograph with the wall counter.

Given the near linear relation between in vivo and in vitro measurements within the low to moderately high flow range, the N-13 ammonia/positron emission tomographic technique will be sensitive for blood flow determinations in patients at rest or during moderate hyperemia as well as in patients with regional perfusion abnormalities. The utility of this technique at higher flow ranges will largely depend on the accuracy of regional tissue concentration measurements with positron emission tomography. The shortcomings with current instrumentation, as outlined earlier, represent a major limitation for the use of this approach to the human heart in which in vivo determination of ventricular wall thickness in exactly the imaged cross section may be limited or impossible. The newer generation positron emission tomographs, however, offer far better temporal and spatial resolutions so that rapid serial electrocardiographic gated cross-sectional imaging will be possible and the loss in count recovery considerably less. For correction of residual count recovery, direct measurements of regional wall thickness may be possible directly from the cross-sectional images. Finally, it may be possible to determine the arterial input function directly from regions of interest assigned to the left ventricular blood pool, thus obviating the need for arterial blood sampling and rendering this approach largely noninvasive.

The potential of N-13 ammonia for external quantification of regional blood flow in human subjects has been indicated in earlier studies (16). Application of this technique in human beings will require rigorous testing. The optimal time for imaging and, hence, determination of segmental tracer tissue concentrations will depend on the time when the tracer has cleared almost completely from blood and when the arterial input function as well as myocardial tracer tissue uptake are completed. The possibility of using positron emitting microspheres for measuring regional blood flow with the arterial reference sampling technique and positron emission tomography (Selwyn et al., unpublished data) would further allow validation of the N-13 ammonia technique against the microsphere technique as the standard.
We thank Anthony Ricci and Gerald Low for their technical assistance, M. Lee Griswold for preparing the illustrations, Norman S. MacDonald, PhD and his Cyclotron staff for producing N-13 ammonia, Kerry Engber for her skillful secretarial assistance and Patrick Welton for his editorial assistance.

References


