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Cardiovascular Effects of Chronic Carbon Monoxide and High-Altitude Exposure

James J. McGrath
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Includes the Commentary by the Institute's Health Review Committee

Research Report Number 27

HEALTH EFFECTS INSTITUTE

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Cardiovascular Effects of Chronic Carbon Monoxide and High-Altitude Exposure

INVESTIGATOR'S REPORT James J. McGrath

HEALTH REVIEW COMMITTEE'S COMMENTARY Health Effects Institute

Cardiovascular Effects of Chronic Carbon Monoxide and High-Altitude Exposure

By James J. McGrath[1]

ABSTRACT

At higher altitudes, ambient carbon monoxide levels are increasing with the number of residents and tourists and their use of motor vehicles and heating devices (such as fireplaces, furnaces, and stoves). Although chronic exposure to carbon monoxide or high altitude causes pronounced cardiovascular changes in humans as well as in animals, there is little information on the effects elicited by these stressors combined. Data from acute studies and theoretical considerations suggest that carbon monoxide inhaled at altitude may be more detrimental than carbon monoxide inhaled at sea level. It is not known, however, if the cardiovascular system adapts or deteriorates with continuous, concurrent exposure to carbon monoxide and high altitude.

Male laboratory rats were exposed for six weeks in steel barometric chambers to altitudes ranging from 3,300 ft (ambient) to 18,000 ft and to concentrations ranging from 0 to 500 parts per million (ppm)[2].

Carbon monoxide had no effect on body weight at any altitude. There was a tendency for hematocrit to increase even at the lowest concentration of carbon monoxide (9 ppm), but the increase did not become significant until 100 ppm. At 10,000 ft, there was a tendency for total heart weight to increase in rats inhaling 100 ppm carbon monoxide. Although its effects on the heart at altitude are complex, carbon monoxide, in concentrations of 500 ppm or less, had little effect on the right ventricle; it did not exacerbate any effects due to altitude. There was a tendency for the left ventricle weight to increase with exposure to 35 ppm carbon monoxide at altitude, but the increase was not significant until 100 ppm carbon monoxide.

Heart rate, blood pressure, cardiac output, and peripheral resistance were unaffected by exposure to 35 ppm carbon monoxide or 10,000-ft altitude singly or in combination. I conclude that six weeks of exposure to 35 ppm carbon monoxide does not produce measurable effects in the healthy laboratory rat, nor does it exacerbate the effects produced by exposure to 10,000-ft altitude.

Basal carboxyhemoglobin (COHb) level (due to endogenous carbon monoxide production) was increased from 0.7 to 1.0 percent at 10,000 ft, and to 1.7 percent at 15,000 ft. This suggests that the high-altitude resident has a greater initial body burden of COHb and will attain the COHb level associated with the National Ambient Air Quality Standard for carbon monoxide more quickly than the sea-level resident.

INTRODUCTION

The physiological responses of humans and animals to chronic exposure to high altitude or carbon monoxide (CO) have been studied intensively, but the responses to these stressors combined have not been investigated. This research was initiated because theoretical and experimental data suggest that acute exposure to CO combined with altitude may be more detrimental to the cardiovascular system than exposure to CO alone, and because there are no data on the effects of chronic exposure to CO at altitude.

OXYGEN TRANSPORT

Oxygen is transported to the tissues by hemoglobin; the amount of oxygen transported depends on the partial pressure of oxygen (P_{O_2}) in the inspired air, the amount of functional hemoglobin, and the percentage of hemoglobin saturated with oxygen. At altitude, the inspired P_{O_2} and the percent of hemoglobin saturated with oxygen are lower than normal, and accordingly, the amount of oxygen delivered to the tissues is less; this results in hypoxic hypoxia. On the other hand, CO, by binding with hemoglobin to form COHb, reduces the amount of functional hemoglobin and makes the hemoglobin-bound oxygen less available to the tissues; this results in anemic hypoxia. Consequently, the effects of breathing CO at altitude might be expected to be at least additive, because the tissue hypoxia resulting from anemic hypoxia (decreased functional hemoglobin) is superimposed on that caused by hypoxic hypoxia (decreased inhaled P_{O_2}).

The study reported here was planned to measure the consequences of the interaction between anemic hypoxia and hypoxic hypoxia in rats breathing CO at altitude for six weeks.

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

CHRONIC EFFECTS OF ALTITUDE

Living at high altitude imposes a hypoxic stress on the body, the magnitude of which is determined by the absolute altitude, as well as the duration of time spent at altitude. However, many physiological changes occur in organisms living at altitude, and some of these changes are considered compensatory. A conceptual framework for these changes and their importance to the organism is provided by Barbashova (1964).

Prominent features of prolonged altitude exposure are increases in hemoglobin concentration and in hematocrit; these changes increase the oxygen-carrying capacity of the blood. However, because of the increased work required to pump the more viscous blood, not everyone agrees that this response is beneficial to the organism. Guyton and Richardson (1961) suggest not only that changes in hematocrit affect the oxygen-carrying capacity of the blood, but also that the blood flow to the tissues may reduce oxygen delivery; this can more than offset the advantage gained by increasing the oxygen-carrying capacity of the blood. Smith and Crowall (1967) conclude that there is an optimal hematocrit at sea level that shifts to a higher value with altitude acclimatization.

A second notable feature of extended stay at altitude is pulmonary vasoconstriction. Constriction of the pulmonary arteries is due to a direct effect of hypoxia on arterial smooth muscle. Some degree of pulmonary vasoconstriction in response to hypoxia may be beneficial, in that it can match perfusion to ventilation within the lung (Reeves et al. 1979). The response is useful in local disease of the lung (for example, pneumonia), because the hypoxic vasoconstrictor mechanism can shunt blood away from diseased areas. But, at altitude, all alveoli are hypoxic, and the vasoconstrictor response is general throughout the lung. Under these conditions, pulmonary blood pressure is increased; this may redistribute blood flow and, at least in humans (because of our upright posture), increase the oxygen-diffusing capacity. Pulmonary hypertension is followed by right ventricular hypertrophy, because of the increased workload imposed on the right ventricle.

Pulmonary vasoconstriction, as well as right ventricular hypertrophy, is most pronounced in relative newcomers to altitude and is less pronounced in long-term residents. In cases of excessive hypoxia, it can lead to right heart failure. Right ventricular hypertrophy has been reported in many animals, including rats, cattle, and birds, as well as in humans.

A third feature of ascent to and residence at altitude relevant to these studies is change in cardiac output. The data are controversial, but it is generally agreed that cardiac output increases on ascent to altitude, but decreases after a few days at altitude (Lenfant and Sullivan 1971). Tachycardia remains, but stroke volume falls, so that cardiac output returns to near normal levels.

Changes in cardiac output during exposure to altitude result from several factors (Lenfant and Sullivan 1971). During the initial stages of exposure to altitude, the predominant factor appears to be increased sympathetic activity; this is reflected in the rise in plasma and urinary catecholamines. The cardiac response is not related to tissue P_{O_2}, because after a few days at altitude the hypoxia remains, but the total blood flow decreases. Others believe that the secondary decrease in cardiac output with prolonged exposure to altitude is caused by a direct depressing effect of hypoxia on the myocardium (Alexander et al. 1967).

Thus, the salient features of prolonged altitude exposure are increased hematocrit, right ventricular hypertrophy, and possible changes in cardiac output. It is not known how these responses would be affected by CO superimposed on the altitude stress. In the studies reported here, this interaction was investigated.

CHRONIC EFFECTS OF CARBON MONOXIDE EXPOSURE

Chronic CO exposure also increases hemoglobin concentration, hematocrit, and erythrocyte count (Wilks et al. 1959; Syvertsen and Harris 1973; Penney and Bishop 1978). The mechanism responsible for these hematological changes is believed to be the same as in chronic altitude exposure (that is, lowered tissue oxygen delivery resulting from elevated COHb levels). Reticulocyte release from the hemopoietic organs, as well as other aspects of erythropoietic activity, is stimulated by erythropoietin released by the kidney; CO exposure enhances erythropoietin production (Syvertsen and Harris 1973; Guidi et al. 1977). Penney and coworkers (1974a) concluded that the threshold for the erythropoietic response is 100 ppm CO.

Blood volume increases with chronic CO exposure, primarily because the circulating red blood cell mass increases. However, plasma volume, which may be depressed in the first few days at altitude, is either unchanged by CO exposure (Parving 1972) or elevated slightly (Wilks et al. 1959).

Early studies reporting cardiac enlargement in response to chronic CO exposure have been verified (Theodore et al. 1971). In rats exposed to 500 ppm CO, heart mass increased rapidly and cardiac lactate dehydrogenase composition changed in parallel with the heart weight changes (Penney et al. 1974b). Penney and coworkers (1974a) concluded that the threshold for cardiac enlargement is near 200 ppm, and, unlike cardiac hypertrophy caused by altitude, which

involves primarily the right ventricle, cardiac hypertrophy caused by CO involves the whole heart.

The hemodynamic consequences of chronic CO exposure have been examined by Penney and coworkers (1979) and by James and associates (1979). In rats exposed to 500 ppm CO for 1 to 42 days (Penney et al. 1979), hematocrit increased from 49.8 to 69.7 percent; cardiomegaly developed; and stroke index, mean stroke power, and mean cardiac output increased; however, total systemic and pulmonary resistances decreased. There were slight, but nonsignificant, changes in left and right ventricular systolic pressures, mean aortic pressure, and maximum left ventricular pressure rate of change (dP/dt); there was no consistent change in heart rate. In chronically instrumented goats exposed to CO for two weeks, James and colleagues (1979) reported no changes in cardiac index or stroke volume; left ventricular contractility and heart rate were unchanged during CO exposure, but were depressed significantly during the first week after the exposure. The differences in the two studies may be attributable, in part, to differences in the intensity of the CO exposure, species differences, and (because the James study was conducted in Denver) exposure to a moderately higher altitude.

Thus, the well-documented physiological responses to chronic CO exposure include increased oxygen-carrying capacity of the blood and cardiac enlargement. The levels of CO reported to elicit these changes are generally high, and there is some question if the changes occurring in the heart can be considered beneficial. In the studies reported here, the CO exposures were more comparable to those encountered in the ambient environment.

CARBON MONOXIDE EXPOSURE AT ALTITUDE

Precise estimates of the number of people exposed to CO at high altitude are not readily available. However, more than 2.2 million people live at altitudes in excess of 5,000 ft (1,524 m), and countless tourists sojourn in high-altitude areas during the summer and winter months (National Research Council 1977). Moreover, there are several factors that tend to exacerbate ambient CO levels at high altitude (Kirkpatrick and Reeser 1976). For example, in mountain recreational communities, automobile emissions are higher; automobiles tuned for driving at 5,280 ft (1,610 m) emit almost 1.8 times more CO when driven at 8,000 ft (2,438 m), and automobiles tuned for sea-level driving emit almost 4 times more CO when driven at 8,000 ft. Furthermore, automobile emissions are increased by driving at reduced speeds along steep grades under poor driving conditions. Therefore, large influxes of tourists, driving automobiles tuned for sea-level conditions into high-altitude resort areas, may drastically increase pollutant levels in general, and CO levels in particular (National Research Council 1977). In addition, population growth in mountain areas is concentrated along valley floors; this factor, combined with the reduced volume of air available for pollutant dispersal, causes pollutants, including CO, to accumulate in mountain valleys. Finally, heating devices (kerosene space heaters and fireplaces) used for social effect, as well as warmth, also contribute to CO emissions in mountain resort areas. As a result of these factors, the National Ambient Air Quality Standard for CO of 9 ppm is exceeded frequently in Denver, CO (altitude 5,280 ft) during the winter months (Haagenson 1979).

The effects of altitude on COHb formation can be predicted from Haldane's first principle (Haldane and Smith 1897):

$$\frac{[COHb]}{[O_2Hb]} = M \frac{P_{CO}}{P_{O_2}}$$

where $[COHb]$ is the blood concentration of carboxyhemoglobin; $[O_2Hb]$ is the blood concentration of oxyhemoglobin; P_{CO} is the partial pressure of CO in blood; P_{O_2} is the partial pressure of O_2 in blood; and M is the Haldane constant.

According to this principle, a P_{CO} will result in a higher percent carboxyhemoglobin (%COHb) at altitude (where P_{O_2} is reduced) than at sea level. Thus, Collier and Goldsmith (1983) calculate that humans exposed to 8 ppm CO will have equilibrium COHb levels of 1.4 percent at sea level and 1.8 percent at 11,741 ft (3,579 m). Interestingly, these workers calculate an increase in COHb at altitude even in the absence of ambient CO (due to Haldane's first principle).

The possibility that CO may pose a special threat at altitude is suggested by Luomanmaki and Coburn (1969), who reported that, during hypoxia, CO shifts out of the blood and into the tissues. Agostoni and associates (1980) presented a theoretical model to support these observations; they developed a series of equations predicting that with decreased venous P_{O_2}, CO moves out of the vascular compartment and into skeletal and heart muscle, increasing the formation of carboxymyoglobin (COMb) in the tissues. Thus, available evidence suggests that CO moves into the extravascular compartment during hypoxemia and causes the COMb:COHb ratio to increase.

Many studies have compared the cardiovascular effects of CO with those of altitude, but there are few studies on the effects of CO at altitude. Increased CO uptake during light activity at 16,000 ft (Forbes et al. 1945), and increased pulse rate in healthy men at 15,000 ft with COHb levels of up to 13 percent (Pitts and Pace 1947), have been reported. Pitts and Pace conclude that, in healthy men, the response to a

1 percent increase in COHb level is equivalent to an increase of 335 ft in altitude.

Weiser and colleagues (1981) studied the effects of CO on aerobic work at altitude; COHb levels of 5 percent impaired work performance to the same extent as at sea level. There were small, but significant, changes in cardiorespiratory function during submaximal exercise and CO exposure; working heart rate increased and postexercise left ventricular ejection time shortened. Exposure to CO also caused a lower anaerobic threshold and a greater minute ventilation at work rates heavier than the anaerobic threshold.

Most physiological data on the effects of combined CO and altitude exposure come from psychophysiological studies, and it is on the basis of these studies that the concept of physiologically equivalent altitudes has been developed. McFarland and coworkers (1944) and McFarland (1970) reported changes in visual sensitivity at COHb concentrations of 5 percent or at an altitude of approximately 8,000 ft. Lilienthal and Fugitt (1946) reported that combined exposure to altitude and CO decreased flicker-fusion frequency. Whereas mild hypoxia alone impaired flicker-fusion frequency, COHb levels of 5 to 10 percent decreased the altitude threshold for onset of impairment to between 5,000 and 6,000 ft.

Thus, although the literature abounds with studies comparing and contrasting CO and altitude exposures, there are few reports on the effects of CO at altitude. There are data to support the concept that the effects of these two hypoxia-inducing conditions are at least additive. The data presented by Luomanmaki and Coburn (1969), however, suggest that the effects may be more than simply additive.

Moreover, it is difficult to assemble a cohesive picture of the potential cardiovascular effects elicited by long-term, low-level CO exposure, because some studies are being challenged and others have used unrealistically high CO levels. In addition, there have been few studies of the potential synergistic effects of CO combined with altitude. In the studies reported here, we followed a more integrated approach by conducting all studies reported here in one animal model, the rat, which was exposed to levels of CO and altitude that are commensurate with human experience.

CARBON MONOXIDE EFFECTS ON THE HEART

The effects of CO on the heart and its association with cardiovascular disease have been described in both human and animal studies. A significant shortening in the time until onset of pain in exercise-induced angina has been reported in human subjects exposed to CO (Anderson et al. 1973; Aronow and Isbell 1973); however, the Aronow and Isbell study has been criticized (Horvath et al. 1983).

In studies on humans undergoing cardiac catheterization, Ayres and associates (1969, 1970) reported that both cardiac output and minute ventilation increased when subjects breathed 50,000 ppm CO. However, when the subjects breathed 1,000 ppm CO, minute ventilation increased, but cardiac output did not. Coronary blood flow increased significantly in patients with noncoronary heart disease, but not in patients with coronary heart disease.

The effects of CO on coronary flow, heart rate, blood pressure, cardiac output, and myocardial oxygen consumption have been described in several studies. The results are somewhat contradictory, but most workers agree that coronary flow increases with exposure to CO (Adams et al. 1973; Young and Stone 1976). However, Einzig and coworkers (1980) demonstrated that whereas both right and left ventricular beds were dilated maximally at COHb levels of 41 percent, the ratio of subendocardial to subepicardial blood flow was reduced. The authors concluded that there is a pronounced underperfusion of the subendocardial layer of the left ventricle, in addition to the global hypoxia associated with CO poisoning. Horvath (1975) reported that coronary blood flow increased in healthy dogs as blood COHb levels increased, but coronary blood flow did not increase in animals with complete atrioventricular block. These results are provocative because they suggest an increased danger from low COHb levels in cardiac-disabled individuals.

Thus, the susceptibility of the cardiovascular system (especially the heart) to CO is well documented: CO increases coronary blood flow, has little effect on heart rate, and, depending on dosage, may decrease blood pressure and myocardial oxygen consumption (Traystman and Fitzgerald 1977; Sylvester et al. 1979). Cardiac output may be increased by high levels of CO, but it is unaffected by low-level exposure. However, most studies of the effects of CO on the cardiovascular system have been conducted for brief periods and have used high levels of CO. The studies reported here were conducted for prolonged periods using more realistic CO exposures.

SPECIFIC AIMS

The objectives of these studies were to determine whether or not CO exposure potentiates the effects of altitude and to determine those levels of CO and altitude at which changes occur. To accomplish this, animals were exposed for six weeks to various combinations of CO and altitude, and the chronic impact of these two stressors on the blood, heart, select organs, hemodynamic performance of the cardiovascular system, and blood gases were assessed.

Specific questions addressed include the following:

1. Does CO further exacerbate the loss in body weight observed at altitude?
2. Does CO further increase erythropoietic activity at altitude?
3. Does CO exacerbate the right ventricular hypertrophy observed at altitude?
4. Does CO cause left ventricular hypertrophy at high altitude, as it does at sea level?
5. Does CO alter hemodynamic performance at altitude?

MATERIALS AND METHODS

ANIMAL MODEL

The rat was used in these studies for several reasons: there is a large, appropriate data base on the response of the rat to chronic altitude and chronic CO exposure; the rat's cardiovascular responses to altitude and CO are similar in many ways to those of humans; it is easily housed in the specialized chambers required for this work; and sufficient numbers can be exposed to the study conditions to determine statistical significance.

The rats used in these studies were either male Fischer-344 (F344), chosen because they are a well-described strain used frequently in toxicological studies, or male Sprague-Dawley, chosen because their greater size facilitates certain surgical procedures; all animals were obtained from Sasco, Inc. (Omaha, NE). The animals were housed in shoebox cages (six per cage); they were fed a commercial diet (Purina Rat Chow, Ralston Purina Co.) and were provided tap water ad libitum. All animals were randomly assigned to treatment groups by a random-numbers table.

Two experimental designs were used. In the first, animals were exposed to four different conditions: ambient altitude (3,300 ft), ambient altitude plus CO, altitude, and altitude plus CO. In the second, animals at the same altitude were exposed to different CO concentrations to determine dose-response relationships. All exposures were continuous for six weeks, except when the chambers were opened, twice weekly for four hours, for animal maintenance. Although most exposures were conducted at the one-hour and eight-hour National Ambient Air Quality Standards for CO of 35 and 9 ppm, respectively, several exposures were conducted at higher CO concentrations (up to 500 ppm) to confirm effects reported by others (Penney et al. 1974a,b).

EXPOSURE SYSTEM

The altitude chamber system consisted of six cylindrical steel chambers connected to a central air supply duct (Figure 1). Each chamber was fitted with a clear, Lucite plastic door for illuminating and viewing the animals; the door was fitted with an O-ring to ensure a gas-tight seal. Air entered the system through a high-efficiency particulate air filter and flowed through each chamber at 55 l/min (16 air changes per hr). Altitude conditions, simulated by a water-sealed pump (Flui-vac, model A10, Atlantic Fluidic) and a system of adjustable globe valves, were measured by an aircraft altimeter. Carbon monoxide, provided in high-pressure, steel cylinders, was introduced into the air-intake side of each chamber via a mass-flow controller system (Matheson Gas, La Porte, TX). Chamber atmosphere was sampled sequentially for one hour from each chamber, including the control (0 ppm) chamber, via a switching solenoid (Chronotrol-Lindberg Enterprises, Inc., San Diego, CA). Carbon monoxide concentrations were measured continuously for one hour with a nondispersive infrared analyzer system (Beckman, Fullerton, CA) and were recorded continually on a strip-chart recorder (Houston Instruments, Austin, TX). The nominal and measured CO concentrations at each altitude are presented in Table 1.

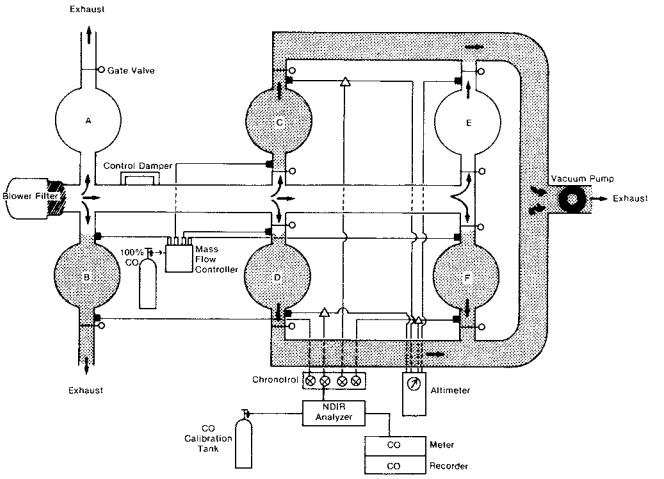

Figure 1. Altitude and CO exposure system.

ANALYTICAL METHODS

The final value for each CO-altitude exposure was achieved in accord with the schema presented in Figure 2. An altitude of 10,000 ft was attained in 2.5 minutes, held for 5 minutes, then increased in 5-minute increments until the desired altitude was achieved. Carbon monoxide concentrations reached 90 percent of their equilibrium values within 7.5 minutes and 100 percent within 10 minutes.

Each week the animals were weighed. Hematocrit and COHb concentrations were measured weekly throughout

Table 1. Chamber Carbon Monoxide Concentrations[a]

Altitude (ft)	9 ppm	35 ppm	50 ppm	100 ppm	500 ppm
3,300	9.0 ± 0.8	35.0 ± 1.0	49.9 ± 1.7	100.9 ± 1.4	501.0 ± 4.5
10,000	9.2 ± 0.7	35.2 ± 1.0	ND[b]	100.3 ± 1.8	ND
15,000	9.0 ± 0.5	ND	ND	100.1 ± 1.1	500.3 ± 2.8
18,000	ND	ND	50.2 ± 3.6	100.8 ± 3.4	500.9 ± 6.3

[a] Values are means ± SD.

[b] ND = not determined.

the exposure on blood samples obtained by snipping the tails immediately after the animals were removed from the chambers. Generally, hemoglobin concentration was also measured weekly. The COHb concentrations reported in Table 2 are those measurements taken at the end of the six-week exposure.

Two methods were used for the COHb determinations. In the first, based on the method of Small and coworkers (1971) and including modifications of Beeckmans (1967), a heparinized whole-blood sample, diluted 1:70 with a 0.04 percent ammonia solution, was placed in a 1-mm path length cuvette, and the absorbance was read on a spectrophotometer (Acta Beckman, Fullerton, CA) calibrated at 4 λ in the Soret band. Based on these measurements, the %COHb was calculated according to the Beer-Lambert law (Ewing 1960). In the second method, based on the method of Rodkey (1970), a gas chromatograph (Carle 211, Hach Company, Loveland, CO) was used to detect low levels of COHb. In this procedure, 100 µl blood was introduced via a gas-tight syringe into a reaction vessel containing 89 µl 1 percent citric acid monohydrate and 178 µl 10 percent sterox. After introducing the sample, 133 µl 3 percent potassium ferricyanide was added and the reaction was allowed to proceed for 10 minutes. At the end of this time, the reaction vessel was switched into the gas chromatograph system through a four-way valve. Carbon monoxide was separated from oxygen, nitrogen, and methane by a 6-ft molecular sieve 5A column operating at 100°C. Water, carbon dioxide, and organic substances that interfere with the performance of the column were removed by a liquid-nitrogen trap. The effluent from the column was mixed with hydrogen, catalytically reduced to methane over a nickel catalyst at 400°C, and passed through a flame ionization detector. The peak area in the chromatogram was determined by integration and, by comparing the peak area with a standard calibration curve, the amount of CO was determined. The concentration of CO in the blood, expressed as %COHb, was obtained from the following relationship:

$$\%COHb = A/Hb \times 1.39 \text{ (ml/g hemoglobin)}$$

where *A* is the volume of CO gas corrected to standard temperature and pressure (STP) conditions, and *Hb* is the amount of hemoglobin in the sample, as determined by the cyanmethemoglobin method. Hemoglobin (1.39 ml/g) is the conversion factor that accounts for the fact that 1 M of hemoglobin with a molecular weight of 64,458 combines with 4 × 22,414 ml of CO under STP conditions. The COHb concentrations at each altitude are presented in Table 2.

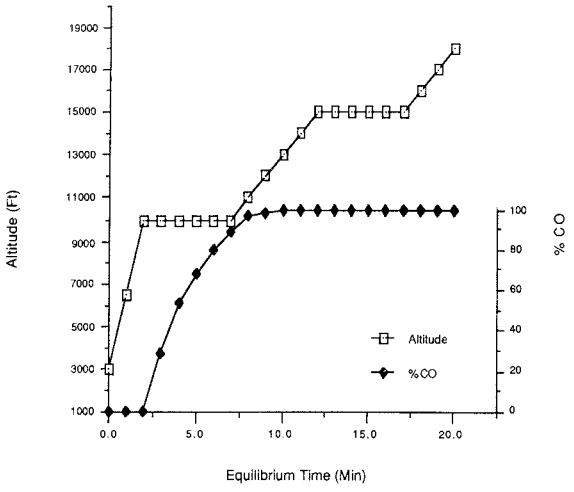

Figure 2. Schema used to reach both CO and altitude equilibria at the start of each experiment.

The animals were killed by an overdose of pentobarbital; the hearts were removed, and the atria, great vessels, and visible fat were trimmed away. The right ventricles were dissected away, and the left ventricles were opened and rinsed. Both ventricles were blotted dry on filter paper (Whatman No. 4) and weighed on a precision balance; weights of the right ventricles and left ventricles plus septa were recorded. The spleens, kidneys, and adrenal and pituitary glands were removed, blotted dry, and weighed. All organs were placed in a vacuum oven at 95°C and dried to constant weight. To correct for variations in animal size, all organ weights were normalized by individual animal body weight.

In the blood-gas studies, arterial pH, partial pressure of

Table 2. Percent Carboxyhemoglobin Levels Corresponding to Given Chamber Carbon Monoxide Concentrations and Altitudes[a]

	Carbon Monoxide Concentration					
Altitude (ft)	0 ppm	9 ppm	35 ppm	50 ppm	100 ppm	500 ppm
3,300	0.6 ± 0.1	0.9 ± 0.1	2.4 ± 0.2	3.7 ± 0.1	8.5 ± 0.4	40.0 ± 0.4
10,000	1.3 ± 0.2	1.8 ± 0.2	3.3 ± 0.2	ND[b]	9.4 ± 0.4	ND
15,000	1.7 ± 0.8	2.1 ± 0.1	ND	ND	10.0 ± 0.5	41.5 ± 0.5
18,000	1.9 ± 0.5	ND	ND	5.0 ± 0.4	10.2 ± 0.7	42.0 ± 0.7

[a] Values are means ± SD.

[b] ND = not determined.

carbon dioxide in the blood (P_{CO_2}) (Torr), and P_{O_2} (Torr) were measured at 37.5°C with a Radiometer blood-gas analyzer (model BMS2-MK3, Radiometer America, Westlake, OH) immediately after the blood samples were obtained. Arterial bicarbonate concentration ($[HCO_3^-]$, mM/l) was calculated with the Henderson-Hasselbalch equation (Peters and Van Slyke 1932). Each animal was removed from the exposure chamber and transferred to a Plexiglas box; to maintain the same blood-gas values for %COHb and P_{O_2} that were present during the exposure, the box was flushed with halothane anesthesia and the appropriate gas mixture.

To implant a catheter for blood sampling, the animals were removed from the box and switched to a face-mask system, by which flow and concentrations of the gas mixtures were maintained. The femoral artery was cannulated, and the skin and underlying tissue were sutured closed with stainless steel wound clips. The animals were given approximately one hour to recover, during which time they showed no visible signs of discomfort other than those associated with being confined in the box.

In the hemodynamic studies, the animals were anesthetized with pentobarbitol, heparinized, and ventilated with air. A midline thoracotomy was performed, and an electromagnetic-flow probe, calibrated previously, was positioned on the ascending aorta. A transducer, inserted in the femoral artery, recorded pressure changes, and signals from the transducer were transmitted to a Physiograph recorder (Beckman, Schiller Park, IL) and monitored with a Data Logging System (Buxco Electronics, Inc., Sharon, CT).

In the electrocardiogram (ECG) studies, a vented box was used to contain the rat and the apparatus required for recording the ECG. A vacuum line was attached to a port on top of the box and the box was placed in an aluminum Faraday cage. The animal and the apparatus could be manipulated via an opening in the side of the box; the opening was covered with a screen during the exposure. The rat was anesthetized with halothane administered via a nose cone; the cone, fabricated from silicone rubber, fit loosely over the rat's nose.

To record the ECG, the rat was placed on a platform and its legs were placed into containers filled with a 10 percent sodium chloride solution. Individual wires from three of the leg containers (right front, left front, and left rear) were fastened to a panel; the panel had connectors to which the corresponding leads from the ECG recorder were attached. The ground lead from the ECG recorder was attached to the Faraday cage.

The ECG recordings were taken in a standard sequence (lead I, lead II, lead III). The mean electrical axis obtained from the recordings of the three common leads was determined by the common triaxial reference system according to the method of Burch and Winsor (1953).

The results were analyzed by one-way analysis of variance (ANOVA) (dose-response studies) followed by Dunnett's test, or by two-way ANOVA. A p value less than 0.05 (two-tailed) was the minimal level of significance.

RESULTS

Carboxyhemoglobin increased with increasing CO concentrations. Nine ppm CO produced a COHb concentration of 0.9 ± 0.1 in rats (measured with a gas chromatograph). The levels of COHb (measured with a spectrophotometer) at 35, 100, and 500 ppm CO were 2.4 ± 0.2, 8.5 ± 0.4, and 40.0 ± 0.4 percent, respectively (Figure 3, Table 2).

OVERVIEW

Table 3 presents all significant changes observed at or below 15,000-ft altitude and 100 ppm CO. Body weight decreased significantly in animals exposed to 15,000-ft altitude. There was no significant difference in body weight due to CO or CO-altitude interaction up to 500 ppm CO and

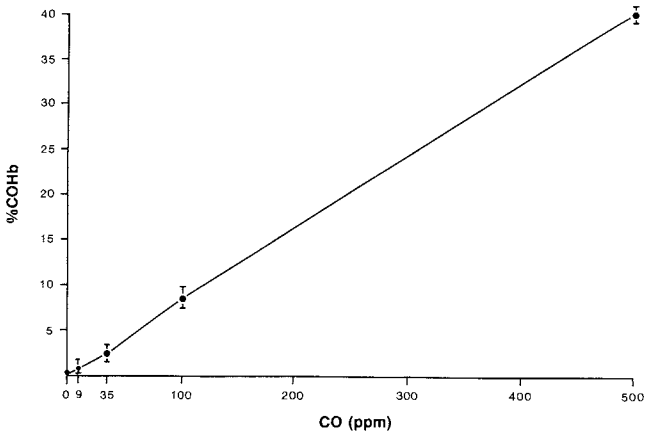

Figure 3. Carboxyhemoglobin concentrations in rats exposed to CO.

18,000-ft altitude. At these levels of CO and altitude, body weight decreased and several animals died.

Hematocrit increased with increasing CO concentrations and with increasing altitude (Figure 4). Hematocrit was significantly higher at 100 ppm CO and above, and at 10,000 ft and above; the interaction between 500 ppm CO and altitude had a significant effect on hematocrit.

Right ventricle weight increased with increasing altitude (Figure 5). In general, right ventricle weight was unaffected by CO exposures up to 500 ppm. In one experiment, however, right ventricle weight was increased by exposure to 100 ppm CO (see, for example, Table 9). The effect of CO-altitude interaction at 15,000 ft and 100 ppm CO was significant. Generally, CO in concentrations less than 100 ppm had no significant effect on right ventricle weight at any altitude.

Left ventricle weight was generally unaffected by altitudes less than 15,000 ft (Figure 6). There was no significant difference in left ventricle weight between groups due to CO or CO-altitude interaction up to 100 ppm CO. Left ventricle weight significantly increased in the groups exposed to 100 ppm CO and 3,300 ft; CO in concentrations below 100 ppm had little effect on left ventricle weight at any altitude.

Total heart weight increased with altitudes in excess of 10,000 ft (Figure 7). There were no significant differences in total heart weight up to 100 ppm CO in the animals exposed to 3,300-ft altitude. Total heart weight was greater at 500 ppm CO in both the 3,300- and 15,000-ft groups.

EXPOSURE TO 35 AND 100 PPM CARBON MONOXIDE AND 10,000-FT ALTITUDE

No significant differences in body weight were caused by 10,000-ft altitude or by 35 or 100 ppm CO (Tables 4 through

Table 3. Summary of Significant Changes at 10,000 and 15,000 Feet and 35 and 100 ppm Carbon Monoxide

Treatment	Increased	Decreased
10,000 ft	Hematocrit Hemoglobin Right ventricle weight	
15,000 ft	Hematocrit Hemoglobin Right ventricle weight Heart weight	Body weight
35 ppm CO	Hemoglobin	
100 ppm CO	Hematocrit Left ventricle plus septum weight Heart weight	
10,000 ft + 35 ppm CO	Hemoglobin further than 10,000 ft alone	
10,000 ft + 100 ppm CO	Hematocrit further than 10,000 ft alone Hemoglobin further than 10,000 ft alone	
15,000 ft + 100 ppm CO	Right ventricle weight further than 15,000 ft alone Heart weight further than 15,000 ft alone	

8). Hematocrit increased with exposure to 10,000 ft, and with exposure to 100 ppm CO. Only in the 100-ppm CO exposure group did the altitude-CO interaction increase the hematocrit. Hemoglobin concentration was generally increased by altitude, by 35 ppm CO, and by CO-altitude interaction.

Although altitude increased right ventricle weight significantly, 35 ppm CO did not affect right ventricle weight, nor

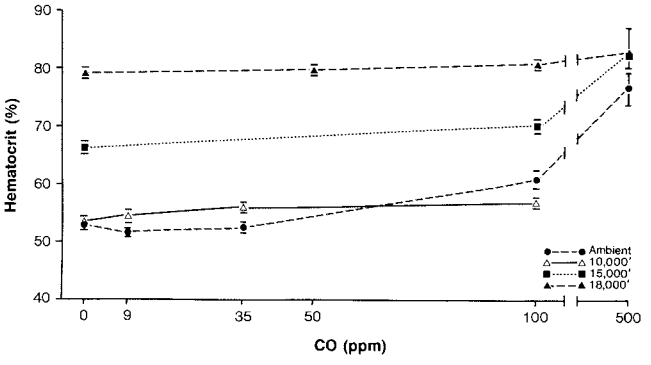

Figure 4. Hematocrit in rats exposed to CO at altitude.

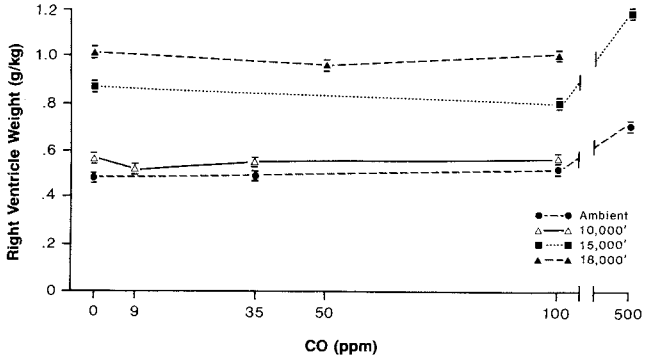

Figure 5. Right ventricle weights in rats exposed to CO at altitude.

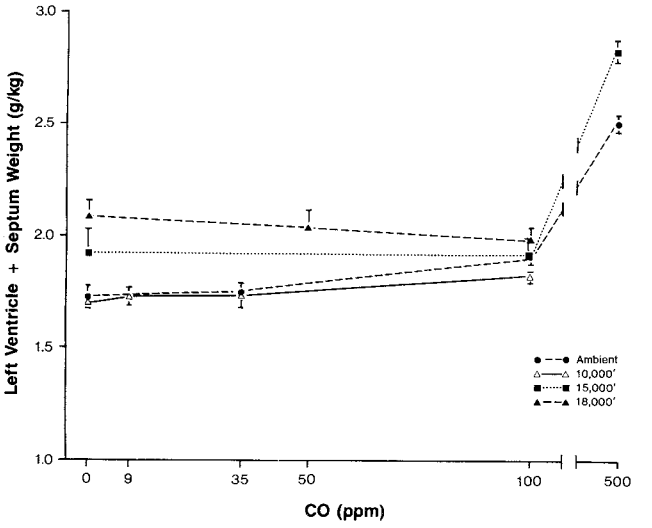

Figure 6. Left ventricle plus septum weights in rats exposed to CO at altitude.

did it exacerbate the effects of altitude on right ventricle weight.

No consistent changes were attributable to CO in any of the hemodynamic parameters examined (Appendix A, Tables A.1 through A.4); there were no consistent changes in heart rate, systolic pressure, diastolic pressure, mean arterial pressure, pulse pressure, cardiac index, or peripheral resistance. Cardiac output and cardiac index were generally depressed by altitude, but these changes were not exacerbated by CO.

EXPOSURE TO 100 PPM CARBON MONOXIDE AND 15,000-FT ALTITUDE

Body weight decreased significantly with exposure to 15,000-ft altitude (Tables 9 through 11). There were no significant differences in body weight between groups due to CO or CO-altitude interaction. Hematocrit was increased by

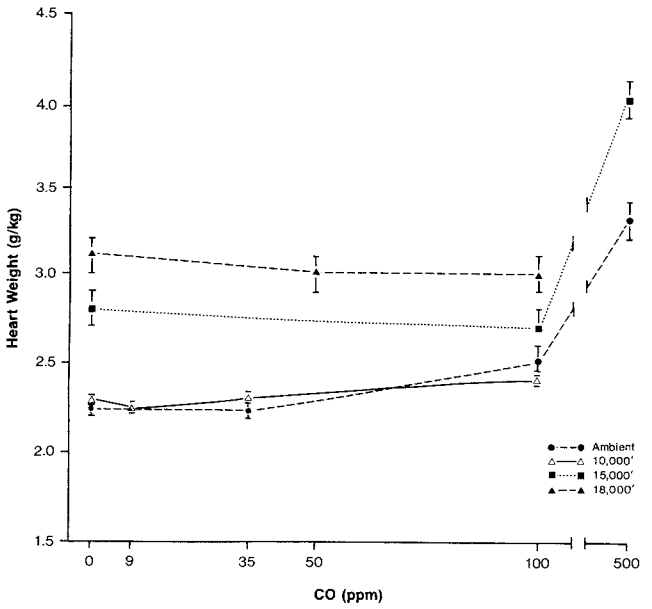

Figure 7. Total heart weight in rats exposed to CO at altitude.

100 ppm CO and by 15,000-ft altitude; there was no significant interaction between CO and altitude on hematocrit. Hemoglobin concentration was increased by altitude. We observed no significant differences in hemoglobin concentration caused by CO or CO-altitude interaction.

Right ventricle weight was generally increased by CO, altitude, and CO-altitude interaction; left ventricle weight was increased by CO, but generally unaffected by altitude or by CO-altitude interaction. Total heart weight was higher in the altitude groups, and tended to be increased by CO and by CO-altitude interaction; however, the differences were significant in only one replicate.

Altitude significantly decreased the P_{O_2}, the P_{CO_2}, and the level of bicarbonate in the blood, but had no effect on pH. Carbon monoxide increased P_{CO_2} significantly and decreased blood pH significantly, but had no effect on P_{O_2} or bicarbonate levels. There was a significant effect of CO-altitude interaction on blood bicarbonate level, but not on P_{O_2}, P_{CO_2}, or pH.

Electrocardiograms were taken on rats exposed to 15,000-ft altitude and 100 ppm CO, and the effects on the mean electrical axis of the heart were assessed. The mean electrical axis was shifted to the left by CO, but to the right by altitude and altitude plus CO; only the right shift by altitude was statistically significant (Table 12).

There were no consistent differences in heart rate due to altitude or CO (Tables A.5 and A.6); in one study, the effect of CO-altitude interaction on heart rate was significant (Table A.5). Systolic and diastolic pressures tended to be

Table 4. Experiment 1: Morphological Parameters in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)
1. 3,300 ft + 0 ppm CO	266.6 ± 8.5 (6)	53.3 ± 0.9 (6)	17.9 ± 0.7 (6)	0.4780 ± 0.0130 (6)	1.7408 ± 0.0601 (6)	2.2185 ± 0.0660 (6)
2. 10,000 ft + 0 ppm CO	269.5 ± 10.8 (6)	55.5 ± 2.0[b] (6)	18.6 ± 0.9 (5)	0.5721 ± 0.0442[b] (6)	1.6040 ± 0.0433[b] (6)	2.1762 ± 0.0856 (6)
3. 3,300 ft + 35 ppm CO	274.2 ± 7.0 (5)	53.3 ± 0.5 (6)	17.7 ± 1.5 (6)	0.4788 ± 0.0258 (5)	1.7577 ± 0.0292 (5)	2.2366 ± 0.0423 (5)
4. 10,000 ft + 35 ppm CO	253.5 ± 6.6 (6)	56.6 ± 0.9 (6)	18.5 ± 1.3 (6)	0.5866 ± 0.0395 (6)	1.6843 ± 0.0212 (6)	2.2709 ± 0.0392 (6)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

Table 5. Experiment 2: Morphological Parameters in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)
1. 3,300 ft + 0 ppm CO	358.0 ± 52.0	50.0 ± 2.0	17.3 ± 1.0	0.554 ± 0.098	1.754 ± 0.073	2.308 ± 0.117
2. 10,000 ft + 0 ppm CO	342.0 ± 39.0	51.0 ± 2.0	20.1 ± 0.8[b]	0.705 ± 0.100[b]	1.800 ± 0.110	2.505 ± 0.110[b]
3. 3,300 ft + 35 ppm CO	328.0 ± 61.0	51.0 ± 2.0	19.1 ± 0.6[c]	0.537 ± 0.040	1.801 ± 0.063	2.338 ± 0.080
4. 10,000 ft + 35 ppm CO	310.0 ± 54.0	54.0 ± 3.0	20.0 ± 1.2[d]	0.619 ± 0.080	1.872 ± 0.127	2.491 ± 0.190

[a] Values are means ± SD. Six animals were used for each value.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

[c] Significant CO effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 2 vs. treatments 3 and 4).

[d] Significant altitude-CO interaction ($p < 0.05$ by two-way ANOVA for treatments 1 and 4 vs. treatments 2 and 3).

Table 6. Experiment 3: Morphological Parameters in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)
1. 3,300 ft + 0 ppm CO	296.7 ± 25.0 (12)	52.8 ± 2.4 (12)	16.2 ± 0.7 (12)	0.445 ± 0.013 (5)	1.691 ± 0.005 (5)	2.214 ± 0.015 (5)
2. 10,000 ft + 0 ppm CO	297.5 ± 13.6 (12)	56.4 ± 1.8[b] (12)	17.2 ± 0.9[b] (12)	0.514 ± 0.014[b] (6)	1.650 ± 0.044 (6)	2.163 ± 0.041 (6)
3. 3,300 ft + 35 ppm CO	299.4 ± 23.0 (12)	53.9 ± 1.0 (12)	16.8 ± 0.6 (12)	0.476 ± 0.015 (12)	1.680 ± 0.026 (12)	2.156 ± 0.038 (12)
4. 10,000 ft + 35 ppm CO	305.1 ± 32.8 (11)	58.3 ± 4.1 (11)	17.3 ± 0.8 (11)	0.517 ± 0.011 (11)	1.624 ± 0.019 (11)	2.141 ± 0.023 (11)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

higher in the altitude groups (Table A.5). There were no differences in systolic or diastolic pressures due to CO. Mean arterial pressure tended to be elevated by altitude, but there was no significant difference in mean arterial pressure due to CO or CO-altitude interaction. Pulse pressure was depressed by altitude in one experiment (Table A.6). There were no significant differences in pulse pressure due to CO or to CO-altitude interaction.

Cardiac output was lower in the altitude groups, but there were no significant differences in cardiac output due to CO or to CO-altitude interaction.

Peripheral resistance was significantly higher in the altitude-exposed groups. There were no significant differences in peripheral resistance due to CO, or to CO-altitude interaction.

Table 7. Experiment 4: Body Weight and Hematocrit in Sprague-Dawley Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)
3,300 ft + 0 ppm CO	403.8 ± 23.1	53.2 ± 1.3
10,000 ft + 0 ppm CO	407.8 ± 9.7	54.7 ± 1.0[b]
10,000 ft + 9 ppm CO	375.8 ± 53.6	58.1 ± 2.3[b]
10,000 ft + 35 ppm CO	405.0 ± 20.3	59.7 ± 2.8[b]
10,000 ft + 100 ppm CO	365.7 ± 20.5	61.5 ± 3.7[c]

[a] Values are means ± SD. Six animals were used for each value.

[b] Significant compared to measurement made at 3,300 ft + 0 ppm CO ($p < 0.05$ by one-way ANOVA).

[c] Significant compared to measurement made at 10,000 ft + 0 ppm CO ($p < 0.05$ by one-way ANOVA).

EXPOSURE TO 500 PPM CARBON MONOXIDE AND 15,000-FT ALTITUDE

Body weight was decreased significantly by 15,000-ft altitude (Table A.7). There were no significant differences in body weight between groups due to CO or to CO-altitude interaction. Hematocrit was increased significantly by 500 ppm CO and by 15,000-ft altitude. The interaction between CO and altitude also had a significant effect on hematocrit.

Right ventricle weight increased significantly by CO and by altitude; there was no significant effect of altitude-CO interaction on right ventricle weight. Left ventricle weight also increased significantly with CO and with altitude; there were no significant differences in left ventricle weight due to CO-altitude interaction. Total heart weight increased also significantly with altitude and with CO; again, there was no significant effect of altitude-CO interaction on total heart weight.

Kidney and spleen weights were increased significantly by altitude and by CO, but there was no significant effect of altitude-CO interaction on kidney or spleen weight. Adrenal gland weight increased significantly by altitude; there were no significant differences in pituitary or adrenal gland weight due to CO or to CO-altitude interaction.

EXPOSURE TO 0, 50, 100, AND 500 PPM CARBON MONOXIDE AND 18,000-FT ALTITUDE

Since this altitude has such a pronounced effect on body weight, all parameters (except body weight and hematocrit) were tested against weight-matched controls for statistical significance. Body weight of animals exposed to 18,000-ft altitude plus CO were tested for significance against animals exposed to 18,000 ft with 0 ppm CO; although body weight tended to be lower, the difference in body weight was statistically significant only in the group exposed to 18,000-ft altitude plus 500 ppm CO (Table A.8). There were no statistically significant differences in hematocrit among groups exposed to altitude, altitude plus 50 ppm CO, altitude plus 100 ppm CO, and altitude plus 500 ppm CO.

Right ventricle and total heart weights were significantly greater in the altitude and altitude plus CO groups, but left ventricle weight was unchanged.

The effects of altitude and CO on cardiac muscle vascularity were assessed. At 18,000 ft, coronary capillarity was increased. However, this increase in capillarity was attenuated by exposure to 50, 100, and 500 ppm CO. At 15,000 ft, there was no increase in coronary capillarity and no effect of CO.

There were no statistically significant differences in heart rate (Table A.9) or systolic pressure among groups. Changes among the groups included increased diastolic pressure at 50 and 100 ppm CO, and increased mean arterial pressure at 0, 50, and 100 ppm CO. Although there was a

Table 8. Experiment 5: Morphological Parameters in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)
10,000 ft + 0 ppm CO	248.3 ± 21.3	53.5 ± 2.4	0.558 ± 0.037	1.800 ± 0.048	2.359 ± 0.051
10,000 ft + 9 ppm CO	249.2 ± 20.9	55.8 ± 2.1	0.526 ± 0.032	1.724 ± 0.094	2.250 ± 0.098
10,000 ft + 35 ppm CO	269.5 ± 17.0	54.8 ± 1.7	0.514 ± 0.025[b]	1.741 ± 0.139	2.254 ± 0.157
10,000 ft + 100 ppm CO	263.5 ± 36.5	57.3 ± 0.6[b]	0.571 ± 0.048	1.820 ± 0.081	2.390 ± 0.084

[a] Values are means ± SD. Six animals were used for each value.

[b] Significant compared to measurement made at 10,000 ft + 0 ppm CO ($p < 0.05$ by one-way ANOVA).

Table 9. Experiment 6: Body Weight, Heart Weight, Hematocrit, and Blood Gases in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH	Bicarbonate (mEq/l)
1. 3,300 ft + 0 ppm CO	249.3 ± 4.2 (12)	52.4 ± 0.6 (12)	0.409 ± 0.044 (6)	1.684 ± 0.067 (6)	2.093 ± 0.107 (6)	78.3 ± 4.2 (20)	34.5 ± 3.44 (20)	7.455 ± 0.022 (20)	23.7 ± 2.2 (20)
2. 15,000 ft + 0 ppm CO	207.0 ± 7.0[b] (12)	65.3 ± 0.7[b] (12)	0.820 ± 0.046[b] (6)	1.785 ± 0.047 (6)	2.605 ± 0.065[b] (6)	44.3 ± 5.1[b] (16)	23.6 ± 3.81[b] (16)	7.466 ± 0.025 (16)	16.6 ± 2.1[b] (16)
3. 3,300 ft + 100 ppm CO	239.0 ± 2.7 (12)	56.8 ± 0.7[c] (12)	0.584 ± 0.019[c] (6)	2.082 ± 0.071[c] (6)	2.666 ± 0.81[c] (6)	77.6 ± 4.5 (22)	35.5 ± 3.3[c] (22)	7.436 ± 0.024[c] (22)	23.3 ± 2.1 (22)
4. 15,000 ft + 100 ppm CO	209.7 ± 5.8 (12)	68.8 ± 0.7 (12)	0.825 ± 0.062[d] (6)	1.921 ± 0.088 (6)	2.745 ± 0.117[d] (6)	43.3 ± 2.2 (5)	28.3 ± 0.8 (5)	7.449 ± 0.023 (5)	19.0 ± 1.1[d] (5)

[a] Values are means ± SD. Number of animals is shown in parentheses.
[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).
[c] Significant CO effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 2 vs. treatments 3 and 4).
[d] Significant altitude-CO interaction ($p < 0.05$ by two-way ANOVA for treatments 1 and 4 vs. treatments 2 and 3).

tendency for pulse pressure to be reduced in the altitude and altitude plus CO groups, there were no statistically significant differences in pulse pressure among groups.

Cardiac output was unchanged among groups, but tended to be lower in the altitude and altitude plus CO groups.

There were no statistically significant differences in peripheral resistance among groups. There was, however, a tendency for peripheral resistance to be elevated in the altitude and altitude plus CO groups.

CARBOXYHEMOGLOBIN STUDIES AT 9 PPM CARBON MONOXIDE AND 10,000- AND 15,000-FT ALTITUDE

At 10,000 ft (Table 13, Figure 8), there was no significant difference in hemoglobin concentration caused by altitude, CO, or CO-altitude interaction, but COHb level increased significantly with altitude, CO, and CO-altitude interaction.

At 15,000 ft, the differences in hemoglobin and COHb concentrations caused by altitude were significant. There was no significant difference in hemoglobin concentration because of CO, but COHb level was significantly higher in the CO group than in the 0 ppm CO group. There was no significant effect of CO-altitude interaction on hemoglobin concentration or COHb level.

CARBOXYHEMOGLOBIN FORMATION UNDER HYPOXIC VERSUS ALTITUDE CONDITIONS

Fischer-344 (F344) rats were exposed to 500 ppm CO either in air (normoxia) or in 10 percent oxygen (hypoxia). The results of these experiments are presented in Table 14. Carboxyhemoglobin levels in rats exposed to 500 ppm CO during hypoxia were significantly higher ($p < 0.05$) than those in rats exposed during normoxia. There was no significant difference between COHb levels in rats exposed to 500 ppm CO at 3,300 ft and those exposed at 15,000 ft.

DISCUSSION AND CONCLUSIONS

A major aim of these studies was to determine if high-altitude residents are at greater risk from the effects of CO than are lowland residents. The basis for concern is that both altitude and CO reduce the amount of oxygen that is available to the tissues. At altitude, the P_{O_2} in the inspired air is reduced; this causes a drop in the P_{O_2} gradient from tracheal air to mixed venous blood and causes tissue hypoxia (hypoxic hypoxia). On the other hand, inhaled CO produces tissue hypoxia by binding with hemoglobin to

Table 10. Experiment 6: Morphological Parameters in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)
1. 3,300 ft + 0 ppm CO	268.2 ± 5.5	55.8 ± 1.0	18.5 ± 0.5	0.449 ± 0.040	1.832 ± 0.160	2.218 ± 0.018
2. 15,000 ft + 0 ppm CO	227.2 ± 2.8[b]	68.4 ± 0.7[b]	22.4 ± 0.7[b]	0.796 ± 0.040[b]	1.963 ± 0.220	2.759 ± 0.250[b]
3. 3,300 ft + 100 ppm CO	266.8 ± 5.4	57.3 ± 0.2[c]	19.6 ± 0.6	0.513 ± 0.050	1.975 ± 0.230	2.488 ± 0.244
4. 15,000 ft + 100 ppm CO	232.2 ± 3.5	69.8 ± 0.6	22.1 ± 0.9	0.726 ± 0.090[d]	2.021 ± 0.200	2.747 ± 0.220

[a] Values are means ± SD. Six animals were used for each value.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

[c] Significant CO effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 2 vs. treatments 3 and 4).

[d] Significant altitude-CO interaction ($p < 0.05$ by two-way ANOVA for treatments 1 and 4 vs. treatments 2 and 3).

Table 11. Experiment 8: Morphological Parameters in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg/bw)
1. 3,300 ft + 0 ppm CO	474.9 ± 36.9 (12)	54.6 ± 5.0 (11)	0.526 ± 0.060 (6)	1.755 ± 1.155 (6)	2.282 ± 0.209 (6)
2. 15,000 ft + 0 ppm CO	443.4 ± 32.8[b] (12)	66.3 ± 3.2[b] (12)	0.876 ± 0.156[b] (6)	1.671 ± 0.098 (6)	2.547 ± 0.195[b] (6)
3. 3,300 ft + 100 ppm CO	472.0 ± 35.9 (12)	59.9 ± 3.7[c] (12)	0.485 ± 0.029 (6)	1.762 ± 0.100 (6)	2.248 ± 0.099 (6)
4. 15,000 ft + 100 ppm CO	440.3 ± 23.4 (12)	67.9 ± 2.4 (12)	0.829 ± 0.107 (6)	1.676 ± 0.185 (6)	2.501 ± 0.266 (6)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

[c] Significant CO effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 2 vs. treatments 3 and 4).

form COHb; COHb reduces the oxygen-carrying capacity of the blood and releases oxygen to the tissues less readily (anemic hypoxia).

An exposure period of six weeks was chosen because previous studies have shown that changes in hematology and cardiac morphology caused by altitude or CO are complete by this time (Jaeger and McGrath 1974, 1975; Penney et al. 1979). Most experiments consisted of four exposure groups: ambient, altitude, ambient plus CO, and altitude plus CO. This design enabled us to determine which effects were attributable to altitude, CO, or CO-altitude interaction. However, few consistent changes were noted using this de-

Table 12. Experiment 9: Mean Electrical Axes in F344 Rats[a]

Treatment	Mean Electrical Axis (degrees)
1. 3,300 ft + 0 ppm CO	40.5 ± 31.5
2. 15,000 ft + 0 ppm CO	67.1 ± 12.5[b]
3. 3,300 ft + 100 ppm CO	27.0 ± 16.6
4. 15,000 ft + 100 ppm CO	65.0 ± 35.7

[a] Values are means ± SD. Twelve animals were used for each value.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

Table 13. Experiments 12 and 13: Hemoglobin and Carboxyhemoglobin Concentrations in F344 Rats[a]

Treatment	Hemoglobin (g/dl)	Carboxy-hemoglobin (%)
Experiment 12		
1. 3,300 ft + 0 ppm CO	18.4 ± 1.3	0.7 ± 0.1
2. 10,000 ft + 0 ppm CO	17.7 ± 3.0	1.3 ± 0.2[b]
3. 3,300 ft + 9 ppm CO	18.9 ± 1.6	1.0 ± 0.1[c]
4. 10,000 ft + 9 ppm CO	18.6 ± 3.1	1.8 ± 0.2[d]
Experiment 13		
1. 3,300 ft + 0 ppm CO	17.5 ± 0.7	0.6 ± 0.0
2. 15,000 ft + 0 ppm CO	22.6 ± 1.7[b]	1.7 ± 0.8[b]
3. 3,300 ft + 9 ppm CO	18.5 ± 0.8	0.9 ± 0.1[c]
4. 15,000 ft + 9 ppm CO	23.4 ± 1.5	2.1 ± 0.1

[a] Values are means ± SD. Six animals were used for each value.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

[c] Significant CO effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 2 vs. treatments 3 and 4).

[d] Significant altitude-CO interaction ($p < 0.05$ by two-way ANOVA for treatments 1 and 4 vs. treatments 2 and 3).

sign, and an effort was made to determine if a standard dose-response paradigm, in which animals are exposed to a single altitude and increasing doses of CO, might be a more sensitive model to detect interactions between altitude and CO and to determine a threshold at which CO effects would become measurable. There were no CO-related changes observed at less than 100 ppm CO, using the dose-response design.

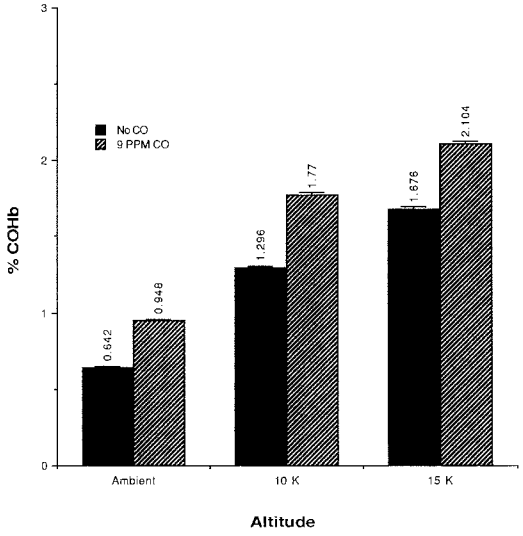

Figure 8. Carboxyhemoglobin concentrations in rats at ambient (3,300-ft), 10,000-, or 15,000-ft altitude and breathing 0 or 9 ppm CO.

Table 14. Experiment 14: Carboxyhemoglobin Levels in F344 Rats Exposed to 500 ppm Carbon Monoxide in Air (Normoxia) or 500 ppm in 10 Percent Oxygen (Hypoxia) and to 500 ppm Carbon Monoxide at 3,300 ft or 15,000 ft[a]

	Percent Carboxyhemoglobin
Normoxia	39.9 ± 2.1
Hypoxia	49.3 ± 1.5[b]
3,300 ft	35.1 ± 1.7
15,000 ft	35.3 ± 3.3

[a] Values are means ± SD.

[b] $p < 0.05$ by Student's t test.

The animals in this study responded to altitude or CO in accord with observations reported previously. Thus, body weight was significantly lower in animals exposed to altitudes in excess of 10,000 ft, but were not affected by exposure to CO in concentrations up to 500 ppm. These observations were anticipated, and are in accord with those reported earlier (McGrath et al. 1973; Chen et al. 1984; Penney 1984). The decreased body weight in altitude-exposed animals is caused by both decreased caloric intake and decreased food utilization (Blume 1983). There was no effect on body weight from CO-altitude interaction; this indicates that CO does not enhance the effect of altitude on caloric intake and food utilization.

Laboratory rats, as well as humans, adapt to long-term hypoxia (caused by altitude or CO) by increasing the oxygen-carrying capacity of the blood. Oxygen-carrying capacity (as measured by hematocrit and hemoglobin concentration) was significantly higher in rats exposed to altitudes in excess of 10,000 ft or to CO concentrations in excess of 35 ppm. These observations were also anticipated, and are in accord with those reported earlier (Musselman et al. 1959; Penney et al. 1974b). The oxygen-carrying capacity of the blood was further increased by CO-altitude interaction. This effect was significant at 10,000 ft and 35 ppm CO. Increasing the oxygen-carrying capacity of the blood in response to chronic hypoxia is thought generally (but not exclusively) to be advantageous at high altitude. Since the hematocrit affects blood flow to the tissues and the oxygen-carrying capacity of blood, very high hematocrits can decrease blood flow to the tissues and can more than offset the increased oxygen-carrying capacity. It is unlikely that the small changes in oxygen-carrying capacity noted at 10,000 ft and 100 ppm CO and below were detrimental.

Constriction of the pulmonary vasculature, pulmonary

hypertension, and consequent hypertrophy of the right ventricle are well-described responses to altitude in humans, as well as in laboratory rats (Grover et al. 1963). The pulmonary hypertension, if excessive, may result in life-threatening pulmonary edema. Right ventricle weight was significantly higher in rats exposed to 10,000 ft or more; these observations were anticipated and are in accord with those reported earlier (Jaeger and McGrath 1973, 1975; Penney et al. 1974a). There were no biologically significant effects on right ventricle weight due to CO in concentrations up to 500 ppm or to CO-altitude interaction; this indicates that CO does not enhance the constricting effect of altitude on the pulmonary vasculature.

Left ventricle weight in laboratory rats, as well as in humans, is not increased by altitude (Van Liere and Stickney 1963; Heath and Williams 1981). Left ventricle weight in rats exposed to any altitude or to CO concentrations up to 100 ppm was not significantly different from that of control rats. These results confirm those of Penney and coworkers (1974a,b), who reported cardiac enlargement in rats exposed to 500 ppm CO and predicted the threshold for cardiac enlargement to be near 200 ppm CO. These workers conclude that the major factor responsible for the CO-induced cardiomegaly is the greater work required to maintain adequate oxygenation of the tissues at high concentrations of CO, and that CO produces a volume overload, rather than a pressure overload, on the heart.

There were few changes in any of the hemodynamic parameters measured. In general, altitude tends to decrease cardiac output and increase peripheral resistance. There were no significant effects on any of these parameters caused by CO in concentrations below 100 ppm.

The most striking observation made in this study was the effect of altitude on COHb level in the absence of exogenous CO. Carboxyhemoglobin levels in animals at altitude are influenced by at least three factors: competition between oxygen and CO for the hemoglobin molecule; increased erythropoietic activity; and an increase in the volume of circulating hemoglobin. At altitude, inspired P_{O_2} is reduced, but endogenous CO production remains the same (or may be increased because of the increased erythropoietic activity that occurs with six weeks of exposure to altitude). The reduced P_{O_2} alters the Haldane relationship so that CO is favored in the competition with oxygen for the hemoglobin molecule. Thus, COHb levels were higher in animals that breathed air at 10,000 and 15,000 ft than they were in animals that breathed air or 9 ppm CO at 3,300 ft. The other factors (increased erythropoietic activity and increased circulating hemoglobin volume) would undoubtedly affect COHb levels, but the design of this experiment did not permit an assessment of their individual influences.

IMPLICATIONS OF THE FINDINGS

These results indicate that there are few consistent morphological, hemodynamic, or hematological alterations in healthy rats, exposed for six weeks to altitudes of 10,000 ft or less and CO concentrations of 100 ppm or less, that cannot be explained by altitude alone. The results are in agreement with findings of other workers (Stupfel and Bouley 1970; Eckhardt et al. 1972; Penney et al. 1974a,b; Penney and Bishop 1978; James et al. 1979), as reviewed by McGrath (1982).

There are, however, several aspects of this study that bear on regulatory issues. Carboxyhemoglobin concentration is viewed as being "the best index of CO exposure" (U.S. Environmental Protection Agency 1979), and most of the regulatory effort regarding CO is directed toward regulating the CO exposure that causes a given COHb level. Endogenous CO production accounts for some 0.5 to 0.9 %COHb at sea level (Luomanmaki and Coburn 1969). Ascending to altitude, in the absence of CO, results in a higher COHb concentration because inspired P_{O_2} (and thus arterial P_{O_2}) is reduced, whereas CO from endogenous sources is at least constant (and may be elevated). Thus, COHb formation is favored, and higher levels of COHb are attained at altitude even if endogenous production of CO is constant. The problem has been reviewed in detail by Collier and Goldsmith (1983).

With increased time spent at altitude, however, erythropoiesis (and presumably red cell destruction) attains a higher equilibrium value and results in a higher hemoglobin concentration. Increased red blood cell destruction provides a greater amount of endogenously produced CO (tending to increase COHb levels), but the increased red blood cell volume provides a larger hemoglobin pool in which the endogenously produced CO may be dissipated (tending to lower COHb levels).

The time course affecting these factors and COHb levels at altitude would also differ. The first factor (immediate ascent to altitude with decreased inspired P_{O_2} and constant endogenous CO production) would act to increase COHb levels immediately. The second and third factors (chronic exposure to altitudes with increased erythropoiesis and red cell destruction, and the increase in size of the circulating hemoglobin pool) would become apparent after several weeks at high altitudes, when a new hemoglobin equilibrium level had become established.

The foregoing observations also suggest that humans with clinical conditions characterized by decreased arterial P_{O_2} and elevated circulating hemoglobin levels (for example, emphysema, congenital heart defects) may be especially sensitive to CO.

In any event, the COHb level for which the National Ambient Air Quality Standard for CO of 9 ppm is established for sea-level residents will be reached more quickly by people at higher altitudes because of their greater initial body burden of COHb.

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APPENDIX A. Cardiovascular and Hemodynamic Parameters

Table A.1. Experiment 1: Cardiovascular and Hemodynamic Parameters in F344 Rats[a]

Treatment	Heart Rate (beats/min)	Systolic Pressure (Torr)	Diastolic Pressure (Torr)	Mean Arterial Pressure (Torr)	Pulse Pressure (Torr)	Cardiac Output (ml/min)	Cardiac Index (ml/min/kg)	Peripheral Resistance (mm Hg/ml/min)
1. 3,300 ft + 0 ppm CO	453.8 ± 37.4	135.0 ± 31.9	83.3 ± 35.1	101.6 ± 33.5	54.7 ± 13.7	45.4 ± 12.7	138.8 ± 46.5	2.3 ± 0.8
2. 10,000 ft + 0 ppm CO	439.8 ± 21.8	134.5 ± 18.5	93.3 ± 17.1	107.1 ± 16.8	41.2 ± 11.3[b]	38.3 ± 2.5	115.7 ± 17.7	2.9 ± 0.8
3. 3,300 ft + 35 ppm CO	468.0 ± 33.3	150.2 ± 19.6	109.5 ± 19.9	123.1 ± 19.4	40.7 ± 8.8[c]	45.7 ± 8.4	130.8 ± 33.5	2.7 ± 0.5
4. 10,000 ft + 35 ppm CO	441.5 ± 16.3	129.5 ± 32.3	97.2 ± 19.7	107.9 ± 23.6	32.3 ± 14.6	33.7 ± 12.8	102.7 ± 37.7	3.7 ± 1.7

[a] Values are means ± SD. Six animals were used for each value.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

[c] Significant CO effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 2 vs. treatments 3 and 4).

Table A.2. Experiment 2: Cardiovascular and Hemodynamic Parameters in F344 Rats[a]

Treatment	Heart Rate (beats/min)	Systolic Pressure (Torr)	Diastolic Pressure (Torr)	Mean Arterial Pressure (Torr)	Pulse Pressure (Torr)	Cardiac Output (ml/min)	Cardiac Index (ml/min/kg)	Peripheral Resistance (mm Hg/ml/min)
1. 3,300 ft + 0 ppm CO	455.0 ± 19.4 (6)	147.0 ± 25.7 (6)	106.0 ± 24.2 (6)	119.7 ± 24.4 (6)	41.0 ± 9.9 (6)	36.1 ± 9.1 (6)	100.8 ± 29.7 (6)	3.4 ± 0.6 (6)
2. 10,000 ft + 0 ppm CO	451.2 ± 67.2 (6)	136.5 ± 26.4 (6)	97.7 ± 29.4 (6)	110.6 ± 28.1 (6)	38.8 ± 9.5 (6)	29.6 ± 6.1[b] (6)	84.0 ± 15.5[b] (6)	3.8 ± 1.2 (6)
3. 3,300 ft + 35 ppm CO	484.4 ± 36.4 (6)	154.6 ± 36.5 (5)	108.4 ± 28.7 (5)	123.8 ± 30.9 (5)	46.2 ± 12.9 (5)	36.6 ± 5.6 (5)	119.8 ± 11.1 (5)	3.4 ± 0.8 (5)
4. 10,000 ft + 35 ppm CO	445.3 ± 36.3 (6)	155.7 ± 22.2 (6)	109.7 ± 22.1 (6)	125.0 ± 21.6 (6)	46.0 ± 9.7 (6)	28.0 ± 5.9 (6)	89.3 ± 22.7 (6)	4.8 ± 1.8 (6)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

Table A.3. Experiment 3: Cardiovascular and Hemodynamic Parameters in F344 Rats[a]

Treatment	Heart Rate (beats/min)	Systolic Pressure (Torr)	Diastolic Pressure (Torr)	Mean Arterial Pressure (Torr)	Pulse Pressure (Torr)	Cardiac Output (ml/min)	Cardiac Index (ml/min/kg)	Peripheral Resistance (mm Hg/ml/min)
1. 3,300 ft + 0 ppm CO	373.6 ± 33.5 (5)	102.6 ± 26.2 (5)	73.2 ± 26.9 (5)	86.3 ± 26.7 (5)	24.4 ± 3.9 (5)	41.8 ± 9.3 (4)	125.3 ± 23.7 (4)	2.2 ± 1.1 (4)
2. 10,000 ft + 0 ppm CO	413.8 ± 30.2[b] (5)	101.8 ± 21.0 (5)	73.4 ± 15.5 (5)	82.9 ± 16.3 (5)	28.4 ± 13.7 (5)	37.1 ± 2.6[b] (5)	103.2 ± 9.3[b] (5)	2.2 ± 0.4 (5)
3. 3,300 ft + 35 ppm CO	383.7 ± 31.9 (11)	107.2 ± 20.0 (11)	78.2 ± 15.7 (11)	87.8 ± 16.8 (11)	29.0 ± 8.3 (11)	39.8 ± 7.5 (11)	115.3 ± 19.5 (11)	2.3 ± 0.7 (11)
4. 10,000 ft + 35 ppm CO	406.0 ± 25.1 (10)	105.2 ± 25.9 (10)	80.8 ± 23.0 (10)	88.9 ± 23.9 (10)	24.4 ± 4.3 (9)	30.8 ± 5.5 (9)	92.2 ± 10.1 (9)	2.9 ± 1.0 (9)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

Table A.4. Experiment 5: Cardiovascular and Hemodynamic Parameters in F344 Rats[a]

Treatment	Heart Rate (beats/min)	Systolic Pressure (Torr)	Diastolic Pressure (Torr)	Mean Arterial Pressure (Torr)	Pulse Pressure (Torr)	Cardiac Output (ml/min)	Cardiac Index (ml/min/kg)	Peripheral Resistance (mm Hg/ml/min)
10,000 ft + 0 ppm CO	442.8 ± 31.1 (6)	145.0 ± 18.5 (6)	117.7 ± 18.2 (6)	126.8 ± 18.1 (6)	27.3 ± 5.1 (6)	48.3 ± 13.7 (6)	167.3 ± 34.1 (6)	2.8 ± 0.8 (6)
10,000 ft + 9 ppm CO	448.2 ± 17.5 (6)	120.2 ± 33.1 (6)	95.5 ± 28.4 (6)	103.7 ± 29.9 (6)	24.7 ± 7.0 (6)	50.4 ± 8.0 (6)	180.2 ± 28.3 (6)	2.2 ± 0.9 (6)
10,000 ft + 35 ppm CO	437.3 ± 35.3 (6)	141.8 ± 26.3 (6)	115.7 ± 27.3 (6)	124.4 ± 26.9 (6)	26.2 ± 3.5 (6)	50.4 ± 6.9 (5)	157.4 ± 17.6 (6)	2.5 ± 0.5 (5)
10,000 ft + 100 ppm CO	447.4 ± 32.7 (5)	156.2 ± 17.5 (5)	126.0 ± 16.4 (5)	136.1 ± 16.6 (5)	30.2 ± 4.2 (5)	49.5 ± 8.1 (5)	158.8 ± 19.0 (5)	2.9 ± 0.6 (5)

[a] Values are means ± SD. Number of animals is shown in parentheses.

Table A.5. Experiment 8: Cardiovascular and Hemodynamic Parameters in F344 Rats[a]

Treatment	Heart Rate (beats/min)	Systolic Pressure (Torr)	Diastolic Pressure (Torr)	Mean Arterial Pressure (Torr)	Pulse Pressure (Torr)	Cardiac Output (ml/min)	Peripheral Resistance (mm Hg/ml/min)
1. 3,300 ft + 0 ppm CO	420.6 ± 23.7 (5)	94.0 ± 28.0 (5)	71.2 ± 26.6 (5)	78.9 ± 26.9 (5)	23.2 ± 4.1 (5)	49.2 ± 14.1 (5)	1.6 ± 0.4 (5)
2. 15,000 ft + 0 ppm CO	398.0 ± 9.8 (4)	124.0 ± 33.0[b] (5)	101.0 ± 32.7[b] (5)	108.5 ± 32.8[b] (5)	23.0 ± 1.71 (5)	46.2 ± 32.8[b] (5)	2.7 ± 0.7[b] (5)
3. 3,300 ft + 100 ppm CO	393.6 ± 26.1 (5)	90.0 ± 19.0 (5)	70.0 ± 20.6 (5)	76.7 ± 19.9 (5)	20.2 ± 3.8 (5)	44.2 ± 17.1 (5)	2.0 ± 1.1 (5)
4. 15,000 ft + 100 ppm CO	435.8 ± 25.5[c] (5)	131.0 ± 47.0 (5)	113.6 ± 47.6 (5)	119.4 ± 47.2 (5)	17.4 ± 6.6 (5)	40.6 ± 5.8 (5)	2.9 ± 1.0 (5)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

[c] Significant altitude-CO interaction ($p < 0.05$ by two-way ANOVA for treatments 1 and 4 vs. treatments 2 and 3).

Table A.6. Experiment 7: Cardiovascular and Hemodynamic Parameters in F344 Rats[a]

Treatment	Heart Rate (beats/min)	Systolic Pressure (Torr)	Diastolic Pressure (Torr)	Mean Arterial Pressure (Torr)	Pulse Pressure (Torr)	Cardiac Output (ml/min)	Peripheral Resistance (mm Hg/ml/min)
1. 3,300 ft + 0 ppm CO	425.7 ± 17.7 (5)	114.0 ± 23.2 (5)	87.2 ± 22.5 (5)	96.1 ± 22.2 (5)	26.8 ± 10.1 (5)	50.4 ± 4.9 (5)	1.9 ± 0.6 (5)
2. 15,000 ft + 0 ppm CO	464.8 ± 52.1 (5)	143.5 ± 13.5 (4)	121.0 ± 9.9 (4)	128.5 ± 10.9 (4)	22.5 ± 5.9[b] (4)	42.0 ± 2.7[b] (4)	3.1 ± 0.5[b] (4)
3. 3,300 ft + 100 ppm CO	467.4 ± 40.2 (5)	131.0 ± 33.5 (5)	97.2 ± 31.4 (5)	108.5 ± 32.0 (5)	33.8 ± 5.4 (5)	46.3 ± 5.3 (5)	2.3 ± 0.6 (5)
4. 15,000 ft + 100 ppm CO	454.3 ± 30.8 (4)	132.8 ± 28.1 (4)	112.5 ± 26.0 (4)	119.3 ± 26.6 (4)	20.3 ± 4.3 (4)	38.0 ± 4.8 (4)	3.1 ± 0.6 (4)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

Table A.7. Experiment 10: Body Weight, Organ Weights, and Hemodynamic Parameters in F344 Rats[a]

	Body Weight (g)	Hematocrit (%)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)	Kidney Weight (g/kg bw)	Spleen Weight (g/kg bw)	Pituitary Weight (g/kg bw)	Adrenal Weight (g/kg bw)
1. 3,300 ft + 0 ppm CO	276.0 ± 14.7 (6)	54.0 ± 1.4 (4)	0.569 ± 0.055 (6)	2.079 ± 0.102 (6)	2.648 ± 0.143 (6)	7.347 ± 0.229 (6)	2.139 ± 0.220 (6)	0.030 ± 0.021 (5)	0.140 ± 0.031 (6)
2. 15,000 ft + 0 ppm CO	248.8 ± 18.7[b] (6)	67.8 ± 2.5[b] (6)	0.999 ± 0.063[b] (6)	2.256 ± 0.31[b] (6)	3.289 ± 0.038[b] (5)	8.259 ± 0.014[b] (6)	2.420 ± 0.345[b] (6)	0.035 ± 0.010[b] (6)	0.191 ± 0.014[b] (6)
3. 3,300 ft + 500 ppm CO	279.5 ± 15.2 (6)	76.6 ± 2.3[c] (5)	0.696 ± 0.059[c] (6)	2.604 ± 0.096[c] (6)	3.299 ± 0.148[c] (6)	8.151 ± 0.551[c] (6)	3.051 ± 0.154[c] (6)	0.035 ± 0.009 (6)	0.143 ± 0.006 (6)
4. 15,000 ft + 500 ppm CO	249.2 ± 18.3 (5)	82.0 ± 2.0[d] (5)	1.179 ± 0.21 (5)	2.850 ± 0.090 (5)	4.029 ± 0.181 (5)	8.970 ± 1.470 (5)	4.091 ± 0.329 (5)	0.037 ± 0.021 (5)	0.177 ± 0.016 (5)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

[c] Significant CO effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 2 vs. treatments 3 and 4).

[d] Significant altitude-CO interaction ($p < 0.05$ by two-way ANOVA for treatments 1 and 4 vs. treatments 2 and 3).

Table A.8. Experiment 11: Body Weight and Hemodynamic Parameters in F344 Rats[a]

Treatment	Body Weight (g)	Hematocrit (%)	Right Ventricle Weight (g/kg bw)	Left Ventricle + Septum Weight (g/kg bw)	Heart Weight (g/kg bw)
3,300 ft + 0 ppm CO	ND[b]	ND	0.487 ± 0.005 (5)	1.835 ± 0.028 (5)	2.323 ± 0.023 (5)
18,000 ft + 0 ppm CO	227.8 ± 22.4 (12)	79.9 ± 1.3 (12)	1.002 ± 0.077[c] (6)	2.090 ± 0.075 (6)	3.092 ± 0.138[c] (6)
18,000 ft + 50 ppm CO	210.8 ± 65.1 (12)	80.0 ± 1.1 (12)	0.961 ± 0.059[c] (5)	2.042 ± 0.081 (5)	3.003 ± 0.131[c] (5)
18,000 ft + 100 ppm CO	222.3 ± 12.2 (12)	81.2 ± 1.4 (12)	1.020 ± 0.055[c] (6)	1.993 ± 0.058 (6)	3.013 ± 0.095[c] (6)
18,000 ft + 500 ppm CO	183.8 ± 20.9[d] (10)	82.6 ± 3.6 (8)	ND	ND	ND

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] ND = not determined.

[c] Significant compared to measurement made at ambient altitude ($p < 0.05$ by one-way ANOVA).

[d] Significant compared to measurement made at 18,000 ft + 0 ppm CO ($p < 0.05$ by one-way ANOVA).

Table A.9 Experiment 11: Cardiovascular and Hemodynamic Parameters in F344 Rats[a]

Treatment	Heart Rate (beats/min)	Systolic Pressure (Torr)	Diastolic Pressure (Torr)	Mean Arterial Pressure (Torr)	Pulse Pressure (Torr)	Cardiac Output (ml/min)	Peripheral Resistance (mm Hg/ml/min)
1. 3,300 ft + 0 ppm CO	430.8 ± 34.8 (5)	117.6 ± 8.5 (4)	93.2 ± 13.6 (5)	79.4 ± 37.9 (5)	24.4 ± 13.3 (5)	46.6 ± 16.5 (5)	2.3 ± 0.8 (5)
2. 18,000 ft + 0 ppm CO	434.6 ± 36.6 (5)	134.8 ± 17.0 (5)	115.6 ± 30.1 (5)	122.1 ± 32.7[b] (5)	19.2 ± 8.5 (5)	37.7 ± 7.3 (5)	3.5 ± 1.5 (5)
3. 18,000 ft + 50 ppm CO	446.6 ± 36.9 (5)	153.2 ± 11.1 (5)	134.6 ± 20.3[b] (5)	140.8 ± 21.6[b] (5)	18.6 ± 7.1 (5)	33.9 ± 4.7 (4)	4.1 ± 1.1 (4)
4. 18,000 ft + 100 ppm CO	459.3 ± 22.0 (6)	152.3 ± 8.9 (6)	133.2 ± 20.2[b] (6)	139.6 ± 20.6[b] (6)	19.2 ± 4.8 (6)	37.1 ± 7.7 (6)	3.9 ± 1.0 (6)

[a] Values are means ± SD. Number of animals is shown in parentheses.

[b] Significant altitude effect ($p < 0.05$ by two-way ANOVA for treatments 1 and 3 vs. treatments 2 and 4).

ABOUT THE AUTHOR

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ABBREVIATIONS

CO	carbon monoxide
COHb	carboxyhemoglobin
%COHb	percent carboxyhemoglobin
COMb	carboxymyoglobin
dP/dt	left ventricular pressure rate of change
ECG	electrocardiogram
F344	Fischer-344 rats
O_2Hb	oxyhemoglobin
P_{CO}	partial pressure of carbon monoxide in blood
P_{CO_2}	partial pressure of carbon dioxide in blood
P_{O_2}	partial pressure of oxygen
ppm	parts per million
STP	standard temperature and pressure

Cardiovascular Effects of Chronic Carbon Monoxide and High-Altitude Exposure

James J. McGrath, Principal Investigator

INTRODUCTION

In the summer of 1983, the Health Effects Institute (HEI) issued a Request for Applications (RFA 83-1) soliciting proposals on "Cardiovascular and Other Health Effects of Carbon Monoxide." In the fall of 1983, Dr. James J. McGrath of Texas Tech University Health Sciences Center, Lubbock, TX, proposed a study entitled "Cardiovascular Effects of Chronic Carbon-Monoxide-Altitude Exposure." The HEI approved the three-year project, which began in February 1984, and authorized expenditure of $239,503. The Investigator's Report was received at the HEI in June 1987, and accepted by the Health Review Committee in April 1988. During the review of the Investigator's Report, the Review Committee and the investigator had the opportunity to exchange comments and to clarify issues in the Investigator's Report and in the Review Committee's Commentary. The Health Review Committee's Commentary is intended to place the Investigator's Report in perspective as an aid to the sponsors of the HEI and to the public.

THE CLEAN AIR ACT

Under Sections 202(a) and 202(b)(1) of the Clean Air Act, the U.S. Environmental Protection Agency (EPA) imposes specific requirements for reductions in motor vehicle emissions of carbon monoxide (and other pollutants). The Act also provides the EPA with limited discretion to modify those requirements. The determination of the appropriate standards for emissions from mobile sources depends in part on an assessment of the risks to health that they present. Research on the health effects of carbon monoxide at altitudes above sea level can contribute to such risk assessment and, therefore, to informed regulatory decision-making.

In addition, Section 109 of the Clean Air Act provides for the establishment and periodic review of National Ambient Air Quality Standards to protect the public health and welfare. A set of primary (health-related) standards currently applies to carbon monoxide. Under Section 109, such standards must allow "an adequate margin of safety." Potential additive effects of carbon monoxide and altitude on human health are relevant to the risk assessments that underlie current and future standards for carbon monoxide. Thus, research on such effects can contribute to a more informed assessment of risks from carbon monoxide and of the margin of safety that is adequate with respect to those risks.

Finally, the legislative history of the Clean Air Act makes it clear that, in setting the National Ambient Air Quality Standards, 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 maximal permissible air level of 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 clear, but the report does specify that "included among those persons whose health should be protected by the ambient air 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 in such a group." In identifying groups potentially particularly sensitive to the health effects of carbon monoxide, the U.S. Environmental Protection Agency (1985) observed: "Visitors to high altitude locations are also expected to be more vulnerable to carbon monoxide health effects due to reduced levels of oxygen in the air they breathe." Thus, research on the potential additive effects of carbon monoxide and altitude can contribute to an assessment of the combined risk to an identifiable group that may be especially vulnerable to the effects of carbon monoxide.

BACKGROUND

Carbon monoxide is a product of incomplete combustion. Motor vehicles are the major source of carbon monoxide in urban air, although combustion of fuel in stationary sources such as industry, solid-waste disposal, and residences also contributes to the atmospheric burden of carbon monoxide.

The EPA has set National Ambient Air Quality Standards for carbon monoxide. These standards include an eight-hour average of 9 ppm and a one-hour average of 35 ppm

carbon monoxide and are based on data generated at sea level. Many citizens of this country, most notably those of the Rocky Mountain states, reside at altitudes much higher than sea level. It is uncertain whether or not the health effects of carbon monoxide would be the same as one ascends to higher altitudes.

The health effects of carbon monoxide arise from interference in the normal oxygen-carrying function of the blood. 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 carbon monoxide is present in the inhaled air, the carbon monoxide and oxygen compete for the oxygen-binding sites on the hemoglobin molecules in the red blood cells. Because the affinity of carbon monoxide for hemoglobin is 240 times greater than that of oxygen, carboxyhemoglobin (COHb) rapidly forms and the oxygen-carrying capacity of the red blood cells is reduced (West 1985). In addition, delivery of oxygen to the tissues is further impaired because, in the presence of carbon monoxide, hemoglobin releases oxygen to the tissues more slowly. This impaired delivery decreases oxygen-dependent cellular respiration and results in tissue hypoxia. Tissue hypoxia may cause transient or permanent damage, especially in those organs that demand high oxygen delivery, such as the brain and heart. The developing fetus also has high oxygen requirements.

Tissue hypoxia initiates a variety of responses to compensate for a decrease in oxygen availability. Studies in both animals and humans have shown that acute exposure to carbon monoxide increases coronary blood flow and heart rate without increasing ventilation rate (Ayres et al. 1970). Blood pressure and vascular resistance also have been shown to fall upon acute carbon monoxide exposure (Penney 1988). Although the effects of chronic exposure on cardiac output are uncertain, most studies indicate that prolonged carbon monoxide exposure leads to increased production of red blood cells (polycythemia), increased cardiac output, and hypertrophy (enlargement) involving the whole heart (Kanten et al. 1983; reviews by McGrath 1982, Penney 1988).

At high altitude, oxygen delivery to the cells is also decreased but for different reasons. Air is less dense at high altitude; therefore, the partial pressure of oxygen (P_{O_2}) in air is lower. Because there are fewer molecules of oxygen available, hemoglobin does not saturate with oxygen. This state, termed "hypoxic hypoxia," also leads to tissue hypoxia. Unlike carbon-monoxide-induced hypoxia in which the arterial P_{O_2} is normal, the P_{O_2} of the arterial blood is reduced in hypoxic hypoxia.

Increased ventilation rate, polycythemia, and right ventricular hypertrophy are also known to occur at high altitude. Cardiac output is believed to increase on ascent to high altitude, but then to decrease after a few days because of pulmonary vasoconstriction (Lenfant and Sullivan 1971). Production of red blood cell 2,3-diphosphoglycerate, which binds to hemoglobin and increases oxygen dissociation, also increases (Ganong 1985).

The effects of exposure to carbon monoxide or altitude have been measured in animal studies using hematological and cardiovascular endpoints, and in controlled human studies using exercise performance and blood monitoring. In the past, the effects of carbon monoxide and of altitude generally have been studied separately.

The acute effects of carbon monoxide and altitude in combination have been examined only to a limited extent. Weiser and coworkers (1978) conducted a clinical study in which aerobic capacity measurements were made in four male subjects who breathed carbon monoxide at high altitude (1,610 m). The group found that work performance was not impaired beyond that reported for sea-level carbon monoxide studies that had used identical COHb levels of 5.1 percent. They did note, however, that carbon monoxide exposure at altitude elevated heart rates and lowered anaerobic threshold (the level of exercise above which aerobic energy production is supplemented with anaerobic mechanisms). Virtually no studies of the long-term effects of combined exposure to carbon monoxide and altitude have been reported.

JUSTIFICATION FOR THE STUDY

Exposure to high levels of carbon monoxide is known to affect cardiac function in animals and humans, but whether or not low levels of carbon monoxide impair cardiac performance is uncertain. The HEI solicited proposals (RFA 83-1) to research the cardiovascular effects of carbon monoxide at or near ambient levels. Studies focusing on susceptible populations, such as persons residing at high altitude, were of interest.

In response to the RFA, Dr. McGrath proposed to expose rats for six weeks to different concentrations of carbon monoxide, and at different altitudes, to determine if the resulting effects of carbon monoxide and altitude were additive. For this purpose, he proposed to examine hemoglobin concentration and hematocrit (the percentage volume of a blood sample occupied by cells), and to assess cardiovascular fitness by measuring heart weight, right and left ventricle weights, cardiac output, heart rate, and blood pressure. In addition, the overall health of the animals was to be monitored. This analysis of multiple physiological parameters in healthy animals after exposure to carbon monoxide at high altitude for six weeks was expected to provide new

information on the combined effects of carbon monoxide and altitude.

SPECIFIC AIMS

The specific aims of this study were to determine if exposure of animals to carbon monoxide exacerbates the following effects, which occur at high altitude: (1) loss of body weight; (2) increase in erythropoietic activity; (3) right ventricular hypertrophy; and (4) alteration of hemodynamic performance. An additional aim was to determine whether or not exposure to high altitude would exacerbate the left ventricular hypertrophy that results from exposure to carbon monoxide at sea level.

STUDY DESIGN

In the first set of experiments, Dr. McGrath used four experimental conditions: control atmosphere (0 ppm carbon monoxide, 3,300 ft); high altitude (10,000 ft) alone; carbon monoxide (35 ppm) at low altitude (3,300 ft); and carbon monoxide (35 ppm) at high altitude (10,000 ft). Four groups of six Fischer-344 rats each were exposed continuously under each of the four conditions for six weeks. When this protocol did not yield consistent or definitive results, a second protocol was employed in which groups of six or more Fischer-344 and six or more Sprague-Dawley rats were exposed for six weeks to 0, 9, 35, 50, 100, or 500 ppm carbon monoxide at altitudes of 3,300, 10,000, 15,000, and 18,000 ft. Sprague-Dawley rats also were chosen for study because their large size facilitated the surgical procedures involved in the hemodynamic studies, which were performed on thoracotomized animals.

Examination of the effects of six-week exposures to carbon monoxide and altitude, separately and in combination, included determination of the hematocrit, hemoglobin, and COHb concentrations for each experimental group. Body weight, and the weights of the entire heart, right ventricle, left ventricle plus septum, spleen, adrenal glands, kidneys, and pituitary gland also were recorded. Blood gas determinations included arterial measurements of pH, bicarbonate concentration, partial pressure of carbon dioxide (P_{CO_2}), and P_{O_2}. In addition, heart rate, systolic and diastolic blood pressures, pulse pressures, cardiac output, and peripheral resistance were measured.

SUMMARY OF INVESTIGATOR'S FINDINGS

The author reported that in the first set of experiments, exposure to carbon monoxide (35 ppm) alone or exposure to high altitude (10,000 ft) alone increased the COHb level, hematocrit, right ventricle weight, and heart rate, but the observed increases were not consistently reproducible.

In the second set of experiments, which were designed to obtain dose-response information, exposure to carbon monoxide at levels in excess of 35 ppm caused increases in the hematocrit and in the weights of the entire heart and the left ventricle plus septum. Exposure at altitudes above 10,000 ft caused loss of body weight, and increase in hematocrit, hemoglobin, and right ventricle weight. The responses elicited by exposure to either carbon monoxide alone or altitude alone were consistent with those previously reported in the literature (Lenfant and Sullivan 1971; Barer et al. 1983; Penney and Baylarian 1987; Penney 1988).

When rats were exposed to both carbon monoxide and altitude combined, no significant additive effects on any of the hematological or cardiovascular parameters monitored were observed at carbon monoxide concentrations below 100 ppm; however, exposure to carbon monoxide at concentrations of 100 ppm or 500 ppm increased certain effects of altitude that were observed at altitudes of 15,000 ft and 18,000 ft. No additive effects of the combined exposure conditions on body weight were noted.

The author draws attention to an unexpected finding in the study; namely, that COHb levels increased with increasing altitude (for example, even in the absence of carbon monoxide exposure, COHb rose from 0.6 percent at the lowest altitude to 1.3 percent at 10,000 ft, 1.7 percent at 15,000 ft, and 1.9 percent at 18,000 ft).

TECHNICAL EVALUATION

METHODS AND STUDY DESIGN

Although many of the experimental procedures that were used to evaluate the effects of exposure to carbon monoxide and altitude were standard techniques, it is not clear that the endpoints chosen for study were appropriate to test the hypothesis that the physiological effects resulting from combined exposure to carbon monoxide and altitude are additive. In addition, few rationale were presented for the selection of the specific combined exposures to carbon monoxide and altitude.

An area of concern relates to the effect of anesthesia on some of the measurements reported. During the continuous six-week exposure, blood samples were taken periodically from unanesthetized animals, but hemodynamic and cardiovascular measurements were performed on open-chested animals that were anesthetized. Under anesthesia, measurement of hemodynamic and cardiovascular responses

may have been altered sufficiently to complicate their interpretation. In addition, pentobarbital anesthesia can cause sympathetic stimulatory effects, which could have accounted in part for the high heart rate and blood pressure seen in control animals (Kanten et al. 1983). Hence, it would have been useful to obtain heart rate and blood pressure on unanesthetized rats, even if mildly restrained, to compare with the values from anesthetized rats. Although the anesthetized rat model has been used in other studies (Penney et al. 1979; Kanten et al. 1983; Penney and Baylerian 1987), no data at present directly compare hemodynamic and cardiovascular measurements in unanesthetized and anesthetized animals.

It should be noted also that the study did not include measurements to determine the level of hypoxia under each experimental condition. An appropriate endpoint for this purpose would have been a determination of mixed venous P_{O_2} taken from the pulmonary arterial blood, which reflects the average P_{O_2} as the blood returns from all the tissues of the body. It is recognized that this would have been a difficult measurement to undertake, perhaps necessitating the shut-down and stabilization of the chamber system; however, the lack of any measure of oxygen delivery to the tissues weakens the inferences that can be drawn from the data.

The author relied on heart weight and other organ weights, normalized to body weight, to assess the effects of carbon monoxide and altitude separately and in combination. Although normalization of heart weight to body weight is often used in studies of this type, the problems associated with the procedure must be kept in mind. Unless the body weights of the animals being compared are unchanging, the heart weight:body weight ratio varies with body weight. Therefore, because stressed animals usually grow more slowly, as noted in this study, the observed increase in heart weight:body weight ratios may not be the direct result of the treatment (Styka and Penney 1978). When body weights are changing, regression relationships based on body weight can be used to predict normal heart weight for comparison.

In addition to hematocrit determinations, blood volume measurements would have been desirable. Blood volume is an important hematological parameter that can be used to compare the responses to carbon-monoxide- or altitude-induced hypoxia. Polycythemia and increased blood volume both serve to reestablish the oxygen-carrying capacity of the blood, but they also alter cardiac dynamics because of changes in blood viscosity. It is still uncertain whether the beneficial effect of increased oxygen-carrying capacity outweighs the detrimental effect of increased blood viscosity (Barer et al. 1983; Davidson and Penney 1988).

The author monitored carbon monoxide exposures by measuring COHb both by spectrophotometry and by gas chromatography; the gas chromatograph was used for the 0- and 9-ppm carbon monoxide exposures only. The use of the more accurate gas chromatograph at the lower COHb concentrations is commendable in view of the evidence that spectrophotometry is less reliable at COHb values below 5 percent (Guillot et al. 1981; Kane 1985). More extensive use of the gas chromatograph would have been even better.

DATA ANALYSIS

The analysis performed by the investigator assumed that each individual animal, housed in a cage containing a total of six animals, was an independent observation. However, when animals are housed together for extensive periods, they can influence the health of one another in ways that may confound the measured endpoints. Therefore, it would have been desirable to house the animals separately.

The second set of experiments, which explored dose-response relations, was analyzed by one-way analysis of variance; however, formal analyses for trends were not performed. Although the relations can be visualized in the graphs provided, statistical analysis of the data, using regression models, linear or otherwise, would have maximized the use of the data in describing dose-response relations.

It appears that many of the observations reported in different tables of the Investigator's Report were obtained from animals exposed to identical carbon monoxide levels and altitudes. It would have been appropriate to have combined the data from the animals exposed to identical conditions, thus enhancing the statistical power of the parameters.

INTERPRETATION OF RESULTS

The original hypothesis held that the physiological effects of exposure to carbon monoxide and altitude would be additive. To test this hypothesis, the investigator generated data to document the effects of carbon monoxide and altitude on several parameters measured in rats, and he concluded that no evidence of additivity between the effects of carbon monoxide and altitude was apparent in any of the endpoints reported. The interpretation of the observed effects would have been aided, however, by a more thorough and rigorous discussion of the biological significance of the parameters measured.

Although organ weights, body weight, hematocrit, blood gases, cardiovascular indices, and heart rate were measured to document the interactive effects of carbon monoxide and altitude exposures, without a measurement of mixed venous P_{O_2}, the actual level of hypoxia under each condition is uncertain. In addition, the choice of anesthesia used may

have obscured the hematological endpoints that were monitored.

The author relied on organ weights and body weight to determine the health effects of carbon monoxide and altitude exposures. Their utility in comparing hypoxic effects is uncertain, particularly because the reported heart weights appear to be dependent on the body weight measurements—increased heart weights were noted when body weights decreased.

Hematological status was to have been assessed through the measurement of hematocrit and peripheral resistance, but the author has provided only minimal interpretation of these measurements; hematocrit increased under some conditions and peripheral resistance was unchanged. With information on vasodilation, blood volume, red blood cell population, or hemoglobin affinity, interesting questions concerning the synergistic effects of carbon monoxide hypoxia and hypoxic hypoxia could have been answered more definitively.

The extent to which results from rodent studies may be extrapolated to humans has long been debated. Justifying the use of rats in this study, the investigator points out the similarity between laboratory rats and humans in responses to altitude and carbon monoxide. Both species undergo polycythemia and experience cardiac changes in response to altitude or carbon monoxide. However, many factors, such as temperature, pH, and hemoglobin variants, influence the affinity of hemoglobin for carbon monoxide, even within the same species. The range of partition-constant values in the Haldane equation (M, a measure of affinity) for human hemoglobin overlaps with those found for rat hemoglobin (in humans, M = 180 to 240; in rats, M = 120 to 230). The author did not determine whether the M values for the hemoglobin in the rat strains used in these studies were within the range of M values for hemoglobin in humans. If they were within range, the use of the rat to model carbon monoxide effects in combination with altitude could have been further justified.

The author states that his "most striking observation" was that COHb levels increased on six-week exposure to altitude in the absence of exogenous carbon monoxide. Although the observed increases were small, they were consistent. The author's suggestion, that they were endogenously produced, is reasonable; however, a more extensive discussion of the biological implications of the increased COHb levels would have been helpful. The hypothesis that the increased carbon monoxide derives from the increased destruction of erythrocytes could have been verified by showing that red blood cell turnover is increased in hypoxic-hypoxia-induced polycythemia.

IMPLICATIONS FOR FUTURE RESEARCH

To assess the effects of carbon monoxide at high altitude in animals, the design of future studies must include clearly defined health endpoints and exposure conditions. Chronic exposure protocols will yield the most valuable information. Adequate documentation of carbon monoxide exposure conditions, especially during the performance of cardiovascular and hematological measurements, is necessary. An issue that is worth considering in this field of research, when it is feasible and ethical to do so, is the measurement of cardiovascular endpoints in both anesthetized and conscious animals in order to determine, and control for, the effect of anesthesia.

CONCLUSIONS

The author has reported that in rats housed for six weeks at altitudes of less than 15,000 ft, the cardiovascular responses are not modified significantly by concentrations of carbon monoxide below 100 ppm. He also has reported that exposure to altitudes of 10,000 ft or higher, in the absence of exogenous carbon monoxide, increases COHb levels; this observation implies that background blood levels of COHb may rise after spending time at higher altitude, independent of the ambient concentration of environmentally produced carbon monoxide. Although the data presented in the report appear to suggest that low levels of carbon monoxide do not aggravate the effects of moderate altitude, interpretation of the data is complicated by limitations in the design of the study and the methods employed. As a result, it would be inappropriate to draw conclusions from this study relating to public health policy.

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LIST OF HEI PUBLICATIONS

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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 Carcinogenecity 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	To be released soon
26	Investigation of a Potential Cotumorigenic Effect of the Dioxides of Nitrogen and Sulfur, and of Diesel-Engine Exhaust, on the Respiratory Tract of Syrian Golden Hamsters	U. Heinrich	May 1989

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The Institute is structured to define, select, support, and review research that is aimed at investigating the possible health effects of mobile source emissions. Its research program is developed by the Health Research Committee, a multidisciplinary group of scientists knowledgeable about the complex problems involved in determining the health effects of mobile source emissions. The Committee seeks advice from HEI's sponsors and from other sources prior to independently determining the research priorities of the Institute.

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Each Investigator's Report is peer-reviewed, generally by a biostatistician and three outside, independent reviewers chosen by the Review Committee. At one of its regularly scheduled meetings, the Review Committee discusses the Investigator's Report. The comments of the Committee and the peer reviewers are sent to the investigator, and he or she is asked to respond to those comments and, if necessary, revise the report. The Review Committee then prepares its Report, which includes a general background on the study, a technical evaluation of the work, a discussion of the remaining uncertainties and areas for future research, and implications of the findings for public health. After evaluation by the HEI Board of Directors, the HEI Research Report, which includes the Investigator's Report and the Review Committee's Report, is then published in monograph form. The Research Reports are made available to the sponsors, the public, and many scientific and medical libraries, and are registered with NTIS.

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