Transmural Gradients in Ventricular Tissue Metabolites Produced by Stopping Coronary Blood Flow in the Dog

By Robert B. Dunn and Douglas M. Griggs, Jr.

ABSTRACT
To determine whether transmural metabolite gradients develop in the contracting, ischemic left ventricle due to factors other than a nonuniform distribution of myocardial blood flow, right and left coronary artery inflow was completely stopped with vessel occluders in open-chest dogs for 15 or 30 seconds before a transmural myocardial tissue sample was obtained for regional analysis of creatine phosphate, adenosine triphosphate (ATP), and lactate. Heart rate was controlled, and the decline in left ventricular systolic pressure during the period in which coronary blood flow was stopped was attenuated by aortic constriction. Studies were also performed in dogs that were (1) pretreated with propranolol, (2) subjected to ventricular fibrillation, and (3) volume loaded. Control studies revealed no transmural metabolite gradients in the normally perfused ventricle, but creatine phosphate was slightly lower in the inner region than it was in the outer and middle ventricular wall regions. With coronary blood flow stopped for 30 seconds, a significant lactate gradient, increasing from the outer to the inner region, was present. Propranolol-treated dogs with their coronary blood flow stopped for 30 seconds also exhibited a lactate gradient, but dogs with ventricular fibrillation and their coronary blood flow stopped for 30 seconds did not. Volume-loaded dogs with their coronary blood flow stopped for only 15 seconds had a significant lactate gradient. Reciprocal gradients occurred in creatine phosphate but not in ATP. The findings suggest that the contracting ventricle uses energy unevenly and that in myocardial ischemia one of the factors causing greater subendocardial vulnerability is a greater energy need in this region.

Previous studies from this laboratory have shown that metabolic changes occurring in the left ventricle because of inadequate coronary blood flow are greater in the subendocardium than they are in the subepicardium (1–4). This difference has been attributed primarily to nonuniform systolic compression of the coronary vessels (5, 6) which results in a greater impairment of subendocardial blood flow than it does of subepicardial blood flow (7, 8). However, the possibility also exists that other factors, such as a higher subendocardial energy requirement (9–12), a greater capacity of subendocardial tissue cells for glycolysis (13, 14), or greater subendocardial beta-adrenergic stimulation, contribute to the uneven metabolic response. The primary purpose of the present study was to investigate these possibilities in open-chest dogs by examining regional metabolite levels in the left ventricle after blood flow had been completely stopped in both the left and right coronary arteries for a brief but metabolically significant interval of time. Heart rate was controlled, and the decline in left ventricular systolic pressure during the period of no coronary blood flow was attenuated by constricting the aorta. Interventions designed to alter ventricular wall stress and other variables which presumably could influence regional myocardial metabolism independently from flow were also employed.

Methods
Studies were performed on male mongrel dogs that had been fasted overnight. The dogs had been screened for microfilaria, medicated against rabies and for intestinal parasites, and maintained on a nourishing diet for at least 30 days. Anesthesia was induced with sodium pentobarbital (30 mg/kg, iv). Additional doses were given as required during the experiment. The trachea was intubated, and respiration was maintained with a Harvard respirator. Supplemental oxygen was added to the inspired air (approximately 10% of the inspired air volume) to ensure a normal arterial oxygen tension. Rectal temperature, measured with a Yellow Springs telethermometer, was maintained between 38 and 39°C by the use of a heating pad. Pressure in the arch of the aorta was monitored through a side-hole polyethylene catheter passed retrograde from the femoral artery. Left ventricular intracavitary pressure was obtained through a needle (thin wall, no. 17) which was later inserted through the left ventricular wall. Statham pressure transducers (P23Db) and an Electronics-for-Medicine (model DR-8) multichannel oscillograph were used to record pressures.

A right thoracotomy was performed, and the origin of the right coronary artery was exposed sufficiently to pass...
CARDIAC METABOLISM WITHOUT CORONARY FLOW

a ligature around it. This ligature was passed through a length of polyethylene tubing to produce a vessel occluder which was later used to stop right coronary blood flow. The sinoatrial node was crushed, and the heart was paced from the right atrial appendage at 150 beats/min.

A left thoracotomy was then performed, the main left coronary artery was dissected close to its origin on the aorta, and a vessel occluder was prepared for this vessel as described earlier for the right coronary artery. The origin of the septal branch distal to the occluder was verified at the end of the experiment. A ligature was also placed around the thoracic descending aorta for later tightening to attenuate the fall in left ventricular systolic pressure that occurs during the period of stopped coronary blood flow. Heparin was administered (350 units/kg, iv), and a blood sample was drawn anaerobically from the aortic catheter for the estimation of arterial pH, carbon dioxide tension (PCO₂), oxygen tension (PO₂), and hematocrit. Blood gas samples were read immediately on an Instrumentation Laboratory blood-gas analyzer (model 113-S1). If required, ventilation and oxygen administration were adjusted to maintain an arterial PCO₂ between 33 and 40 mm Hg and a PO₂ between 90 and 110 mm Hg. A final arterial blood gas sample was taken immediately prior to the experimental period. An arterial sample was also drawn and precipitated with cold 6% perchloric acid for subsequent determination of lactate (15). Dogs having an arterial lactate level greater than 1.75 mM were eliminated from the study. This level produced by stopping coronary blood flow. The mean arterial lactate level for the dogs included in the study was 0.96 ± 0.04 (SE) mM.

EXPERIMENTAL PROTOCOLS

After being prepared as just described, the dogs were studied in one of four series of experiments. The protocols for the different experiments were as follows.

Series 1.—A transmural tissue sample of the left ventricle was obtained before coronary blood flow was disturbed (controls) or after coronary blood flow had been stopped for 15 or 30 seconds. Right coronary blood flow was stopped first and within 10 seconds left coronary blood flow was stopped. Timing began after left coronary flow had been stopped and thus represents the period during which the left ventricle received no flow. Ordinarily, the right coronary artery does not supply the left ventricle in the dog, but flow was stopped in it to prevent collateral flow from reaching the left ventricle after the left coronary artery had been occluded. In preliminary studies, it was observed that 14C-antipyrine injected into the root of the aorta was occasionally detected in left ventricular tissue when only the left coronary artery was occluded, but it was never seen when both vessels were occluded.

Series 2.—Propranolol (d,l-propranolol hydrochloride) was given intravenously in a dose of 0.75 mg/kg. With this dose, a normally potent beta-adrenergic dose of isoproterenol (0.5 μg/kg administered in 60 seconds) caused a barely detectable increase in left ventricular dP/dt. Thirty minutes later, a transmural tissue sample was obtained from control dogs and from dogs whose coronary blood flow had been stopped for 30 seconds.

Series 3.—At the instant at which left coronary blood flow was stopped, the left ventricle was electrically fibrillated; a transmural tissue sample was obtained 30 seconds later.

Series 4.—Fresh donor blood (600 ml in 8–12 minutes) was administered via a femoral venous catheter. If at the end of the infusion left ventricular end-diastolic pressure was less than 10 mm Hg, an additional amount of Dextran 70 was given. Dextran was utilized in approximately 40% of the cases and produced, on the average, a 9% reduction in hematocrit. In two dogs, a left ventricular end-diastolic pressure of 10 mm Hg was not achieved; these animals were rejected. Within the following 5–10 minutes, a transmural tissue sample was taken from control dogs and from dogs whose coronary blood flow had been stopped for 15 or 30 seconds.

In all experiments, tissue sampling was performed high on the left ventricular wall slightly lateral to the crux formed by the anterior descending and circumflex branches of the left coronary artery using a cylindrical cutting tool mounted in an electric hand drill (Fig. 1). The stainless steel device consisted of two parts, a solid inner shaft (10 mm in diameter) and a movable outer shell held in place by spring-loaded ball bearings in the shaft. The shell extended 18 mm beyond the tip of the shell, creating a hollow recess for receiving the tissue sample. Passing through the center of this recess was an 18-gauge, 1.5-inch needle which was attached to the shaft and extended beyond the sharpened rim of the shell. During penetration of the ventricle, this needle acted as a guide and a skewer on which the cut tissue sample became impaled. A plastic sleeve with a flared end located at the base of the needle served as a stop for the tissue sample. When the tool was withdrawn, the sample was exposed by grasping the outer shell and retracting it toward the head of the drill. In the same motion, the sample was placed between two large aluminum blocks attached to heavy metal tongs and precooled in liquid nitrogen for compressing the sample. The total

![Diagram of the ventricular tissue-sampling tool with the outer shell in place ready for sampling. The overall length of the shaft, including the needle, was 20 cm, the diameter of the shaft was 1.0 cm, the length of the shell was 11 cm, and the outer diameters of the shell were 1.30 cm and 1.55 cm.](image-url)

Circulation Research, Vol. 37, October 1975
HEMODYNAMIC DATA

Results

HEMODYNAMIC DATA

Figure 2 is a plot of the hemodynamic data obtained in the four series of experiments described in Methods. The means ± se for left ventricular systolic and end-diastolic pressures obtained in all groups of dogs are shown. The open triangles on the far left depict the pressures obtained under control conditions in the group subjected to ventricular fibrillation (series 3). The solid symbols on the left depict the pressures obtained in the untreated (series 1), the propranolol-treated (series 2), and the volume-loaded (series 4) control groups. Both the systolic and end-diastolic pressures were elevated in the volume-loaded control group. The solid symbols joined by lines depict the pressures obtained at intervals after coronary blood flow was stopped for either 15 or 30 seconds in the different groups of dogs composing the series 1, 2, and 4 experiments. A series 2 group with their coronary blood flow stopped for 15 seconds was not included in the study. In every group in which coronary blood flow was stopped, the pressures prior to flow stoppage (0 seconds) were similar to those obtained in the appropriate control group for that series. After coronary blood flow had been stopped, the ventricular systolic pressure generally was well maintained for the first 15–20 seconds, but it tended to decline progressively thereafter despite near complete or complete tightening of the constrictor on the thoracic aorta. A gradual increase occurred in left ventricular end-diastolic pressure in all groups after coronary blood flow had been stopped. This increase caused end-diastolic pressure to be abnormally elevated, with the series 1 groups exhibiting the lowest and the series 4 groups the highest end-diastolic pressures. The series 2 group exhibited an end-diastolic pressure between those of the other two groups.

BLOOD DATA

The arterial blood gas sample taken immediately prior to the stoppage of coronary blood flow or the tissue sampling in control dogs exhibited a range for $PcO_2$ between 34 ± 1 and 39 ± 1 mm Hg and pH between 7.348 ± 0.020 and 7.385 ± 0.007 (means ±

STATISTICAL METHODS

An investigation of the tissue data revealed that some of the samples had non-Gaussian characteristics that could lead to erroneous conclusions when normal theory-dependent procedures were used. Therefore, all results were analyzed by nonparametric statistical methods (18). Within each series of experiments, outer, middle, and inner tissue metabolite values were tested with the Friedman two-way analysis of variance by ranks. If a statistically significant value ($P < 0.05$) was found, individual differences were analyzed by the Wilcoxon matched pairs test. Among the dog groups within a series of experiments, outer, middle, and inner region values were tested to determine any changes induced by stopping coronary blood flow. The Jonckheere’s test was applied to the groups of series 1 and 4, and the Wilcoxon rank sum test was applied to those of series 2 and 3. The control values and the values between groups of dogs in the different experimental series were compared by the Wilcoxon rank sum test. This test was also used to compare blood gases, pH, and hematocrit among the groups.
TISSUE DATA

The myocardial tissue metabolite data for all groups of dogs included in the series 1-4 experiments are presented in Table 1. A summary of the significant findings and pertinent statistical information not included in the table are presented in the following sections.

Series 1 (Untreated Dogs).—Under control conditions of normal coronary perfusion, creatine phosphate was slightly less in the inner ventricular region than it was in either the outer or the middle ventricular region. None of the regional creatine phosphate levels were as high as the normal level reported by Pool et al. (19), who were able to achieve a level of 13.6 ± 1.3 μmoles/g by reducing the tissue sampling time to within 1 second.

With coronary blood flow stopped for 15 seconds, the tissue lactate levels were higher than the control levels, and the inner region level was higher than that in the outer and the middle region. Creatine phosphate levels were lower than control levels in all three regions, and the inner region level remained below that in the outer and the middle regions.

With coronary blood flow stopped for 30 seconds, the tissue lactate levels were further elevated, and a transmural gradient, increasing from the outer to the inner region, was present. Creatine phosphate levels were low in all three regions, and there were no regional differences.

Series 2 (Propranolol-Treated Dogs).—In the group with normal coronary perfusion, the tissue findings were essentially the same as those obtained in the series 1 group with normal coronary perfusion. However, the inner region creatine phosphate level was different only from that in the middle region rather than both the middle and the outer region. Also, ATP was slightly lower in the outer region than it was in the other two regions.

With coronary blood flow stopped for 30 seconds, the tissue lactate levels were increased above control levels, and, as in the comparable series 1 group, there was a transmural gradient increasing from the outer to the inner region. The tissue lactate levels in each of the three regions were lower than those present in the comparable series 1 group (P < 0.05). Creatine phosphate levels were below control levels, but, in contrast to the previous results, there was a transmural gradient decreasing from the outer to the inner region. This gradient was established because the outer and middle region levels were not as low in this group as they were in the comparable series 1 group.

Series 3 (Ventricular Fibrillation).—Thirty seconds after simultaneously stopping coronary blood flow and inducing ventricular fibrillation, the lactate levels were increased in all three regions, but, in contrast to results obtained in the contracting, pressure-generating ventricle, there was no transmural lactate gradient. The regional lactate levels were uniformly elevated to about that of the outer region in the series 1 group with flow stopped for 30 seconds. Creatine phosphate levels were below control levels, and there were no regional differences. ATP was below control levels in the outer region, and this level was also lower than that in the middle or the inner region.

Series 4 (Volume-Loaded Dogs).—In the group with normal coronary perfusion the data were similar to those obtained in the comparable series 1 and 2 groups. However, the middle region creatine phosphate level was significantly higher than that obtained in this region in the comparable series 1 and 2 groups (P < 0.05).

With coronary blood flow stopped for 15 seconds, the tissue lactate levels were increased, and, in contrast to the results obtained in the comparable series 1 group, a transmural lactate gradient was present. The inner region lactate level was significantly higher than that of this region in the comparable series 1 group (P < 0.05). Also, creatine phosphate, which was below control levels in all regions, demonstrated a transmural gradient, decreasing from the outer to the inner region.

With coronary blood flow stopped for 30 seconds, the lactate levels were further elevated; they were higher than those for the same region in the comparable series 1 group (P < 0.05). The middle region value was particularly increased, resulting in a level which was not different from that in the inner region. The outer region level was significantly lower than that in the middle and the inner region. ATP was below control levels in all three regions, and the outer region level was lower than that in either the middle or the inner region. Also the inner region level was below that in the middle region. Creatine phosphate levels were uniformly low.

To ensure that the transmural metabolite gradi-
### TABLE 1

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Control (N = 11)</th>
<th>15-second stoppage (N = 7)</th>
<th>30-second stoppage (N = 8)</th>
<th>P§</th>
<th>Series 2</th>
<th>Control (N = 7)</th>
<th>30-second stoppage (N = 7)</th>
<th>P§</th>
<th>Series 3</th>
<th>30-second stoppage (N = 8)</th>
<th>P§</th>
<th>Series 4</th>
<th>Control (N = 7)</th>
<th>15-second stoppage (N = 7)</th>
<th>30-second stoppage (N = 6)</th>
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All values are means ± SE. NS = not significant.

* Outer (O), middle (M), and inner (I) regions compared with each other.
† P < 0.01.
‡ P < 0.05.
§ 15-second and 30-second groups compared with the control group; P values denote the significance of the progressive difference with time.
|| 30-second group compared with the control group in the same series.
§ 30-second group compared with the series 1 control group.
ents were not due to uneven perfusion of the myocardium with well-oxygenated blood remaining in the left coronary artery after coronary blood flow had been stopped, additional results were obtained in a modified experiment in which, on stopping coronary blood flow, the left coronary artery was vented distal to the vessel occluder and then suctioned. This method of decompressing the occluded vessel and removing the residual volume of well-oxygenated blood was employed in two dogs in which coronary blood flow was stopped for 30 seconds. Both dogs exhibited transmural lactate gradients and creatine phosphate and ATP levels similar to those obtained in the comparable series 1 group. These results essentially ruled out uneven perfusion of the myocardium with well-oxygenated residual blood as the explanation for the metabolic findings in the series 1, 2, and 4 experiments.

Additional results were obtained in a modified experiment in which, after coronary blood flow had been stopped, left ventricular end-diastolic pressure was controlled during the subsequent 30-second period of no coronary blood flow by withdrawing blood from the cannulated left atrium with a variable-speed pump. A portion of the withdrawn blood was rerouted via polyethylene tubing to the left subclavian artery to help maintain aortic pressure. Results were obtained in three dogs with left ventricular systolic and end-diastolic pressures adequately controlled for the 30-second period (time averaged pressures: systolic = 113 ± 5 mm Hg, end-diastolic = 6.9 ± 0.9 mm Hg). The tissue data revealed a transmural lactate gradient in all three dogs (outer region = 1.85 ± 0.32 μmoles/g, middle region = 2.19 ± 0.37 μmoles/g, and inner region = 2.53 ± 0.39 μmoles/g) and a slight transmural creatine phosphate gradient in all three dogs (outer region = 4.32 ± 0.54 μmoles/g, middle region = 3.73 ± 0.45 μmoles/g, and inner region = 3.65 ± 0.30 μmoles/g). ATP levels were essentially uniform (outer region = 5.27 ± 0.12 μmoles/g, middle region = 5.40 ± 0.15 μmoles/g, and inner region = 5.33 ± 0.24 μmoles/g). These results indicated that the elevated left ventricular end-diastolic pressure which occurred after coronary blood flow was stopped in the series 1, 2, and 4 experiments was not essential to the positive metabolic findings in those experiments.

**Discussion**

In a previous study on regional myocardial metabolism from this laboratory, we reported that the tissue levels of pyruvate, lactate, and ATP were essentially uniform in the outer, middle, and inner regions of the normally perfused canine left ventricle, whereas significant transmural gradients compatible with increasing tissue hypoxia from epicardium to endocardium occurred in these metabolites in the experimentally underperfused ventricle (2). These findings were consistent with an earlier study on regional myocardial blood flow in which flow was noted to be uniform in the outer and inner ventricular regions under normal conditions but relatively less in the inner region under ischemic conditions (8). Both studies supported the concept that the subendocardium is more vulnerable to hypoxia than is the subepicardium because of a transmural gradient in myocardial tissue pressure (5, 6) which imposes a nonuniform type of extravascular resistance on the coronary circulation. Additional studies from this laboratory (1, 3, 4, 8) and other laboratories (7, 20-25) have added considerable support to the hypothesis that the greater vulnerability of the subendocardium to hypoxic injury is due to a nonuniform distribution of myocardial blood flow in the underperfused ventricle.

The possibility that this hypothesis is too simplistic to account entirely for the uneven metabolic changes occurring in the underperfused ventricle is suggested from the literature. Reports that tangential wall stress in the left ventricle is unevenly distributed transmurally (26) and greater at the endocardial than at the epicardial surface (9-12) suggest that the energy need of the contracting ventricle is uneven and greatest in the subendocardium. If this difference does indeed exist, then a given reduction in coronary blood flow should result in a greater disparity between oxygen supply and demand in the subendocardium, thereby increasing subendocardial hypoxia and anaerobic glycolysis. Along a different line, evidence that the glycogen content (13) and the activity of several glycolytic enzymes (14) are highest in the subendocardium suggests that subendocardial anaerobic metabolism may be enhanced due to a greater capacity of the tissue cells in this region for glycolytic activity. An additional consideration, not prompted by prior experimental evidence, but one that we felt should be tested, is the possibility that sympathetic nerve stimulation or hypoxia-induced catecholamine release (27) could be greater in the subendocardium, causing more marked metabolic changes there. The present study was undertaken to examine these possibilities.

The results clearly indicate that the production of lactate and the depletion of creatine phosphate...
are not uniform across the myocardium when the contracting left ventricle is deprived of all antegrade coronary blood flow for a brief but metabolically significant interval of time. Transmural gradients in one or both of these substrates, indicating greater metabolic activity in the inner ventricular region, were observed in most instances when coronary blood flow was stopped in the contracting ventricle for either 15 or 30 seconds.

The occurrence in the propranolol-treated dogs of well-developed lactate and creatine phosphate gradients after coronary blood flow had been stopped is against the possibility that an uneven form of beta-adrenergic stimulation takes place in the ischemic myocardium which, in turn, causes greater metabolic activity in the inner ventricular region. Although complete beta-adrenergic blockade in the myocardium could not be assured, achievement of less than complete blockade should have altered the magnitude of the transmural lactate gradient if the proposed mechanism were real. However, the results revealed essentially the same quantitative difference between the outer and inner regional lactate levels in the untreated and the propranolol-treated dogs after coronary blood flow was stopped for 30 seconds, indicating no change in the magnitude of the lactate gradient. The fact that the metabolite changes in all three regions were less pronounced in the propranolol-treated dogs than they were in the untreated dogs with similar ventricular loading conditions and identical heart rates indicates that the increment in myocardial energy requirement related to beta-adrenergic stimulation of the myocardium was appreciable in the untreated dogs. These findings are consistent with those of others suggesting a difference in the level of beta-adrenergic activity in anesthetized and unanesthetized dogs (28, 29). These results also demonstrate the protective role of propranolol in reducing tissue changes in the ischemic myocardium (30).

In contrast to the attenuating effects on myocardial metabolism of pretreating the dogs with propranolol, volume loading the dogs caused the metabolic changes produced by stoppage of coronary blood flow to be accentuated. The presence of fully developed transmural gradients in lactate and creatine phosphate when flow was stopped for only 15 seconds and the significant changes found in all metabolites, including ATP, when flow was stopped for 30 seconds indicate that the metabolic changes in the myocardium were intimately related to the energy needs of the ventricle as regulated by the existing loading conditions. The significantly elevated ventricular end-diastolic and systolic pressures produced by volume loading undoubtedly resulted in a substantially increased wall stress, which is a primary determinant of the myocardial energy needs (31). The elevated end-diastolic pressure might be of particular significance if increasing ventricular filling pressure preferentially moves the inner region sarcomeres closer to the peak of their active length-tension curve, as suggested by Yoran et al. (32).

The rise in ventricular end-diastolic pressure after coronary blood flow was stopped represented an alteration in ventricular loading conditions from those present in the control situation. The change in end-diastolic pressure was physiologically significant, resulting in an abnormally elevated pressure in dogs which had a normal pressure before coronary blood flow was stopped. In the additional experiments in which this variable was controlled, transmural metabolite gradients developed after coronary blood flow was stopped even when the end-diastolic pressure was maintained within normal limits. This finding adds support to the hypothesis that ventricular contraction is associated with uneven metabolic activity in the presence of a normal wall stress.

The fact that no transmural gradients in lactate or creatine phosphate were found in the fibrillated ventricle indicates that these metabolite gradients are, indeed, dependent on some nonuniform function of the contracting ventricle, such as its wall stress. The presence of the uniformly elevated tissue lactate levels and the uniformly depressed creatine phosphate levels in the outer, middle, and inner regions of the fibrillated ventricle also signifies that the findings in the ischemic contracting ventricle are not due to intrinsic regional differences in glycolytic capacity (13, 14) or to a tissue $P_{O_2}$ gradient purported to exist in the normal heart (33-37). The finding of a slightly lower creatine phosphate level in the inner region of the well-perfused ventricle, which has been reported by Boerth et al. (38), however, does constitute evidence of regional differences in energy metabolism within the normal ventricle and further supports the hypothesis that energy utilization is uneven in the presence of a normal wall stress.

Acknowledgment

The authors wish to acknowledge the able technical assistance of Mr. Phillip Psaltis.

References

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Transmural gradients in ventricular tissue metabolites produced by stopping coronary blood flow in the dog.
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doi: 10.1161/01.RES.37.4.438
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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