THE hypothesis that adenosine regulates coronary flow (Berne, 1963) predicts that there is a unique, causal relationship between cardiac oxygen usage, M\textsubscript{VO}\textsubscript{2}, cardiac muscle adenosine levels ([Ado]), and coronary vascular resistance (R) in open-chest, anesthetized dogs tested the hypothesis that adenosine is a physiological regulator of coronary flow. Experiments using each dog as its own control showed that [Ado] varied directly with M\textsubscript{VO}\textsubscript{2} as the latter changed spontaneously or in response to atrial pacing, paired pacing, aortic constriction, or \beta-adrenergic blockade. In turn, R varied inversely with changes in [Ado]. Stimulating M\textsubscript{VO}\textsubscript{2} with isoproterenol significantly increased the slope of the regression of [Ado] on M\textsubscript{VO}\textsubscript{2} as well as of R\textsuperscript{-1} on [Ado]. The effect of \beta-adrenergic stimulation on [Ado] is unexplained, but its effect on R\textsuperscript{-1} seems to reflect the combined effects of adenosine and direct \beta-adrenergic coronary relaxation. These results support the hypothesis that adenosine mediates the coronary flow responses to changes in M\textsubscript{VO}\textsubscript{2}.

M\textsubscript{VO}\textsubscript{2} and R are each the resultant of a number of factors whose influences may vary from one animal to the next and could contribute variation comparable to those due to the interventions themselves, the experimental design employed each dog as its own control. This design depended in turn on a muscle-sampling technique which permitted repeated heart muscle biopsy and an adenosine assay sensitive enough to measure the small amounts of adenosine in these samples. Owing to post-sampling changes in adenosine content, these data provided only indirect estimates of true muscle adenosine content. Nonetheless, M\textsubscript{VO}\textsubscript{2}, [Ado], and R\textsuperscript{-1} were significantly covariant, consistent with the possibility that these variables are related causally.

**Methods**

Conditioned mongrel dogs of either sex weighing 11-16 kg were premedicated with morphine sulfate (3 mg/kg subcut) and anesthetized 30 minutes later with \alpha-chloralose (110 mg/kg, iv). The chest was opened through the left 4th interspace and the left coronary artery was perfused via a Gregg cannula with blood from the left common carotid artery. An electromagnetic flowmeter interposed in the perfusion line and an electromanometer connected to this line monitored coronary flow and perfusion pressure, respectively. A pointed-tip polyethylene side-hole catheter inserted transepicardially into
Experimental Protocol

Coronary flow and perfusion pressure were recorded continuously throughout each experiment. A pair of 20-second coronary occlusions separated by 5–10 minutes established the zero flow baseline and provided an estimate of peak reactive hyperemia flow, which served as an index of coronary reactivity. After a 10-minute recovery period, control samples of arterial and coronary venous blood were collected anaerobically and stored in ice. A sample of left ventricular myocardium then was obtained with the device described by Pool et al. (1968). Within 0.7 second from the beginning of biopsy, as measured with a stopwatch, the sample was transferred to a beaker containing 2-methylbutane which previously had been frozen in liquid nitrogen and thawed partially just prior to biopsy. At this temperature, 2-methylbutane is a viscous syrup containing some solid methylbutane ice. The beaker containing the tissue sample was stored in liquid N2 until the tissue was processed. Bleeding at the biopsy site was controlled by inserting a wooden peg into the wound, brief pressure and mechanical flow zero references were recorded, and after reactive hyperemia subsided, cardiac oxygen usage was varied by one of five interventions: (1) atrial pacing at 20–70 beats/min above the prevailing heart rate, (2) paired right ventricular pacing at rates between 90 and 165 beats/min, (3) constriction of the descending thoracic aorta to raise proximal aortic pressure by 30–60 mm Hg, (4) intracoronary infusion of isoproterenol at approximately 0.05 μg/min, or (5) the administration of L-propranolol, 1 mg/kg iv. Except in the case of propranolol, where the long duration of action required that it be the final intervention whenever it was employed, the type and order of the intervention were randomized.

After 5 minutes (20–30 minutes in the case of propranolol) to establish the new steady state of MVO2, blood and tissue sampling and registration of pressure and flow zero references were repeated. The intervening stimulus was withdrawn, and after 10–15 minutes, a second set of control and intervention samples were obtained. After a total of two or three interventions, each preceded by control observations, the dog was killed by a cardiac injection of a saturated solution of KCl. The left coronary artery and coronary sinus were dissected to ensure proper cannula placements, and the left ventricle was trimmed of fat and contiguous structures, and weighed.

The time necessary to transfer the muscle sample into the coolant is an inherent source of artefactual change in adenosine content. Two experiments assessed the importance of this error. The first study compared adenosine levels in samples cooled immediately (0.5–0.7 second) with those of samples injected into coolant after a deliberate delay of up to 5 seconds. The second experiment compared the adenosine level in a sample obtained by the drill with that of muscle sampled <10 seconds later by means of metal tongs cooled in boiling N2. To minimize the hemodynamic fluctuations caused by biopsy and possible effects on the adenosine levels in the subsequent sample (Fig. 3), these hearts were paced electrically at 20–30 beats/min above the spontaneous rate.

Tissue Processing and Chemical Analysis

Blood PO2, PCO2, and pH were estimated with a Corning model 161 analyzer calibrated with a reference gas mixture between each sample. Duplicate estimates of PO2 and PCO2 differed by 1 mm Hg or less and of pH by less than 0.05 unit. Blood oxygen content was estimated with a Lex-O-Con TL fuel cell; duplicates agreed within 0.1 ml/dl.

The transmural samples of frozen myocardium, which were typically 5–10 mm long and 0.5–0.7 mm in diameter, were quickly weighed to the nearest 0.1 mg on a Cahn Model 7500 millibalance located in a 4°C room. The frozen tissue was transferred immediately to a homogenizer tube containing 0.5 ml ice-cold 0.6 N HClO4 and [2, 8, 5'-3H]adenosine recovery standard (approximately 5,000 dpm). Puriﬁcation consisted of homogenizing the tissue, collecting the supernatant after centrifugation, and re-extracting the pellet with a second 0.5-ml aliquot of HClO4. The combined supernatants were adjusted to pH 5.5 (methyl yellow end point) and precipitated KClO4 was centrifuged off. The supernatant then was adjusted to pH 3.5 (phenol red end point) and mixed with one-tenth volume of 0.16 M triethylammonium acetate, pH 3.5. Adenosine was separated on columns of Aminex A-6 by the method of Schrader et al. (1978). Phenol red is not retained on Aminex A6, but methyl yellow is bound tightly, so that with repeated use the columns become deep red at pH 3.5. Performance is not affected, however, as judged by the complete separation of [3H]adenosine from either [14C]adenine or [3H]5'-AMP. An aliquot of the adenosine-containing eluate was counted for [3H]adenosine activity to calculate recovery and the remainder was dried at 40°C in a stream of filtered air. Preliminary experience showed the necessity of prompt workup of the tissue samples. Storage of frozen tissue for days or weeks at -15°C, -60°C, or -90°C resulted in large (10- to 100-fold), time-dependent increases in adenosine
levels. Accordingly, samples were carried through Aminex chromatography and dried within 4 hours after they were obtained. Adenosine levels in the dried eluates were stable for 4–6 weeks at −15°C. For analysis, the sample was reconstituted in 1 ml 0.1 M Tris-HCl, pH 7.5, containing 1 mm EDTA and the adenosine content in 50-μl aliquots estimated in duplicate by a radioligand-binding assay (Olsøn et al., 1978). The useful range of this assay (radioligand displacement between 10 and 90%) is 1–16 pmol adenosine. A program-adjusted log-logit transformation (“PALL”; Halch et al., 1976) was used to generate the best fit for the standard curve. Quality control was monitored by the method of Rodbard et al. (1968). The coefficients of between-assay and within-assay variation for the 4.1 and 10.2 pmol standards run with accepted assays of the present group of samples were 3.5 and 3.6%, respectively. Adenosine concentration in each tissue sample was corrected for the recovery of [3H]adenosine.

**Experimental Design and Data Analysis**

The design of these experiments to test Berne’s hypothesis incorporates one of its implicit assumptions, that MVO₂ is the independent variable. These experiments test three derivative hypotheses: (1) under control conditions, spontaneous variations in MVO₂ cause proportional changes in [Ado]; (2) these changes in [Ado] cause inversely proportional changes in R; and (3) these proportional changes are maintained when MVO₂ is perturbed experimentally.

This design recognizes that the heart has a basal rate of oxygen usage which is independent of the oxygen usage related to work (McKeever et al., 1958; Braunwald et al., 1976). Whether there are “basal” values of [Ado] and a basal coronary resistance characteristic of quiescent heart muscle is uncertain. By comparing changes in the three variables, this design is free of any assumptions about their basal values. These expressions are consistent with models in which the cardiac adenosine pool is exclusively extracellular (Berne et al., 1979) or consists of extracellular and intracellular compartments (Schrader et al., 1976; Olsøn et al., 1978), but does assume in the latter case that changes in the two compartments are proportional.

The use of a technique permitting repeated sampling of myocardium with minimal effect on blood pressure, coronary flow, or heart rate (Pool et al., 1968) and a sensitive assay for adenosine exploits the power of an experimental design in which each animal serves as its own control.

Experiments were included in the data analysis if both the control and experimental observations for each perturbation of MVO₂ met the following criteria: (1) coronary perfusion pressure ≥80 mm Hg, (2) peak reactive hyperemia to control coronary flow ratio ≥2, (3) tissue immersion in coolant occurred ≤0.7 second after sampling, and (4) acceptable adenosine assay quality control data and adenosine recovery ≥80%.

To normalize dog-to-dog variation in coronary flow due to differences in heart size, coronary flow and data derived from this variable are expressed per 100 g LV weight. MVO₂ was calculated as the product of coronary flow multiplied by arterio-coronary venous oxygen content difference. Coronary vascular resistance was calculated as the quotient of perfusion pressure divided by coronary flow. Cardiac muscle adenosine levels are expressed in units of nmol/g LV weight.

If the frequency distribution of control hemodynamic data suggested a normal distribution, they were described as mean ± SEM and, if not, as the range and modal values. Since each dog served as its own control, a t-test for paired samples assessed the hemodynamic effects of the interventions used to alter MVO₂. The covariance of [Ado] and R⁻¹, respectively, with MVO₂ and of R⁻¹ with [Ado], was examined by standard least squares correlation and regression formulas. The slopes of these regressions provided estimates of average ΔR⁻¹/ΔMVO₂, Δ[Ado]/ΔMVO₂, and ΔR⁻¹/Δ[Ado]. A t-test comparing the slopes of the regressions characterizing the relationships between ΔMVO₂, Δ[Ado], and ΔR⁻¹ during interventions with those during the control tested the hypothesis that the interventions were quantitatively equivalent to the spontaneous variations during the control state. As a measure of variance, this test employed Sₒ, calculated as Sₒ = (∑X²/σ)⁰.⁵, where X is the mean of all X. The possibility that the various interventions were not quantitatively assessed and also by calculating the ratios of these increments for each experiment, then evaluating differences in group means by one-way analysis of variance employing Tukey’s w test.

**Results**

Control heart rates ranged between 72 and 231 beats/min and were distributed about modal values of 125–135 and 195–205 beats/min. Failure of morphine/chloralose anesthesia to produce a slow heart rate in some dogs appears to explain this wide range and bimodal distribution. Coronary flow averaged 103.6 ± 4.6 ml/min per 100 g, perfusion pressure 112 ± 3 mm Hg, arterial oxygen content 19.0 ± 0.6 ml/dl. Peak reactive hyperemia blood flow rate averaged 338 ± 50% of control. The effects of the five interventions on these variables are summarized in Table I.

Figures 1 and 2 summarize the assessment of the muscle sampling method used in this study. Figure 1 shows the effect of retarded tissue cooling on adenosine levels. During the first 2 seconds after biopsy, adenosine levels rose rapidly to a peak value of 26 nmol/g but fell precipitously thereafter to levels slightly lower than those in samples cooled with a minimum of delay. Since adenosine concentration increased predictably during the first second
after biopsy, the concentration in samples cooled immediately could serve as an index of the true concentration at the moment of biopsy if the scaling factor were known. The experiments shown in Figure 2 verify that the amount of adenosine found in drill samples is indeed proportional to, but approximately 10 times greater than, the amount found in muscle samples obtained a few moments later with tongs. Other experiments employed four dogs to test the possibility that repeated biopsy systematically altered adenosine levels. Adenosine levels in the six samples from each dog were 7.9 ± 0.3, 8.2 ± 0.2, 10.1 ± 0.6, and 7.9 ± 0.3 nmol/g LV. To test the hypothesis that repeated sampling affected adenosine levels, each biopsy was considered a treatment. The adenosine levels in each treatment group were 9.8 ± 0.8, 8.0 ± 0.9, 9.3 ± 1.5, 8.3 ± 0.5, 9.0 ± 1.7, and 9.4 ± 0.9 nmol/g LV. Analysis of variance rejected the hypothesis that significant treatment effects existed, F = 0.04, P > 0.5 for 5 and 3 d.f.

Figure 3 illustrates a typical experiment. Biopsy usually caused 1-2 premature ventricular contractions and transiently perturbed perfusion pressure and coronary flow. Electrical pacing prevented these hemodynamic transients, a result that we

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**TABLE 1** Effect of Interventions on Hemodynamic and Metabolic Variables

<table>
<thead>
<tr>
<th>Intervention</th>
<th>n</th>
<th>HR beats/min</th>
<th>C</th>
<th>Δ</th>
<th>BP (mm Hg)</th>
<th>C</th>
<th>Δ</th>
<th>C</th>
<th>Δ</th>
<th>CBF (ml/100 g per min)</th>
<th>C</th>
<th>Δ</th>
<th>ΔAVO₂ (ml O₂/dl)</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial pacing</td>
<td>4</td>
<td>98 ± 17</td>
<td>44*</td>
<td>119 ± 13</td>
<td>1</td>
<td>84.6 ± 7.7</td>
<td>20.8†</td>
<td>8.9 ± 0.2</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paired pacing</td>
<td>8</td>
<td>135 ± 14</td>
<td>-11</td>
<td>116 ± 7</td>
<td>-1</td>
<td>94.0 ± 9.7</td>
<td>25.4§</td>
<td>8.1 ± 0.5</td>
<td>1.1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic constriction</td>
<td>7</td>
<td>150 ± 12</td>
<td>-84§</td>
<td>106 ± 5</td>
<td>44§</td>
<td>100.7 ± 12.3</td>
<td>69.3§</td>
<td>9.1 ± 0.7</td>
<td>-2.5‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>7</td>
<td>138 ± 16</td>
<td>10</td>
<td>117 ± 5</td>
<td>-2</td>
<td>108.8 ± 6.0</td>
<td>47.7§</td>
<td>8.5 ± 0.6</td>
<td>-1.2§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>8</td>
<td>148 ± 16</td>
<td>-56§</td>
<td>105 ± 5</td>
<td>-4</td>
<td>99.8 ± 10.6</td>
<td>-29.7§</td>
<td>9.7 ± 0.6</td>
<td>-0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: n, Number of observations; HR, heart rate; BP, blood pressure; CBF, coronary flow; ΔAVO₂, coronary arteriovenous oxygen difference; C, group mean of control observations; Δ, mean within-group change from control.

Significance of change from control, as determined by paired t-test:

* 0.05 > P > 0.02; † 0.02 > P > 0.01; § 0.01 > P > 0.001; ‡ 0.001 > P.

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**FIGURE 1** Effect of delayed cooling of drill sample on myocardial adenosine content. Samples either were cooled immediately (0.5-second point) or immersion in coolant was delayed deliberately for the interval shown on the abscissa. See text for additional discussion.

**FIGURE 2** Relationship between adenosine levels in heart muscle samples obtained by cooled tongs (abscissa) and samples obtained by drill and immediately cooled in 2-methylbutane cooled to 118°K (ordinate). The linear regression equation describing this relationship, \[ \text{Ado}\text{Drill} = 10.3 \times \text{Ado}_{\text{tongs}} - 2.3, r = 0.86, \] suggests that the two estimates differ systematically.

**FIGURE 3** Reproduction of an experimental record from a typical experiment. Left panel: Control observations. After sampling arterial and coronary venous blood (not shown), myocardium was sampled at a, followed by registration of pressure and flow zeros. At b, atrial pacing was begun. The right panel was abstracted from the experimental record during pacing 8 minutes later. At c, myocardium was sampled and, at d, pacing stopped. Note that electrical pacing eliminated the evanescent changes in blood pressure and coronary flow due to sampling. PP, perfusion pressure; CBF, coronary blood flow rate.
TABLE 2 Covariance of $\text{MVO}_2$, [Ado], and $R^{-1}$

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Non-$\beta$-adrenergic Stimulation</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>$n = 34$</td>
<td>$n = 27$</td>
<td>$n = 7$</td>
</tr>
<tr>
<td>$R^{-1}$</td>
<td>$0.0492 \text{ MVO}_2 + 0.397$</td>
<td>$0.069 \text{ MVO}_2 - 0.018$</td>
<td>$0.123 \text{ MVO}_2 + 0.100$</td>
</tr>
<tr>
<td>$r$</td>
<td>$0.643$</td>
<td>$0.956$</td>
<td>$0.834$</td>
</tr>
<tr>
<td>95% CL slope</td>
<td>$0.0263 - 0.0641$</td>
<td>$0.2 &gt; P &gt; 0.1$</td>
<td>$0.001 &gt; P$</td>
</tr>
<tr>
<td>$[\text{Ado}]$</td>
<td>$0.619 \text{ MVO}_2 + 1.35$</td>
<td>$0.788 \text{ MVO}_2 - 0.933$</td>
<td>$1.06 \text{ MVO}_2 - 0.231$</td>
</tr>
<tr>
<td>$r$</td>
<td>$0.663$</td>
<td>$0.842$</td>
<td>$0.700$</td>
</tr>
<tr>
<td>95% CL slope</td>
<td>$0.374 - 0.864$</td>
<td>$0.2 &gt; P &gt; 0.1$</td>
<td>$0.02 &gt; P &gt; 0.01$</td>
</tr>
<tr>
<td>$R^{-1}$</td>
<td>$0.0458 [\text{Ado}] + 0.512$</td>
<td>$0.0558 [\text{Ado}] + 0.958$</td>
<td>$0.0873 [\text{Ado}] + 0.0816$</td>
</tr>
<tr>
<td>$r$</td>
<td>$0.607$</td>
<td>$0.834$</td>
<td>$0.894$</td>
</tr>
<tr>
<td>95% CL slope</td>
<td>$0.0248 - 0.0668$</td>
<td>$0.4 &gt; P &gt; 0.3$</td>
<td>$0.01 &gt; P &gt; 0.001$</td>
</tr>
</tbody>
</table>

Abbreviations: $r =$ regression coefficient, 95% CL of estimated slope, calculated as described in the text; $P$, probability that the estimated slope differs from control.

exploited in the assessment of post-sampling changes in adenosine content described above.

A total of 34 pairs of observations, 1 from each of 3 dogs, 2 from each of 5 dogs, and 3 from each of 7 dogs, met all the criteria for inclusion in the study. Figures 4–8 and Table 2 summarize the observations as $\text{MVO}_2$, [Ado], and $R^{-1}$ varied spontaneously during the control state and in response to the interventions changing $\text{MVO}_2$.

$R^{-1}$ was significantly covariant with $\text{MVO}_2$ during the control state and, as shown in Figure 4, during the interventions. As the resultant of superimposing interventions having directionally opposite effects on $\text{MVO}_2$ upon control values which themselves varied spontaneously, it was possible to examine these relationships over a 4-fold range of $\text{MVO}_2$ between 5.8 and 24.5 ml O$_2$/min per 100 g. Analysis of variance showed that the interventions did not exert equipotent effects on $\Delta R^{-1}/\Delta \text{MVO}_2$, F-4.94, $P > 0.005$ for 29 and 4 d.f. Isoproterenol had a uniquely large effect, whereas the other interventions were equivalent to each other and to the spontaneous variations of the control state.

Figures 5 and 6 examine the relationships between [Ado] and $\text{MVO}_2$, and Figures 7 and 8 those of $R^{-1}$ and [Ado]. In both instances, significant treatment effects were attributable to $\beta$-adrenergic...
stimulation by isoproterenol, whereas the other interventions were quantitatively equivalent to each other and also to the control state (Table 2). In 3 of the 34 trials, increasing MVO₂ was associated with a fall in [Ado]. These exceptional results could not be excluded from the data analysis because the physiological experiments and the chemical assays met all the quality control criteria, nor were they attributable to a particular type of intervention.

During control observations, spontaneous variations in MVO₂ accounted for a relatively small fraction of the variation in [Ado] and, in turn, [Ado] for a relatively small fraction of the variation in R⁻¹, r² = 0.44 and 0.37, respectively. This is not surprising in view of the relatively narrow range spanned by the dependent variables, the number of physiological factors that may affect them, and the fact that each of these estimates was the product of three or four chemical or physiological measurements, each with its own experimental error. That the degree of covariance was higher during interventions is consistent with the hypothesis that the changes in these variables may be related causally.

Discussion

This study supports Berne’s hypothesis that adenosine produced in proportion to cardiac oxygen demand regulates coronary vascular resistance to satisfy this demand (Berne, 1963; Rubio and Berne, 1975). Most experimental tests of this hypothesis have examined the effect of ischemia or hypoxia on cardiac purine metabolism and coronary flow (Katori and Berne, 1966; Rubio et al., 1969; Rubio and Berne, 1970; Olsson, 1970; Fox et al., 1974; Olsson et al., 1978). Reports dealing with the relationships of oxygen usage, adenosine metabolism, and coronary resistance in normally oxygenated hearts have begun to appear only recently. Wiedmeier and Spell (1977) found that the amount of adenosine recovered in the coronary venous effluent of guinea pig Langendorf heart preparations was proportional to oxygen usage as the latter increased in response to histamine, epinephrine, or norepinephrine administration. Foley et al. (1978) showed that increasing cardiac work by aortic constriction raises cardiac muscle adenosine levels. Watkinson et al. (1979) employed the adenosine concentration in pericardial superfusates to estimate indirectly cardiac muscle levels in conscious dogs. This adenosine index varied in proportion to the heart rate X blood pressure product during stellate ganglion stimulation and treadmill exercise. Subsequently, Miller et al. (1979) showed in open-chest dogs that pericardial superfusate adenosine concentration and left circumflex coronary flow varied directly with MVO₂ as the latter was increased in response to stellate ganglion stimulation. McKenzie et al. (1979) raised cardiac oxygen usage of open-chest dogs up to 4-fold by isoproterenol infusions or aortic constriction. Cardiac muscle adenosine levels varied directly and coronary vascular resistance varied inversely as functions of oxygen usage. This study of McKenzie et al. and the present study appear to be the only ones that examine, directly, simultaneously, and in blood-perfused hearts, the relationships between oxygen usage, estimated cardiac muscle adenosine concentration, and coronary vascular resistance, the three critical variables in the adenosine hypothesis.

Although our observations provide new quantitative evidence that the relationships between cardiac oxygen usage, adenosine content, and coronary resistance do not seem to be accidental, we wish to emphasize that they do not discriminate between causality and coincidence. This determination awaits identification of the mechanism(s) by which adenosine initiates coronary relaxation.

The availability of a technique for repeatedly sampling muscle from the heart and the development of an assay sensitive enough to estimate the adenosine content of these small (5–10 mg) samples allowed us to exploit the power of an experimental design in which each dog served as its own control and in which different interventions could be com-
pared in the same animal. This important advantage was offset by an equally important drawback common to similar studies (Foley et al., 1979; McKenzie et al., 1979), namely, post-sampling artifactual changes in adenosine content. These investigators sampled cardiac muscle with the device invented by Dunn and Griggs (1975), which removes a cylindrical sample of tissue several millimeters in diameter that is then frozen by clamping between metal blocks cooled in boiling N₂. Sample manipulation is reported to incur a delay of up to 3 seconds before the onset of cooling.

In the present study, the adenosine content of the cardiac muscle samples obtained as controls averaged 9.3 ± 0.51 nmol/g, which is several-fold higher than the values we find in samples obtained by direct biopsy with metal tongs cooled to the temperature of boiling N₂ (Olsson et al., 1978) and probably about twice as high as the value of 0.02 nmol/mg protein reported by Foley et al. (1979) when the different reference measurements are reconciled.

We believe that continued net, non-steady state adenosine production during the interval between sampling and the cooling of the drill sample explains the difference between the two biopsy methods. This interpretation is supported by two lines of experimental evidence developed in the preliminary evaluation of methodology: (1) deliberately increasing the interval between sampling and immersion of the sample in 2-methylbutane caused a time-dependent increase in adenosine content followed, to our surprise, by a precipitous fall after 2 sec of delay; and (2) the amount of adenosine recovered from drill samples was closely proportional to the amount found in samples obtained moments later with metal tongs. Although we lack a precise explanation for the post-sampling time course of the changes in adenosine content of drill samples, it seems reasonable that this reflects non-steady state changes in the rates of adenosine production and degradation, influenced perhaps by frictional heating of the tissue sample during biopsy and radiative and conductive cooling during the interval between biopsy and immersion in the coolant.

Assigning these elevated values to delayed cooling is also consistent with the physicochemical properties of 2-methylbutane that determine its efficiency as a coolant. At cryogenic temperatures, the heat capacity of 2-methylbutane is 4.22 J/°K per mol, roughly an order of magnitude lower than those of the metals commonly used to fabricate biopsy tongs (Ag, Al, Cu, Fe). Data on the thermal conductivity of 2-methylbutane are not available, but the conductivities of other hydrocarbons, generally <1 J/g per hr per cm per °K, are 2-3 orders of magnitude lower than metals (Handbook Chem. Phys., 1979). Thus, in addition to the delay in the onset of cooling, tissue in contact with 2-methylbutane cools more slowly than if it were in contact with metal. The estimates of Allard et al. (1979) for samples 1.5 mm in diameter place an upper bound on the additional time necessary to cool our tissue samples (<1 mm in diameter) to 0°C at ≤ 0.29 second, substantially longer than the value of 0.08 second obtained with tongs (Wollenberger et al., 1960).

Our experiments show that, by rigorously controlling the sampling procedure, it is possible to reduce the artifactual changes in adenosine content due to delayed cooling to a systematic error and make these estimates reasonably valid indices of the true adenosine levels prevailing in these hearts at the moment of biopsy. The 1-mm drill used for biopsy yielded samples whose radii (the diffusion path of heat) varied over the relatively narrow range of 0.25-0.35 mm. After much practice it was possible to standardize the sampling procedure so that immersion in the coolant occurred between 0.5 and 0.7 second after biopsy. Since the coolant was thawed only partially, its temperature, the melting point of 2-methylbutane (118°K), was constant from experiment to experiment. The extraction and chromatographic separation of adenosine from other tissue components was accomplished within 3 or 4 hours, thus avoiding the further artifactual changes that seem to occur with storage.

Detailed analysis of the effects of the various interventions used to change MVO₂ identified a disproportionately large effect of isoproterenol on the ratios ∆[Ado]/MVO₂, ∆R⁻¹/∆[Ado] and, consequently, ∆R⁻¹/MVO₂. To our knowledge, the observation that isoproterenol stimulates adenosine production out of proportion to its effect on MVO₂ has not been reported previously. We cannot offer an explanation for this finding at this time. The enhanced effect of isoproterenol on R⁻¹ relative to its effect on [Ado] is probably the resultant of direct β-adrenergic coronary vasodilation additive to coronary relaxation mediated by adenosine. That we did not observe the converse; i.e., a fall in R⁻¹ out of proportion to the change in MVO₂, is probably an effect of anesthesia, since chloralose and halothane anesthetics greatly reduce basal β-adrenergic activity in the heart (Vatner and Braunwald, 1975).

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Circ Res. 1980;47:875-882
doi: 10.1161/01.RES.47.6.875

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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