IT IS well known that the autonomic nervous system may influence the development of cardiac dysrhythmias. Experimental observations in dysrhythmias. The electrophysiological basis for these autonomic effects has not been elucidated completely. Two studies have shown no or minimal influence of the autonomic nervous system on conduction delays in the acute (Hope et al., 1974) and the subacute phase (El-Sherif, 1978) of coronary artery occlusion in the dog.

During preliminary studies on electrophysiolog-
Secobarbital and α-Chloralose Anesthesia

Nineteen mongrel dogs weighing 10–26 kg were anesthetized and artificially ventilated with room air. Nine dogs received secobarbital (30 mg/kg, iv), and 10 dogs received morphine sulfate (2.25 mg/kg, sc) and α-chloralose (50–100 mg/kg, iv). Administration of α-chloralose was repeated as necessary; no measurements were obtained for at least 15 minutes after drug administration. A heating blanket maintained normal body temperature. A carotid artery was cannulated to monitor the systemic arterial pressure. A jugular vein was used to administer the anesthetics and 0.9% normal saline solution (150 ml/hr) to replace fluid lost during the experiment. Lead II of the electrocardiogram was monitored constantly. A brachial artery and a femoral artery were cannulated to collect arterial blood during measurement of regional myocardial blood flow.

The chest was opened via a left thoracotomy or midline sternotomy, and the heart was exposed and cradled in a pericardial sling. The sinus node region was crushed to slow the atrial rate. The left atrium was cannulated to monitor the systemic arterial pressure. A jugular vein was used to administer the anesthetics and 0.9% normal saline solution (150 ml/hr) to replace fluid lost during the experiment. Lead II of the electrocardiogram was monitored constantly. A brachial artery and a femoral artery were cannulated to collect arterial blood during measurement of regional myocardial blood flow.

Electrocardiogram Recording and Analysis

In each dog, three 22-gauge decapolar plunge needle electrodes were placed at least 1.5 cm apart near the center of the potentially ischemic zone of the anterior left ventricular wall, and a fourth plunge electrode was inserted in the high lateral left ventricular wall, well removed from potential ischemic zone. The recording electrodes on the needle were 0.05 mm in diameter and 1 mm apart. The needle electrodes were inserted perpendicular to the epicardium. Two adjacent electrodes, located close to the subepicardium and subendocardium, were selected on each needle to record bipolar subepicardial and subendocardial activation. The signals were amplified (Medical Electronics Consulting Associates), filtered between 12 and 500 Hz, displayed on a storage oscilloscope (Tektronix 5A18N), and simultaneously recorded on a strip chart recorder (Honeywell) at a paper speed of 400 mm/sec.

Electrograms from one cardiac cycle before occlusion, and during occlusion at the beginning of the microsphere injection, were selected from each recording site. Only electrograms that did not change throughout the entire control period, and for at least five consecutive cardiac cycles at the beginning of the microsphere injection, were selected. In absence of coronary occlusion, the electrograms were stable and the electrogram characteristics that were measured varied by less than 5% over several hours. The data were rejected if the plunge needle electrode became dislodged during the coronary artery occlusion or if the electrogram contour changed from one beat to the next.

The changes in amplitude, time-to-onset, time-to-major-peak, time-to-end (total time), and duration of each electrogram were measured, using a computerized data acquisition system consisting of a Tektronix 4010-1 graphics terminal and tablet, and associated software as previously reported (Ruffy et al., 1979). All points to be measured were chosen by the same investigator and were measured with an accuracy of 0.25 mm (0.6 msec). Repeated measurements by the same investigator varied by less than 1%.

The data were normalized by expressing the ischemia-induced electrogram changes as a percent of the control values measured immediately prior to coronary artery occlusion.

Measurements of Regional Myocardial Blood Flow

Myocardial blood flow was measured by carbonized microspheres (7–10 μm in diameter) labeled with 51Cr or 141Ce. For each blood flow determination, between 2 × 10⁶ and 3 × 10⁶ microspheres were injected over a 10-second period, followed by a 5-ml saline flush. The microspheres were obtained as 1 mCi of nuclide suspended in 10 ml of 10% dextran to which 1 drop of Tween-80 had been added (Minnesota Mining and Manufacturing Company). Starting at least 10 seconds before injection and continuing for at least 60 seconds after injection, blood was withdrawn at the rate of 10 ml/min.
with an infusion/withdrawal pump (Harvard Apparatus Company, model 940) from the brachial and femoral arterial cannulae. Prior to injection, the vial containing the microspheres was agitated vigorously, ultrasonically and mechanically, for approximately 10 minutes. Microscopic examination of spheres treated in this manner showed a greater than 95% dispersion.

After the animal had been killed, the heart was excised totally and sliced into six slices perpendicular to its long axis. In each slice, the right ventricle was separated from the left ventricle, and each ring of left ventricle was cut and counted as described previously (Ruffy et al., 1979). Each subepicardial and subendocardial recording site was located precisely and labeled for later identification. Reference blood samples were divided and counted. Standard techniques were used for isotope separation.

Myocardial perfusion was calculated using the formula BFm = (Cm X 100 BFr)/Cr in which BFm = myocardial blood flow (ml/min X 100 g), Cm = counts/g of myocardium, BFr = reference blood flow (the rate of withdrawal from the reference artery), and Cr = the total counts in the reference blood. For each flow measurement, a normal zone (core area) value was derived from the mean flow measurement in eight epicardial and eight endocardial sections of the posterior left ventricular wall. Blood flow outside of the core area was expressed as a percentage of the core area flow.

Study Protocol

After heparin (300 U/kg) administration, acute myocardial ischemia was produced by complete occlusion of the left anterior descending (LAD) coronary artery 1-2 cm distal to the origin of the anterior septal artery. The first occlusion served to determine the time needed to develop alterations in the bipolar electrograms and to establish the approximate boundaries of the ischemic zone. The occlusion then was released. If ventricular fibrillation occurred, direct current defibrillation was performed within 15 seconds using 10-20 joules. A recovery period of at least 30 minutes was allowed between LAD occlusions. All initial occlusions lasted less than 6 minutes.

Just before the second coronary artery occlusion, a control recording of the electrograms was obtained. The LAD then was occluded for 2-5 minutes and electrograms were monitored continuously. The use of such short-term occlusions reduced the chance of ventricular dysrhythmias. When marked electrogram alteration had taken place, or 1 minute before the expected onset of ventricular fibrillation (as determined by the first LAD occlusion), electrograms were recorded and microspheres were injected simultaneously. The arterial occlusion was maintained during the entire microsphere sampling period, and the data were excluded if ventricular fibrillation occurred before completion of the arterial blood withdrawal. After completion of the blood flow determination, the animal was killed by inducing ventricular fibrillation electrically, and the heart was removed and studied as described above.

Data Analysis

The extent of changes in bipolar electrograms at each recording site was compared to the regional blood flow, as a percent of the core area flow, measured at that site. The results obtained from the group of dogs anesthetized with sevoflurane were compared with results obtained from the dogs anesthetized with a-chloralose.

The data were analyzed statistically using the analysis of variance, Mann-Whitney U-test, and the Spearman rank order correlation coefficient. The analysis of variance was used to define the significance of difference between the mean values of groups of observations. Because of the heterogeneity of the data, in some cases the analysis of variance was replaced with the nonparametric equivalent, Mann-Whitney U-test. The Mann-Whitney U-test was used whenever the data groupings resulted in populations that grossly violated the homogeneity of variance assumption as shown by Bartlett’s test and Cochran’s test. The Spearman rank order correlation coefficient was used since the relationship between the electrophysiological variables and the regional myocardial blood flow may not be linear (Ruffy et al., 1979).

Reproducibility Study

The second group of experiments was designed to establish quantitatively the stability of the changes in electrogram characteristics and in regional myocardial blood flow which took place during short, serial coronary artery occlusions. These feasibility studies were required prior to doing the experiments (next three paragraphs) in which we evaluated the effects of interruption of autonomic nerves on the electrogram changes occurring during consecutive, short-term occlusions of the coronary artery. Fourteen dogs anesthetized with morphine sulfate (2.25 mg/kg) and a-chloralose (50-100 mg/kg), were prepared as described above. Four consecutive periods of coronary artery occlusion (of less than 5 minutes) were performed, separated by at least 30 minutes of recovery. The first occlusion again served to determine the time needed to develop alterations in the bipolar electrograms and to establish the approximate boundaries of the ischemic zone. Thus, the reported measurements were collected during the second, third, and fourth periods of arterial occlusion. Regional myocardial blood flow measurements were obtained during each period of occlusion using microspheres labeled with 82Sr, 141Ce, and 46Sc, according to the method described above. The epicardial and endocardial measurements during each occlusion were grouped and the data for normal zone and ischemic zone were analyzed with a correlated t-test. In the same dog, each occlusion was of identical duration and
all the electrograms were obtained at precisely the same time after arterial occlusion.

** Interruption of the Vagosympathetic Trunks and Stellate Ganglia 

Twenty-two mongrel dogs weighing between 14 and 23 kg were anesthetized with morphine sulfate (2.25 mg/kg) and α-chloralose (50–100 mg/kg), and prepared as described above, with the following differences. The left atrium was not cannulated since myocardial blood flow was not measured in this group of animals. To slow the sinus node without crushing the atrium and epicardial nerves coursing to the ventricles, the sinus nodal artery was cannulated and verapamil (0.25–0.5 mg) was injected once or twice during the study but not within 30 minutes of data collection (Zipes and Fischer, 1974).

** Study Protocol 

Acute myocardial ischemia was produced by complete occlusion of the left anterior descending coronary artery for periods of 1.5 to 4 minutes, followed by at least 30 minutes of recovery. As in the protocols described above, the first occlusion was not included in the collection of data. The next occlusion was used to obtain control measurements of the ischemia-induced changes in electrograms. In group I dogs, during recovery from the control occlusion, the right and left vagosympathetic trunks were ligated and cut at the cervical level, and the occlusion repeated 30 minutes later. During recovery from this occlusion, the stellate ganglia were decentralized by tying one ligature around the proximal and another around the distal end of the ganglion, and the occlusion repeated. In group II dogs, the order of autonomic interruption was reversed: the stellate ganglia were decentralized after the control occlusion, and the vagosympathetic trunks were interrupted after the next occlusion. In any single dog, each coronary arterial occlusion was of identical duration, and all the recordings were obtained at precisely the same time after the occlusion. The data were rejected if ventricular fibrillation occurred at any time during the experiment.

** Data Collection and Analysis 

In each dog, electrograms were recorded as described above and submitted to the same computerized analysis. Because of its lack of contribution to the analysis in earlier studies (Ruffy et al., 1979) measurement of time-to-the major-peak was omitted in this study. The data were analyzed by correlated t-test.

** Results 

** Secobarbital and α-Chloralose Anesthesia 

The mean duration of occlusion, pacing cycle length, and mean systemic pressure before occlusion and before release were the same in dogs anesthetized with secobarbital and in dogs anesthetized with α-chloralose. The average blood flow reduction from the core mean flow for all of the pooled subepicardial (50% ± 9 vs. 48% ± 6) and subendocardial (66% ± 22 vs. 45% ± 6) samples was similar in the two groups. However, the changes in time-to-onset (6% ± 2 vs. −1% ± 1), time-to-peak (11% ± 2 vs. 0.1% ± 1) and total time (14% ± 3 vs. 1.5% ± 0.5) were significantly greater in the secobarbital group than in the α-chloralose group at the subepicardium (Fig. 1A). Such differences were not found at the subendocardium (Fig. 1B).

** Reproducibility of the Study 

Figure 2, A and B, summarizes the subepicardial and subendocardial measurements made during three consecutive coronary artery occlusions. A small but systemic decrease in regional myocardial blood flow to the ischemic zone relative to the core area was measured from one occlusion to the other and was statistically significant (in the paired sense). At the subepicardium the flow values were 29 ± 5, 27 ± 4, and 25 ± 4% core area during the first, second, and third occlusion, respectively (Fig. 2A). At the subendocardium, these values were 23 ± 5, 18 ± 4, and 17 ± 5% core area (Fig. 2B). However, treated as a whole, no reduction in mean value of regional myocardial blood flow occurred from one occlusion to the next, in either the ischemic or normal zone. No difference was found in the changes of any electrogram characteristics in the ischemic zone. Small changes in amplitude were measured in the normal subepicardial zone where a statistically significant difference was noted between the second and third occlusion (5% ± 3 vs. −10% ± 4) (Fig. 2B).

** Interruption of Vagosympathetic Trunks and Stellate Ganglia 

** Group I Dogs 

Following interruption of the vagosympathetic trunks, the changes in total time and duration of the electrograms recorded in the subepicardial ischemic zone became significantly greater than those observed during the control occlusion (Figs. 3 and 4A). Furthermore, stellate ganglia decentralization produced a return of the ischemia-induced conduction delays to their values measured during the occlusion which preceded the vagotomy. For total time, the values were 7 ± 3, 12 ± 3 and 6 ± 3% control value during the first, second, and third occlusion, respectively, and for duration these values were 31% ± 8, 63% ± 17, and 21% ± 9 (Fig. 4A).

At the subendocardium, the ischemia-induced changes in time intervals were uniformly smaller than at the subepicardium, and there was no statistically significant difference in their mean values following autonomic interruption (Fig. 4B).
Small changes in time intervals and electrograms were measured in the subepicardial and subendocardial normal zone. Interruption of the autonomic nerves did not significantly modify these changes.

**Group II Dogs**

Following decentralization of the stellate ganglia, the ischemia-induced changes in subepicardial electrograms were regularly lessened when compared to the control occlusion (Figs. 5 and 6A). Furthermore, when bilateral vagotomy was added, the changes tended to remain as after sympathetic interruption, although the changes in electrogram total time and duration decreased still further. For total time, the values were 7 ± 2, 3 ± 2, and 2 ± 2% control value during the first, second, and third occlusion, respectively. For duration, these values were 66% ± 13, 46% ± 14, and 43% ± 14. In addition, there was a significantly smaller loss of amplitude following sympathetic interruption (Fig. 6A). At the subendocardium (Fig. 6B), the same trend was observed for electrogram duration in the ischemic zone (22 ± 8, 6 ± 6 and 6 ± 7% control value).

In these experiments, small changes in electrogram characteristics also were measured in the normal zone, and autonomic interruption did not significantly modify these changes.

**Discussion**

Three major observations can be made from these studies. First, α-chloralose and secobarbital exert different effects on myocardial activation during acute ischemia. Second, altering the tonic influence of the two limbs of the autonomic nervous system affects myocardial activation during ischemia. These two observations may be related. Third, ischemia-induced changes in bipolar electrograms during serial short-term occlusions of the left anterior descending coronary artery are reproducible, with only a slight decrease in the relative blood flow to the ischemic zone from one occlusion to the next.

**Effects of Secobarbital and α-Chloralose Anesthesia**

The greater ischemia-induced changes in myocardial activation in animals anesthetized with secobarbital than in those anesthetized with α-chloralose may result from several factors, including a direct effect of the anesthetic on the electrophysiological and/or metabolic properties of the ischemic myocardium. However, the two agents are considered to modify unevenly the tonic influence of the autonomic nervous system. Recent studies have demonstrated that pentobarbital inhibits the release of acetylcholine from the postganglionic parasympathetic neuron of the heart (Lindmar et al., 1979). Conversely, α-chloralose, is known to preserve the tonic parasympathetic tone to a greater degree (Van Citters et al., 1964). These considerations led us to hypothesize that interruption of the vagal nerves might exacerbate, whereas...
Figure 2. A: Regional myocardial blood flow and subepicardial electrogram changes in three consecutive coronary artery occlusions. B: Regional myocardial blood flow and subendocardial electrogram changes in three consecutive coronary artery occlusions.
interruption of the stellate ganglia might attenuate, the activation changes in response to acute myocardial ischemia.

**Sympathetic-Parasympathetic Interruption**

The contribution of the autonomic nervous system in modulating the development of cardiac dysrhythmias of ischemic heart disease has been well established by many experimental and clinical studies. However, the mechanisms underlying the vagal and sympathetic influence on the electrical stability of the ischemic myocardium still are poorly understood. Our study may provide an explanation for the tolerance of animals with sympathetic interruption to acute myocardial ischemia and, in particular, for their reduced propensity to develop fatal ventricular dysrhythmias (Cox et al., 1936; Harris et al., 1951, Schaal et al., 1969). The results are also consonant with the detrimental effect of parasympathetic pharmacological blockade on the electrical stability of the ischemic myocardium (Goldstein et al., 1973; Kent et al., 1973; Corr and Gillis, 1974; Harrison et al., 1974; Myers et al., 1974; Brooks et al., 1978). Furthermore, it may be that the beneficial effects of parasympathetic tone are to oppose or modulate the prevailing sympathetic influence (Kolman et al., 1975; Martins and Zipes, 1980), a hypothesis consistent with the lack of difference in ischemia-induced activation changes that followed vagal interruption when it was performed after sympathetic interruption.

In a study of the development of conduction delays and ventricular tachycardia during acute experimental myocardial ischemia, Hope et al. (1974) found no apparent difference between dogs with an intact autonomic nervous system and dogs that had undergone sympathectomy. However, only four sympathectomized animals were studied and, in contrast to our protocol, each dog did not serve as its own control. Use of the latter method enabled us to demonstrate the independent effect of autonomic nervous interruption. Due to the variable extent of ischemia-induced condition delay between dogs, it might be difficult to document that sympathectomy exerted an effect, unless a large number of dogs are used. In another study, El-Sherif (1978) found no direct vagal effect, but did find a small direct sympathetic effect on intraventricular conduction in the infarction zone 3 to 7 days after occlusion of the left anterior descending coronary artery in dogs. The absence of a cholinergic influence may relate to the fact that El-Sherif examined the effects of the autonomic nervous system 3 to 7 days after coronary ligation, whereas our measurements were made within minutes of the onset of coronary occlusion. If vagus nerves travel in the endocardium (Kent, 1974; Martins and Zipes, 1980b), it is possible that they die in the late myocardial infarction period, along with death of subendocardial muscle, and no longer innervate the surviving epicardial layers 3 to 7 days after coronary occlusion.

Our protocol was not designed to investigate the actual development of ventricular dysrhythmias be-
Figure 4: A: Subepicardial electrogram changes during coronary artery occlusion in protocol 1. Columns indicate mean value ± SEM. ANS = autonomic nervous system. Δ = change measured during arterial occlusion. B: Subendocardial electrogram changes during coronary artery occlusion in protocol 1. Columns indicate mean value ± SEM. ANS = autonomic nervous system. Δ = change measured during arterial occlusion.
Figure 5 Analog recordings at the subepicardium during protocol 2. The order of denervation has been reversed. Following decentralization of the stellate ganglia, the changes in electrogram induced by coronary artery occlusion are attenuated and remain so after vagotomy. The normal zone electrograms show only minimal changes.

fore and after autonomic neural decentralization because we used occlusions of such short duration as to ensure complete recovery following restoration of blood flow. However, several laboratories have demonstrated a correlation between the development of measurable subepicardial ventricular conduction delays taking place during acute coronary artery occlusion and the occurrence of ventricular tachycardia and ventricular fibrillation (Williams et al., 1974; Scherlag et al., 1974; Elharrar et al., 1977). Therefore our data suggest that a possible protective effect of bilateral stellectomy in experimental acute myocardial ischemia when heart rate is fixed may be the lesser severity of intraventricular conduction delays taking place in the ischemic zone. Conversely, the detrimental effect of parasympathetic blockade may be the production of greater conduction delays from an enhanced, unopposed sympathetic tonic influence, as well as the removal of primary cholinergic influence (Kent et al., 1974). Since we did not correlate electrogram changes with the development of ventricular dysrhythmias, these conclusions are speculative.

The mechanisms underlying the effects of sympathetic tone on ischemia-induced conduction delays remain to be determined. In our experiments the heart rate was kept constant. However, other determinants of myocardial oxygen consumption, such as contractility and afterload, were not controlled. Variations in oxygen demand, partially dependent on the degree of sympathetic tone, may explain the variations in the degree of electrogram alterations. Similarly, the differential effect of autonomic interruption on subepicardial and subendocardial electrograms in this study may depend in part on the resistance of the Purkinje fibers compared to the myocardium to various stresses (Bagdonas et al., 1961; Lundsgaard-Hansen et al., 1967; Friedman et al., 1973; Lazzara et al., 1973; Gilmour and Zipes, 1980).

However, changes in myocardial oxygen demand/supply relationship may not be the sole explanation for our observations. A direct effect of the autonomic nervous system on electrical activation of ischemic myocardial tissue may play a role. Furthermore, other electrophysiological phenomena that are known to be important in the development of myocardial electrical instability, such as dispersion in the recovery of excitability, also have been found to be modified by variation in autonomic neural influence, at least in normal myocardium (Han and Moe, 1964). Finally, it is possible that alterations in autonomic tone produced changes in regional myocardial blood flow that accounted for these observations. We did not measure regional myocardial blood flow during the autonomic interruption in order to keep the design of the experiments simple. Such studies still need to be done.

Considerations of the Model

Our reasons for using a short-term occlusion have been stated previously (Ruffy et al., 1979). In these studies we found that the changes in electrogram characteristics produced by acute myocardial ischemia were quantitatively reproducible, making this an excellent model in which to study the influence of interventions on activation of the ischemic myocardium. The only statistically significant difference observed was in the change in amplitude of the normal zone electrograms between the second and the third occlusions. We think that this finding was fortuitous.
Figure 6: A: Subepicardial electrogram changes during coronary artery occlusion in protocol 2. Columns indicate mean value ± SEM. ANS = autonomic nervous system. Δ = change measured during arterial occlusion. B: Subendocardial electrogram changes during coronary artery occlusion in protocol 2. Columns indicate mean value ± SEM. ANS = autonomic nervous system. Δ = change measured during arterial occlusion.

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Influence of secobarbital and alpha-chloralose, and of vagal and sympathetic interruption, on left ventricular activation after acute coronary artery occlusion in the dog.

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Circ Res. 1981;48:884-894
doi: 10.1161/01.RES.48.6.884

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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