# CHAPTER 5

# *Pharmacokinetics and Mechanisms of Action of Carbon Monoxide*

## 5.1 Introduction

Basic research on the physiology, pharmacokinetics, and toxicology of carbon monoxide (CO) that ended in the late seventies was followed by studies focused primarily on the cardiopulmonary effects of CO as an ambient air pollutant. Although research in this area continues, more recent studies have refocused on the mechanisms of action and pathophysiological effects of CO at a cellular level and on its role as a cytotoxic agent and neural messenger. In this chapter, the sections discussing basic pharmacokinetics draw heavily from Chapter 9 of the previous CO criteria document (U.S. Environmental Protection Agency, 1991). However, all sections were revised and consolidated, many were expanded, and several new sections were added. In particular, sections on tissue production and metabolism of CO and intracellular effects of CO have been revised extensively and expanded. The new section on conditions affecting uptake and elimination of CO discusses the influence of physical activity, altitude, physical characteristics, and health status on carboxyhemoglobin (COHb) formation. Also, new sections on the mechanisms of CO and a review of the developing concepts have been added.

Although the focus of this document is on the effects of ambient and near ambient levels of CO leading to low COHb levels ($\leq$5%), this chapter discusses, where appropriate, findings of a selected number of human studies carried out at moderate COHb levels ($\leq$20%). Also discussed are observations from a limited number of relevant animal studies at even higher COHb levels. The purpose for the inclusion of such observations from human studies at higher CO concentrations, and animal studies in general, is to facilitate the understanding of CO kinetics, related pathophysiologic processes, and mechanisms of cytotoxicity. Despite much higher CO uptake and elimination rates in animal species than in humans, primarily because of substantially higher ventilation rates, the laboratory animal data still fill, although only partially, the knowledge gaps for which no human data are available in these areas of research. Over the range of CO concentrations that are most relevant experimentally to typical environmental CO exposures (e.g., 50 to 500 ppm), the rate of both CO uptake and elimination in mammals is inversely proportional to body mass (i.e., the smaller the animal, the faster the rate [Klimisch et al., 1975; Tyuma et al., 1981]). Over this same range of CO concentrations, the most widely used predictive model of COHb formation, the Coburn-Forster-Kane (CFK) equation, accurately predicts the resulting COHb levels not only in human subjects, but also in laboratory rats and mice (Tyuma et al., 1981; Benignus and Annau, 1994; Kimmel et al., 1999). Thus, despite many well identified interspecies differences in the toxicokinetics of CO, the basic mechanisms of CO toxicity between laboratory animals and humans are similar and, in many respects, close to identical. Although a more detailed discussion of interspecies differences as they relate to humans may aid in interpretation of data and elucidation of mechanisms, it is not essential for understanding the material presented in this chapter and is well beyond the scope of this document (see Chapter 1). Despite interspecies differences, especially in the uptake and elimination kinetics of CO, extrapolation of observations from animals to man as applied in this chapter, even with its many assumptions, may be useful

in identifying potential pathophysiologic and histotoxic processes associated with ambient or near ambient CO exposure.

## 5.2 Absorption, Distribution, and Pulmonary Elimination

### 5.2.1 Pulmonary Uptake

Although CO is not one of the respiratory gases, the similarity of physico-chemical properties of CO and oxygen ($O_2$) permits an extension of the findings of studies on the kinetics of transport of $O_2$ to those of CO. The rate of formation and elimination of COHb, its concentration in blood, and its catabolism is controlled by numerous physical factors and physiological mechanisms. The relative contribution of these mechanisms to the overall COHb kinetics will depend on the environmental conditions, the physical activity of an individual, and many other physiological processes, some of which are complex and still poorly understood (see Section 5.4 for details). All of the pulmonary uptake occurs at the respiratory bronchioles and alveolar ducts and sacs. The rate of CO uptake depends on the rate of COHb formation. At the low concentration of CO in inhaled air, the rate of uptake and the rate of COHb formation could, for all practical purposes, be considered to be qualitatively the same.

#### 5.2.1.1 Mass Transfer of Carbon Monoxide

The mass transport of CO between the airway opening (mouth and nose) and the red blood cell (RBC) hemoglobin (Hb) is predominantly controlled by physical processes. The CO transfer to the Hb-binding sites is accomplished in two sequential steps: (1) transfer of CO in a gas phase, between the airway opening and the alveoli, and (2) transfer in a "liquid" phase, across the air-blood interface, including the RBC. In the gas phase, the key mechanisms of transport are convective flow, by the mechanical action of the respiratory system, and diffusion in the acinar zone of the lung (Engel et al., 1973). Subsequent molecular diffusion of CO across the alveolo-capillary membrane along the CO pressure gradient, plasma, and RBC is the virtual mechanism of the liquid phase. The principal transport pathways and body stores of CO are shown in Figure 5-1 (Coburn, 1967).

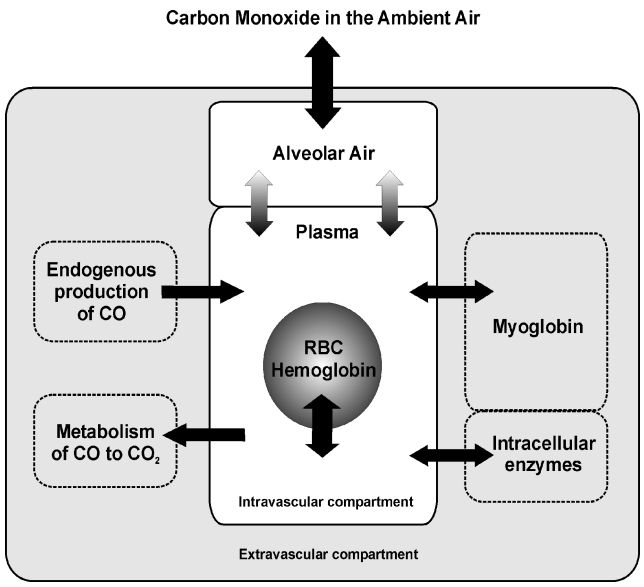


*Figure 5-1. Diagrammatic presentation of CO uptake and elimination pathways and CO body stores.*
Source: Adapted from Coburn (1967).

#### 5.2.1.2 Effects of Dead Space and Ventilation/Perfusion Ratio

The effectiveness of alveolar gas exchange depends on effective gas mixing and matching of ventilation and perfusion. During normal tidal breathing, the inhaled gas is not distributed uniformly across the tracheobronchial tree. With increased inspiratory flow, as during exercise, intrapulmonary gas distribution becomes more uniform, but gas concentration inhomogeneity still will persist. Considering that almost 90% of gas is contained within the acinar zone of the lung, any increase in gas inhomogeneity in this terminal region will have about the same negative effect as an additional increase in the alveolar dead space or a decrease in the alveolo-capillary diffusion capacity (Engel et al., 1973).

The inefficiency of gas mixing and a consequent decrease in the effectiveness of alveolar gas exchange is aggravated by ventilation/perfusion ($\dot{V}_A/\dot{Q}$) mismatch. Because of the gravity dependence of ventilation and even more of perfusion in an upright posture, regional $\dot{V}_A/\dot{Q}$ ratios will range from 0.6 (at the base of the lung) to 3.0 (at the apex), the overall value being 0.85. As a result, the $\dot{V}_A/\dot{Q}$ ratio is the principal variable controlling gas exchange, and any inequalities not only will impair transfer of gases to the blood but also will interfere with unloading of gases from the blood into the alveolar air. In humans, a change in posture to recumbent or exercise will increase the uniformity of $\dot{V}_A/\dot{Q}$ ratios and promote more efficient gas exchange, whereas increased resting lung volume, increased airway resistance, decreased lung compliance, and, generally, any lung abnormality will aggravate $\dot{V}_A/\dot{Q}$ ratio inequality.

The simplest indicator of the $\dot{V}_A/\dot{Q}$ ratio inequalities is the volume of physiological dead space ($V_D$), which comprises both the anatomical and alveolar dead space. The alveolar dead space results from reduced perfusion of alveoli, relative to their ventilation (Singleton et al., 1972). Both right-to-left and physiological shunts under normal conditions contribute little to $\dot{V}_A/\dot{Q}$ inequality (West, 1990a). An increase in tidal volume or respiratory frequency, or both, will increase moderately to substantially the $V_D$ in healthy subjects and in individuals with lung function impairment, respectively (Lifshay et al., 1971).

#### 5.2.1.3 Lung Diffusion of Carbon Monoxide

The next step in the transfer of gases across the alveolar air-Hb barrier is accomplished by gas diffusion, which is an entirely passive process. To reach the Hb-binding sites, CO and other gas molecules have to diffuse across the alveolo-capillary membrane, through the plasma, across the RBC membrane, and, finally, into the RBC stroma before reaction between CO and Hb can take place. The molecular transfer across the membrane and the blood phase is governed by general physico-chemical laws, particularly by Fick's first law of diffusion (West, 1990b). The exchange and equilibration of gases between the two compartments (air and blood) is very rapid. The dominant driving force is a partial pressure differential of CO across this membrane; for example, inhalation of a bolus of air containing levels of CO above blood baseline rapidly increases blood COHb. The rapidity of CO binding to Hb keeps a low partial pressure of CO within the RBC, thus maintaining a high pressure differential between air and blood and consequent diffusion of CO into blood. Subsequent inhalation of CO-free air reverses the gradient (higher CO pressure on the blood side than alveolar air), and CO is released into alveolar air. The air-blood gradient for CO pressure is usually much higher than the blood-air gradient; therefore, CO uptake will be a proportionately faster process than CO elimination. The rate of CO release also will be affected by back pressure from endogenous production of CO.

Diurnal variations in CO diffusion capacity of the lung ($D_LCO$) related to variations in Hb concentration have been reported in normal, healthy subjects (Frey et al., 1987). Others found the changes to be related also to physiological factors such as oxyhemoglobin ($O_2Hb$), COHb, partial pressure of alveolar carbon dioxide ($CO_2$), ventilatory pattern, $O_2$ consumption, blood flow, functional residual capacity, etc. (Forster, 1987). Diffusion capacity seems to be relatively independent of lung volume within the mid-range of vital capacity. However, at extreme volumes, the differences in diffusion rates could be significant; at total lung capacity, diffusion is higher, whereas, at residual volume, it is lower than the average (McClean et al., 1981). In a supine position at rest, $D_LCO$ has been shown to be significantly higher than that at rest in a sitting position (McClean et al., 1981). Carbon monoxide diffusion capacity increases with exercise, and, at maximum work rates, diffusion will be maximal regardless of body position. This increase is attained not only by increases in both the diffusing capacity of the alveolar-capillary membrane and the pulmonary capillary blood flow (Stokes et al., 1981) but also by increased $\dot{V}_A/\dot{Q}$ uniformity (Harf et al., 1978). Under pathologic conditions, where several components of the air-blood interface may be affected severely, and the $\dot{V}_A/\dot{Q}$ ratio inequality also may increase (as in emphysema, and fibrosis, or edema), both the uptake and elimination of CO will be affected (Barie et al., 1994).

### 5.2.2 Tissue Uptake

#### 5.2.2.1 The Lung

Although the lung in its function as a transport system for gases is exposed continuously to CO, very little CO actually diffuses into the lung tissue itself (as dissolved CO), except for the alveolar region where it diffuses across the lung tissue and into blood. The epithelium of the conductive zone (nasopharynx and large airways) presents a significant barrier to diffusion of CO. Therefore, diffusion and gas uptake by the tissue, even at high CO concentration, will be slow; most of this small amount of CO will be dissolved in the mucosa of the airways. Diffusion into the submucosal layers and interstitium will depend on the concentration and duration of CO exposure and on the relative surface area. Experimental exposures of the oronasal cavity in monkeys to very high concentrations of CO (>400 ppm) for a very short period of time (5 s) increased the blood COHb level to <3.5%. Comparative exposures of the whole lung, however, elevated COHb to almost 60% (Schoenfisch et al., 1980). Thus, diffusion of CO across the airway mucosa will contribute little, if at all, to overall COHb concentration.

#### 5.2.2.2 The Blood

The rate of CO binding with Hb is about 20% slower, and the rate of dissociation from Hb is an order of magnitude slower than are these rates for $O_2$. However, the CO chemical affinity (represented by the Haldane coefficient, M) for Hb is about 218 (210 to 250) times greater than that of $O_2$ (Roughton, 1970; Rodkey et al., 1969). Under steady-state conditions (gas exchange between blood and atmosphere remain constant), one part of CO and 218 parts of $O_2$ would form equal parts of $O_2Hb$ and COHb, which would be achieved by breathing air containing 21% oxygen and 650 ppm CO. Moreover, the ratio of COHb to $O_2Hb$ is proportional to the ratio of their respective partial pressures, PCO and $PO_2$. The relationship between the affinity constant M and $PO_2$ and PCO, first expressed by Haldane (1897-1898), has the following form:

$$COHb / O_2Hb = M \times (PCO / PO_2). \quad (5\text{-}1)$$

At equilibrium, when Hb is maximally saturated by $O_2$ and CO at their respective gas tensions, the M value for all practical purposes is independent of pH, $CO_2$, temperature, and 2,3-diphosphoglycerate (Wyman et al., 1982; Grønlund and Garby, 1984).

Under dynamic conditions, competitive binding of $O_2$ and CO to Hb is complex; simply said, the greater the number of heme molecules bound to CO, the greater is the affinity of free hemes for $O_2$. However, CO not only occupies $O_2$-binding sites, molecule for molecule, thus reducing the amount of available $O_2$, but also alters the characteristic relationship between $O_2Hb$ and $PO_2$, which, in normal blood, is S-shaped. Figure 5-2 illustrates the basic mechanisms of CO toxicity operating at any CO concentration. The a and a′ points represent the arterial values of $PO_2$. The v represents the venous $PO_2$ of healthy individuals after extraction of 5 vol % of $O_2$. With increasing concentration of COHb in blood, the dissociation curve is shifted gradually to the left, and its shape is transformed into a near rectangular hyperbola. Because the shift occurs

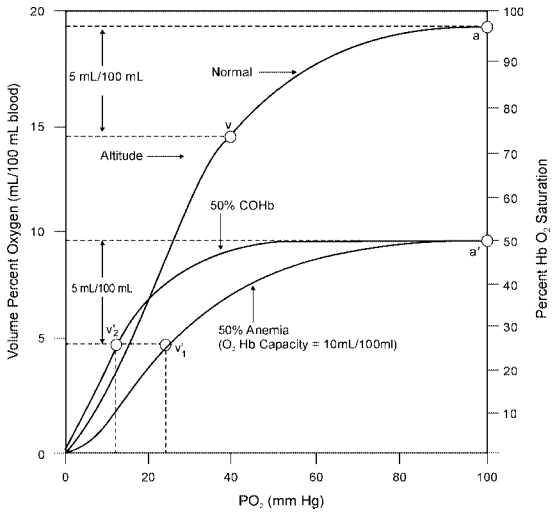


*Figure 5-2. Oxyhemoglobin dissociation curve of normal human blood, of blood containing 50% COHb, and of blood with only 50% Hb because of anemia. See the text for additional details.*
Source: U.S. Environmental Protection Agency (1991).

over a critical saturation range for release of $O_2$ to tissues, a reduction in $O_2$Hb by CO binding will have more severe effects on the release of $O_2$ than the equivalent reduction in Hb caused by anemia. Thus, in an acute anemia patient (50% of Hb) at a venous $PO_2$ of 26 torr ($v_1'$), 5 vol % of $O_2$ (50% desaturation) was extracted from blood, an amount sufficient to sustain tissue metabolism. In contrast, in a person poisoned with CO (50% COHb), the venous $PO_2$ will have to drop to 16 torr ($v_2'$; severe hypoxia) to release the same, 5 vol % $O_2$. Any higher demand on $O_2$ under these conditions (e.g., by exercise) might result in brain oxygen depletion and loss of consciousness of the CO-poisoned individual.

Because so many cardiopulmonary factors determine COHb formation, the association between COHb concentration in blood and duration of exposure is not linear but S-shaped. With progression of exposure, the initial slower COHb formation gradually accelerates, but, as COHb approaches equilibrium, the build-up slows down again. The S-shape form becomes more pronounced with higher CO levels or with exercise (Benignus et al., 1994; Tikuisis et al., 1992).

As Figure 5-1 shows, CO not only is exchanged between alveolar air and blood but also is distributed by blood to other tissues. Studies on dogs (Coburn, 1967; Luomanmäki and Coburn, 1969) found that, over the range of 2 to 35% COHb, an average of 77% of total body CO remains in the vascular compartment. The rest of CO diffused to extravascular tissues, primarily skeletal muscle where it is bound to myoglobin (Mb). Compared to dogs, the extravascular CO stores in men are smaller and account for 10 to 15% of total body CO, and less than 1% of the body CO stores appears to be physically dissolved in body fluids (Coburn, 1970a). Similar to animals, no shift between blood and extravascular compartments in men was found at low (<4%) COHb.

#### 5.2.2.3 Heart and Skeletal Muscle

Myoglobin, as a respiratory hemoprotein of muscular tissue, will undergo a reversible reaction with CO in a manner similar to $O_2$. Greater affinity of $O_2$ for Mb than Hb (hyperbolic versus S-shaped dissociation curve) is, in this instance, physiologically beneficial because a small drop in tissue $PO_2$ will release a large amount of $O_2$ from oxymyoglobin. The main function of Mb is thought to be a temporary store of $O_2$ and to act as a diffusion facilitator between Hb and the tissues (Peters et al., 1994).

Myoglobin has a CO affinity constant approximately eight-times lower than Hb (M = 20 to 40 versus 218, respectively) (Haab and Durand-Arczynska, 1991; Coburn and Mayers, 1971). As with Hb, the combination velocity constant between CO and Mb is only slightly lower than that for $O_2$, but the dissociation velocity constant is much lower than that for $O_2$. The combination of greater affinity (Mb is 90% saturated at $PO_2$ of 20 mmHg) and lower dissociation velocity constant for CO favors retention of CO in the muscular tissue. Thus, a considerable amount of CO potentially can be stored in the skeletal muscle (Luomanmäki and Coburn, 1969). The binding of CO to Mb (carboxymyoglobin [COMb]) in heart and skeletal muscle in vivo has been demonstrated at levels of COHb below 2% in heart and 1% in skeletal muscle (Coburn, 1973; Coburn and Mayers, 1971). At rest, the COMb/COHb ratio (0.4 to 1.2) does not increase with an increase in COHb up to 50% saturation and appears to be independent of the duration of exposure (Sokal et al., 1984). During exercise, the relative rate of CO binding increases more for Mb than for Hb, and CO will diffuse from blood to skeletal muscle (Werner and Lindahl, 1980); consequently, the COMb/COHb will increase for both skeletal and cardiac muscles (Sokal et al., 1986). A similar shift in CO has been observed under hypoxic conditions because a fall in myocyte intracellular $PO_2$ below a critical level will increase the relative affinity of Mb to CO (Coburn and Mayers, 1971). Consequent reduction in $O_2$ storage capacity of Mb may have a profound effect on the supply of $O_2$ to the tissue. Apart from Hb and Mb, other hemoproteins will react with CO; however, the exact role of such compounds on $O_2$-CO kinetics still needs to be ascertained. For more discussion on this topic, see Section 5.6.1.

#### 5.2.2.4 The Brain and Other Tissues

The concentration of CO in brain tissue has been found to be about 30- to 50-times lower than that in blood. During the elimination of CO from the brain, the above ratio of concentrations was still maintained (Sokal et al., 1984). However, the energy requirement of brain tissue is very high and varies greatly with local metabolism. Because oxygen demand also is coupled to local functional activity, which at times may be very high, and because the brain's oxygen storage is minimal, any degree of hypoxia if uncompensated will have a detrimental effect on brain function. The primary effects of low ambient concentrations of CO on other organs (e.g., liver, kidney) is via hypoxic mechanisms (see Section 6.6).

### 5.2.3 Pulmonary and Tissue Elimination

An extensive amount of data available on the rate of CO uptake and the formation of COHb contrast sharply with the limited information available on the dynamics of CO washout from body stores and blood. Although almost all of the studies investigating CO elimination pattern and processes were done at moderate COHb levels ($\leq$20%), the physiologic mechanisms involved in CO elimination kinetics also are effected at lower blood COHb, including levels resulting from ambient exposures ($\leq$5%). The elimination rate of CO from an equilibrium state will follow a monotonically decreasing, second-order (logarithmic or exponential) function (Pace et al., 1950). The rate, however, may not be constant when the steady-state conditions have not yet been reached. Particularly after very short and high CO exposures, it is possible that COHb decline could be biphasic, and it can be approximated best by a double-exponential function; the initial rate of decline or "distribution" might be considerably faster than the later "elimination" phase (Wagner et al., 1975). The reported divergence of the COHb decline rate in blood and in exhaled air suggests that the CO elimination rates from extravascular pools are slower than those reported for blood (Landaw, 1973). Although the absolute elimination rates are associated positively with the initial concentration of COHb, the relative elimination rates appear to be independent of the initial concentration of COHb (Wagner et al., 1975).

The same factors that govern CO uptake will affect CO elimination. This suggests that the CFK model (see Section 5.5.1) may be suitable to predict CO elimination as well. Surprisingly, few studies tested this application. When breathing air, the CFK model predicted very well the COHb decline. However, at a higher partial pressure of $O_2$ in humidified inspired air ($P_IO_2$) or under hyperbaric $O_2$ conditions, the key CFK equation parameters, particularly the $D_LCO$ value, must be adjusted for hyperoxic conditions so that CFK will predict more accurately the elimination of CO (Tikuisis, 1996; Tikuisis et al., 1992; Tyuma et al., 1981). The half-time of CO disappearance from blood under normal recovery (air) showed a considerable between-individual variance. For COHb concentrations of 2 to 10%, the half-time ranged from 3 to 5 h (Landaw, 1973); others reported the range to be 2 to 6.5 h for slightly higher initial concentrations of COHb (Peterson and Stewart, 1970). The CO elimination half-time in nonsmokers is considerably longer in men (4.5 h) than in women (3.2 h). During sleep, the elimination rate slowed in both sexes, but, in men, it became almost twice as slow (8.0 h) as during waking hours. Although no ventilation variables were obtained during the study, the day-to-night differences have been attributed to lower ventilation rates at sleep. The authors speculate that the sex differences in elimination half-time are related to the skeletal muscle mass and intrinsically to the amount of Mb (Deller et al., 1992). The half-time elimination rate appears to be independent of the CO exposure source (e.g., fire, CO intoxication). Normobaric $O_2$ administered to fire victims and CO-poisoned individuals resulted in about the same CO elimination half-time, 91 and 87 min, respectively (Levasseur et al., 1996).

Increased inhaled concentrations of $O_2$ accelerated elimination of CO; by breathing 100% $O_2$, the half-time was shortened by almost 75% (Peterson and Stewart, 1970). The average half-life of COHb in individuals with very low COHb level (1.16%) breathing hyperbaric $O_2$ was 26 min, compared with 71 min when breathing normobaric $O_2$ (Jay and McKindly, 1997). The elevation of $PO_2$ to 3 atm reduced the half-time to about 20 min, which is approximately a 14-fold decrease over that seen when breathing room

air (Britten and Myers, 1985; Landaw, 1973). Although the washout of CO can be somewhat accelerated by an admixture of 5% $CO_2$ in $O_2$, hyperbaric $O_2$ treatment is more effective in facilitating displacement of CO. Therefore, hyperbaric oxygen is used as a treatment of choice in CO poisoning. A mathematical model of COHb elimination that takes into account $P_IO_2$ has been developed but not yet validated (Singh et al., 1991; Selvakumar et al., 1993).

## 5.3 Tissue Production and Metabolism of Carbon Monoxide

In the process of natural degradation of RBC Hb to bile pigments, a carbon atom is separated from the porphyrin nucleus and, subsequently, is catabolized by heme oxygenase (HO) into CO. The major site of heme breakdown and, therefore, the major production organ of endogenous CO is the liver (Berk et al., 1976). The spleen and the erythropoietic system are other important catabolic generators of CO. Because the amount of porphyrin breakdown is stoichiometrically related to the amount of endogenously formed CO, the blood level of COHb or the concentration of CO in the alveolar air have been used with mixed success as quantitative indices of the rate of heme catabolism (Landaw et al., 1970; Solanki et al., 1988). Diurnal variations in endogenous CO production are significant, reaching a maximum around noon and a minimum around midnight (Levitt et al., 1994; Mercke et al., 1975a). Week-to-week variations of CO production are greater than day-to-day or within-day variations for both males and females (Lynch and Moede, 1972; Mercke et al., 1975b).

Any disturbance leading to accelerated destruction of RBCs and accelerated breakdown of other hemoproteins would lead to increased production of CO. Hematomas, intravascular hemolysis of RBCs, blood transfusion, and ineffective erythropoiesis all will elevate COHb concentration in blood. In females, COHb levels fluctuate with the menstrual cycle; the mean rate of CO production in the premenstrual, progesterone phase is almost doubled (Delivoria-Papadopoulos et al., 1974; Mercke and Lundh, 1976). Neonates and pregnant women also showed a significant increase in endogenous CO production related to increased breakdown of RBCs. Degradation of RBCs under pathologic conditions such as anemia (hemolytic, sideroblastic, and sickle cell), thalassemia, Gilbert's syndrome with hemolysis, and other hematological diseases also will accelerate CO production (Berk et al., 1974; Solanki et al., 1988). In patients with hemolytic anemia, the CO production rate was 2- to 8-times higher, and blood COHb concentration was 2- to 3-times higher than in healthy individuals (Coburn et al., 1966). Anemias also may develop under many pathophysiologic conditions characterized by chronic inflammation, such as malignant tumors or chronic infections (Cavallin-Ståhl et al., 1976) (see also Section 5.4.3).

Not all endogenous CO comes from RBC degradation. Other hemoproteins, such as Mb, cytochromes, peroxidases, and catalase, contribute approximately 20 to 25% to the total amount of CO (Berk et al., 1976). Approximately 0.4 mL/h of CO is formed by Hb catabolism, and about 0.1 mL/h originates from non-Hb sources (Coburn et al., 1963, 1964). This will result in a blood COHb concentration between 0.4 and 0.7% (Coburn et al., 1965).

A large variety of drugs will affect endogenous CO production. Generally, any drug that will increase bilirubin production, primarily from the catabolism of Hb, will promote endogenous production of CO. Nicotinic acid (Lundh et al., 1975), allyl-containing compounds (acetamids and barbiturates) (Mercke et al., 1975c), diphenylhydantoin (Coburn, 1970b), progesterone (Delivoria-Papadopoulos et al., 1974), and contraceptives (Mercke et al., 1975b) all will elevate tissue bilirubin and, subsequently, CO production.

Another mechanism that will increase CO production is a stimulation of HO and subsequent degradation of cytochrome P-450-dependent, mixed-function oxidases. Several types of compounds, such as a carbon disulfide and sulfur-containing chemicals (parathion and phenylthiourea), will act on different moieties of the P-450 system leading to an increase in endogenous CO (Landaw et al., 1970). Other sources of CO involving HO activity include auto-oxidation of phenols, photooxidation of organic compounds, and

lipid peroxidation of cell membrane lipids (Rodgers et al., 1994). The P-450 system also is involved in oxidative dehalogenation of dihalomethanes, widely used solvents in homes and industry (Kim and Kim, 1996). Metabolic degradation of these solvents and other xenobiotics results in the formation of CO that can lead to very high (>10%) COHb levels (Manno et al., 1992; Pankow, 1996).

Ascent to high elevations will increase the endogenous level of COHb in both humans and animals (McGrath, 1992; McGrath et al., 1993). The baseline COHb level has been shown to be positively dependent on altitude (McGrath, 1992). Assuming the same endogenous production of CO at altitude as at sea level, the increase in COHb most likely is consequent to a decrease in $PO_2$ (McGrath et al., 1993). Whether other variables, such as an accelerated metabolism or a greater pool of Hb, transient shifts in body stores, or a change in the elimination rate of CO are contributing factors, remains to be explored. Animal studies suggest that the elevated basal COHb production is not a transient phenomenon but persists through a long-term adaptation period (McGrath, 1992).

In recent years, new discoveries in molecular biology identified the CO molecule as being involved in many physiological responses, such as smooth muscle relaxation, inhibition of platelet aggregation, and as a neural messenger in the brain (for details, see Sections 5.6 and 5.7). Most recently, several studies reported yet another function of CO, that of a possible marker of inflammation in individuals with upper respiratory tract infection (Yamaya et al., 1998) and bronchiectasis (Horvath et al., 1998a) and in asthmatics (Zayasu et al., 1997; Horvath et al., 1998b). In the Zayasu et al. (1997) study, the investigators found that exhaled concentrations of CO in asthmatics taking corticosteroids were about the same as in healthy individuals (1.7 and 1.5 ppm, respectively), whereas, in asthmatics who did not use corticosteroids, the average CO concentration was 5.7 ppm. The authors speculate that one of the anti-inflammatory effects of corticosteroids is the down-regulation of HO. Whether asthmatics have an increased COHb level was not measured in this study or reported in other studies. Patients with chronic inflammatory lung disease, such as bronchiectasis may produce a substantial amount of CO (e.g., 11.8 ppm). As with asthma, induction of heme oxygenase appears to be the primary mechanism involved in the production of CO (Horvath et al., 1998a,b). Critical illness also seems to be associated with elevated production of CO (Meyer et al., 1998). When compared with controls, ill patients (not characterized) had higher COHb in both arterial and central venous blood not attributable to an elevated inspired concentration of $O_2$ used to treat patients. Moreover, the higher COHb in arterial blood than in central venous blood measured in both ill and control individuals has lead the authors to speculate that a positive arterio-venous COHb difference results from the up-regulation of the inducible isoform of heme oxygenase (HO-1) in the lung and subsequent production of CO (see Section 5.6.4).

## 5.4 Conditions Affecting Carbon Monoxide Uptake and Elimination

### 5.4.1 Environment and Activity

During exercise, increased demand for $O_2$ requires adjustment of the cardiopulmonary system, so that an increased demand for $O_2$ is met with an adequate supply of $O_2$. Depending on the intensity of exercise, the physiologic changes may range from minimal, involving primarily the respiratory system, to substantial, involving extensively the respiratory, cardiovascular, and other organ systems, inducing local as well as systemic changes. Exercise will improve the $\dot{V}_A/\dot{Q}$ ratio in the lung, increase the respiratory exchange ratio (RER), increase cardiac output, increase $D_LCO$, mobilize RBC reserves from the spleen, and induce other compensatory changes. Heavy exercise will cause a decrease in plasma volume, leading to hemoconcentration and a subsequent decrease in blood volume. Of the many mechanisms operating during exercise, the two most important physiologic variables are (1) the alveolar ventilation ($\dot{V}_A$) and (2) cardiac output. Although some physiologic changes during exercise may impair CO loading into blood (e.g., relative decrease in $D_LCO$ during severe exercise), the majority of the changes will facilitate CO transport. Thus, by increasing gas exchange efficiency, exercise also will promote CO uptake. Consequently, the rates

of CO uptake and COHb formation will be proportional to the intensity of exercise. During a transition period from rest to exercise while exposed to CO (500 ppm/10 min), the diffusing capacity and CO uptake were reported to rise faster than $O_2$ consumption for each exercise intensity (Kinker et al., 1992).

Apart from physiological factors, the concentration of CO, as well as the rate of change of CO concentration in an individual's immediate environment, can have a significant impact on COHb. For example, at intersections with idling and accelerating cars, pedestrians will be exposed for a short period of time to higher CO concentrations than those present at other places on the same street. Around home, an individual working with a chain saw, lawnmower, or other gasoline-powered tools will be exposed transiently to higher, and occasionally to much higher (e.g., breathing near the exhaust of a chain saw), concentrations of CO (up to 400 ppm) (Bünger et al., 1997). In indoor environments, exposure to elevated CO from unventilated gas appliances or from environmental tobacco smoke may increase transiently the COHb level of a previously unexposed individual. Occupationally, there are many instances and conditions under which workers may be exposed briefly to moderate-to-high levels of CO from operating equipment or other sources. Despite the shortness of each exposure episode, such transients may result in a relatively high COHb concentration. As an example, exposure for 5 min or less of a resting individual to 7,600 ppm CO in inhaled air will result in almost 20% COHb (Benignus et al., 1994). On repeated brief exposures to high CO, the COHb will increase further until the concentrations in inhaled CO and in blood reach equilibrium. Once the distribution of CO within body stores is complete, the COHb will remain constant, unless the ambient CO concentration changes (either up or down) again. As is the CO uptake, so is the elimination of CO from blood governed by the gas concentration gradient between blood and alveolar air. However, the elimination of CO from blood is a much slower process (see Section 5.2.3) and, therefore, will take many hours of breathing clean air before the baseline COHb value is reached.

Recently, a unique source of CO exposure was identified. It has been found repeatedly that the use of volatile anesthetics (fluranes) in closed-circuit anesthetic machines, when $CO_2$ absorbent (soda lime) is dry, can result in a significant production of CO caused by a degradation of the anesthetic and subsequent exposure of a patient to CO (up to 7% COHb) (Woehlck et al., 1997a,b).

### 5.4.2 Altitude

Altitude may have a significant influence on the COHb kinetics (U.S. Environmental Protection Agency, 1978). These changes are consequent to compensatory and adaptive physiologic mechanisms. At sea level, at a body temperature of 37 °C, barometric pressure ($P_B$) of 760 torr, and air (gas) saturated with water vapor (BTPS conditions) the $P_IO_2$ is 149 torr. At an altitude of 3,000 m (9,840 ft; $P_B$ = 526 torr), the $P_IO_2$ is only 100 torr, resulting in an acute hypoxic hypoxia. Direct measurements of blood gases on over 1,000 nonacclimatized individuals at this altitude found the partial pressure of $O_2$ in alveolar air to be only 61 torr (Boothby et al., 1954). The hypoxic drive will trigger a complement of physiological compensatory mechanisms (to maintain $O_2$ transport and supply), the extent of which will depend on elevation, exercise intensity, and the length of a stay at the altitude. During the first several days, the pulmonary ventilation at a given $O_2$ uptake (work level) will increase progressively until a new quasi-steady-state level is achieved (Bender et al., 1987; Burki, 1984). The $D_LCO$ will not change substantially at elevations below 2,200 m but was reported to increase above that altitude, and the spirometric lung function will be reduced as well (Ge et al., 1997). The maximal aerobic capacity and total work performance will decrease, and the RER will increase (Horvath et al., 1988). Redistribution of blood from skin to organs and within organs from blood into extravascular compartments, as well as an increase in cardiac output, will promote CO loading (Luomanmäki and Coburn, 1969). Because of a decrease in plasma volume (hemoconcentration), the Hb concentration will be higher than at sea level (Messmer, 1982). The blood electrolytes and acid-base equilibrium will be readjusted, facilitating transport of $O_2$. Thus, for the same CO concentration as at sea level, these compensatory changes will favor CO uptake and COHb formation (Burki, 1984). By the same token, the adaptive changes will affect not only CO uptake but CO

elimination as well. Carboxyhemoglobin levels at altitude have been shown to be increased in both laboratory animals and humans (McGrath, 1992; McGrath et al., 1993). Breathing CO (9 ppm) at rest at altitude has produced higher COHb levels than those at sea level (McGrath et al., 1993). Surprisingly, exercise in a CO atmosphere (50 to 150 ppm) at altitude appeared either to suppress COHb formation or to shift the CO storage, or both. The measured COHb levels were lower than those found under similar conditions of exercise and exposure at sea level (Horvath et al., 1988).

The short-term acclimatization (within a week or two) will stabilize the compensatory changes. During a prolonged stay at high altitude (over a few months), most of the early adaptive changes gradually will revert to the sea level values, and long-term adaptive changes, such as an increase in tissue capillarity and Mb content in the skeletal muscle, begin to take place. Smokers appear to tolerate short-term hypoxic hypoxia caused by high altitude (7,620 m [25,000 ft]) much better than nonsmokers, who experience more severe subjective symptoms and a greater decline in task performance (Yoneda and Watanabe, 1997). Perhaps smokers, because of chronic hypoxemia (because of chronically elevated COHb), develop partial tolerance to hypoxic hypoxia. Although the mechanisms of COHb formation in hypoxic hypoxia and CO hypoxia are different, the resultant decrease in $O_2$ saturation and activation of compensatory mechanisms (e.g., an increased cerebral blood flow) appear to be at least additive (McGrath, 1988). Psychophysiological studies, in particular, seem to support the possibility of physiological equivalency of hypoxic effects, whether induced by altitude at equlibrium or ambient CO concentration. However, it must be remembered that, although some of the mechanisms of action of hypoxic hypoxia and CO hypoxia are the same, CO elicits other toxic effects not necessarily related to $O_2$ transport mechanisms (Ludbrook et al., 1992; Zhu and Weiss, 1994). Recently, Kleinman et al. (1998) demonstrated that the effects of CO and simulated altitude were not synergistic but additive.

### 5.4.3 Physical Characteristics

Physical characteristics (e.g., sex, age, race, pregnancy) are not known to directly modify the basic mechanisms of CO uptake and COHb formation and elimination. However, the baseline values of many cardiopulmonary variables that may influence COHb kinetics are known to change with physical characteristics.

The CO uptake and elimination rates either at rest or with exercise decrease with age. During the growing years (2 to 16 years of age), the COHb elimination half-time increases rapidly with age in both sexes and is relatively shorter for boys than for girls. Beyond teenage years, the half-time for CO elimination continues to grow longer but at a lower rate. In contrast to the adolescent period, the COHb half-life during the adult years was found to be persistently shorter (≈6%) in females than that in males (Joumard et al., 1981). Furthermore, it has been well established that the $D_LCO$ decreases with age (Guénard and Marthan, 1996). The rate of $D_LCO$ decline is lower in middle-aged women than it is in men; however, at older ages, the rates evened and are about the same for both sexes (Neas and Schwartz, 1996). The decrease in $D_LCO$, combined with an increase in $\dot{V}_A/\dot{Q}$ mismatch, which increases with age, means that it will take longer to both load and eliminate CO from blood.

In pregnancy, increased requirement for iron and hemodilution may lead to iron deficiency and anemia (for further details see Sections 6.4 and 7.7.1). Pregnant women who smoked showed a more pronounced shift of the $O_2$ dissociation curve to the left (≈5% COHb) than one would expect from the same COHb concentration in nonpregnant women. Thus, increased $O_2$ affinity, combined with decreased $O_2$-carrying capacity of blood of CO-exposed women, may promote fetal hypoxia (Grote et al., 1994). Animal studies found that protein deficiency in pregnant mice had no modulating effect on maternal COHb but resulted in a greater concentration of placental COHb (Singh et al., 1992, 1993; Singh and Moore-Cheatum, 1993).

Young women were found to be more resistant to altitude hypoxia than were men, but the physiological factors for this difference remain unexplored (Horvath et al., 1988). Carboxyhemoglobin

levels, although elevated at altitude, were found to be about the same for both males and females (McGrath et al., 1993).

Whether the dynamics of COHb formation and elimination or the absolute COHb levels for the same exposure conditions are different in any way between races have not been studied. Blacks have lower diffusion capacity than whites (Neas and Schwartz, 1996), which transiently will slow CO loading and unloading. It also is well documented that the black population has a higher incidence of sickle cell anemia, which may be a risk factor for CO hypoxia (see Section 5.4.4 below).

### 5.4.4 Health Status

An individual with any pathophysiologic condition that reduces the blood $O_2$ content will be at a greater risk from CO exposure because additional reduction in the $O_2$-carrying capacity of blood resulting from COHb formation will increase hypoxemia. Depending on the severity of initial hypoxia, exposure to CO may lower the $O_2$ content to the point where $O_2$ delivery to the tissues becomes insufficient.

One group of disorders that encompasses a range of etiologically varied diseases characterized by a reduction in total blood Hb and subsequent insufficiency to meet $O_2$ demands are the anemias. Anemia is a result of either impaired formation of RBCs or increased loss or destruction of RBCs. The former category includes disorders of altered $O_2$ affinity, methemoglobinemias, and diseases with functionally abnormal and unstable Hb. By far, the most prevalent disorder in this group is a single-point mutation of Hb, causing sickle cell diseases, the most typical of which is a sickle cell anemia. The $O_2$-carrying capacity of individuals afflicted with sickle cell anemia is reduced not only because of a smaller amount of Hb, but also the $O_2$ dissociation curve is shifted to the right, reducing the $O_2$ affinity as well. Initial compensation involves primarily the cardiovascular system. The cardiac output will increase as both heart rate and stroke volume increase.

The opposite condition of anemia is polycythemia, an increased number of RBCs in blood. Although in polycythemia the total amount of Hb generally is elevated, under certain conditions the arterial $O_2$ saturation may be decreased, leading to a higher risk of additional hypoxia when exposed to CO (Foster et al., 1978; Stork et al., 1988).

A distinctive characteristic of chronic obstructive pulmonary disease (COPD) is increased $V_D$ and $\dot{V}_A/\dot{Q}$ inequality (Marthan et al., 1985). Subsequently, impaired gas mixing because of poorly ventilated lung zones will result in decreased arterial $O_2$ saturation and hypoxemia. These pathophysiologic conditions will slow both CO uptake and elimination. Any COHb formation will further lower the $O_2$ content of blood and increase hypoxemia. Because COPD patients very often operate at the limit of their $O_2$ transport capability, exposure to CO may severely compromise tissue oxygenation.

Because $O_2$ extraction by the myocardium is high, a greater $O_2$ demand by the myocardium of healthy individuals is met by an increased coronary blood flow. Patients with coronary artery disease (CAD) have a limited ability to increase coronary blood flow in response to increased $O_2$ demand during physical activity. If this compensatory mechanism is further compromised by decreased $O_2$ saturation from CO inhalation, the physical activity of patients with CAD may be restricted severely consequent to more rapid development of myocardial ischemia.

Individuals with congestive heart failure, right-to-left shunt in congenital heart disease, or cerebrovascular disease also may be at a greater risk from CO exposure because of already compromised $O_2$ delivery.

## 5.5 Modeling Carboxyhemoglobin Formation

### 5.5.1 The Coburn-Forster-Kane and Other Regression Models

#### 5.5.1.1 Empirical Regression Models

The most direct approach to establishing a prediction equation for COHb is to regress observed COHb values against the concentration and duration of exogenous CO exposure. Inclusion of other predictors such as initial COHb level and $\dot{V}_A$ generally will improve the precision of the predictions. Most of the CO regression models are purely empirical and have no physiological basis. Their applicability therefore is limited to more or less exact conditions that were used to collect the data on which they are based.

Peterson and Stewart (1970) developed a regression equation for estimating percent COHb following a 15-min to 8-h exposure of resting nonsmokers to moderate levels of CO (25 to 523 ppm):

$$\text{Log \% COHb} = 0.858 \text{ Log CO} + 0.630 \text{ Log t} - 0.00094\ t' - 2.295, \quad (5\text{-}2)$$

where CO refers to the concentration of CO in inhaled ambient air in parts per million, t is the exposure duration in minutes, and t′ is the postexposure time in minutes (set to 0 until the end of exposure). Data from a subsequent study were used to derive a new empirical formula for much higher concentrations of CO (1,000 to 35,600 ppm) but much shorter exposure times (45 s to 10 min) (Stewart et al., 1973). These equations still are used occasionally in field conditions to quickly estimate COHb concentration.

To predict changes in COHb as a function of ambient CO concentration in an urban setting, Ott and Mage (1978) developed a linear differential equation where only ambient CO concentration varied with time. All other parameters were empirically derived constants. With this simple model, they were able to show that the presence of CO spikes in data averaged over hourly intervals may lead to underestimating the COHb concentration by as much as 21% of the true value. Consequently, they recommended that monitored CO be averaged over 10 to 15 min periods. Based on a similar approach, other empirical models have been developed but not validated (Chung, 1988; Forbes et al., 1945). Comparison of predicted COHb values by these two models revealed a progressive divergence of the estimated COHb curves between models as exposure (100 ppm) progressed, with absolute differences approaching almost 7% COHb. Such wide variations in predicted COHb best demonstrate the inaccuracy of these types of models when applied outside of a narrowly defined range and make their utility in practical applications questionable (Tikuisis, 1996).

Several more sophisticated mathematical models have been developed to predict COHb as a function of exposure time (Singh et al., 1991; Sharan et al., 1990) or altitude (Selvakumar et al., 1992). The physiological variables used by Peterson and Stewart (1970) were employed to test these models and compare the results to the CFK predictions. The agreement between predicted COHb values by these models and the CFK model was very good; however, these theoretical models have not been validated by experimental studies.

#### 5.5.1.2 Linear and Nonlinear Coburn-Forster-Kane Differential Equations

In 1965, Coburn, Forster, and Kane developed a differential equation to describe the major physical and physiological variables that determine the concentration of COHb in blood for the examination of the endogenous production of CO. The equation, referred to as the CFK model, either in its original form or adapted to special conditions is still much in use today for the prediction of COHb consequent to inhalation of CO. Equation 5-3 represents the linear CFK model that assumes $O_2Hb$ is constant:

$$V_b \frac{d[COHb]_t}{dt} = \dot{V}_{CO} - \frac{[COHb]_0 P_{\bar{C}}O_2}{[O_2Hb]M} \left( \frac{1}{\frac{1}{D_L CO} + \frac{1}{\dot{V}_A}} \right) + \left( \frac{P_I CO}{\frac{1}{D_L CO} + \frac{1}{\dot{V}_A}} \right), \quad (5\text{-}3)$$

where $V_b$ is blood volume in milliliters; $[COHb]_t$ is the COHb concentration at time t in milliliters CO per milliliter blood, standard temperature and pressure, dry (STPD); $\dot{V}_{CO}$ is the endogenous CO production rate in milliliters per minute, STPD; $[COHb]_0$ is the COHb concentration at time zero in milliliters CO per milliliter blood, STPD; $[O_2Hb]$ is the oxyhemoglobin concentration in milliliters $O_2$ per milliliter blood, STPD; $P_{\bar{C}}O_2$ is the average partial pressure of $O_2$ in lung capillaries in millimeters of mercury; $\dot{V}_A$ is the alveolar ventilation in milliliters per minute, STPD; $D_L CO$ is the lung diffusing capacity of CO in milliliters per minute per millimeter of mercury, STPD; and $P_I CO$ is the CO partial pressure in inhaled air in millimeters of mercury. The model also assumes an instant equilibration of gases in the lung, COHb concentration between venous and arterial blood, and COHb concentrations between blood and extravascular tissues. Because $O_2$ and CO combine with Hb from the same pool, higher COHb values do affect the amount of Hb available for bonding with $O_2$. Such interdependence can be modeled by substituting (1.38 Hb – [COHb]) for $[O_2Hb]$, where Hb refers to the number of grams of Hb per milliliter of blood (Tikuisis et al., 1987a). The CFK differential equation (Equation 5-3) then becomes nonlinear:

$$\frac{d[COHb]_t}{dt} = \frac{\dot{V}_{CO}}{V_b} + \frac{1}{V_b \beta} \left( P_I CO - \frac{[COHb]_0 P_{\bar{C}}O_2}{[O_2Hb]M} \right), \quad (5\text{-}4)$$

where $\beta$ is $(1/D_L CO) + (P_B - 47)/\dot{V}_A$, and $P_B$ is the barometric pressure in millimeters of mercury. The nonlinear CFK model is more accurate physiologically but has no explicit solution. Therefore, interactive or numerical integration methods must be used to solve the equation (Muller and Barton, 1987; Johnson et al., 1992). One of the requirements of the method is that the volumes of all gases be adjusted to the same conditions (e.g., STPD) (Muller and Barton, 1987; Tikuisis et al., 1987a,b).

In general, the linear CFK equation is a better approximation to the nonlinear equation during the uptake of CO than during the elimination of CO. As long as the linear CFK equation is used to predict COHb levels at or below 6% COHb, the solution to the nonlinear CFK model will deviate no more than ±0.5% COHb (Smith, 1990). Over the years, it has been empirically determined that minute ventilation and the $D_L CO$ have the greatest influence on the CO uptake and elimination. The relative importance of other physiologic variables will vary with exposure conditions and health status. A comprehensive evaluation of fractional sensitivities of physiologic variables for both the linear and nonlinear CFK equations shows that each variable will exert its maximal influence at different times of exposure (McCartney, 1990). The analysis found that only the fractional concentration of CO in inhaled air, in parts per million ($F_I CO$), and $V_{CO}$ will not affect the rate at which equilibrium is reached. Figure 5-3 illustrates the temporal changes in fractional sensitivities of the principal determinants of CO uptake for the linear form of the CFK equation; THb is the total blood concentration of Hb. The fractional sensitivity of unity means that, for example, a 5% error in the selected variable induces a 5% error in predicted COHb value by the nonlinear model.

#### 5.5.1.3 Confirmation Studies of Coburn-Forster-Kane Models

Since the publication of the original paper (Coburn et al., 1965), several investigators have tested the fit of both the linear and nonlinear CFK model to experimental data using different CO exposure

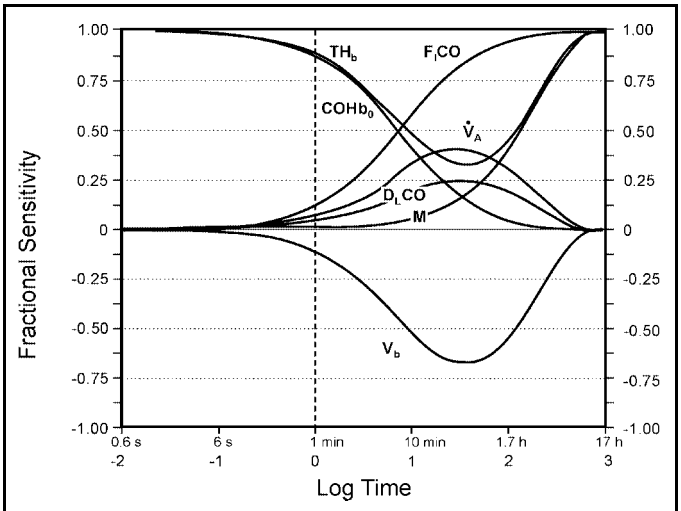


*Figure 5-3. Plot of fractional sensitivities of selected variables versus time of exposure (see text for details).*
Source: Modified from McCartney (1990).

profiles, a variety of experimental conditions, and different approaches to evaluating the parameters of the model. In all of these studies, almost all of the physiologic coefficients either were assumed or estimated based on each individual's physical characteristics; the COHb values were measured directly and also calculated for each individual.

Stewart et al. (1970) tested the CFK linear differential equation on 18 resting, healthy subjects exposed to 25 different CO exposure profiles for periods of 0.5 to 24 h and to CO concentrations ranging from 1 to 1,000 ppm. In a later study, they tested the nonlinear CFK equation on 22 subjects at various levels of exercise while being exposed to up to 200 ppm CO for up to 5.25 h (Peterson and Stewart, 1975). From the obtained values, they concluded that, either at rest or with exercise, the agreement between the predicted and measured COHb values was good (correlation coefficient [r] > 0.74).

The first study to test both the linear and nonlinear CFK models for CO uptake and elimination in pedestrians and car passengers exposed to ambient CO levels in a city was conducted by Joumard et al. (1981). The two cohorts exposed for 2 h to street and traffic concentrations of CO, respectively, comprised 73 nonsmokers (18 to 60 years of age). Blood COHb samples were taken only at the beginning and the end of each journey, where the COHb value reached 2.7%, on average. Both equations performed well in estimating accurately COHb levels, although the value for males was underestimated slightly.

The predictive strength of the CFK model under variable CO concentrations was tested by Hauck and Neuberger (1984). A series of experiments was performed on four subjects exposed to a total of 10 different CO exposure profiles at several exercise levels, so that each exposure was a unique combination of CO concentration and exercise pattern. The ventilation and COHb values (measured and calculated) were obtained at 1-min intervals. The agreement between measured and predicted COHb under these varied conditions was very good; the mean difference was only 7.4% of the nominal (maximal predicted) value.

A series of studies has tested the accuracy of the CFK equation under transient exposure conditions that would violate several assumptions of the CFK model, specifically the assumption of a single, well-mixed vascular compartment. These studies were designed to simulate everyday conditions (e.g., environmental, occupational, military) that may involve frequent but brief (75 s to 5 min) exposures to high (667 to 7,500 ppm) CO concentrations at rest and with exercise. Moreover, the experiments were designed to test the accuracy of the CFK equation under transient exposure conditions during the CO uptake and early elimination phases from arterial and venous blood. Attempts were made to measure directly some of the key physiologic parameters of the CFK equation for each subject (Tikuisis et al., 1987a,b; Benignus et al., 1994). The studies have shown that during and immediately following exposure, the arterial COHb was considerably higher (1.5 to 6.1%), and the venous COHb was considerably lower (0.8 to 6%) than the predicted COHb. The observed COHb differences between arterial and venous blood ranged from 2.3 to 12.1% COHb among individuals (Benignus et al., 1994). The overprediction of venous COHb increased during exercise (≈10% of the true value). Provided that the total CO dose (concentration × time) is the same and within the time constant for the CO uptake and elimination, the COHb value was found to be the same, regardless of the pattern of exposure. Because $\dot{V}_A$ affects both the equilibrium and the rate at which

it is achieved, inconsistencies in the estimates or conversion of gas volumes (ATPS and BTPS to STPD) will affect the predicted values. The interindividual and intraindividual disparities between measured and predicted COHb values were attributed primarily to delays in mixing of arterial and venous blood and differences in cardiac output; but, other factors, such as lung wash-in, also contribute to this phenomenon. Modification of the CFK equation by adjusting for regional differences in blood flow produced a model that predicted with much greater accuracy both the arterial (<0.7% COHb difference) and venous (<1% COHb difference) COHb during transient uptake and elimination of CO from blood (Smith et al., 1994).

Although the CO concentrations used in these studies are several orders of magnitude higher than the usual CO concentrations found in ambient air, under certain conditions (see Section 5.4.1), people can be exposed briefly (<10 min) to such (or even higher) levels of CO in their immediate environment. Because the physiologic mechanisms (but not the kinetics) of COHb formation are independent of CO concentration, high COHb transients, particularly in at-risk individuals, could be of clinical importance. Even briefly, higher arterial COHb may lead to functional impairment of the hypoxia-sensitive heart and brain (see Sections 5.2.2.3 and 5.2.2.4). In these situations, the predicted instantaneous arterial COHb level will be substantially underestimated.

#### 5.5.1.4 Application of Coburn-Forster-Kane Models

To obviate measurements of CFK equation parameters, many of which are complex techniques, attempts were made to simplify the CFK equation, because it may be difficult or even impossible to measure directly some of these parameters, particularly during physical activity. In one study, by relating physiological parameters to the $O_2$ uptake by the body, which was in turn related to an activity level, a simplified linear form of the CFK model was developed (Bernard and Duker, 1981). The model was used subsequently to draw simple nomograms of predictive relationships between pairs of variables, but the accuracy of the nomograms was not tested experimentally.

The need for more accurate COHb prediction under more complex physiologic or exposure conditions requires either modification or expansion of the CFK model. Benignus (1995) combined a physiological model of respiratory gas exchange, MACPUF (Ingram et al., 1987), with the CFK model. The new model allows for continuous output and input of 60 cardiopulmonary variables, including $F_ICO$. The usefulness of the model is particularly in its ability to continuously update COHb concentration in response to dynamically changing physiologic parameters. The model also allows COHb prediction under conditions that otherwise would be very difficult to duplicate in the laboratory.

A fundamental modification of the CFK model was made by Hill et al. (1977) to study the effects of CO inspired by the mother on the level of fetal COHb. The Hill equation combines the CFK equation (for maternal COHb) with a term denoting COHb transfer from a placenta into the fetus. Comparative evaluation of predicted and measured fetal COHb concentrations under time-varying and steady-state conditions in both women and animals showed acceptable agreement only under steady-state conditions (Hill et al., 1977; Longo and Hill, 1977).

As mentioned in Section 5.5.1.3, Smith et al. (1994) expanded the CFK model to allow for prediction of arterial and venous COHb during transient CO uptake and early elimination phases. The model incorporated regional differences in blood flow, particularly in the forearm, because the forearm is used most frequently for blood sampling. This more elaborate model performed extremely well in predicting blood COHb. Although the model was validated on a small number of subjects using the same experimental setting, the validation was not performed under more demanding conditions of physical activity and varying CO concentrations.

To accurately predict COHb in individuals exposed to dihalomethanes, which are a source of endogenous CO (see Section 5.3), the CFK model was extended to account for the CO production caused by oxidation of a parent chemical (Andersen et al., 1991). The model developed and validated on rats employed a variety of exposure scenarios to dichloromethane. It subsequently was tested on six volunteers

exposed to dichloromethane, and, after adjustment of a few parameters, the COHb level was predicted remarkably well. After further validation, this model has potential use in predicting accurately COHb caused by exogenous and endogenous CO originating from different sources (e.g., Hb degradation, metabolism of dihalomethanes, inhaled CO).

Reexpression of the solution of the CFK model from percent COHb to parts per million of CO allows the examination of a variety of CO concentration profiles, while keeping a simple preselected target COHb as a constant. Application of the transformed model to urban hourly averaged CO concentrations that just attained alternative 1-h and 8-h CO National Ambient Air Quality Standards (NAAQS) showed that, depending on the air quality pattern used, between 0.01 to 10% of the population may exceed a target 2.1% COHb level in blood without ambient CO concentrations ever exceeding the standard. By including transients, the models predicted COHb more accurately, particularly when built into the 8-h running averages (Venkatram and Louch, 1979; Biller and Richmond, 1982, 1992). Actually, the ambient CO concentrations could be averaged over any time period less than or equal to the half-life of COHb (Saltzman and Fox, 1986).

## 5.6 Intracellular Effects of Carbon Monoxide

### 5.6.1 Introduction

Traditional concepts for CO pathophysiology have been based on the high affinity of CO for deoxyhemoglobin and consequent reduction of $O_2$ delivery. This mechanism is relevant for high CO concentrations, but it is less likely to be relevant to the concentrations of CO currently found in the ambient environment. This section will summarize recently published information on biochemical mechanisms that is not related to an impairment of oxygen delivery from elevations in COHb. Some of the studies outlined in this section were done with cells in culture and others with laboratory rats. To be relevant to human exposures from environmental contamination, it is important to note what concentrations of CO are likely to occur in vivo. Lung parenchyma represents a special situation where cells may be exposed to ambient CO without the reduction in concentration associated with Hb-bound CO. Elsewhere in the body, only a fraction of COHb will dissociate to elevate extravascular CO concentrations. This elevation is in the range of approximately 2 to 10 nmol when the COHb concentration is from 0.8 to 3.8% (Coburn, 1970a; Göthert et al., 1970). The COHb values near steady-state conditions in laboratory rats are close to values for humans (Kimmell et al., 1999). This strengthens the potential for human relevance in recent animal studies that show that newly identified biochemical mechanisms do have adverse physiological effects. However, caution still is warranted because direct evidence for the occurrence of these mechanisms in humans has not been shown.

### 5.6.2 Inhibition of Hemoprotein Function

Carbon monoxide can inhibit a number of hemoproteins found in cells, such as Mb, cytochrome c oxidase, cytochrome P-450, dopamine β hydroxylase, and tryptophan oxygenase (Coburn and Forman, 1987). Inhibition of these enzymes could have adverse effects on cell function.

Carbon monoxide acts as a competitive inhibitor, hence biological effects depend on the partial pressures of both CO and $O_2$. The cellular hemoprotein with the highest relative affinity for CO over that for $O_2$ is Mb. Carbon monoxide will inhibit Mb-facilitated oxygen diffusion, but physiological compromise is seen only with high concentrations of COMb. Wittenberg and Wittenberg (1993) found that high-energy phosphate production was inhibited in isolated cardiac myocytes, maintained at a physiologically relevant oxygen concentration, when COMb exceeded 40%. The authors estimated that formation of sufficient COMb to impair oxidative phosphorylation in vivo would require a COHb level of 20 to 40%.

Coefficients for binding CO versus $O_2$ among cytochrome P-450-like proteins vary between 0.1 and approximately 12, and there have been recent discussions suggesting that CO-mediated inhibition of these

proteins could cause smooth muscle relaxation in vivo (Coburn and Forman, 1987; Wang et al., 1997a; Wang, 1998). The issue relates to inhibition of cytochrome P-450-dependent synthesis of several potent vasoconstricting agents (Wang, 1998). Vasodilation has been shown via this mechanism with high concentrations of CO (ca. 90,000 ppm) (Coceani et al., 1988). It is unclear, however, whether this could arise under physiological conditions and CO concentrations produced endogenously. The competition between CO and $O_2$ for cytochrome c oxidase was well outlined in the previous review (U.S. Environmental Protection Agency, 1991), but some additional information has been published since then. Based on its Warburg partition coefficient of between 5 and 15, CO binding is favored only in situations where oxygen tension is extremely low (Coburn and Forman, 1987). Carbon monoxide binding to cytochrome c oxidase in vivo will occur when COHb is high (ca. 50%), a level that causes both systemic hypotension as well as impaired oxygen delivery (Brown and Piantadosi, 1992). Mitochondrial dysfunction, possibly linked to cytochrome inhibition, has been shown to inhibit energy production, and it also may be related to enhanced free radical production (Piantadosi et al., 1995, 1997a). There has been no new information published since the last air quality criteria document that pertains to the effects of CO on dopamine β hydroxylase or tryptophan oxygenase.

### 5.6.3 Free Radical Production

Laboratory animal studies indicate that nitrogen- and oxygen-based free radicals are generated in vivo during CO exposures. Exposure to CO at concentrations of 20 ppm or more for 1 h will cause platelets to become a source of the nitric oxide free radical (•NO) in the systemic circulation of rats (Thom et al., 1994; Thom and Ischiropoulos, 1997). Studies with cultured bovine pulmonary endothelial cells have demonstrated that exposures to CO at concentrations as low as 20 ppm cause cells to release •NO, and the exposure will cause death by a •NO process that is manifested 18 to 24 h after the CO exposure (Thom et al., 1997; Thom and Ischiropoulos, 1997). The mechanism is based on elevations in steady-state •NO concentration and production of peroxynitrite (Thom et al., 1994, 1997). Peroxynitrite is a relatively long-lived, strong oxidant that is produced by the near diffusion-limited reaction between •NO and superoxide radical (Huie and Padmaja, 1993).

The mechanism by which CO concentrations of 11 nmol or more cause an elevation of steady-state •NO concentrations appears to be based on altered intracellular "routing" of •NO in endothelial cells and platelets. It is well established that the association and dissociation rate constants of •NO with hemoproteins exceed the rate constants for $O_2$ or CO (Gibson et al., 1986). However, Moore and Gibson (1976) found that when CO was incubated with nitrosyl (•NO)-Mb or •NO-Hb, CO slowly displaced the •NO. Carbon monoxide replacement occurred even when there was excess •NO-heme protein, and replacement rates were enhanced by increasing the CO concentration or by carrying out the reaction in the presence of agents such as thiols, which will react with the liberated •NO. These conditions, including the presence of thiols, exist in cells exposed to environmentally relevant concentrations of CO. Exposures to up to 1,070 nmol CO do not alter the rate of production of •NO by platelets and endothelial cells, yet liberation of •NO was enhanced by CO (Thom and Ischiropoulos, 1997; Thom et al., 1994; Thom et al., 1997).

Carbon monoxide will increase the concentration of •NO available to react with in vivo targets in both lung and brain, based on electron paramagnetic resonance studies with rats exposed to 50 ppm CO or more (Ischiropoulos et al., 1996; Thom et al., 1999a). The concentrations of nitric oxide synthase isoforms in lung were not altered because of CO, and the mechanism for elevation in •NO was thought to be the same as that found in isolated cells (Thom et al., 1994, 1997). Exposure to 50 to 100 ppm CO also will increase hydrogen peroxide ($H_2O_2$) production in lungs of rats (Thom et al., 1999a). The phenomenon depended on •NO production, as it was inhibited in rats pretreated with $N^{\omega}$nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor. Production of •NO-derived oxidants also is increased in CO-exposed rats, based on measurements of nitrotyrosine, a major product of the reaction of peroxynitrite with proteins (Ischiropoulos et al., 1996; Thom et al., 1998, 1999a,b).

The mechanism for enhanced $H_2O_2$ production in lungs of CO-exposed rats is not clear. It is possible that •NO or peroxynitrite may perturb mitochondrial function. Peroxynitrite inhibits electron transport at complexes I through III, and •NO targets cytochrome oxidase (Cassina and Radi, 1996; Lizasoain et al., 1996; Poderoso et al., 1996). It is important to note, however, that alterations in mitochondrial function and an increase of cellular $H_2O_2$ were not found in studies where cultured bovine endothelial cells were exposed to similar CO concentrations (Thom et al., 1997). An alternative possible mechanism to mitochondrial dysfunction is that exposure to CO may inhibit antioxidant defenses. Mechanisms linked to elevations in •NO could be responsible for inhibiting one or more enzymes. Nitric oxide-derived oxidants can inhibit manganese superoxide dismutase and glyceraldehyde-3-phosphate dehydrogenase and deplete cellular stores of reduced glutathione (Ischiropoulos et al., 1992; Luperchio et al., 1996).

Exposure to high CO concentrations (2,500 to 10,000 ppm) cause mitochondria in brain cells to generate hydroxyl-like radicals (Piantadosi et al., 1995, 1997a). An additional source of partially reduced $O_2$ species found in animals exposed to CO is xanthine oxidase. Conversion of xanthine dehydrogenase, the enzyme normally involved with uric acid metabolism, to xanthine oxidase, the radical-producing form of the enzyme, occurred in the brains of rats exposed to approximately 3,000 ppm CO (Thom, 1992). Lower CO concentrations did not trigger this change. Therefore, xanthine oxidase is unlikely to be a free radical source following exposures to CO at concentrations found in ambient air. Moreover, enzyme conversion was not a primary effect of CO; rather, it occurred only following sequestration and activation of circulating leukocytes (Thom, 1993).

### 5.6.4 Stimulation of Guanylate Cyclase

In recent years, CO has been demonstrated to play a physiological role in vasomotor control and neuronal signal transduction (Morita et al., 1995; Ingi et al., 1996). Carbon monoxide is produced endogenously by oxidation of organic molecules, but the predominant source is from the degradation of heme (Rodgers et al., 1994). The rate-limiting enzyme for heme metabolism is heme oxygenase (HO), which converts heme to biliverdin, free iron, and CO. Three isoforms of HO have been characterized. The HO-1 is an inducible enzyme found in vascular endothelial cells, smooth muscle cells, bronchoalveolar epithelium, and pulmonary macrophages. The HO-1 is induced by its substrate, heme, as well as •NO, $H_2O_2$, several cytokines, and lipopolysaccharide (Arias-Díaz et al., 1995; Durante et al., 1997; Morita et al., 1995; Motterlini et al., 1996). The HO-2 is a constitutive enzyme found in certain neurons within the central nervous system, testicular cells, and vascular smooth muscle cells (Marks, 1994). Little is known about HO-3, which recently was identified in homogenates from a number of organs (McCoubrey et al., 1997).

A main physiological role for CO is thought to be regulation of soluble guanylate cyclase activity. Both CO and •NO can activate guanylate cyclase, although activation by CO is approximately 30-fold lower (Stone and Marletta, 1994). In neuronal cells possessing both heme oxygenase and nitric oxide synthase, regulation of cyclic guanosine monophosphate (cGMP) synthesis is mediated in a reciprocal fashion by producing either CO or •NO (Ingi et al., 1996; Maines et al., 1993). A compensatory interrelationship between nitric oxide synthase and heme oxygenase also has been found in endothelial cells and activated macrophages, although its functional significance is unknown (Kurata et al., 1996; Seki et al., 1997). In macrophages, cGMP synthesis promotes chemotaxis, and cGMP-mediated synthesis and secretion of tumor necrosis factor α has been linked to both CO and •NO (Arias-Díaz et al., 1995; Belenky et al., 1993). Carbon monoxide causes smooth muscle relaxation by stimulating soluble guanylate cyclase (Utz and Ullrich, 1991; Wang et al., 1997b). Smooth muscle relaxation also may occur because of activation of calcium dependent potassium channels, although this effect may be linked to guanylate cyclase activity (Trischmann et al., 1991; Wang et al., 1997a). Carbon monoxide-mediated smooth muscle relaxation is involved with control of microvascular hepatic portal blood flow (Goda et al., 1998; Pannen and Bauer,

1998) and suppressing contractions in the gravid uterus (Acevedo and Ahmed, 1998). It also may play a role in gastrointestinal motility (Farrugia et al., 1998).

## 5.7 Mechanisms of Carbon Monoxide Toxicity

### 5.7.1 Alterations in Blood Flow

Carbon monoxide from environmental pollution may exert similar effects in vivo to those of endogenously produced CO, because the nanomolar tissue concentrations resulting from inhalation of CO are comparable or greater than concentrations produced by cells possessing heme oxygenase. Liver parenchyma has been estimated to generate approximately 0.45 nmol CO/gram liver/min (Goda et al., 1998). Carbon monoxide synthesis by smooth muscle cells is approximately 8 pmol/mg protein/min for human aorta and 23 to 37 pmol/mg protein/min for rat aorta (Cook et al., 1995; Grundemar et al., 1995). Carbon monoxide production by unstimulated pulmonary macrophages is 3.6 pmol/mg protein/min, and, after stimulation with lipopolysaccharide, it increases to about 5.1 pmol/mg protein/min (Arias-Díaz et al., 1995). The rate of synthesis of CO varies widely for nerve cells. Cerebellar granule cells generate approximately 3 fmol/mg protein/min, olfactory nerve cells produce 4.7 pmol/mg protein/min, and rat cerebellar homogenates can generate as much as 56.6 pmol/mg protein/min (Ingi and Ronnett, 1995; Ingi et al., 1996; Maines, 1988; Nathanson et al., 1995).

Vasodilation is a well-established effect caused by exposure to environmental CO. At high CO concentrations, on the order of 500 to 2,000 ppm, the mechanism is related to impairment of $O_2$ delivery (Kanten et al., 1983; MacMillan, 1975). However, a portion of the observed increases in cerebral blood flow are independent of perturbations in $O_2$ supply (Koehler et al., 1982). In a setting where cellular oxidative metabolism was not impaired by CO, elevations in cerebral blood flow appeared to be mediated by $^{\bullet}NO$ (Meilin et al., 1996). Whether the mechanism was the same as that outlined in the section above, which causes oxidative stress, remains to be determined.

It is unclear whether disturbances in vascular tone by environmental CO is a generalized, systemic response, and the impact of variables such as the duration of exposure have not been adequately investigated. Although cerebral vasodilation mediated by $^{\bullet}NO$ was reported with exposures to 1,000 ppm CO, that level of exposure did not alter pulmonary vasoconstriction in an isolated-perfused rat lung model (Cantrell and Tucker, 1996). Exposure to 150,000 ppm CO caused no changes in pulmonary artery pressure in isolated blood-perfused lungs, although CO did inhibit hypoxic pulmonary vasoconstriction (Tamayo et al., 1997). Humans exposed to CO for sufficient time to achieve COHb levels of approximately 8% were not found to have alterations in forearm blood flow, blood pressure, or heart rate (Hausberg and Somers, 1997).

Animals exposed to high CO concentrations (e.g., 3,000 to 10,000 ppm) have diminished organ blood flow, which contributes to CO-mediated tissue injury (Brown and Piantadosi, 1992; Ginsberg and Meyers, 1974; Okeda et al., 1981; Song et al., 1983; Thom, 1990). The mechanism is based on CO-mediated hypoxic stress and cardiac dysfunction; therefore, these effects do not arise at CO concentrations relevant to ambient air quality.

### 5.7.2 Mitochondrial Dysfunction and Altered Production of High-Energy Intermediates

When exposed to 10,000 ppm CO, rats exhibit impaired high-energy phosphate synthesis and production of hydroxyl free radicals because of mitochondrial dysfunction (Brown and Piantadosi, 1992; Piantadosi et al., 1995). Exposure to 2,500 ppm CO also will cause hydroxyl radicals to be produced, apparently by mitochondria, because of a process that could not be related to hypoxic stress (Piantadosi et al., 1997a). Evidence for mitochondrial dysfunction has not been observed in vivo at lower CO concentrations. However, under conditions of high metabolic demand, exposure to even 1,000 ppm CO

in the absence of an overt hypoxic stress will result in impaired energy production in brain (Meilin et al., 1996).

Carbon monoxide binding to mitochondrial cytochromes of respiring cells in vitro has been documented only when either the CO concentration was extraordinarily high, or $O_2$ tension was extremely low, such that the $CO/O_2$ ratio exceeded 12:1 (Coburn and Forman, 1987). Following CO exposure and removal to fresh air, CO diffuses out from cells, and mitochondrial function is restored. This process is enhanced by inspiration of hyperbaric oxygen (Brown and Piantadosi, 1992). Studies in mice indicate that high CO concentrations inhibit synthesis of high-energy phosphates during exposure to 5,000 ppm CO for 15 min and these changes do not persist following removal to fresh air (Matsuoka et al., 1993). In summary, mitochondrial dysfunction and impaired high-energy phosphate synthesis have been shown by several independent laboratories to occur during exposures to high CO concentrations. Current information suggests that this alteration does not occur at CO concentrations relevant to ambient air quality, and that changes in energy production are not persistent for long periods of time following CO exposure.

### 5.7.3 Vascular Insults Associated with Exposure to Carbon Monoxide

There are two primary variables that impact on CO toxicity. One is the concentration of CO, the other is the duration of exposure. Traditionally, these two variables have been viewed as merely alternative ways of elevating COHb concentration in the body. The concentration of CO breathed dictates the duration of exposure required to achieve a particular blood level of COHb or tissue level of CO. This view is predicated on the notion that CO pathophysiology is determined by its binding to one or another hemoprotein and to inhibition of oxygen delivery or oxidative metabolism.

There is a substantial body of literature to suggest that, at least with regard to vascular effects, the duration of exposure has a greater impact on the magnitude of CO pathophysiology than what is predicted based on the concentration of CO that is inspired. For example, the lungs are the first site for potential action of environmental CO. Results from investigations have been conflicting regarding the risk for pulmonary injury from CO. Because of the lack of consensus and also the absence of a recognized biochemical mechanism, low concentrations of CO have been viewed as posing little risk to lung physiology (U.S. Environmental Protection Agency, 1991, 1992). When animals have been exposed to high CO concentrations sufficient to raise COHb levels to 56 to 90%, exposures have lasted for only minutes because of the hypoxic stress. In these studies, evidence of increased capillary permeability was inconsistent (Fein et al., 1980; Niden and Schulz, 1965; Penney et al., 1988), and no other alterations in lung physiology were detected (Fisher et al., 1969; Robinson et al., 1985; Shimazu et al., 1990; Sugi et al., 1990). In contrast, when human beings or experimental animals were exposed to CO for many hours at a time, capillary leakage of macromolecules from the lungs and systemic vasculature has been documented, but the presence of hypoxic stress was questioned (Kjeldsen et al., 1972; Maurer, 1941; Parving et al., 1972; Siggaard-Andersen et al., 1968).

In light of the physiological role for CO in vasomotor control, protracted exposures may be prone to disturb vascular homeostasis, giving rise to pathophysiological responses. Monkeys exposed to 250 ppm CO for 2 weeks exhibited coronary artery damage consisting of subendothelial edema, fatty streaking, and lipid-loaded cells (Thomsen, 1974). This study and others (Armitage et al., 1976; Davies et al., 1976; Turner et al., 1979; Webster et al., 1968) have suggested a link between atherosclerosis and chronic CO exposure. However, other studies have failed to find evidence for an association (Hugod et al., 1978; Penn et al., 1992).

Carbon monoxide may cause vascular insults. Leakage of albumin and leukocyte sequestration have been shown following exposures of rats to 50 ppm or more for 1 h, and the process is mediated by $^{\bullet}NO$-derived oxidants (Ischiropoulos et al., 1996; Thom, 1993; Thom et al., 1998, 1999a,b). Brain oxidative stress associated with this mechanism has been shown with rats exposed to 1,000 to 3,000 ppm CO for 1 h (Ischiropoulos et al., 1996; Thom, 1993). However, it is unclear whether the flux of $^{\bullet}NO$, resulting from

exposures to lower CO concentrations contribute to oxidative or nitrosative stress in vivo. Important differences in the patterns of leakage from pulmonary and systemic vascular beds suggest that they may be caused by different mechanisms. For example, systemic vascular leakage was present for several hours after CO exposure, and the leakage resolved within 18 h, whereas pulmonary vascular leakage was not measurable until 18 h after CO exposure, and it resolved by 48 h. Both pulmonary and systemic vascular leakage occurred after hour-long exposures to CO, but not when exposures lasted for only 30 min, and vascular changes were not different whether rats were exposed to just 50 ppm or as much as 1,000 ppm CO. These are recent observations, and further investigations are required before their relevance to environmental CO contamination can be assessed adequately. Moreover, it should be emphasized that the vascular leakage observed in lungs and systemic microvasculature following exposures to CO at concentrations as low as 50 ppm may have no pathophysiological impact if regional lymphatics can sustain a higher flow so that edema does not occur (Thom et al., 1998, 1999a,b).

## 5.8 Other Effects of Carbon Monoxide

Among the most concerning pathophysiological effects of CO is its propensity for causing brain damage. There has been considerable effort focused on potential mechanisms for this process. With regard to ambient air standards, however, it is important to note that recent studies were done with high CO concentrations. Carbon monoxide poisoning is not a "pure" pathological process, as injuries may be precipitated by a combination of cardiovascular effects linked to hypoperfusion or frank ischemia, COHb-mediated hypoxic stress, and intracellular effects, including free radical production and oxidative stress. For example, CO poisoning causes elevations of glutamate and dopamine in experimental models and human fatalities (Arranz et al., 1997; Ishimaru et al., 1991, 1992; Nabeshima et al., 1990, 1991; Newby et al., 1978; Piantadosi et al., 1997b). These elevations occur because of the CO-associated cardiovascular compromise and, possibly, other direct CO-mediated effects. Based on the effects of agents that block the N-methyl-D-aspartate (NMDA) receptor, elevations of glutamate in experimental CO poisoning have been linked to a delayed type (but not an acute type) of amnesia, to loss of CA1 neurons in the hippocampus of mice, and to loss of glutamate-dependent cells in the inner ear of rats (Ishimaru et al., 1991, 1992; Liu and Fechter, 1995; Nabeshima et al., 1990, 1991). Antioxidants can protect against CO-mediated cytotoxicity of glutamate-dependent nerve cells (Fechter et al., 1997). Mechanisms of glutamate neurotoxicity include excessive calcium influx, free-radical-mediated injury that may include calcium-calmodulin-dependent activation of cytosolic NO synthase, and lipid peroxidation. Moderate stimulation by excitatory amino acids may cause mitochondrial dysfunction with impaired synthesis of adenosine triphosphate and production of reactive $O_2$ species (Beal, 1992). Cell death can be through necrosis or programmed cell death, depending on the intensity of the stimulus (Gwag et al., 1995). There also may be a synergistic injury with other forms of oxidative stress because reactive $O_2$ species can intensify excitotoxicity (Bridges et al., 1991; Pellegrini-Giampietro et al., 1990). Glutamate also can injure cells in the central nervous system that do not have NMDA receptors by competing for cysteine uptake, which inhibits synthesis of glutathione (Lipton et al., 1997; Murphy et al., 1989; Oka et al., 1993).

## 5.9 Summary

The most prominent pathophysiological effect of CO is hypoxemia caused by avid binding of CO to Hb. Formation of COHb reduces $O_2$-carrying capacity of blood and impairs release of $O_2$ from $O_2$Hb to tissues. Failure of vasodilation to compensate causes tissue hypoxia. In addition to tissue hypoxia, ultimate diffusion of CO to cells may affect adversely their function. The brain and heart tissues are particularly sensitive to CO-induced hypoxia and cytotoxicity. The rate of COHb formation and elimination depends on many physical and physiological factors. The same factors that govern CO uptake determine CO

elimination as well. The flow of CO between blood and either alveolar air or the tissues, and vice versa, is governed by the CO concentration gradient between these compartments and becomes the rate-determining step in the mass transfer of CO for COHb formation and elimination. Because of a small blood-to-air CO gradient and tight binding of CO to Hb, the elimination half-time is quite long, varying from 2.0 to 6.5 h. Apart from the CO concentration in ambient air, the principal determinants of CO uptake are minute ventilation and lung diffusion capacity. Thus, any physiological conditions that affect these variables (e.g., exercise, age) also will affect the kinetics of COHb. Both the physical and physiological variables have been integrated into many empirical and mathematical models of COHb formation and elimination under static and dynamic conditions of ambient CO concentration and physiologic function. The nonlinear CFK equation is the most widely used predictive model of COHb formation, and it still is considered the best all-around model for COHb prediction. Altitude may have a significant influence on COHb kinetics. The effects of hypoxic hypoxia (altitude) and CO-induced hypoxia appear to be additive. Adaptation to altitude will moderate COHb formation. In addition to exogenous sources of CO, the gas also is produced endogenously through catabolism of Hb, metabolic processes of drugs, and degradation of inhaled solvents and other xenobiotics. This last source may lead to very high (up to 50%) COHb concentrations. Many disorders, particularly anemias of any etiology, will predispose affected individuals to CO hypoxia. Furthermore, patients with a variety of cardiopulmonary diseases (e.g., COPD, CAD) and chronic inflammatory diseases may be at increased risk because of elevated COHb.

Apart from impaired $O_2$ delivery to the tissues because of COHb formation, recent studies of CO pathophysiology suggest cytotoxic effects independent of $O_2$. New investigations have expanded the understanding of CO in two areas. First, there is a growing recognition of the role that CO may play in normal neurophysiology and in microvascular vasomotor control. The impact of CO from ambient air on these processes has not been investigated adequately; hence, there is insufficient information available to influence decisions on ambient air quality standards. The second area of investigation of CO is related to its propensity for causing free-radical-mediated changes in tissues. Mechanisms for these changes have been linked both to mitochondria and to a CO-mediated disturbance of intracellular "buffering" of endogenously generated free radicals (e.g., ·NO). The role these mechanisms play in pathophysiology currently is being investigated. Where dose-response studies are available, the concentrations of CO that cause adverse effects in animals exceed current NAAQS.

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