

HEALTH EFFECTS INSTITUTE

Acute Effects of Carbon Monoxide on Cardiac Electrical Stability

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**Includes the Commentary by the Institute's
Health Review Committee**

Research Report Number 35

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Acute Effects of Carbon Monoxide on Cardiac Electrical Stability

Richard L. Verrier¹, Alex K. Mills, William A. Skornik

ABSTRACT

The objective of this project was to determine the effects of acute carbon monoxide exposure on cardiac electrical stability. To obtain a comprehensive assessment, diverse biological models were employed. These involved cardiac electrical testing in the normal and ischemic heart in anesthetized and conscious dogs. The experimental plan was designed both to examine the direct effects of carbon monoxide exposure on the myocardium and to evaluate possible indirect influences through alterations in platelet aggregability or changes in central nervous system activity in the conscious animal.

Our results indicate that exposure to relatively high levels of carbon monoxide, leading to carboxyhemoglobin concentrations of up to 20 percent, is without significant effect on ventricular electrical stability. This appears to be the case in the acutely ischemic heart as well as in the normal heart. It is important to note that the total exposure period was in the range of 90 to 120 minutes. The possibility that longer periods of exposure or exacerbation from nicotine in cigarette smoke could have a deleterious effect cannot be excluded.

We also examined whether or not alterations in platelet aggregability due to carbon monoxide exposure could be a predisposing factor for cardiac arrhythmias. A model involving partial coronary artery stenosis was used to simulate the conditions under which platelet plugs could lead to myocardial ischemia and life-threatening arrhythmias. We found no changes either in the cycle frequency of coronary blood flow oscillations or in platelet aggregability during carbon monoxide exposure. Thus, carbon monoxide exposure does not appear to alter platelet aggregability or its effect on coronary blood flow during stenosis.

In the final series of experiments, we examined the effects of carbon monoxide exposure in the conscious state. The rationale was to take into consideration possible adverse consequences mediated by the central nervous system. We found no adverse effects on cardiac excitable properties in response to either a 2-hour- or 24-hour-exposure paradigm. This appears to argue against major deleterious influences of carbon monoxide exposure as a result of direct myocar-

dial actions or indirect actions mediated through effects on central nervous system activity.

INTRODUCTION

Carbon monoxide (CO)² is an important constituent of air pollution and tobacco smoke. It has been implicated in the pathogenesis of pulmonary and cardiovascular disease (Haggard 1921; Middleton et al. 1961; Goldsmith and Landaw 1968; Goldsmith 1970; Ball and Turner 1974; Aronow 1975, 1983; Kuller et al. 1975; Radford 1976; Weir and Fabiano 1982). Komatsu (1955) reported that Japanese workers who manufacture tatami mats in tightly sealed rooms heated by charcoal fires achieve carboxyhemoglobin (COHb) levels of up to 30 percent. He noted an unusual frequency of angina attacks in this group and found that 18 percent of this population is afflicted with a specific syndrome characterized by dyspnea, angina, cardiomegaly, and arrhythmias ("Shinshu myocardiosis"). Mosinger and colleagues (1969) reviewed 88 cases of CO poisoning and found sinus tachycardia in 50 percent, extrasystoles in 20 percent, repolarization abnormalities in 15 percent, and conduction disturbances in 6 percent of the cases.

Epidemiologic studies have established that cigarette smoking increases the risk of death from coronary artery disease (Shafer et al. 1965; Kannel et al. 1966; Cohen et al. 1969; Kaufman et al. 1983). However, major questions relating to the potential hazard of CO in general, and to its cardiovascular effects in particular, remain unanswered. For example, the relative contributions of nicotine and CO to the cardiac effects of tobacco smoke have not been determined. If CO predisposes the heart to arrhythmias, does this increase the risk of sudden death, and, if so, what is the precise electrophysiologic mechanism? Although sudden cardiac death is a major health problem in industrialized countries, claiming over 600,000 lives annually in the United States alone, no systematic studies on the role of CO have been carried out to date. It is possible that CO exerts a profibrillatory action; however, the limited data available on this subject are conflicting (Bellet et al. 1972; DeBias et al. 1976; Aronow et al. 1978).

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² A list of abbreviations appears at the end of this report for your reference.

The present research plan addressed this issue by systematically exploring the effects of CO inhalation on myocardial excitability and coronary hemodynamic function in a canine model.

SPECIFIC AIMS

The first phase of this research was designed to ascertain whether or not CO influences electrical stability in the normal or compromised heart in chloralose-anesthetized dogs. Myocardial ischemia was selected as the main predisposing factor because of its widespread occurrence and clinical relevance. The first biological model involved a 10-minute period of coronary artery occlusion to induce acute myocardial ischemia. This model was selected because it exhibits a time course of vulnerability to ventricular fibrillation that coincides closely with the clinical experience relevant to sudden cardiac death. Specifically, within 2 to 3 minutes of coronary artery occlusion, there is a 30 to 40 percent decrease in the fibrillation threshold. Thereafter, despite continued obstruction of the vessel, the threshold returns to the preocclusion level because of the opening of preformed collateral channels. These observations, which are based on our previous studies (Axelrod et al. 1975; Corbalan et al. 1976; Lown and Verrier 1976; Lombardi et al. 1983), provide the frame of reference for the present investigation.

The experimental model for the second phase entailed inducing critical coronary artery stenosis to determine whether or not CO alters myocardial perfusion through an influence on platelet aggregability. We have demonstrated that a critical coronary artery stenosis induces a gradual decline of coronary blood flow over 5 to 10 minutes, followed abruptly (usually in less than 5 seconds) by recovery of flow to the initial level (Lombardi et al. 1983).

These changes may lead to myocardial ischemia and may reduce the vulnerable-period threshold for ventricular fibrillation. Considerable evidence implicates aggregation and disaggregation of platelets in these phenomena. It was uncertain, however, if spontaneous fluctuations in autonomic nervous system activity could alter the pattern of cyclical coronary flow changes during partial stenosis. To resolve this issue, we studied the effects of autonomic neural ablation in chloralose-anesthetized dogs. Bilateral cervical stellectomy reduced the frequency and the magnitude of coronary blood flow changes. In two of five dogs in which cyclical flow changes were reduced or abolished by decentralizing the stellate ganglia, electrical stimulation of the main body of the left ganglion reestablished the oscillations (Raeder et al. 1982). A 5-minute infusion of epinephrine provoked coronary blood flow changes in all animals

for a period of 5 to 10 minutes. Blockade of muscarinic receptors by atropine resulted in a significant attenuation of flow changes, which may have been due, at least in part, to a direct effect of atropine on platelets. Thus, there are multiple mechanisms, acting both directly on platelets and indirectly through the autonomic nervous system, by which CO could alter the frequency and severity of the cyclical oscillations. The third phase of experiments addressed this question.

In the final phase of experiments, conscious, chronically instrumented animals were utilized to determine whether or not CO might mediate an effect on cardiac electrical stability through an influence on the central nervous system. Accordingly, we determined whether or not CO influences cardiac electrical stability in the conscious state. The main rationale for these investigations is that CO may affect the behavior of the animal and the functioning of its central nervous system in a way that renders the heart electrically unstable. This was assessed by determining the repetitive extrasystole threshold, which has a consistent relationship to the threshold for fibrillation and which can be employed to circumvent the disruptive effects of cardiac resuscitation. Thus, the influence of CO can be tested during the resting state in an exposure chamber.

The fundamental principle guiding these studies is that the use of diverse biological models is likely to provide a comprehensive appraisal of the effects of acute CO exposure on the electrophysiologic function of the heart.

MATERIALS AND METHODS

EFFECTS OF ACUTE CARBON MONOXIDE EXPOSURE ON ELECTRICAL STABILITY OF THE NORMAL HEART

Preparation

Mongrel dogs of either gender, weighing 16 to 22 kg, were used. Anesthesia was induced with intravenous alpha-chloralose, 100 mg/kg of body weight (10 percent weight per volume; Sigma Chemical Co., St. Louis, MO). Additional alpha-chloralose was administered intermittently to maintain a constant level of anesthesia. The animal was intubated with a cuffed endotracheal tube and ventilated with a mixture of room air and oxygen. A Harvard respirator (Harvard Apparatus, South Natick, MA) was used to maintain normal levels of oxygenation (partial pressure of oxygen in arterial blood [PaO₂] at 80 to 120 mm Hg) and acid-base balance (arterial pH at 7.35 to 7.45). The femoral vessels were cannulated with polyethylene catheters, and arterial

pressure was measured using a Statham transducer (Schlumberger Industries, Oxnard, CA) with a recorder from American Optical (Lexington, MA).

Assessment of the Ventricular Fibrillation Threshold

Cardiac electrical testing was accomplished according to methods previously developed in this laboratory (Lown and Verrier 1976; Matta et al. 1976; Kowey et al. 1983a). Two intracavitary catheters were implanted, one to record the intracavitary electrogram and the other to deliver both pacing and test stimuli. The recording catheter (4F bipolar temporary pacing catheter; USCI, Radiological System, Billerica, MA) was bound to the pacing catheter (6901; Medtronic, Minneapolis, MN) so that its distal electrode terminated 3 cm proximally to the pacing catheter. The entire unit was then passed through a jugular vein to the right ventricular apex under fluoroscopic guidance. The recording catheter was used with a forelimb clip electrode to provide a continuous oscilloscopic display of the intracavitary electrogram. The distal pole of the pacing catheter was made cathodal.

Pacing pulses of 2-msec duration were delivered at twice the pacing threshold by an American Optical battery-powered pacemaker. A pacing rate of 180 beats per minute (bpm) was utilized in all experiments. Test stimuli of 5-msec duration were delivered using a constant-current square-wave pulse generator (Grass Instrument Co., Quincy, MA) with an electrically isolated output. The timing of the stimuli was controlled by a digital timer with a crystal-controlled time base having an accuracy of 0.01 percent or better. Timing was synchronized from the pacemaker stimulus. Test stimuli were delivered after every 10th to 15th paced beat and were immediately followed by inhibition of the pacemaker output for 3 seconds.

The ventricular fibrillation threshold was determined as follows. Electrical diastole was scanned with a test stimulus of 5-msec duration at an initial current of 6 mA, beginning at the apex of the T wave and proceeding by 5-msec decrements until the border of the absolute refractory period was reached. If ventricular fibrillation was not induced, the current output was increased in 2-mA steps and the scanning was repeated until ventricular fibrillation occurred.

Defibrillation was accomplished, usually within 5 seconds, by a DC pulse (100 to 150 w-sec capacity discharge) from an American Optical cardioverter, delivered through a pair of copper plates previously fastened to the thorax.

Using this methodology we have found, in studies carried out over the course of more than a decade, that in a well-maintained anesthetized preparation, the base-line fibrillation threshold does not fluctuate more than 5 to 8

mA over the course of several hours. Thus, it is possible to detect reliably the influence of interventions that alter the threshold by 20 percent or more.

Protocol for Acute Carbon Monoxide Exposure in Normal Dogs

Control values for heart rate, blood pressure, refractory period, vulnerable period, and ventricular fibrillation threshold were obtained prior to exposure to CO. After base-line measurements were taken while the dog breathed room air, a 90- to 120-minute exposure to CO was carried out. Carbon monoxide at 500 parts per million (ppm), mixed with room air, was used to ventilate the animals. The expired gas was diluted in a large volume, then exhausted via a fan to the atmosphere outside the laboratory. Up to the exhaust point the apparatus was completely sealed, and the laboratory air was checked at frequent intervals to exclude the possibility of CO leakage.

Electrical testing was repeated and arterial blood samples were drawn at three 30- to 40-minute intervals during the exposure to verify the increasing levels of COHb. Blood samples were iced immediately for determination of COHb levels. An interval of approximately 5 to 8 minutes was allowed after each fibrillation/defibrillation before beginning the next set of determinations.

All COHb values were determined using a gas chromatographic procedure (Model 104 FID; Byron Instruments, Raleigh, NC). This method was selected over spectral-analysis-based CO-oximetry because of its superior accuracy in the low range of COHb concentrations (that is, 5 percent or below). Blood samples for COHb determination were taken from indwelling femoral cannulas. According to this method, any CO present in an aliquot of blood contained in a sealed reaction vial was released from COHb in the blood when a chemical releasing agent, potassium ferricyanide (K_3FeCN_6) solution, combined with a hemolyzing agent (Triton X-100), was added to the vial. The resulting CO vapor collected in the headspace of the vial and was analyzed by gas chromatography.

A pair of special needles was used to penetrate the septum of the vial containing CO in the headspace. Zero-grade carrier air picked up the CO in the headspace and flowed out of the vial into a chromatographic stripper column (Chromosorb 106; Supelco, Bellefonte, PA). Carbon monoxide was allowed to pass through the stripper column and into a molecular sieve separation column (Byron). In the separating column, CO was isolated from all potential interfering gases. From the separating column, the CO flowed downstream into a nickel catalytic reducer (Byron), where it was converted to methane (CH_4).

The CO in the form of methane was then measured in the flame ionization detector (Byron), resulting in electrical signals that were amplified by an electrometer amplifier (Byron). The area of the CO peak, and its peak height, were analyzed and compared with CO standards. Total hemoglobin concentration, an integral part of the COHb in blood, was determined in an aliquot of blood with an oxidizing and cyanating reagent (Drabkin's solution). Hemoglobin species in the blood sample were converted to cyanmethemoglobin (CNMetHb), and its absorbance was determined at $\lambda = 540$ nm. Expired CO levels were continuously monitored using an Ecolyzer CO analyzer (Model 2000; National Draeger, Pittsburgh, PA) and were recorded on a Bascom-Turner plotter/recorder (Model 4120; Bascom Instruments, Norwood, MA).

INFLUENCE OF CARBON MONOXIDE EXPOSURE ON VULNERABILITY IN THE ISCHEMIC HEART

Preparation

The preparation and electrical testing procedures were similar to those described above. The differences related to the implantation of a balloon occluder around a coronary artery. The left side of the chest was opened, and an incision was made between the fourth and fifth ribs to allow the heart to be exposed. A 2-cc Barger balloon (Brunswick Corp., North Quincy, MA) was placed around the left anterior descending coronary artery, just distal to the first diagonal branch, for subsequent coronary artery occlusion.

Electrical Testing

Electrical testing was similar to the procedure described above, with the exception that an electrical stimulus of 10-msec duration was employed to scan the vulnerable period in 10-msec intervals. The use of a longer test stimulus duration (compared to 5 msec in normal hearts) was selected because it permits more expeditious scanning of the vulnerable period during the time-limited interval of vulnerability observed during coronary artery occlusion. Consequently, the longer duration of the stimulus and its greater energy content result in lower base-line values. Thus, the fibrillation threshold values in Table 1 for 5-msec impulses in normal dogs are higher than those in Table 2 for 10-msec impulses in control animals. These base-line levels are in accord with our previously published reports (Matta et al. 1976; Kowey et al. 1983a). Defibrillation was carried out with a 20- to 50-sec DC pulse delivered through a pair of 6-cm² electrode paddles applied directly to the heart.

Experimental Design

As before, control values were obtained for heart rate, blood pressure, refractory period, vulnerable period, and ventricular fibrillation threshold. The balloon occluder was then inflated, and electrical testing was repeated beginning 2 minutes into occlusion. After 10 minutes of occlusion, the balloon was deflated. This was followed by approximately 40 minutes of exposure to CO at 500 ppm, using the apparatus described above. At the end of the exposure period, electrical testing was repeated for control and coronary artery occlusion. The dogs then underwent another 40-minute exposure. This procedure was repeated for a total of three exposures. Arterial blood samples were drawn for COHb determination 2 minutes after coronary occlusion began. The exposure times were chosen in an attempt to achieve COHb levels of approximately 4 to 10 percent, 11 to 15 percent, and 16 to 20 percent for the three exposures.

EFFECTS OF CARBON MONOXIDE EXPOSURE ON CORONARY ARTERY BLOOD FLOW DURING CRITICAL STENOSIS

Preparation

This model was developed by Folts and coworkers (1976) to study the clinical conditions of coronary spasm or severe coronary artery disease. Essentially, a high-grade obstruction is produced by applying a plastic cylinder to a coronary artery. As has been shown by Folts and colleagues (1982) and in our laboratory (Raeder et al. 1982), platelet thrombi aggregate and disaggregate at such stenoses, resulting in cyclical coronary blood flow changes and episodes of myocardial ischemia. This model is highly relevant for the proposed studies, because the heart becomes electrically unstable at the nadir of coronary blood flow and immediately after its reestablishment (Kowey et al. 1983b).

Instrumentation

The animals were premedicated with morphine sulfate (3 mg/kg of body weight intramuscularly) and anesthetized 1 hour later with alpha-chloralose (100 mg/kg of body weight intravenously). Ventilation was carried out through a cuffed endotracheal tube to maintain normal oxygenation (arterial PaO₂ at 80 to 120 mm Hg) and acid-base balance (arterial pH at 7.35 to 7.45). The femoral vessels were cannulated with polyethylene catheters. The electrocardiogram (ECG) was monitored using standard skin electrodes. A left thoracotomy was performed through the fifth intercostal space, and the heart was exposed. The circumflex coronary artery was dissected out to provide a long uniform segment, and

small side branches were tied, if necessary. An electromagnetic flow probe was placed proximally around the artery. A plastic cylinder, 2.5 mm in length, was then placed around the coronary artery distal to the flow probe. The size of the occluder was chosen to produce a narrowing of the vessel of approximately 60 to 80 percent and establish a condition in which blood flows normally through the artery, but the reactive hyperemic response is abolished. This may require one or more replacements of the occluder. Internal diameters of the occluders ranged from 1.4 to 1.8 mm. Once the occluder was in place, a series of spontaneous cyclical blood flow reductions could usually be observed. This phenomenon was attributed to platelets plugging and unplugging the partially stenosed coronary artery.

Protocol for Testing Effects of Carbon Monoxide Exposure on Cyclical Changes in Coronary Blood Flow

Control values were obtained for COHb and platelet aggregability. Once a stable cycle frequency of 5 to 10 minutes was established, the dogs were exposed to 500 ppm CO for 60 to 120 minutes. Heart rate, arterial blood pressure, and coronary blood flow were recorded on a Western Graphtec recorder (Chrono-log Corp., Havertown, PA). Arterial blood samples were drawn for determination of COHb and platelet aggregability 2 minutes after coronary artery occlusion began. After exposure was completed, the dogs were ventilated with an increased inspired oxygen concentration (40 to 60 percent) to reduce the COHb.

All COHb values were determined using gas chromatography. Expired CO levels were continuously monitored using the Ecolyzer CO analyzer and recorded on the Bascom-Turner plotter/recorder. Platelet aggregation was studied using the *in vitro* turbidimetric or optical density technique of Born (1962). Platelet-rich plasma was prepared from citrated blood by centrifugation. Changes in light transmission through the samples of platelet-rich plasma were continuously recorded on a strip-chart recorder (Chrono-log Corp.). This unit records the aggregation of platelets as a curve representing a decrease in light transmittance through platelet-rich plasma with the progression of aggregation. We used adenosine 5'-diphosphate as the aggregating agent.

CARBON MONOXIDE EXPOSURE AND CARDIAC VULNERABILITY IN THE CONSCIOUS DOG

Carboxyhemoglobin Equilibrium Considerations

The uptake of exogenous CO by the blood increases with the concentration of CO, the length of exposure, and the ventilatory rate. When the concentration remains constant,

a state of equilibrium is reached in which the partial pressure of CO (P_{aCO}) in the pulmonary capillary blood is almost equal to that in the alveolar and ambient air.

Since the first extensive study on the rate of uptake of CO by normal men (Forbes et al. 1945), many models have been proposed to describe the relationship between inspired CO and the resulting COHb level. Some models are relatively simple, while others consider many physiologic parameters governing this process. The approach involving the most physiologic parameters appears to be the model developed by Coburn, Forster, and Kane, and therefore called the CFK equation (Coburn et al. 1965). Available data suggest that this model describes well the uptake and excretion of CO.

Using data from a series of human exposures to CO, Peterson and Stewart (1975) found that the CFK equation appears to predict COHb saturations for both men and women, exercising or at rest, when exposed to steady CO concentrations of 50, 100, and 200 ppm for 0.33 to 5.25 hours. The rate of uptake of CO observed is fairly constant with respect to blood COHb until about one-third of the equilibrium value is reached, and then this uptake proceeds at a slower and slower pace. Human exposure to 30 ppm CO has shown that about 80 percent of the equilibrium value of 5 percent COHb is reached within 4 hours, and the remaining 20 percent is achieved slowly over the next 8 hours.

The CFK equation can be used, therefore, to relate COHb saturation to exposure duration and concentration, and to describe the effect of several variables on the rate of CO uptake and on equilibrium COHb levels. Results with this model show that equilibrium is approached very slowly at low CO concentrations, taking about 24 hours at 25 ppm. Furthermore, if CO concentration and exposure duration are constant, then only barometric pressure, the affinity constant (M), and the oxygen concentration appear to have an effect on COHb levels at equilibrium. All other variables exert at least some effect, however, on the rate at which equilibrium is approached.

If we assume that the affinity ratio for the dog is approximately the same as for the human ($M = 218$), then we can estimate that exposure of a previously nonexposed dog to 25 to 50 ppm CO for about 8 hours or more would result in a blood COHb level of about 4 to 7 percent under equilibrium conditions.

Preparation

Mongrel dogs of either gender, weighing 14 to 20 kg, were used. Approximately three to five days prior to testing, an 8.5 French introducer catheter was placed in a jugular vein via a small incision in the neck during a brief period of general anesthesia induced with intravenous methohexital

(1 ml/2.25 kg of body weight). Then the animal was allowed to recover. On the day of testing, a transvenous bipolar pacing/pulsing catheter was inserted under fluoroscopic control, through the introducer catheter already in place. The dogs were conscious for this procedure and were manually restrained as necessary. Surface ECG pads were placed, and external defibrillation paddles were strapped in position.

Individual animals then were placed in a 1.2-m³ stainless-steel and Lucite environmental exposure chamber in an air-conditioned room in which chamber temperature was maintained at 22° ± 1°C and relative humidity at 60 ± 5 percent. The atmosphere in the chamber was controlled by flowing either filtered air or air with CO added at a gas flow rate of 100 L/minute. Chamber pressure was maintained at a net negative static pressure of 0.5 inches of water. The flow of air also served as a source of random noise to mask external sounds. The monitors were connected to an external ECG and pacemaker prior to sealing the chamber.

Cardiac Testing

Previously reported work has demonstrated that the ventricular repetitive extrasystole threshold consistently occurs at approximately two-thirds that of the ventricular fibrillation threshold, and therefore can be used to estimate the ventricular fibrillation threshold accurately (Matta et al. 1976; Kowey et al. 1983a). Utilizing this methodology, we were able to avoid subjecting the animals to repeated fibrillation and defibrillation. The ventricular repetitive extrasystole threshold was determined by the single-stimulus method, employing a 10-msec constant-current stimulus. During electrical testing, the heart rate was maintained at 180 beats per minute by ventricular pacing (Figure 1). The vulnerable period was examined at 5-msec increments beginning at the border of the T wave. The current was then increased in 2-mA steps and scanning was continued until the occurrence of repetitive extrasystoles.

The repetitive extrasystole threshold was judged by the occurrence of extrasystoles during at least two out of three trials at a given current and interval, and the lowest stimulus intensity that elicited this response was taken as the repetitive extrasystole threshold value.

Protocol for Acute Carbon Monoxide Exposure

Control values for heart rate, refractory period, vulnerable period, and repetitive extrasystole threshold were obtained prior to exposure to CO. A 90- to 120-minute exposure to CO then was begun. Carbon monoxide at 200 to 500 ppm was added to the filtered air inflow to the chamber. Gas was exhausted from the chamber to the atmosphere outside the

laboratory. Carbon monoxide levels inside the chamber were continuously monitored using the Ecolyzer CO analyzer and the Bascom-Turner plotter/recorder. The laboratory air was checked at frequent intervals to exclude the possibility of any leakage. Electrical testing was repeated and blood samples were drawn for determination of COHb levels at three 30- to 40-minute intervals during the exposure. All COHb values were determined by gas chromatography. An interval of approximately 5 to 6 minutes was allowed between each set of determinations.

Protocol for Chronic Exposure

The basic procedure was similar to those described above except for the following modifications.

Because of the fewer number of determinations, defibrillation paddles were not strapped to the dogs' chests; however, external defibrillatory paddles were available in case of inadvertent fibrillation.

Control values were obtained for heart rate, refractory period, vulnerable period, and repetitive extrasystole threshold. The dogs were then exposed to 25 to 50 ppm CO for approximately 24 hours while in the chamber. Food and water were provided. We felt that this type of experiment should provide steady-state conditions, and that equilibrium concentrations of COHb would be achieved. After 24 hours, cardiac electrical testing was performed, and a blood sample for COHb determination was drawn.

ANIMAL CARE

Harvard School of Public Health is accredited by the American Association for Accreditation of Laboratory Animal Care and meets all standards prescribed in the *Guide for the Care and Use of Laboratory Animals* (U.S. Department of Health and Human Services 1985). All animal facilities are administered to ensure compliance with federal, state, and Harvard standards for the humane care and use of animals through a program of veterinary care, inspection, and oversight. Animal care and welfare are the charges of the Harvard Medical Committee on Animals. In addition, local facilities are guided and monitored in daily activities by departmental animal-use committees. The procedures to avoid unnecessary discomfort, pain, or injury to animals are those prescribed in the aforementioned *Guide*. Additional detailed protocols for anesthesia, analgesia, tranquilization, euthanasia, or restraint have been developed and promulgated by the Committee on Animals.

Healthy dogs ranging in age from two to seven years were provided by Harvard University's Animal Resources Center. The animals selected were free of parasitic worms and re-

INSTRUMENTATION FOR CARDIAC ELECTRICAL TESTING

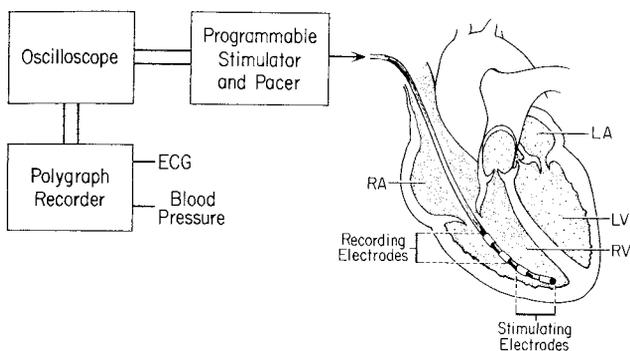


Figure 1. Experimental preparation for determining the ventricular fibrillation threshold. The measurements were made using a quadripolar electrode catheter, placed in the apex of the right ventricle (RV) via the jugular vein. Pacing and test stimuli were delivered through the distal pair of electrodes. The intracavitary ECG was recorded from the proximal set of electrodes (Saini and Verrier 1989).

spiratory infections. They were housed in the same building where the definitive investigations were carried out. All surgical procedures were performed using aseptic techniques, at appropriate planes of anesthesia. Recovery from anesthesia was closely monitored in our veterinary intensive care unit. Special care was taken to administer necessary analgesic medication and prophylactic antibiotics. Subsequent therapy was guided by recordings of rectal temperature, white cell counts, and so forth, as directed by the resident veterinary staff. Minor surgical procedures were carried out under local anesthesia with lidocaine. The requirements outlined in "Guiding Principles in the Care and Use of Animals" (American Physiological Society 1989) and *Guide for the Care and Use of Laboratory Animals* (U.S. Department of Health and Human Services 1985) were strictly adhered to in all phases of the investigation.

RESULTS

EFFECTS OF ACUTE CARBON MONOXIDE EXPOSURE ON ELECTRICAL STABILITY OF THE NORMAL HEART

The influence of a wide range of COHb concentrations on the excitable properties of the normal heart was investigated throughout 90 to 120 minutes of exposure to 500 ppm CO. The determined variables were heart rate, mean arterial blood pressure, effective refractory period, vulnerable-period timing in the cardiac cycle, and the ventricular fibrillation threshold. Seven dogs were studied. One animal aspirated gastric fluids during the induction of anesthesia,

leading to unstable values and death. The results were not included in the data base. The data from the remaining dogs are given in Table 1 and Figures 2 and 3 (see also Mills et al. 1986).

Data were examined by analysis of variance. Carboxyhemoglobin concentrations were categorized as shown in Figures 2 and 3, and analyses were done controlling for the fact that each dog contributed a number of data points. None of the measured parameters was altered during the time of exposure ($p > 0.10$). From these findings, we conclude that inhalation of CO at relatively high levels does not alter the excitable properties of the normal heart.

INFLUENCE OF CARBON MONOXIDE EXPOSURE ON VULNERABILITY IN THE ISCHEMIC HEART

This series of experiments was designed to examine the influence of CO on the heart that has been sensitized by myocardial ischemia. Eleven dogs were studied. However, four of the dogs died as a result of technical difficulties prior to the culmination of testing, so complete data are available for seven animals. The specific problems resulting in death were aspiration of gastric fluids and ventricular fibrillation that could not be terminated by countershock. The latter difficulty is not uncommon when modeling acute coronary artery occlusion.

Data were examined by analysis of variance for effects of COHb concentration and for occlusion or nonocclusion, controlling for the fact that each animal contributed a number of data points. Although the ventricular fibrillation threshold consistently fell during coronary artery occlusion ($p < 0.05$), no statistically significant alterations were detectable in ventricular fibrillation threshold resulting from CO exposure ($p > 0.1$). This was true whether absolute values or the decrements in the thresholds were compared. These results are summarized in Table 2 and Figures 4 and 5 (see also Mills et al. 1987).

EFFECTS OF CARBON MONOXIDE EXPOSURE ON CORONARY ARTERY BLOOD FLOW DURING CRITICAL STENOSIS

Carbon monoxide exposure was carried out for 60 to 120 minutes and the cyclical reductions in blood flow were monitored. Generally, the reductions in coronary blood flow were followed by spontaneous complete or partial restorations in flow, but in some cases it was necessary to tap the occluder manually in order to restore vessel patency. Under these conditions, we intervened in order to avoid the development of irreversible myocardial ischemia.

After the exposure was completed, the inspired oxygen

Table 1. Effects of Carboxyhemoglobin Level on Cardiac Electrical Stability in Normal Dogs^a

	Carboxyhemoglobin Level			
	0 to 5 Percent	6 to 10 Percent	11 to 15 Percent	16 to 20 Percent
Heart rate (bpm)	108 ± 10	114 ± 19	112 ± 14	101 ± 16
Mean arterial blood pressure (mm Hg)	107 ± 8	104 ± 14	107 ± 11	103 ± 12
Effective refractory period (msec)	144 ± 5	156 ± 10	152 ± 7	162 ± 8
Vulnerable period (msec)	156 ± 6	165 ± 11	162 ± 8	174 ± 9
Ventricular fibrillation threshold (mA)	34 ± 2	35 ± 4	38 ± 3	38 ± 3

^a Values are expressed as means ± SEM. *n* = six dogs. A 5-msec impulse was used as the testing stimulus.

concentration was increased to facilitate unloading of COHb and to ascertain whether or not any changes might be reversed with discontinuation of the CO. Ten dogs were studied, but the model of cyclical coronary blood flow changes could be elicited in only seven animals. A representative tracing is shown in Figure 6. Data were examined by analysis of variance, which controlled for the fact that each dog contributed a number of data points. The results are shown in Table 3. No consistent change in either cycle period of coronary blood flow oscillations or platelet aggregability was found (*p* > 0.1). This suggests that CO does not have a major effect on platelet function in the model of coronary stenosis.

CARBON MONOXIDE EXPOSURE AND CARDIAC VULNERABILITY IN THE CONSCIOUS DOG

Acute Experiments

Six dogs were studied. The data from these experiments

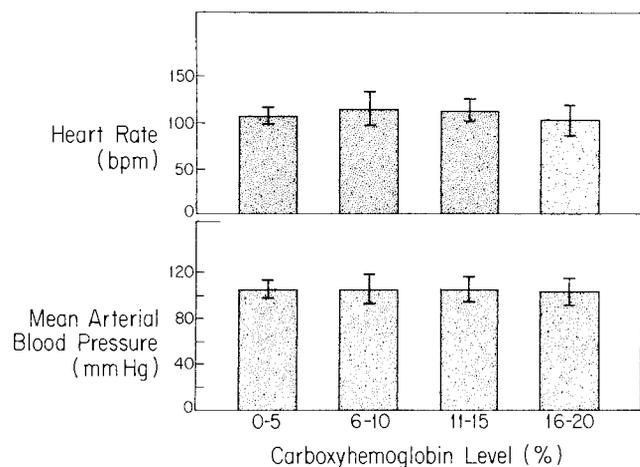


Figure 2. Effects of a 90- to 120-minute exposure to 500 ppm CO in six chloralose-anesthetized dogs. There were no significant changes in either heart rate or arterial blood pressure over the entire experimental period. Values are expressed as means ± SEM.

are presented in Table 4. The data were examined by analysis of variance, which controlled for the fact that each dog contributed a number of data points. No significant changes were found in any of the measured variables (*p* > 0.1). It is important to note that since the repetitive extrasystole threshold is two-thirds of the fibrillation threshold, the base-line values as reported in Table 4 are lower than those indicated in the earlier tables. Thus, the frame of reference

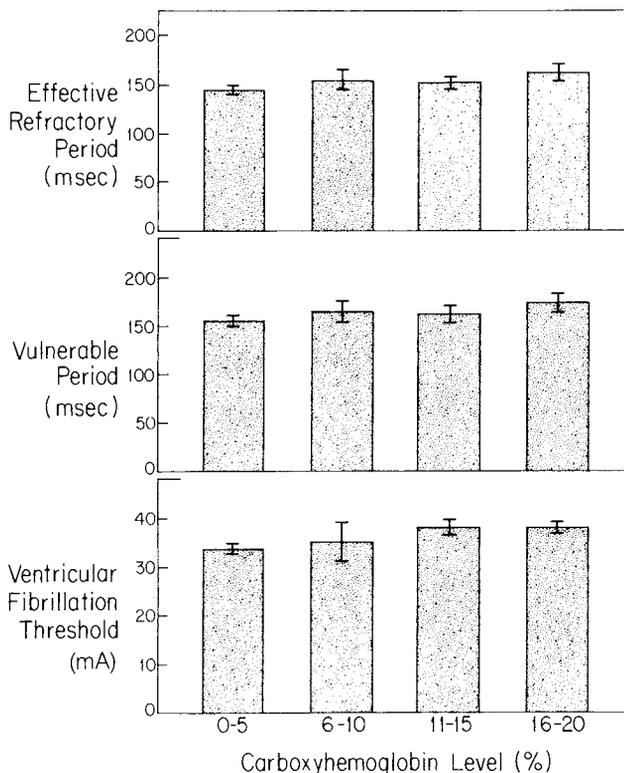


Figure 3. Influence of a 90- to 120-minute exposure to 500 ppm CO in the six chloralose-anesthetized dogs referred to in Figure 2. The parameters illustrated are effective refractory period, timing of the vulnerable period in the cardiac cycle, and the ventricular fibrillation threshold. Despite the wide range of COHb levels (0 to 20 percent), there were no significant changes in any of the electrophysiologic parameters. Heart rate was maintained constant by ventricular pacing during all electrophysiologic determinations.

Table 2. Effects of Carboxyhemoglobin Level on Cardiac Electrical Stability in Dogs with and Without Coronary Artery Occlusion^a

	Carboxyhemoglobin Level							
	0 to 5 Percent		6 to 10 Percent		11 to 15 Percent		16 to 20 Percent	
	Control	Occluded	Control	Occluded	Control	Occluded	Control	Occluded
Heart rate (bpm)	138 ± 2	134 ± 3	129 ± 5	122 ± 5	117 ± 5	120 ± 6	135 ± 8	134 ± 5
Mean arterial blood pressure (mm Hg)	97 ± 2	93 ± 3	108 ± 5	101 ± 5	109 ± 5	102 ± 6	105 ± 8	93 ± 5
Effective refractory period (msec)	130 ± 1	134 ± 2	137 ± 3	145 ± 3	137 ± 2	143 ± 3	130 ± 4	130 ± 3
Vulnerable period (msec)	144 ± 1	147 ± 1	152 ± 2	158 ± 3	155 ± 2	154 ± 3	151 ± 4	143 ± 3
Ventricular fibrillation threshold (mA)	25 ± 1	17 ± 1	24 ± 1	21 ± 2	23 ± 1	18 ± 2	27 ± 2	17 ± 2

^a Values are expressed as means ± SEM. *n* = seven dogs. A 10-msec impulse was used as the testing stimulus.

is different but the ability to detect a change is not lessened. Furthermore, as was the case in our methodology study (Matta et al. 1976; Kowey et al. 1983a), the within-group variability is small, underscoring the reliability and consistency of the results.

Twenty-Four-Hour Exposure Experiments

Six dogs were studied. The data from these experiments are presented in Table 5. The results indicate that in the range of COHb tested, CO has no apparent deleterious influences on cardiac vulnerability, as evidenced by the fact that the main indicator of ventricular vulnerability, the repetitive extrasystole threshold (Lown and Verrier 1976;

Matta et al. 1976; Kowey et al. 1983a), was unaltered by CO exposure. The changes in refractoriness (that is, effective refractory period and vulnerable-period timing), though statistically significant, are not biologically significant because they are discordant with the definitive marker, which is the repetitive extrasystole threshold.

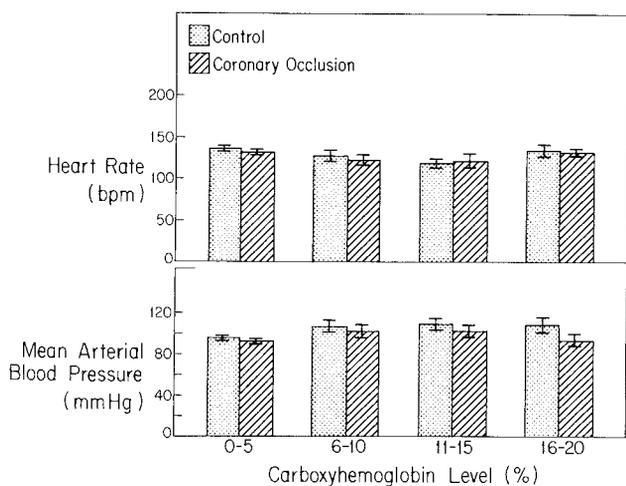


Figure 4. Effects of a 90- to 120-minute exposure to 500 ppm CO on heart rate and mean arterial blood pressure in seven chloralose-anesthetized dogs before and after two minutes of left anterior descending coronary artery occlusion. Carbon monoxide exposure did not produce any statistically significant changes in either variable.

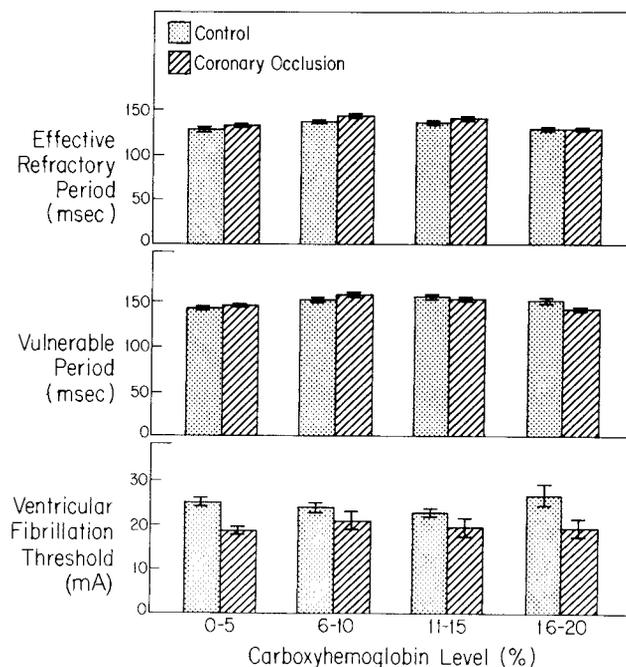


Figure 5. Effect of acute CO exposure on effective refractory period, timing of the vulnerable period, and ventricular fibrillation threshold before and during coronary artery occlusion. Coronary artery occlusion resulted in a decrease in ventricular fibrillation threshold, as anticipated. However, the magnitude of this effect was not altered by CO exposure. The other two parameters were not affected by CO exposure either before or after obstruction of the coronary vessel.

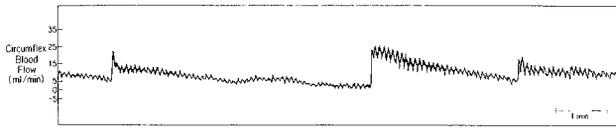


Figure 6. Representative tracing of the cyclical coronary blood flow changes produced by critical stenosis of the left circumflex coronary artery. Note that there are cycles of progressive reductions in blood flow. In each case, the period of low coronary blood flow ends abruptly. As discussed in the text, previous data from this and other laboratories suggest that the changes in flow are due to aggregation and disaggregation of platelets.

DISCUSSION AND CONCLUSIONS

The overall objective of this project was to determine the effects of acute CO exposure on cardiac electrical stability. To obtain a comprehensive assessment, diverse biological models were employed. These involved cardiac electrical testing in the normal and ischemic heart in anesthetized and conscious dogs. The experimental plan was designed both to examine the direct effects of CO exposure on the myocardium and to evaluate possible indirect influences through alterations in platelet aggregability or changes in central nervous system activity in the conscious animal.

EFFECTS OF CARBON MONOXIDE EXPOSURE ON ELECTRICAL STABILITY OF THE NORMAL AND ISCHEMIC HEART

Information is scarce regarding the effects of CO on the electrophysiologic characteristics of the normal heart. DeBias and coworkers (1976) exposed monkeys to CO at 100 ppm for 6 hours. After the COHb level increased to 9.3 percent, the threshold for transthoracic induction of ventricular fibrillation declined significantly. Because of the use of alternating current applied through the chest wall, these data are difficult to interpret and require confirmation.

Bellet and coworkers (1972) ventilated intact dogs with the smoke of three cigarettes for 10 minutes and found a

substantial decline in the ventricular fibrillation threshold with both transthoracic and epicardial electrical testing. However, the substantial nicotine content of the cigarettes (2 mg per cigarette) was likely to have caused a release of catecholamines (Cryer 1980) sufficient to account for the enhanced vulnerability to ventricular fibrillation.

Our working hypothesis at the outset of this project was that in the normal heart, electrical stability would be high and a substantial vasodilator reserve would be present to insure adequate delivery of oxygen to the myocardium. Thus, we anticipated that environmental levels of CO would not lower the vulnerable-period threshold sufficiently to result in cardiac arrhythmias. The findings reported herein confirm this hypothesis. Namely, acute CO exposure, resulting in a relatively wide range of COHb plasma levels up to 20 percent, was completely without effect on cardiac excitable properties in the normal heart. This conclusion was based on measurements of sensitive parameters, including effective refractoriness, timing of the vulnerable period in the cardiac cycle, and the ventricular fibrillation threshold. There were also no statistically significant changes in heart rate or arterial blood pressure.

Does CO exposure affect vulnerability to arrhythmias in a heart in which myocardial perfusion is compromised by acute coronary artery occlusion? Despite a large body of evidence linking CO to the provocation of angina pectoris (Middleton et al. 1961; Beard and Wertheim 1967; Cohen et al. 1969; Aronow 1975; Kuller et al. 1975; Becker and Haak 1979; Cosby and Bergeron 1980; Kaufman et al. 1983; Sekiya et al. 1983), surprisingly little is known about its effect on cardiac electrophysiology during myocardial ischemia. Aronow and coworkers (1978) reported, in a study of 21 anesthetized dogs ventilated with 100 ppm CO for 2 hours, that concomitant with an elevation of the COHb level to 6.34 percent, the ventricular fibrillation threshold declined from 12.8 mA to 8.1 mA. One animal fibrillated spontaneously after 100 minutes of CO inhalation. No other electrophysiologic or hemodynamic data were reported.

In their study referred to above, DeBias and colleagues

Table 3. Platelet Aggregability and Cycle Length of Coronary Blood Flow Changes as Functions of Carboxyhemoglobin Level^a

	Carboxyhemoglobin Level			
	0 to 5 Percent	6 to 10 Percent	11 to 15 Percent	16 to 20 Percent
Platelet aggregability (percent light transmission)	45 ± 25	43 ± 27	48 ± 28	51 ± 29
Cycle length (min)	7.7 ± 2.4	9.2 ± 4.4	7.7 ± 3.3	7.3 ± 1.5

^a Values are expressed as means ± SEM. *n* = seven dogs.

Table 4. Effects of Acute Carbon Monoxide Exposure in Conscious Dogs^a

	Carboxyhemoglobin Level			
	0 to 5 Percent	6 to 10 Percent	11 to 15 Percent	16 to 20 Percent
Heart rate (bpm)	91 ± 24	101 ± 13	110 ± 13	103 ± 13
Effective refractory period (msec)	108 ± 14	110 ± 8	110 ± 8	115 ± 8
Vulnerable period (msec)	122 ± 12	122 ± 6	120 ± 7	122 ± 7
Repetitive extrasystole threshold (mA)	12 ± 3	10 ± 2	11 ± 2	11 ± 2

^a Values are expressed as means ± SEM. *n* = six dogs.

(1976) examined the effect of 6 hours of inhalation of 100 ppm CO on the ventricular fibrillation threshold in monkeys with myocardial infarction. They found that CO induced a significant fall in the fibrillation threshold, similar to that in normal animals. In their experiments in dogs with acute myocardial infarction, Bellet and coworkers (1972) found that the ventricular fibrillation threshold was reduced substantially following ventilation with cigarette smoke.

A contrary view is suggested by the studies of Foster (1981). He subjected dogs to 6-minute occlusions of the left anterior descending coronary artery. Ischemic conduction delay was measured at COHb levels of 10.4 percent. The results indicate that CO neither enhances ventricular tachycardia induced by spontaneous ischemia nor promotes reentry by delaying impulse conduction.

The results of the present study are in general agreement with the findings of Foster (1981). Specifically, we found no effect of CO exposure on any of the parameters studied, even during the acute phase of coronary artery occlusion. These included ventricular refractoriness, timing of the vulnerable period, and the ventricular fibrillation threshold.

Table 5. Effects of Twenty-Four-Hour Exposure to Carbon Monoxide in Conscious Dogs^a

	Base Line ^b	24 Hours ^c
Carboxyhemoglobin (percent)	0 ± 0	9.7 ± 1.6
Heart rate (bpm)	106 ± 15	103 ± 3
Effective refractory period (msec)	103 ± 6	115 ± 2 ^d
Vulnerable period (msec)	114 ± 4	124 ± 2 ^d
Repetitive extrasystole threshold (mA)	11 ± 1	12 ± 1

^a Values are expressed as means ± SEM.

^b *n* = 3 for base-line measurements.

^c *n* = 6 for 24-hour measurements.

^d *p* < 0.05 using two-sample *t* test.

Thus, on the basis of these data, we conclude that acute CO exposure causes no apparent deleterious effects on cardiac electrical stability in the normal or ischemic heart. However, this does not preclude the possibility that more sustained exposure or higher COHb levels might be injurious. Finally, the additional component of nicotine may be an exacerbating factor that might account for a difference between our results and those of Bellet and colleagues (1972).

EFFECTS OF CARBON MONOXIDE EXPOSURE ON CORONARY ARTERY BLOOD FLOW DURING CRITICAL STENOSIS

One mechanism whereby CO exposure could be detrimental is through an adrenergically mediated increase in platelet aggregability (Kowey et al. 1983b). Such an effect could be particularly hazardous in a heart in which the coronary circulation is compromised by stenosis. As has been shown by Folts and associates (1982) and in our laboratory (Raeder et al. 1982), platelet thrombi aggregate and dis-aggregate at arterial stenoses, resulting in cyclical coronary blood flow changes and episodes of myocardial ischemia.

Our findings indicate that CO exposure has no effect on either the cycle period of coronary flow oscillations or platelet aggregability. This suggests that exposure to relatively high levels of CO, leading to COHb plasma concentrations of up to 20 percent, does not impair the flow of blood through stenosed coronary arteries, according to the protocol described in the Materials and Methods section. It is important to reemphasize, however, that higher levels of CO or the addition of nicotine as contained in cigarette smoke could lead to a different outcome. In fact, Folts and Bonebrake (1982) have reported that the inhalation of cigarette smoke by dogs results in an increase in cyclical coronary blood flow changes. This effect appears to be mediated in part by increased alpha-adrenergic receptor activity, because it can be blocked by intravenous administration of the alpha-adrenergic blocking agent phentolamine.

INFLUENCE OF CARBON MONOXIDE EXPOSURE IN CONSCIOUS ANIMALS

In a final series of experiments, we examined whether or not CO might alter cardiac electrical stability in the conscious state. The main rationale for these investigations is that CO may affect the function of the central nervous system in a way that renders the heart electrically unstable (MacFarland et al. 1944; Schulte 1963; Beard and Wertheim 1967; Lown et al. 1973).

In human studies, concern has arisen that driving ability could be impaired by inhalation of CO emitted by automobiles. This has prompted studies of psychomotor function, which have revealed that even low levels of COHb may have an adverse impact (MacFarland et al. 1944; Schulte 1963; Beard and Wertheim 1967). However, the influence of the pollutant on the integrity of the autonomic nervous system is virtually unexplored. Given the pivotal role of the sympathetic and parasympathetic nervous systems in circulatory control and in the genesis of malignant arrhythmias (Corbalan et al. 1976; Lown and Verrier 1976), this issue merited further study.

Two protocols were tested. The first consisted of a 90- to 120-minute exposure to 200 to 500 ppm CO. The second entailed exposure to 25 to 50 ppm CO over a 24-hour period. Neither experimental protocol resulted in any significant alterations in ventricular vulnerability despite relatively high plasma concentrations of COHb. Heart rate was also not altered significantly. Taken in sum, these findings indicate no apparent deleterious influences of CO on the heart's excitable properties, neither direct effects on the myocardium nor indirect effects through an alteration in central nervous system activity.

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PUBLICATIONS RESULTING FROM THIS RESEARCH

Mills AK, Gideonse NL, Skornik WA, Verrier RL. 1986. Acute effects of carbon monoxide on cardiac electrical stability (abstract). *Clin Res* 34:860A.

Mills AK, Skornik WA, Valles LM, O'Rourke JJ, Hennessey RM, Verrier RL. 1987. Effects of carbon monoxide on cardiac electrical properties during acute coronary occlusion (abstract). *Fed Proc* 46:336.

ABBREVIATIONS

bpm	beats per minute
CH ₄	methane
CNMetHb	cyanmethemoglobin
CO	carbon monoxide
COHb	carboxyhemoglobin
ECG	electrocardiogram
K ₃ FeCN ₆	potassium ferricyanide
<i>M</i>	affinity constant
NAAQS	National Ambient Air Quality Standard
P _{aCO}	partial pressure of carbon monoxide
P _{aO₂}	partial pressure of oxygen in arterial blood
ppm	parts per million
SEM	standard error of the mean

INTRODUCTION

In the summer of 1984, the Health Effects Institute (HEI) issued a Request for Applications (RFA 84-2) soliciting proposals for "Acute Effects of Carbon Monoxide on Cardiac Rhythm." In response to this RFA, Richard L. Verrier, of the Department of Nutrition, Harvard University School of Public Health, Boston, MA, submitted a proposal entitled "Acute Effects of Carbon Monoxide on Cardiac Electrical Stability." The HEI approved the fifteen-month project, which began in September 1985. Total expenditures were \$267,445. The Investigators' Report was received at the HEI in October 1987 and was accepted by the Health Review Committee in July 1988. During the review of the Investigators' Report, the Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Review Committee's Commentary. The Commentary is intended to place the Investigators' Report in perspective, as an aid to the sponsors of the HEI and to the public.

REGULATORY BACKGROUND

The Clean Air Act mandates that the U.S. Environmental Protection Agency (EPA) establish primary standards for air pollutants based on health effects and at levels "requisite to protect the public health . . . allowing an adequate margin of safety." The legislative history of the Act makes it clear that in setting the National Ambient Air Quality Standards (NAAQS), the EPA is required to consider the health of particularly sensitive subgroups of the population. The Senate report on the legislation states: "An ambient air quality standard . . . should be the maximum permissible air level of an air pollution agent or class of such agents (related to a period of time) which will protect the health of any group of the population" (U.S. Senate 1970).

The identification of such groups is not clearly defined, but the Senate report does specify that "included among those persons whose health should be protected by the ambient standard are particularly sensitive citizens (such as bronchial asthmatics and emphysematics) who in the normal course of daily activity are exposed to the ambient environment." The report further states that "in establishing an ambient standard necessary to protect the health of these persons, reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group."

The current primary NAAQS for carbon monoxide (CO) is 9 parts per million (ppm), averaged over eight hours, and

35 ppm, averaged over one hour, both not to be exceeded more than once a year. Established in 1971, this standard was based on a study by Beard and Wertheim (1967) that reported an effect on the central nervous system (impairment in discrimination of time intervals) resulting from low-level CO exposures that achieved carboxyhemoglobin (COHb) levels of 2 to 3 percent. However, several subsequent studies failed to replicate the Beard and Wertheim findings (Stewart et al. 1970; O'Donnell et al. 1971; Otto et al. 1979).

During the 1970s, three studies reported that relatively low levels of CO exposure (50 and 100 ppm for one to four hours) aggravated the symptoms associated with cardiovascular disease. The studies by Aronow and Isbell (1973) and by Anderson and coworkers (1973) reported more rapid onset of pain in patients with angina, and another study by Aronow and coworkers (1974) reported a similar result in patients with intermittent claudication (ischemia of the muscles due to sclerosis with narrowing of the arteries). Following these reports, the EPA proposed to lower the one-hour standard from 35 ppm to 25 ppm. This lower standard was never implemented, in part because of controversy related to the studies by Aronow. In 1985, the EPA decided not to revise the existing standard (U.S. Environmental Protection Agency 1985) but is, at present, in the process of reviewing several human health effects studies that have been completed as part of their mandated periodic reevaluation of the criteria pollutant standards (Sheps et al. 1987; Adams et al. 1988; Allred et al. 1989a,b; Kleinman et al. 1989).

The known ability of CO to interfere with tissue oxygen delivery could account for the exacerbation of cardiac symptoms. Whether or not CO-induced hypoxia also causes cardiac disease, or induces potentially life-threatening effects, such as arrhythmias, has not been well studied. Investigations of the arrhythmia-producing effects of CO exposure in experimental models of cardiovascular disease have the potential to contribute knowledge useful in evaluating probable health effects in humans. This information is essential for informed regulatory decision making required by the Clean Air Act.

SCIENTIFIC BACKGROUND

CARDIAC ARRHYTHMIAS IN MYOCARDIAL ISCHEMIA

Heart disease, of which the most prevalent form is ischemic heart disease (commonly referred to as coronary artery disease), is the leading cause of disability and death in in-

dustrialized nations (Levy and Feinleib 1984). Ischemic heart disease refers to a spectrum of clinical disorders of the heart resulting from an imbalance between the myocardial need for oxygen and the adequacy of the blood supply (Robbins and Cotran 1979). Reduction in coronary blood flow is almost always the cause of this imbalance and is most often due to atherosclerosis, the formation of lipid deposits in the large- and medium-sized arteries. Occlusion of the coronary artery by lipid deposits results in inadequate delivery of blood, and hence oxygen, to the myocardial tissue. The resulting myocardial ischemia can be exacerbated further by increased oxygen demand, as occurs with exercise, or by impairment in the oxygen-carrying capacity of the blood, as occurs with anemia or CO-induced hypoxia. Severe myocardial ischemia can lead to myocardial infarction (heart attack).

In the normal human heart, the heartbeat originates in the sinoatrial node, and each myocardial cell then is activated in an organized, programmed sequence via the heart's specialized conduction system. Abnormal cardiac rhythms may result whenever interruptions in the blood supply and oxygen delivery occur, due to disordered impulse formation or altered conduction within the myocardial tissue, or both. In abnormal conditions, an ectopic focus (a focus other than the sinoatrial node) may discharge, resulting in a beat that occurs before the next expected normal beat and transiently interrupts the cardiac cycle (an extrasystole or premature beat). Conduction delay and block in ischemic myocardial tissue can also cause arrhythmias. Such a block usually occurs by partial obstruction of the blood flow due to either atheroma (lipid deposits characteristic of atherosclerosis) or thrombosis (a clot formed from the constituents of blood).

A rapid and irregular discharge of impulses arising from ectopic foci or impaired conduction, or both, can produce atrial or ventricular fibrillation. When extrasystoles initiate fibrillation within the ventricles, the heart is unable to pump blood effectively throughout the systemic circulation and death results (Ganong 1985). Ventricular fibrillation is the most serious arrhythmia that can occur as a consequence of ischemic heart disease (Goldman 1979), and it causes more than 80 percent of the deaths due to heart attacks.

Much of what is known about derangements of cardiac rhythm has come from animal models of myocardial ischemia. As early as 1894, Porter noted that "fibrillar contractions" were often a result of coronary artery occlusion and that irregular cardiac rhythm preceded terminal ventricular fibrillation (Porter 1894). Waldo and Kaiser (1973) found that in dogs with coronary artery ligation, areas of myocardial ischemia had periods of localized fibrillation. Citing

animal studies in both the dog and the pig, investigators have noted that myocardial ischemia affects the electrophysical properties of ventricular myocardial cells, leading to an electrical instability of the myocardium (reviewed by Lazzara et al. 1978; Janse and Kleber 1981; Motte et al. 1984; Lazzara and Scherlag 1988). Extracardiac factors have also been shown to influence cardiac rhythm; stimulation of the cardiac sympathetic nerves can induce irregular heartbeats, and in the ischemic myocardium, can be sufficient stimulus to induce fibrillation (Han et al. 1964; Lown and Verrier 1976). Thus, studies in animal models suggest that myocardial ischemia predisposes the heart to premature ventricular beats that can lead to fibrillation.

The dog has been the model of choice for studies of myocardial ischemia because the normal canine coronary vascular system resembles that of humans who have developed coronary artery disease. Specifically, humans with severe ischemia can avert myocardial infarction by the use of collateral vessels that are able to take over blood flow lost by occluded coronary arteries (Alpert and Braunwald 1984). In the healthy human heart, virtually no functional intercoronary channels exist; however, severe or advanced coronary stenosis (narrowing of the coronary arteries) induces progressive enlargement of small arterial collaterals and development of new collateral channels from preexisting capillaries. These new blood vessels are critical in preventing ventricular fibrillation (Berne and Levy 1981; Alpert and Braunwald 1984; Epstein et al. 1989). In contrast, the normal dog heart has coronary collateral vessels that link the branches of the major coronary arteries, thereby providing immediate epicardial collateral flow after acute coronary artery occlusion. Because coronary stenosis induces the development of collateral vessels in humans, many researchers believe the dog is the best available animal model to study human coronary artery disease (Davis et al. 1983; Gross 1985a,b; Roy and Short 1986); others, however, prefer the pig as a model because, compared to dogs, atherosclerotic plaques in swine more closely resemble those in humans (Eckstein 1954; Brooks et al. 1975; Gross 1985a,b).

ASSAYS OF VENTRICULAR VULNERABILITY

Studies conducted more than forty years ago demonstrated that ventricular fibrillation could be induced in animals by delivering a relatively low-energy discharge during the vulnerable phase of the cardiac cycle (Wiggers et al. 1940). The ventricular fibrillation threshold is defined as the lowest intensity of an electrical stimulus that will induce ventricular fibrillation (Axelrod et al. 1975). The ventricular fibrillation threshold is believed to reflect the vulnerability of the ventricles to ventricular fibrillation.

However, a number of problems, including humane considerations, have been identified regarding the use of ventricular fibrillation threshold as a cardiological probe (Matta et al. 1976).

An alternative method to study ventricular vulnerability without inducing fibrillation is the repetitive extrasystole threshold, defined as the lowest electrical stimulus that will evoke multiple extrasystoles (Han 1969; Lown et al. 1973; Rabinowitz et al. 1975; Matta et al. 1976; Kowey et al. 1983). Repetitive extrasystoles can be induced by lower electrical stimulation than that which induces ventricular fibrillation (Berne and Levy 1981). Because repetitive extrasystoles have been observed to precede the occurrence of ventricular fibrillation, it has been suggested that the threshold for repetitive extrasystoles can reliably predict the vulnerability of the canine ventricle to fibrillation (Kowey et al. 1983).

CARDIOVASCULAR EFFECTS OF CARBON MONOXIDE

The adverse health effects of CO exposure are thought to arise largely from interference with the normal oxygen-carrying function of the blood. Under normal conditions, inhaled oxygen diffuses rapidly across the alveolar walls of the lung and binds to hemoglobin inside the red blood cells. The oxyhemoglobin complex is transported in the blood, and the oxygen is eventually released into the tissues. If CO is present in the inhaled air, the CO and oxygen compete for the oxygen-binding sites on the hemoglobin molecules. Because the affinity of hemoglobin for CO is 240 times greater than for oxygen, COHb forms and the oxygen-carrying capacity of the red blood cells is reduced. In addition, delivery of oxygen to the tissues is further impaired because, in the presence of CO, hemoglobin releases oxygen to the tissues more slowly. The resulting tissue hypoxia may cause transient or permanent damage, especially in those organs that demand high oxygen delivery, such as the brain and heart.

Tissue hypoxia in the myocardium initiates a variety of compensatory responses, such as increased cardiac output and vasodilation, that are directed toward increasing the rate of oxygen delivery. Because of their limited ability to increase blood flow, individuals with coronary artery disease comprise a subpopulation that is potentially susceptible to adverse health effects when breathing low levels of CO, (U.S. Environmental Protection Agency 1985).

Clinical and animal studies have produced limited information with regard to the potential arrhythmogenic effects of CO. In men with coronary artery disease, exposure to low levels of CO (resulting in 2 to 4 percent COHb) while exercising decreased the time to the onset of ischemic ST-

segment changes and the time to onset of angina (Allred et al. 1989a,b). No human studies, however, have demonstrated an arrhythmogenic effect of CO exposure in normal subjects or in subjects with coronary artery disease and no base-line ectopy (Hinderliter et al. 1989).

A limited number of investigations of cardiac vulnerability to ventricular fibrillation, under conditions of acute and chronic CO exposure, have been conducted in laboratory animals. The results of these studies are equivocal. In agreement with the studies in human subjects, abnormal electrocardiograms have been reported in dogs (Preziosi et al. 1970; Sekiya et al. 1983) and Cynomolgus monkeys (DeBias et al. 1973) exposed to low levels of CO. DeBias and co-workers (1976) also reported that the threshold for trans-thoracic induction of ventricular fibrillation was reduced in normal monkeys and in animals with experimental myocardial infarcts. A reduced ventricular fibrillation threshold was also reported in normal dogs and in dogs with acute myocardial infarction (Aronow et al. 1978, 1979). In contrast to these reports, other investigators have found no effects of CO exposure on ventricular arrhythmias or ischemic conduction delay (Foster 1981).

JUSTIFICATION FOR THE STUDY

The effects of breathing CO on cardiac function have been examined in a number of experimental models. It is not known, however, whether or not CO affects prefibrillatory action in normal subjects or in subjects with cardiovascular disease. The HEI solicited proposals under RFA 84-2, "Acute Effects of Carbon Monoxide on Cardiac Rhythm," for research on the effects of CO, at or near ambient levels, on myocardial excitability. Investigations using animal models of susceptible human populations, clinical studies, or epidemiological studies of susceptible individuals were all considered relevant.

After reviewing the proposals submitted under RFA 84-2, the Institute decided to support four research projects, two studies to determine the effects of CO exposure on cardiac electrical stability in animal models, and two human clinical studies to evaluate the effects of CO exposure on subjects with ischemic heart disease and ventricular arrhythmias. By supporting diverse biological models and end points, the Institute's goal was to develop a comprehensive research program to determine the effects of CO exposure on the occurrence of arrhythmias and on susceptibility to ventricular fibrillation.

R.L. Verrier and his colleagues proposed to study the effects of CO exposure on ventricular electrical stability and

myocardial perfusion in normal dogs and in dogs with experimental myocardial ischemia. The HEI Research Committee recommended that Verrier and his colleagues begin their study with relatively high levels of CO that would produce 20 percent COHb concentrations before they tested animals at the proposed levels of 5 and 10 percent COHb. Because no significant effects of exposure to high levels of CO on ventricular electrical stability were found in the acutely ischemic or the normal canine heart, the HEI and the investigators agreed not to test the animals at lower COHb levels.

GOALS AND OBJECTIVES

The overall objective was to determine whether or not CO exposure influences cardiac electrical stability in the acutely ischemic and in the normal canine heart. To achieve this goal, Verrier and his colleagues determined the effect of CO exposure on the threshold of electrical stimulation for ventricular fibrillation in normal anesthetized dogs and anesthetized dogs with experimental myocardial ischemia. In addition, the threshold for repetitive extrasystoles was measured in conscious, resting animals to evaluate the influence of CO on central nervous system-mediated effects on cardiac electrical stability.

STUDY DESIGN

Instruments were placed in the hearts of mongrel dogs to record their intracavitary electrocardiogram, to permit delivery of both the pacing and test stimuli, and to assess their base-line heart rate, blood pressure, refractory period, vulnerable period, and ventricular fibrillation threshold or repetitive extrasystole threshold.

The effect of CO exposure (500 ppm CO resulting in 20 percent COHb) was tested in four subgroups of dogs. Effects were evaluated using two experimental models of myocardial ischemia, acute coronary occlusion and partial coronary stenosis. Three subgroups of animals were exposed to CO for 40 to 120 minutes under alpha-chloralose anesthesia and tested for changes in their ventricular fibrillation threshold. These subgroups included: (1) normal dogs; (2) dogs with acute myocardial ischemia (a ten minute period of coronary artery occlusion induced by implanting a balloon occluder around a coronary artery and inflating the occluder before CO exposure); and (3) dogs with partial stenosis (induced by applying a plastic cylinder to a coronary artery to narrow the vessel 60 to 80 percent before CO exposure). Coronary blood flow and platelet aggregability

were also measured in the latter group. A fourth subgroup of animals was conscious during both short-term (90 to 120 minutes) exposure to CO (200 to 500 ppm resulting in 0 to 20 percent COHb), and long-term (24 hours) exposure to CO (25 to 50 ppm leading to 9.7 percent COHb). The conscious animals were monitored for changes in their ventricular repetitive extrasystole threshold.

TECHNICAL EVALUATION

ATTAINMENT OF STUDY OBJECTIVES

The major goals of this study were met successfully.

ASSESSMENT OF METHODS AND STUDY DESIGN

The models used in these experiments are among the best currently available to study myocardial ischemia. The studies focused on appropriate electrophysiologic and hemodynamic parameters, which were measured in the presence and absence of CO.

In each experimental protocol, base-line measurements were made on all variables. No sham controls were used, however; that is, no animals were instrumented for measurements of cardiac parameters and not exposed to CO. They were omitted to keep the number of laboratory animals to a minimum. The authors have used these methods for many years, and in their report cite that this instrumentation leads to a stable base line. We do not know, however, whether or not prolonged instrumentation or the other procedures used in this study influence the response to CO; it would have been desirable to establish this in the early phase of the study.

Determination of the threshold for ventricular fibrillation induced by an electrical stimulus is a well-established procedure for determining the vulnerability of laboratory animals to cardiac arrhythmias. The use of the threshold for repetitive extrasystoles as a measure of ventricular vulnerability is, however, controversial. Ventricular fibrillation was not preceded by repetitive extrasystoles in approximately 10 percent of the animals in one study (Matta et al. 1976). Furthermore, under conditions of total coronary occlusion, increased blood potassium levels, or pharmacologic intervention, the threshold for repetitive extrasystoles and the threshold for ventricular fibrillation did not change in parallel, nor was ventricular fibrillation preceded by repetitive extrasystoles (Logic 1973; Jaillon et al. 1980; Kowey et al. 1983). Such dissociation of the repetitive extrasystole and

ventricular fibrillation events after pharmacologic or other intervention suggests that they may reflect different electrophysiologic phenomena.

The threshold for repetitive extrasystoles, rather than the threshold for ventricular fibrillation, was used by the investigators as an endpoint in nonanesthetized animals to avoid subjecting conscious dogs to repeated fibrillation and defibrillation. If the investigators had also measured the threshold for repetitive extrasystoles in the anesthetized dogs, then they would have been able to compare the two procedures in the same animal.

STATISTICAL METHODS

The number of animals used in each group (six or seven) was relatively small; it is, however, consistent with the standard practice in this field of research. The authors have indicated that the methodology can detect differences in the threshold of 20 percent or more, which would have biological significance. A presentation of statistical power calculations would have been desirable to convince the reader that the sample size was sufficiently large.

The results are presented in the form of tables of data and graphic representations of mean responses (\pm standard errors). The graphs provide clear, concise answers to the original questions. The parameters of effective refractory period, vulnerable period, and threshold for ventricular fibrillation were determined by assessing the amount of current required to achieve each of the endpoints. Representative on-line experimental analog records were not available, because it is standard practice to read the current values directly. Appendices containing such primary data would have documented the consistency and reproducibility of the measured parameters. Although within each group relatively little variation from animal to animal (as indicated by the small standard errors) was reported, it would have been useful if all measurements of the threshold for ventricular fibrillation had been plotted against time lapses as the CO levels progressively increased.

More details of the statistical analyses could have been presented in the Investigators' Report. For example, the data in Figure 3 suggest a slight increase in the threshold for ventricular fibrillation with increasing COHb. From the description given, however, it cannot be verified whether this was a real effect or was due to chance. The error bars do not help in this regard because they apply to the cross-sectional data and do not take into account the repeated measures of the design.

For much of the data presented in the Investigators' Report, statistical analyses for trends would have been appro-

priate. Most statistical analysis programs test the hypothesis that all responses at different exposure levels are equal, a hypothesis that is less precise than testing for a specific trend in the response. However, nonsignificance of the statistical test of an equality hypothesis does not assure that trends in the response relation do not exist. This apparently anomalous situation arises because statistical tests directed toward examining trends are more powerful than those that are directed toward equality of response to all levels of exposure. Thus, in the current study, even though tests for equality of response to CO exposure indicate nonsignificance, the presence of a trend cannot be ruled out.

INTERPRETATION OF RESULTS

The results suggest that exposure to relatively high levels of CO, resulting in COHb concentrations of 20 percent has no apparent acute effects on the threshold for ventricular fibrillation for normal dogs or for dogs with experimentally induced acute myocardial ischemia. Furthermore, 20 percent COHb does not appear to alter the coronary blood flow or platelet aggregability. In studies in conscious dogs, no deleterious effect of either a 2- or 24-hour exposure was seen on cardiac excitability, as measured by the threshold for repetitive extrasystoles.

The investigators anesthetized the animals with alpha-chloralose after premedication with morphine sulfate, but they do not comment upon the possible effects of the anesthesia on the cardiovascular parameters that were monitored. Alpha-chloralose is the primary anesthetic agent used in acute cardiovascular studies in animals in which the effects of the autonomic nervous system are to be tested. However, Wenger and colleagues (1984; reviewed by Holzgrefe et al. 1987) demonstrated that the threshold for ventricular fibrillation following coronary artery reperfusion was significantly lower in alpha-chloralose-anesthetized dogs than in animals anesthetized with pentobarbital. It has also been reported that the outcome of cardiovascular studies may be different when conducted in conscious animals (Bolli et al. 1986). In the current study, because each animal served as its own control, it is uncertain whether or not the anesthesia affected the physiologic parameters measured.

From the statistical analyses presented, the data suggest no significant changes in the thresholds for ventricular fibrillation or repetitive extrasystoles upon exposure to CO. As discussed earlier, previous studies examining the effect of CO exposure on cardiac arrhythmias in animals with experimentally induced myocardial infarction have been equivocal (DeBias et al. 1976; Aronow et al. 1978).

An implicit assumption in this study is that any hypoxia-inducing mechanism leading to reversible angina is also a mechanism that would lead to arrhythmias. Although there is some evidence for this in humans (Kerin et al. 1979; Carboni et al. 1987), it is limited and still needs to be examined in terms of what is known about CO-induced effects in humans with coronary artery disease. Given the relatively small size of the sample that was used, and the statistical issues noted above, the results of this study should be interpreted with caution and cannot be extrapolated directly to the potential arrhythmogenic effects of CO exposure in human patients with coronary artery disease.

IMPLICATIONS FOR FUTURE RESEARCH

The results from this study highlight a need to evaluate carefully the role of CO-induced hypoxia in relation to ventricular arrhythmias and fibrillation and to determine whether or not arrhythmia induced by conditions of ischemia can be aggravated by exposure to CO. These data need to be considered together with the results of other studies that use different end points and different models.

Given the practical and ethical difficulties associated with conducting research on the cardiovascular system in humans, carefully controlled animal studies will continue to be necessary to establish whether or not CO exposure is a risk factor in humans with cardiovascular disease. The animal species studied should demonstrate the clinical and physiologic alterations seen in humans with ischemia. Also, the cardiac excitability measurements should be relevant to effects measured in humans. Exposure protocols should be representative of human exposure scenarios: short-term peak CO exposures, exposures with exercise, and sustained exposures to low levels of CO. Confounding effects of anesthesia need to be understood in order to extend the results of animal studies to health effects in humans with ischemia. Finally, complementary cellular studies could help to clarify the mechanisms of CO-induced cardiac toxicity.

CONCLUSIONS

Under the experimental conditions used in this study, CO exposure leading to COHb levels of 20 percent did not alter the susceptibility of the canine heart to rhythm disturbances, nor did it alter hemodynamic properties or platelet aggregability in narrowed coronary arteries. The results of this study suggest that exposure of the normal or ischemic canine heart to reasonably high concentrations of CO has

no demonstrable effects on cardiac electrical stability. Care must be taken in extrapolating these results to human subjects with coronary artery disease. The lack of an effect in the dog model may be due to the possibility that CO exposure does not induce arrhythmogenic behavior in this species, to the insensitivity of the animal model, or to other experimental factors.

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Special Reports

Title	Publication Date
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research	September 1985
Automotive Methanol Vapors and Human Health: An Evaluation of Existing Scientific Information and Issues for Future Research	May 1987
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research (Supplement)	January 1988

Research Reports

Report No.	Title	Principal Investigator	Publication Date
1	Estimation of Risk of Glucose 6-Phosphate Dehydrogenase-Deficient Red Cells to Ozone and Nitrogen Dioxide	M. Amoruso	August 1985
2	Disposition and Metabolism of Free and Particle-Associated Nitropyrenes After Inhalation	J. Bond	February 1986
3	Transport of Macromolecules and Particles at Target Sites for Deposition of Air Pollutants	T. Crocker	February 1986
4	The Metabolic Activation and DNA Adducts of Dinitropyrenes	F.A. Beland	August 1986
5	An Investigation into the Effect of a Ceramic Particle Trap on the Chemical Mutagens in Diesel Exhaust	S.T. Bagley	January 1987
6	Effect of Nitrogen Dioxide, Ozone, and Peroxyacetyl Nitrate on Metabolic and Pulmonary Function	D.M. Drechsler-Parks	April 1987
7	DNA Adducts of Nitropyrene Detected by Specific Antibodies	J.D. Groopman	April 1987
8	Effects of Inhaled Nitrogen Dioxide and Diesel Exhaust on Developing Lung	J.L. Mauderly	May 1987
9	Biochemical and Metabolic Response to Nitrogen Dioxide-Induced Endothelial Injury	J.M. Patel	June 1987
10	Predictive Models for Deposition of Inhaled Diesel Exhaust Particles in Humans and Laboratory Species	C.P. Yu	July 1987
11	Effects of Ozone and Nitrogen Dioxide on Human Lung Proteinase Inhibitors	D.A. Johnson	August 1987
12	Neurotoxicity of Prenatal Carbon Monoxide Exposure	L.D. Fechter	September 1987
13	Effects of Nitrogen Dioxide on Alveolar Epithelial Barrier Properties	E.D. Crandall	October 1987
14	The Effects of Ozone and Nitrogen Dioxide on Lung Function in Healthy and Asthmatic Adolescents	J.Q. Koenig	January 1988
15	Susceptibility to Virus Infection with Exposure to Nitrogen Dioxide	T.J. Kulle	January 1988
16	Metabolism and Biological Effects of Nitropyrene and Related Compounds	C.M. King	February 1988

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Research Reports

Report No.	Title	Principal Investigator	Publication Date
17	Studies on the Metabolism and Biological Effects of Nitropyrene and Related Nitro-polycyclic Aromatic Compounds in Diploid Human Fibroblasts	V.M. Maher	March 1988
18	Respiratory Infections in Coal Miners Exposed to Nitrogen Oxides	M. Jacobsen	July 1988
19	Factors Affecting Possible Carcinogenicity of Inhaled Nitropyrene Aerosols	R.K. Wolff	August 1988
20	Modulation of Pulmonary Defense Mechanisms Against Viral and Bacterial Infections by Acute Exposures to Nitrogen Dioxide	G.J. Jakab	October 1988
21	Maximal Aerobic Capacity at Several Ambient Concentrations of Carbon Monoxide at Several Altitudes	S.M. Horvath	December 1988
22	Detection of Paracrine Factors in Oxidant Lung Injury	A.K. Tanswell	February 1989
23	Responses of Susceptible Subpopulations to Nitrogen Dioxide	P.E. Morrow	February 1989
24	Altered Susceptibility to Viral Respiratory Infection During Short-Term Exposure to Nitrogen Dioxide	R.M. Rose	March 1989
25	Acute Effects of Carbon Monoxide Exposure on Individuals with Coronary Artery Disease	HEI Multicenter CO Study Team	November 1989
26	Investigation of a Potential Cotumorogenic Effect of the Dioxides of Nitrogen and Sulfur, and of Diesel-Engine Exhaust, on the Respiratory Tract of Syrian Golden Hamsters	U. Mohr (U. Heinrich)	May 1989
27	Cardiovascular Effects of Chronic Carbon Monoxide and High-Altitude Exposure	J.J. McGrath	July 1989
28	Nitrogen Dioxide and Respiratory Infection: Pilot Investigations	J.M. Samet	September 1989
29	Early Markers of Lung Injury	J.N. Evans	September 1989
30	Influence of Experimental Pulmonary Emphysema on Toxicological Effects from Inhaled Nitrogen Dioxide and Diesel Exhaust	J.L. Mauderly	October 1989
31	DNA Binding by 1-Nitropyrene and Dinitropyrenes in Vitro and in Vivo: Effects of Nitroreductase Induction	F.A. Beland	November 1989
32	Respiratory Carcinogenesis of Nitroaromatics	R.C. Moon	April 1990
33	Markers of Exposure to Diesel Exhaust in Railroad Workers	M.B. Schenker	September 1990
34	Metabolic Activation of Nitropyrene and Diesel Particulate Extracts	A.M. Jeffrey	July 1990

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