INFLUENCE OF CARBON MONOXIDE ON CARDIAC DYNAMICS IN NORMAL AND CARDIOVASCULAR STRESSED ANIMALS

INSTITUTE OF ENVIRONMENTAL STRESS

UNIVERSITY OF CALIFORNIA

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ABSTRACT

A new method for determining carbon monoxide (CO) in minute quantities (100-200 µl) was developed. This is probably the most accurate procedure available at the present time. Elimination rates of CO in anesthetized, spontaneously breathing dogs was found to be biphasic. The initial distribution curve was exponential, followed by an elimination curve which was linear. Prediction equations for elimination of CO from blood were developed. A method was developed for accurately adjusting a subject's blood carboxyhemoglobin (HbCO) level and then maintaining this level during rest or exercise. The effects of low concentrations of HbCO on exercise performance (submaximal levels) were determined. No substantial decrements were observed. Cardiovascular responses, particularly coronary blood flow, of the dog to various levels of HbCO (6.2 to 35.6%) were determined. When CO was given as a bolus (within 2-3 minutes) coronary blood flow increased progressively as HbCO levels increased. This increase was accompanied by decreased arterial and coronary sinus oxygen tensions and decreased myocardial oxygen extraction. Animals exposed for 1 hour to a constant level of HbCO had the anticipated increase in coronary blood flow maintained for the duration of the exposure. Animals exposed to 100 ppm CO for 4 hours each day, 5 days a week for 6 weeks (HbCO levels approximately 10%) showed no evidence of adaptation. Their responses to an acute exposure to 10% HbCO did not differ from those observed in animals with no prior exposure to ambient carbon monoxide. A most suggestive study was conducted on animals in whom a complete atrioventricular (AV) block was produced. These animals were maintained by cardiac pacemakers. Following exposure to 6-7% HbCO in contrast to normal control animals, coronary blood flow failed to increase as anticipated. These data are suggestive of the potential danger of low levels of HbCO to cardiac disabled individuals.

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ACKNOWLEDGEMENTS

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CONCLUSIONS

1. Carbon monoxide inhalation by normal animals resulting in increased levels of carboxyhemoglobin (6 to 35%) increased coronary blood flow, decreased availability of oxygen to the myocardium (heart muscle), and decreased the oxygen tensions of both arterial and coronary sinus blood.

2. Animals with myocardial disturbances, acquired complete atrioventricular block, failed to respond to a low level of HbCO (6 to 7%) as had normal animals, suggesting that animals with myocardial impairments could be at increased risk breathing polluted (CO) air.

3. Disappearance of carbon monoxide from blood was biphasic, with an initial rapid rate followed by a slower rate which was partially dependent upon the absolute level of carboxyhemoglobin.

4. Animals performing submaximal levels of work showed minor impairments in cardiovascular function with levels of HbCO from 8.4 to 20.8%.

5. Animals chronically exposed to ambient air having 100 ppm of carbon monoxide responded to an acute exposure of CO which raised the level of HbCO to the same degree (10%) as was present at the end of each daily exposure had cardiorespiratory responses similar to those observed in non-exposed animals. No significant acclimation effects were observed except for a slightly higher hemoglobin after 6 weeks exposure.

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6. A sensitive and highly accurate method for measuring blood carbon monoxide concentrations was developed. Minimal quantities of blood (100 to 200 µl) are required for this measurement.

RECOMMENDATIONS

1. Animal studies provide an excellent model for evaluating CO effects on the cardiorespiratory system and should be utilized for clarification of the effects of ambient CO levels on these systems.

2. Additional studies should be initiated to determine the influence of low concentrations of carboxyhemoglobin on animals with additional myocardial disturbances to determine if these other CHD states or peripheral vascular diseases also result in an inadequate response to carbon monoxide hypoxia. Suggestive data from human studies indicates that there is a necessity to obtain such more precise information.

3. In view of the demonstrated responses of myocardial tissues and the suggestive psychophysiological changes in central nervous system function, studies on cerebral circulation and metabolism could provide a scientific basis for the implications that the CNS is the other (besides the myocardium) most sensitive tissue to carboxyhemoglobin-induced hypoxia.

4. Longer chronic exposures (of up to 1 year) to low levels of ambient CO up to 50 ppm should be supported to answer the question as to whether or not there is an adaptation to this ambient pollutant.

BODY OF REPORT

INTRODUCTION

Over a hundred years elapsed between the discovery of the chemical composition of carbon monoxide and the first definitive study of the physiological effects of this gas indicating the interrelationships of hemoglobin, oxygen and carbon monoxide (13).* On the basis of early work it was assumed that no harm to the organism would ensue if carboxyhemoglobin (HbCO) levels were below 15-20%. Chiodi et al. (8) demonstrated significant cardiorespiratory responses when HbCO concentrations were raised above 20% but lower concentrations generally failed to produce demonstrable effects. On the other hand, Ayres et al. (5-7) have shown that short exposures to a high level of ambient CO induced undesirable cardiac and oxygen uptake problems in both normal and coronary heart disease patients as well as normal dogs. Further confirmation of these effects have been reported by Aronow et al. (3, 4) and Knelson et al. (2). These studies suggested that individuals with coronary artery disease became anaerobic at levels of HbCO as low as 5% being unable, in some cases, to increase coronary blood flow in response to tissue hypoxia. Other studies have shown that the presence of HbCO at levels approximating 5% would induce changes in higher central nervous system function (14) as well as reducing the capacity to perform maximal aerobic work (15). Thomsen and Kjeldsen (16, 23) observed myofibrillar degeneration and myelin body formation in mitochondria of rabbits exposed to 100 ppm of carbon monoxide for 4 hours, an exposure sufficient to raise carboxyhemoglobin

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* References in this section are to list on pp. 116-118.

concentrations to 8-9% saturation.

Carbon monoxide interferes with myocardial oxygen delivery by decreasing effective oxygen capacity, decreasing arterial oxygen tension, and shifting the oxyhemoglobin dissociation curve to the left, leading to a lower venous oxygen tension for the same degree of extraction. Carbon monoxide may also interfere with facilitated diffusion of oxygen through myoglobin and may alter the function of cytochrome A3. All of these inequalities may be compensated in the normal heart by appropriate increase in coronary blood flow (11). Permutt and Fahri (12) have calculated that, when HbCO levels were approximately 5%, resting coronary blood flow must increase some 20% in order to prevent myocardial ischemia. Ayres et al. (5-7) reported that in patients without coronary heart disease given a bolus of CO that resulted in HbCO levels to 8.9% (2-4, 12, 13, 15, 23), coronary blood flow increased (44%), myocardial lactate extraction decreased, central venous blood P_{O_2} decreased, and cardiac output increased. Patients with CHD under the same CO load also increased their coronary blood flow although decreases in P_{csO_2} and lactate extraction occurred. Both Ayres et al. (6) and Adams et al. (1) found that concentrations of HbCO up to 23% resulted in increased coronary flow. Coronary arteriovenous oxygen differences declined while coronary sinus saturation increased. Myocardial oxygen uptake decreased suggesting negative feedback to maintain cellular oxygen tensions. However, Ayres employed a massive bolus technique by which animals breathed a gas mixture containing 50,000 ppm over a period of 0.5 to 2.0 minutes and Adams et al. allowed their dogs to breathe 1,500 ppm for a period of 30 minutes. These variations in both the mode of administration and the quantities of CO given have

precluded the development of a clear concept of the effects of CO on the cardiovascular system.

METHODS, RESULTS, AND DISCUSSION

*I. Rapid, Accurate Technique for Determination of Carbon Monoxide in Blood**

The need for a rapid, accurate technique for the analysis of carbon monoxide in blood has led to the development of a wide variety of techniques (Table 1). The major difficulty in blood carbon monoxide analysis has been that the more accurate techniques (1-4) are the most difficult and time-consuming; whereas, the simpler methods (5, 6) are very rapid but not as sensitive. A recent report of a National Academy of Sciences committee (7) emphasized not only the general inadequacies of the existing methodology for carbon monoxide (CO) analysis in blood but also the necessity for development of an adequate technique in order to assess the influence of low levels of carboxyhemoglobin on human performance.

Gas chromatography has proven to be a very accurate method for quantification of blood gases and several chromatographic techniques have been developed for CO analysis (3, 8, 9). However, they involved cumbersome and time-consuming techniques for gas release and elution of these gases onto the chromatographic columns. The most common method of effecting release and elution has been the coupling of a manometric apparatus to the sampling valve of the chromatograph (8, 9). Hackney (10) recently reported a modification of a vortex extractor which should simplify this process considerably. Modification of this procedure to permit accurate and rapid determination of CO in whole blood with adequate sensitivity at various levels of carboxyhemoglobin is presented.

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* See pages 30 and 31 for literature references applicable to this section.

TABLE 1

REPRESENTATIVE TECHNIQUES FOR ANALYSIS OF CO IN BLOOD

Source	Method	Sample Vol (ml)	Resolution* (ml/dl)	Sample Analysis Time (min)	Coefficient† Variability
Gasometric					
Horvath & Roughton (4)	Van Slyke	1.0	0.03	15	6%
Roughton & Root (2)	Syringe-Capillary	0.5	0.02	30	2-4%
Optical					
Coburn, et al. (1)	Infrared	2.0	0.006	30	1.8%
Small, et al. (6)	Spectrophotometric	0.1	0.08	10	
Mass, et al. (5)	Co-oximeter	0.4	0.10	3	
Chromatographic					
McCredie & Jose (8)	Thermal Conductivity	1.0	0.005	20§	1.8%
Collison, et al. (3)	Flame Ionization	0.1	0.002	20	1.8%
Ayres, et al. (9)	Thermal Conductivity	1.0	0.001	30	2.0%
Dahms & Horvath	Thermal Conductivity	0.25	0.006	3	1.7%

* Smallest detectable difference between duplicate determinations.
† Calculated based on samples containing less than 2.0 ml/dl CO.
§ Estimated from literature.

(a) METHODS

The gas chromatograph, vortex extractor, and sampling valve have been described in greater detail elsewhere (10). A standard 1-mv full-scale recorder with a rapidly changeable range selector as described by Hamilton (11) were used in these experiments. The range selector functioned as a zero suppression device which improved the resolution of peak heights as well as providing for high levels of sensitivity at both low and high levels of HbCO.

(1) Extraction Procedures

The blood gases were extracted in a 1.8-ml glass vial* (10 mm x 25 mm) sealed with a rubber stopper† clamped onto the vial with an aluminum retainer.† Prior to sealing, the following were placed inside the vial: an acid-washed (0.1 N HCl) magnet§ (1.5 x 8 mm), one drop of 2-octanol, 0.1 ml of 0.5 M lactic acid, and 0.1 ml of saponin-ferricyanide solution [2 g saponin + 8 g K_3 Fe $(CN)_6$ in 42 ml water]. The sealed vial was purged with the carrier gas, helium, for 3 minutes while the contents were stirred rapidly so as to produce a vortex. The purge gas flow rate was maintained in excess of 100 ml/min.

The blood sample, 100 or 250 µl, was added to the purged vial. The microsyringe was filled by either of two procedures: (1) by fitting a slightly extruded section of tubing partially into the barrel opening and orally aspirating the sample into the dry

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* Wheaton Glass Company, Millville, New Jersey.

† The West Company, Phoenixville, Pennsylvania.

§ Permag Pacific, Los Angeles, California.

syringe; or (2) by placing a 1-cm^2 piece of 1/16-inch rubber sheeting over the microsyringe needle and placing the needle of the microsyringe into the sample syringe until the hub of the sample syringe was sealed against the rubber sheet on the microsyringe. The microsyringe was filled by injecting the blood from the sample syringe into the microsyringe. With both methods of filling the microsyringe plunger was repleaced into the barrel ejecting the excess blood through the needle leaving only the desired sample volume to be place in the vial. The vial was placed on the vortex extractor for three minutes after which time the gas phase was eluted onto the columns by activating the gas sampling valve on the chromatograph. After the gas phase was eluted the valve position could be changed and another vial placed on the extractor without affecting the performance of the chromatograph. The purging system has been separated from the extractor permitting simultaneous extraction and purging, thus, the interval between analyses was further reduced.

(2) Detection and Separation

Following elution from the sample vial the sample and the carrier gas travel through three feet of hypodermic tubing to the gas sampling valve through the injection port of the chromatograph and onto the strippers and columns. The strippers and columns are serially connected in the following sequence:

Water stripper - 12 inches - 1/4" O.D., filled with 10-20 mesh Drierite;

Column 1 - 3 feet - 1/4" O.D., filled with 80-100 mesh Porapak Q;

CO_2 stripper - 12 inches - 1/4" O.D., filled with 13X molecular sieve;

Column 2 - 12 feet - 1/4" O.D., filled with 30-60 mesh 13X molecular sieve.

The sample components pass by hot wire thermal conductivity detectors after leaving Column 1 and second set of detectors after Column 2.

The chromatograph was operated under the following conditions: oven temperature, 70°C; detector current, 125 ma; and a column driving pressure of 24 psi and a helium flow rate of 100 ml/min. These conditions were chosen to adequately separate the peaks and permit an analysis time of approximately 3.5 minutes.

(3) Calibration of Chromatograph

The chromatograph was calibrated by injecting a 250-µl sample of a standard gas of known composition (1% or 20% CO balance N_2-Primary Standard grade tanks) into a purged vial containing the normal reagents plus a volume of distilled water equal to the normal sample volume. Quantification of the gas sample was possible with the use of a gas-tight microsyringe (Hamilton, GF-1750 SNCH) which was filled from a gas cylinder containing dry gas. Care was taken not to handle the syringe excessively so the gas in the syringe would remain at room temperature permitting correction of the volume to standard conditions. The vial containing the calibration gas was then treated as a normal sample. The chromatograph could also be calibrated with a blood sample containing a known amount of carbon monoxide. Calibration was required only once during an 8-hour period as no change in calibration gas or blood gas response was observed during this length of operation.

(4) Gasometric Method

The reference technique for determining the carbon monoxide content in blood was the Horvath and Roughton (4) modification of

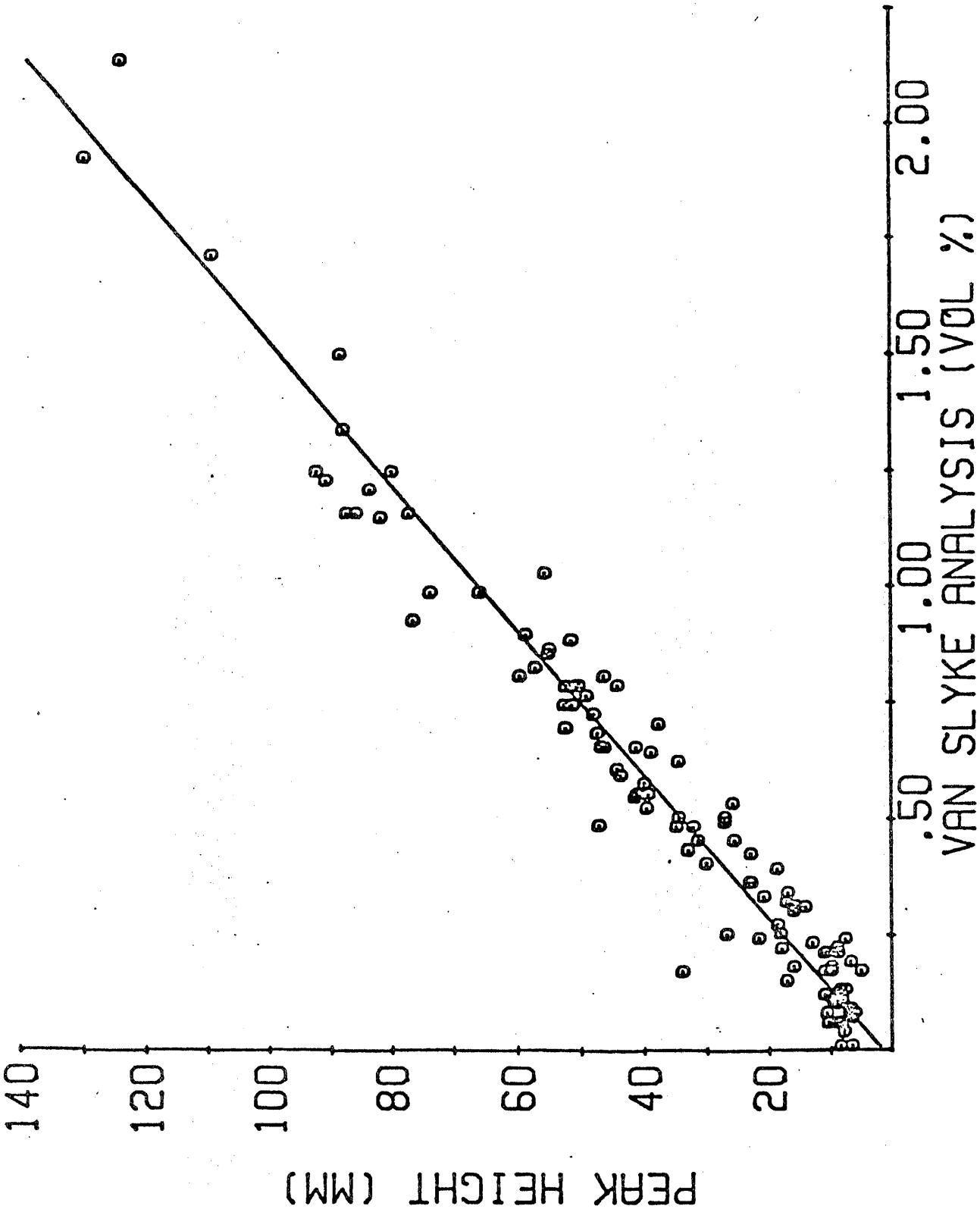
the Van Slyke method and the microsyringe procedure of Roughton and Root (2). In the gasometric technique the sample volume was reduced to 1 ml and the amount of sodium dithionite was reduced to 25 mg. Larger quantities of dithionite lead to the production of hydrogen sulfide gas and erroneous readings. The c correction was determined by substituting 1 ml of distilled water in place of the blood sample in the normal method. The P_1 and P_2 readings were made at 0.5 ml. The required agreement between duplicates was 0.10 ml/dl although the average difference between duplicates was less than the accepted limit.

The comparison of the chromatographic technique with the standard gasometric method was carried out on anaerobically collected venous blood. The samples were simultaneously analyzed by both methods. These blood samples were taken from smokers and nonsmokers exposed to various levels of carbon monoxide so that the entire range of expected control resting carboxyhemoglobin values would be included (0.4 - 10%).

The use of peak height to represent carbon monoxide concentration is more convenient than peak area when using chromatographic methods. The simplicity of conversion of peak height to concentration and the avoidance of the problems inherent in integrating peak areas led to the investigation into the feasibility of using peak height with this analytical method. The peak height linearity was initially established on blood samples containing carbon monoxide concentrations over the range of 0.05 - 2.00 ml/dl (Fig. 1, $r = 0.978$). The linearity found in blood was further established on gas samples containing 1, 9.9, and 99.5% carbon monoxide. The 99.5% CO sample represented four to five times the expected quantity of carbon monoxide

FIGURE 1

Comparison of Chromatographic Peak Height
With Carbon Monoxide Content Determined Manometrically
On 104 Samples Over the Range of 0.3 to 10% HbCO.
Correlation Coefficient = 0.978.



in a blood sample, therefore peak height can be used with this method over the entire physiological range of carbon monoxide contents.

Peak height has been reported to be less accurate than peak area (15), therefore, peak height and peak area were compared on 144 blood samples. The range selector was not utilized for this group of samples, which contained 0.10 to 1.70 ml/dl CO. A linear response between peak height and peak area was found (Fig. 2) on 144 separate blood samples giving a correlation coefficient of 0.994 adequately substantiating the validity of using peak height to represent carbon monoxide concentration with this technique. The use of peak height to represent CO content places a restriction on the method of calibration of the chromatograph. All calibration gas samples must be treated identically to blood samples; that is, injected into the sample vial rather than directly into the injection port of the chromatograph. Mechanical factors in the vortex extractor promote peak spreading. Injecting identical volumes of gas into the sample vial and into the injection port of the chromatograph resulted in a 10% reduction in peak height with an insignificant change in peak area.

The accuracy of the chromatographic technique was established by comparing the chromatographic and gasometric carbon monoxide contents over a limited range of 0.08 to 1.92 ml/dl and over the larger range of 0.5 to 84% carboxyhemoglobin (Figs. 3A and 3B, respectively). Each data point in Figs. 3A and 3B represents the mean value of duplicate determinations by each technique. The correlation coefficient on the 90 blood samples containing from 0.08 to 1.92 ml/dl CO was 0.984 between

FIGURE 2

Comparison of Chromatographic Peak Height With Peak Area
On 144 Samples Containing from 0.10 to 1.70 ml/dl (vol %) Carbon Monoxide.
Correlation Coefficient = 0.994.

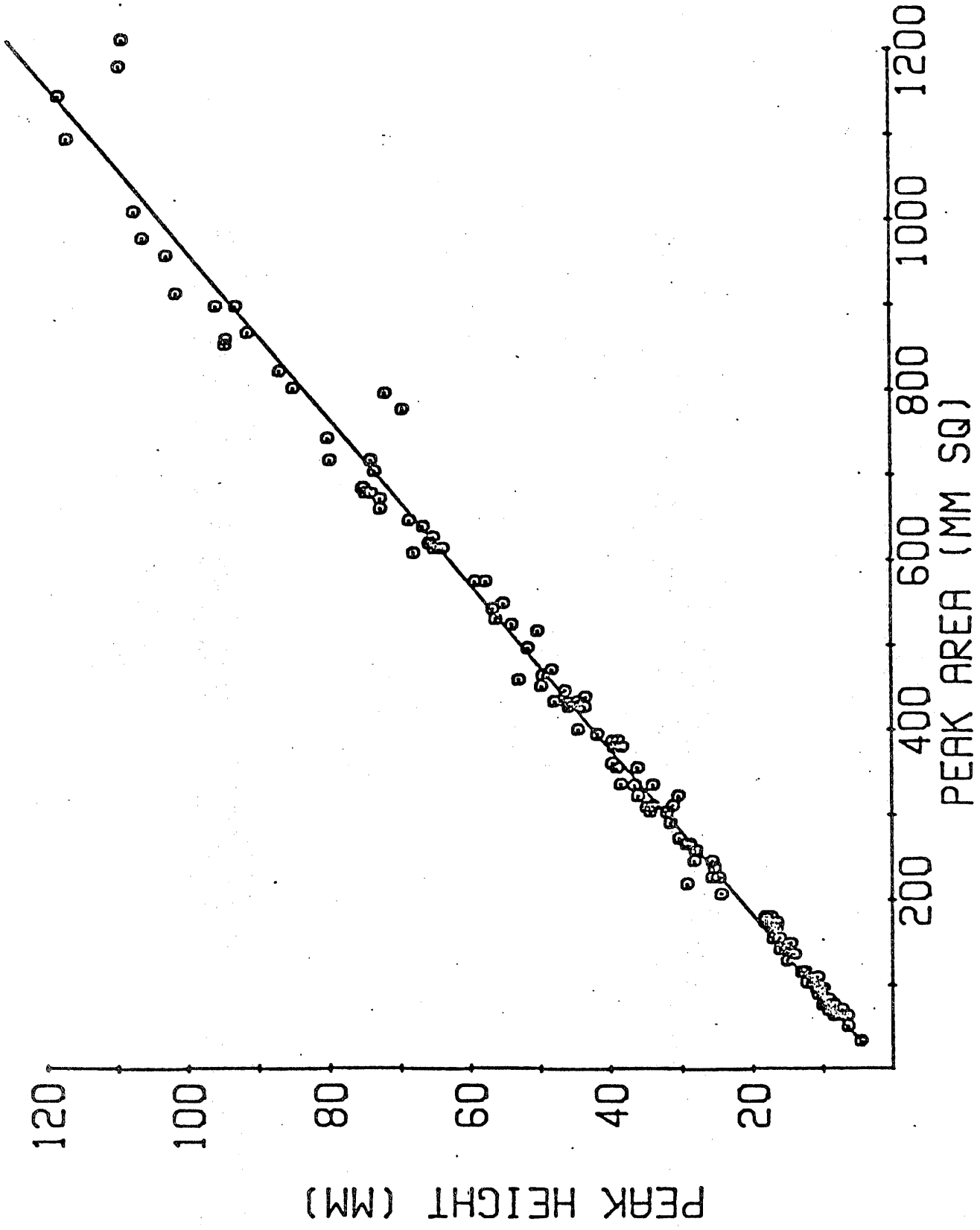
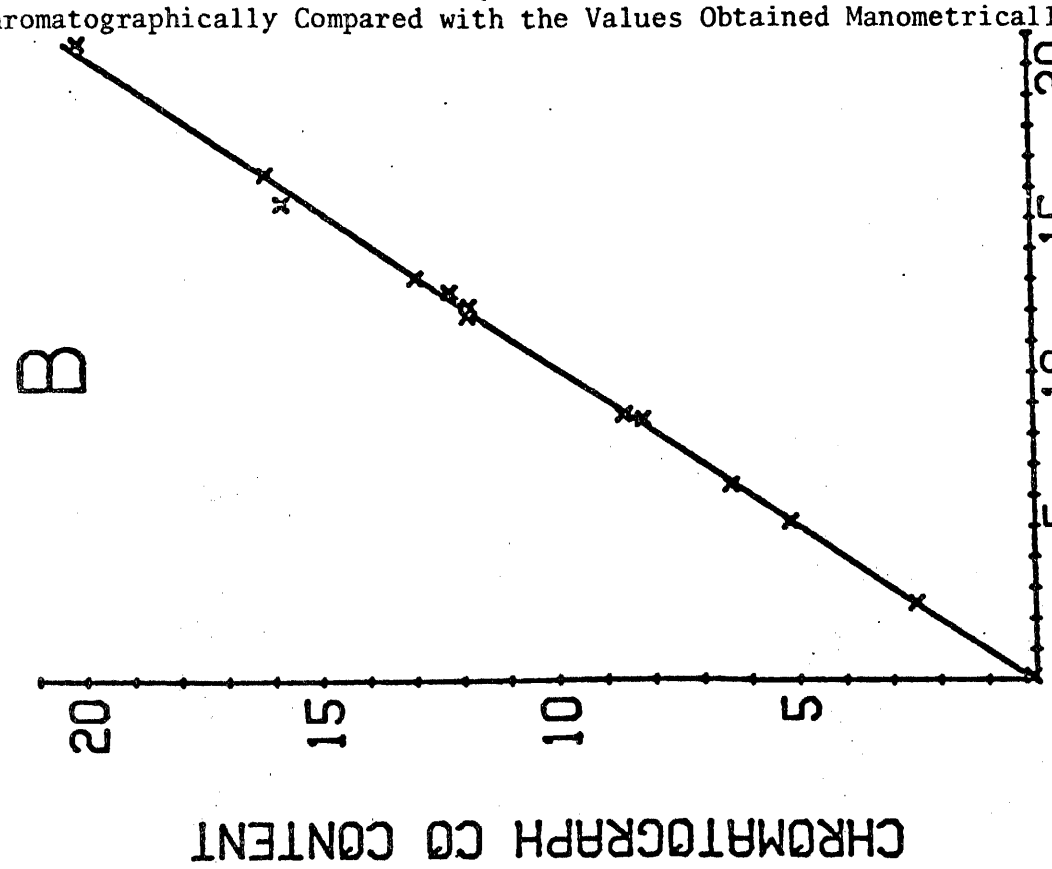
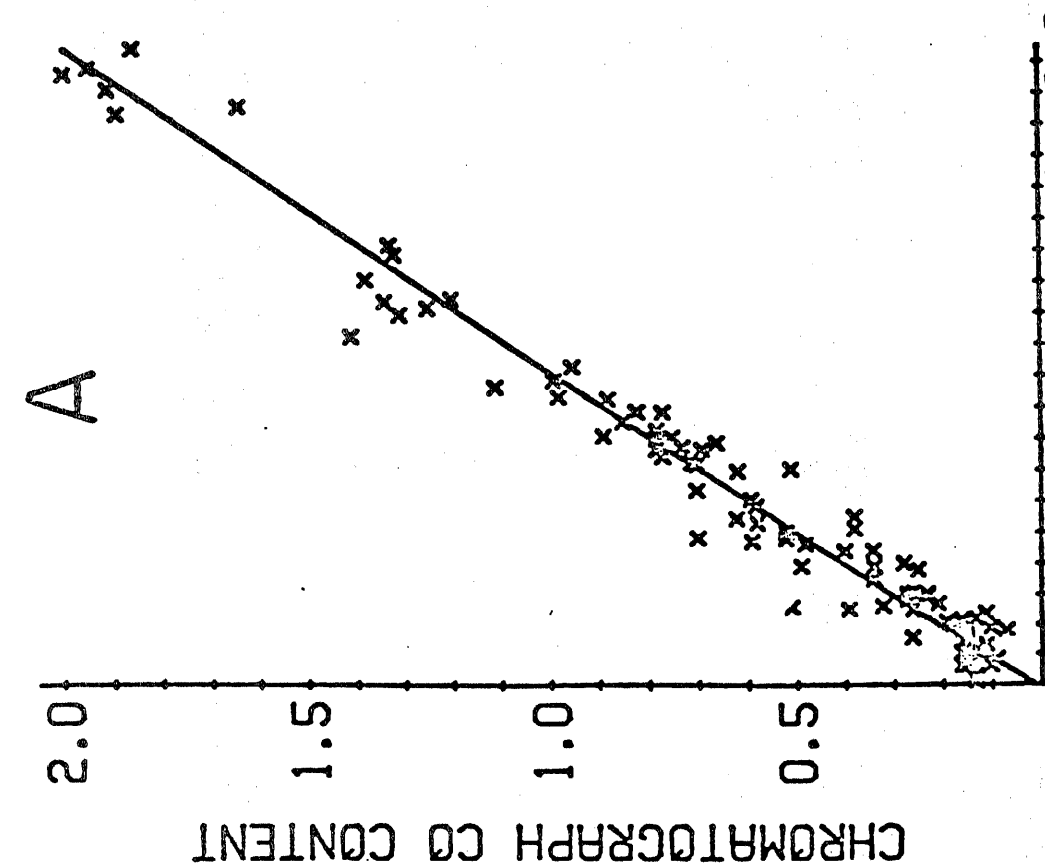


FIGURE 3

The Carbon Monoxide Contents (ml/dl) of Blood Samples Determined Chromatographically Compared with the Values Obtained Manometrically.



B. Results on samples with CO contents over the entire range of possible values; $y = 0.985x + 0.05$, $r = 0.999$.



A. Results on samples from normal nonsmokers and smokers; $y = 0.982x + 0.009$, $r = 0.983$.

the two methods with a linear regression of chromatographic CO = 0.98154 (Van Slyke CO) + 0.0091 ml/dl. The correlation coefficient of chromatographic CO content and Van Slyke CO content was slightly higher than the 0.978 for the comparison of peak height to Van Slyke CO content. This discrepancy was due to the slight variability in the day-to-day calibration of the chromatograph. This accuracy was substantiated over the entire range (Fig. 3B) as the difference between the absolute values of each method did not exceed 2% of any gasometric value.

Reproducibility was determined by repeated analysis of individual samples and by the comparison of the duplicate determinations during routine use of the technique. The conventional representation of reproducibility of repeated analysis is the coefficient of variability or the standard deviation as a percentage of the mean (12). Two samples, one from a nonsmoker and one from a smoker, were analyzed ten times each. These samples contained mean concentrations of CO of 0.130 and 1.303 ml/dl with standard deviations of 0.0066 and 0.022 ml/dl respectively. The coefficients of variability were then 5.1 and 1.7% respectively. This reproducibility is in good agreement with the coefficients of variability reported by Collison et al. (3), Ayers et al. (9), Coburn et al. (1), and McCredie and Jose (8) (Table 1). Another procedure utilized to estimate reproducibility was the determination of the standard error of the estimate between duplicate analyses. The standard error of the estimate was obtained by the product of the standard deviation of the mean and the square root of one minus the square of the correlation coefficient (12). The standard error of the estimate for the chromatographic technique over a range

of CO contents of 0.091 to 2.000 ml/dl was 0.0131 and for the gasometric method on the same samples, 0.0889. This suggests that the scatter of points about the regression line in Fig. 3A was primarily due to the variability of the gasometric method.

The greater coefficient of variability in the sample containing 0.130 ml/dl carbon monoxide (5.1%) was due in part to recorder insensitivity at the sensitivity (1 mv full scale) used during standard operation. The variability in the peak heights from this blood sample was very near the limits of error involved in reading the record. In order to determine the variability of the method apart from this recording variable, the sensitivity of the recorder was increased by a factor of 2.5, i.e., 0.40 ml full scale. No detectable drift or base line noise was observed at this higher sensitivity. This same blood sample (0.130 ml/dl) was then repeatedly analyzed and the coefficient of variability was reduced to 3.4%. Therefore, one of the limitations of this technique lies in the recorder sensitivity.

All of the above mentioned comparisons were carried out on 100 µl of whole blood which gives an acceptable level of accuracy and reproducibility, although the resolution capability was not maximized. This resolution capability has been enhanced by increasing the sample volume to 250-µl. Using 250-µl samples required the establishment of a linear response of peak height to sample volume if the data presented above would be expected to pertain to the larger sample as well. The linearity between sample volume and peak height (CO content) in sample volumes from 25 to 250 µl is shown in Table 2. This linearity was predictable, given sufficient excess of reagents, from the results.

TABLE 2

EFFECT OF VARYING SAMPLE VOLUME
ON RESPONSE OF THE CHROMATOGRAPH

Sample Vol (µl)	Peak Height as a Percentage of the 100-µl Sample	
	CO	O_2
100	100	100
254	253	253
200	201	200
150	150	148
50	49	49
25	25	26
10	9	12

presented in Fig. 1B. Based on routine use of the 250-µl sample volume, replication has been consistently obtained to within 0.004 mv of output signal with a sensitivity of 0.015 ml/dl per 0.01 mv. Therefore, the resolution with a 250-µl sample was 0.006 ml/dl. A similar resolution, 0.005 ml/dl, was obtained during the experiment with increased recorder sensitivity.

The efficiency of the vortex extraction procedure in the removal of dissolved gas was of primary concern. To answer this question 10 ml of fresh plasma treated with 1 ml of 3% K_3 Fe $(CN)_6$ was equilibrated with 99.5% CO for 30 minutes. During the equilibration period the gas phase was replaced at 10-minute intervals. The plasma was equilibrated in an oiled, heparinized, 30-ml glass syringe which was rotated in a water bath at 24°C. Initial elution of the plasma sample vial gave an average of 2.13 ml/dl dissolved CO compared with the theoretical value of 2.11 ml/dl for water (13). These results give a Bunsen solubility coefficient at 24°C of 0.0220 ml CO/ml water per atm CO compared to the theoretical value of 0.0218 (13). The gas phases from the plasma samples were eluted onto the chromatograph a second time and no CO was detected. Thus, the vortex method of extraction removed essentially all dissolved carbon monoxide from the liquid phase of the extraction vial. These findings were predictable due to the large gas phase (1.4 ml at 40 psia) compared to the volume of dissolved CO being released from the plasma samples (0.0055 ml). Under these conditions at equilibrium the liquid phase in the vial would contain 0.006 ml/dl dissolved CO. It is then reasonable that essentially all the dissolved CO would have appeared to have been released in the first elution.

There often arises the need for storage and transport of blood specimens and, therefore, a time delay in the analysis of the blood sample for CO content. A series of samples were stored for different lengths of time in order to determine the stability of carboxyhemoglobin. Samples were heparinized, analyzed, and stored anaerobically in oiled glass syringes at room temperature (20 to 25°C) in the dark for as long as three weeks. There was no loss of CO during three weeks of storage in samples containing from 0.24 to 6.00 ml/dl HbCO. This stability of carboxyhemoglobin confirms the earlier findings of Collison et al. (3) and Blackmore (14) over a shorter time period.

The advantages of this method compared to previously reported techniques can be seen in the various aspects of the representative methods shown in Table 1. This technique's primary advantage lies in its ease and speed of operation without loss of accuracy or precision. A single sample can be analyzed in 3.5 minutes and replicated in a total of 7 minutes. A technician can be taught to run the apparatus and obtain the accuracy required in less than one day. The analysis of 50 samples with this precision (judged by replicates) has routinely been accomplished by one technician in an 8-hour day. This method compares with the speed of the optical methods and with the reproducibility of the other chromatographic methods (Table 1). Therefore, this technique meets the need for a rapid, easy to perform, and accurate method of carbon monoxide analysis in blood.

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*II. Carbon Monoxide Elimination**

Description and documentation of the rates of uptake, degradation, and elimination of air pollutants by living organisms are basic both to the treatment or prevention of their pathological effects and to the development of health and safety tolerance limits of exposure to air pollutants. Since much of the research on carbon monoxide intoxication has been done using experimental animals, the following report describes the elimination of carbon monoxide from anesthetized, spontaneously breathing dogs which were being used to study the effects of carbon monoxide on the cardiovascular system.

(a) METHODS

Nineteen male mongrel dogs, weighing between 20-38 kg, were anesthetized by intravenous administration of pentobarbital sodium (25 mg/kg). Studies were made on two occasions separated by 3-4 weeks. In some instances these were duplicate studies and in others different carboxyhemoglobin (HbCO) levels were produced. A cuffed endotracheal tube was inserted to ensure a patent airway and to enable the measurement of minute ventilation. Catheters were positioned by fluoroscopic guidance into the right and left ventricles. Cardiac output was determined by the dye dilution method using indocyanin green.** Throughout each experiment minute ventilation was measured using a Parkinson-Cowen dry gasometer (1) and a low-resistance valve. Rectal temperature was measured with an indwelling thermistor.

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* See page 42 for literature references applicable to this section.

** Cardiogreen, kindly supplied by Hynson, Westcott and Dunning, Inc., Baltimore, Maryland.

Mean carboxyhemoglobin levels of 6, 10, 14, 23, and 36% were produced in separate groups of dogs by giving them mixtures of 1-6% carbon monoxide (CO) in air to breathe for 3 minutes. Blood samples, minute ventilation, and cardiac outputs were obtained from each dog prior to and 7, 24, 40, 55, 70, and 85 minutes after CO administration. In several experiments additional blood samples were taken immediately following CO administration and 1, 2, 3, 5, 14, and 20 minutes after CO administration. During the course of 38 experiments more than 300 blood samples were analyzed for hemoglobin by the cyanomethemoglobin method and carbon monoxide content by the method of Dahms and Horvath (2), which was periodically checked by Van Slyke analysis (3). HbCO levels in the blood were expressed as a percentage of the oxygen carrying capacity of hemoglobin (%HbCO). Correlation and regression techniques were used in the statistical analysis of the data.

(b) RESULTS

Fig. 4 presents the arterial blood %HbCO levels in five groups of dogs prior to and 7, 24, 40, 55, 70, and 85 minutes after carbon monoxide administration. The mean values are shown only at times that blood samples were obtained from all dogs in each group. There were 10 dogs in the group with the lowest mean %HbCO level and 8, 4, 7, and 7 dogs, respectively, in the groups with higher %HbCO levels. Absolute elimination rates were more rapid in dogs having higher initial HbCO levels than in those with lower HbCO levels.

A linear relationship existing between arterial blood %HbCO and minute ventilation ($r = 0.796$, Standard Error of Estimate, $S_{yx} = 0.6828$, $P < 0.001$) can be expressed as

$$\dot{V}\ (BTPS) = 0.0881\ \%HbCO + 5.5608 \qquad (1)$$

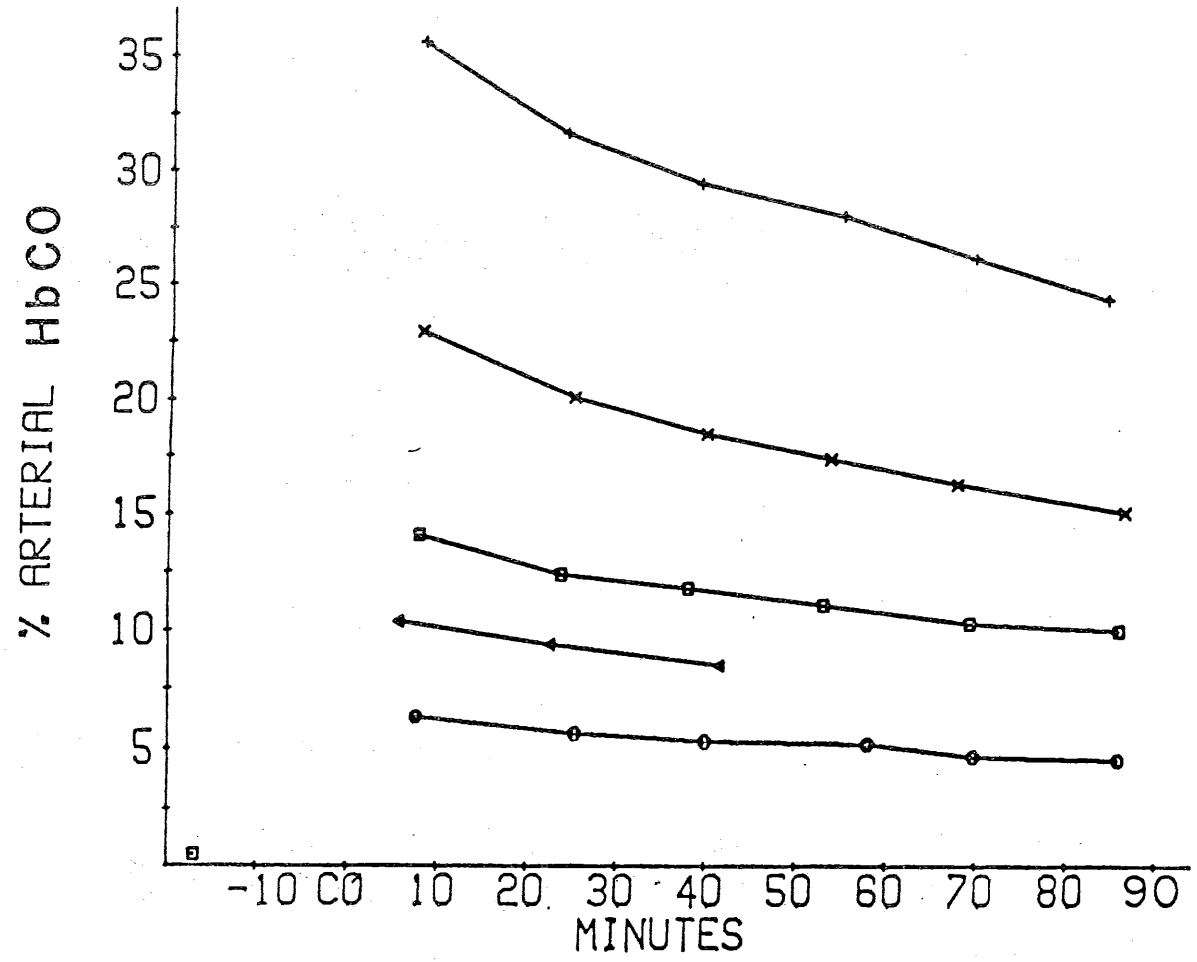

FIGURE 4

Changes in Arterial Blood Percent Carboxyhemoglobin (%HbCO) vs. Time Following Inhalation of 1-6% Carbon Monoxide for 3 Minutes. The Coefficients of Variation (S.D./$\bar{X}$) for Mean Values Were 0.20, 0.18, 0.10, 0.15 and 0.16, Respectively, for Groups with the Lowest Through the Highest %HbCO Levels.

Mean cardiac output levels ranged from 3.2-4.2 liters/min for individual groups. There was no significant relationship between %HbCO levels and cardiac output levels, which remained constant throughout each experiment.

The rates of decline in the %HbCO levels shown in Fig. 4 were compared by expressing each value as a percentage of the %HbCO value measured 24 minutes after CO administration [100 ($\%HbCO_t/\%HbCO_{24}$)], where $\%HbCO_t$ is the %HbCO at any given time after CO administration. This procedure was utilized since the percentage decline in %HbCO levels after approximately 15 minutes appeared to be linear and independent of the actual blood %HbCO level. A mathematical adjustment was made in order to show the linear elimination phase beginning from an initial value of 100% immediately following the administration of CO ($\%HbCO_{I_e}$) and all subsequent blood levels were recalculated in relationship to this initial value [100 ($\%HbCO_t/\%HbCO_{I_e}$)]. After calculating the regression for those values obtained between 15-90 minutes post exposure, all values from zero to 90 minutes were adjusted upward by the difference between the Y-intercepts and 100% of the post-exposure %HbCO level (Fig. 5). Consequently, during the early minutes following CO inhalation, values of blood HbCO were in excess of 100% of the HbCO level of blood equilibrated with all body compartments. The linear relationships extended back to time zero show the hypothetical CO elimination rates from dogs with equilibrated blood %HbCO levels.

The decline in %HbCO levels in anesthetized dogs was apparently biphasic, an initial rapid decline occurring within the first 15 minutes followed by a slower decline throughout the remainder of

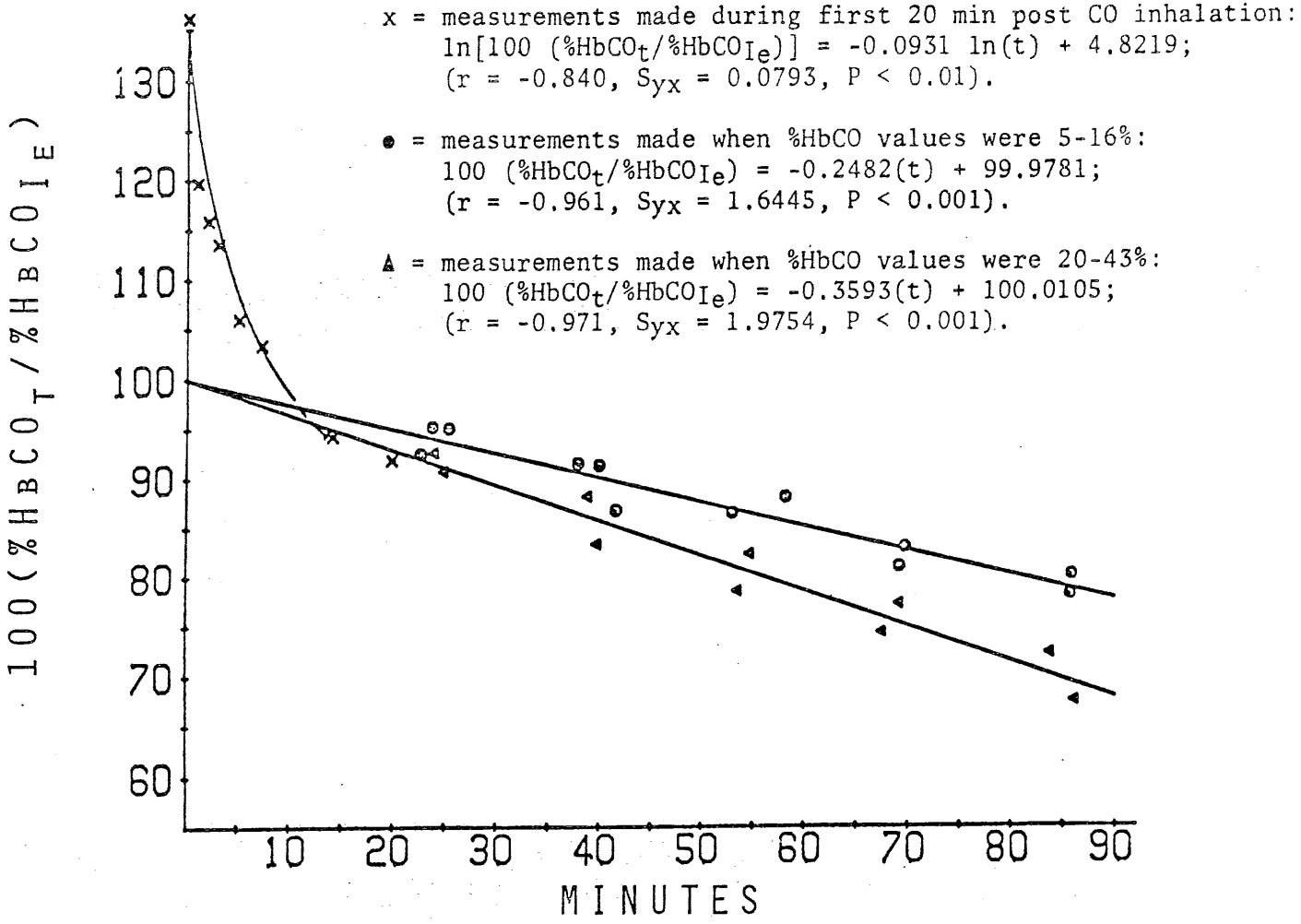

FIGURE 5

Ratio of Changes in Arterial Blood Percent Carboxyhemoglobin (%HbCO) as a Function of an Assumed Instantaneous Equilibrium at the End of Inhalation ($\%HbCO_{I_e}$) (see text).

the experiment. Regression analysis of individual values from 14 experiments obtained during the first 20 minutes after CO administration showed an exponential relationship between the percent of the initial equilibration level of %HbCO [100 ($\%HbCO_t/\%HbCO_{I_e}$)] and post-exposure time (t) in minutes ($r = -0.840$, $S_{yx} = 0.0793$, $P < 0.01$):

$$\ln[100\ (\%HbCO_t/\%HbCO_{I_e})] = -0.0931\ \ln(t) + 4.8219 \quad (2)$$

The secondary slow component of the decline in carboxyhemoglobin levels (15-90 minutes) was linearly related to post-exposure time. The percentage of initial %HbCO levels in individual animals can be predicted during this period as follows ($r = -0.819$, $S_{yx} = 6.4593$, $P < 0.001$):

$$100\ (\%HbCO_t/\%HbCO_{I_e}) = -0.2729(t) + 99.9886 \quad (3)$$

There was no difference among the mean rates of decline in %HbCO in the three groups of dogs with the lowest %HbCO levels (5-16%) or the two groups with the highest %HbCO levels (20-43%). Therefore, the mean data from the three groups with low %HbCO levels (22 experiments) were combined and compared with the combined data from the two groups with the highest %HbCO levels (14 experiments). Statistical analysis revealed a significant difference between these two levels of HbCO ($P < 0.01$). The decline in %HbCO levels in groups of dogs can be predicted with greater confidence than individual values by using Eq. (4) for dogs with low %HbCO (5-16%) and Eq. (5) for those with higher %HbCO levels (20-43%):

$$100\ (\%HbCO_t/\%HbCO_{I_e}) = -0.2482(t) + 99.9781 \quad (4)$$

$(r = -0.961,\ S_{yx} = 1.6445,\ P < 0.001)$

$$100\ (\%HbCO_t/\%HbCO_{I_e}) = -0.3593(t) + 100.0105 \quad (5)$$

$(r = -0.971,\ S_{yx} = 1.9754,\ P < 0.001)$

(c) DISCUSSION

The biphasic decline in arterial blood %HbCO levels shown in Fig. 5 appeared to be due to the sequential occurrence of two unrelated processes. Since carbon monoxide was administered in high concentrations over a 3-minute period, CO was rapidly absorbed and tightly bound to the hemoglobin molecules in the circulating red blood cells. The initial rapid decline in arterial blood %HbCO levels (distribution phase) was probably partly related to distribution of CO from the circulating blood to splenic blood, myoglobin, and cytochrome enzymes. Elimination of CO via the lungs also occurred during the distribution phase, but at a much slower rate. The distribution phase, which lasted approximately 15-20 minutes, was followed by a slower linear decline in arterial %HbCO levels (elimination phase). The elimination phase probably reflected the rates of release of CO from hemoglobin and myoglobin, pulmonary diffusion, and ventilation. In normal healthy dogs the rates of release of CO from hemoglobin and myoglobin would be the primary determinants of the CO elimination rate.

The initial carbon monoxide distribution curve has not been recognized in most previous studies because the emphasis of these studies has been on the long-term elimination of carbon monoxide, CO was administered in lower doses over longer periods of time, or blood was not sampled as frequently during the early transient phase

as it was in the present investigation. Previous investigators have reported exponential carboxyhemoglobin elimination curves over many hours for awake human subjects (4, 5). The data obtained between 15-90 minutes after CO administration in the present study can be fitted to a semilogarithmic relationship as shown by Eq. (6) for dogs with 5-16% HbCO and Eq. (7) for dogs with 20-43% HbCO levels.

$$\ln[100\ (\%HbCO_t/\%HbCO_{I_e})] = -0.0029(t) + 4.6052 \qquad (6)$$

$(r = -0.958,\ S_{yx} = 0.0195,\ P < 0.001)$

$$\ln[100\ (\%HbCO_t/\%HbCO_{I_e})] = -0.0045(t) + 4.6052 \qquad (7)$$

$(r = -0.968,\ S_{yx} = 0.0262,\ P < 0.001)$

However, after the initial distribution of CO to the various body compartments had neared completion, the carboxyhemoglobin elimination curves in anesthetized dogs during short periods of time may be effectively described as a linear function (Eq. (3), (4), and (5)). The linear Eq. (4) and (5) are easier to use than Eq. (6) and (7), and probably provide a more accurate description of CO elimination over short periods of time. Since the observations were made on anesthetized dogs, however, they do not necessarily describe the CO elimination rates in awake dogs. If carbon monoxide had been administered over a much longer period of time, the distribution phase may not have been seen since the distribution of CO may have been essentially complete by the end of the administration period. Godin and Shephard (6) calculated carboxyhemoglobin levels from the partial pressure of CO in equilibrated expired air. The subjects were followed for 30 minutes with data obtained at 6-minute intervals. These investigators

showed that following a 2-6 minute exposure to CO the decline in %HbCO was moderately faster during the first 18 minutes of the 30-minute observation period following CO administration and proposed a model for CO elimination which recognized an initial period of distribution of CO to all body compartments. However, clear separation of the two components was not possible from their data due to infrequent sampling and the large potential error related to calculating the blood %HbCO from the CO concentration in expired air.

The data from the present study indicated that the elimination phase of the decline in the %HbCO level was related more strongly to the physical characteristics of CO affinity to hemoglobin and myoglobin, and pulmonary diffusion than to minute ventilation or to cardiac output. Cardiac outputs remained at control levels of 3.2-4.2 liters/min throughout the period of study and minute ventilation was increased only slightly in the two groups of dogs with the highest %HbCO levels. The mean decline in %HbCO showed a higher correlation with the physical variable of post-exposure time alone ($r > 0.96$) as compared to a correlation of $r = 0.80$ between arterial blood %HbCO and minute ventilation. On the basis of linear declines in blood %HbCO levels, dogs with low (5-16%) and high (20-43%) HbCO levels eliminated 50% of their total CO load in 190 ± 6.4 and 134 ± 5.3 ($\bar{X} \pm S_{xy}$) minutes, respectively. The differences between the CO elimination rates of these subgroups appeared to be due to their different ventilations and, consequently, their ventilation/perfusion ratios. Mean cardiac outputs were similar and constant for these groups but the greater minute ventilations of the latter group resulted in a greater ventilation/perfusion ratio. The dogs with the lower

%HbCO levels had a mean ventilation/perfusion ratio of 1.85 and those with the higher %HbCO levels had a mean ventilation/perfusion ratio of 2.35. Greater minute ventilations and higher %HbCO levels in the latter group would also produce a greater diffusion gradient for CO to pass out of the blood and be eliminated in the lungs. Pulmonary diffusion capacities and blood volumes were not measured in this study but since many of the dogs were exposed to both low and high CO levels, there was no reason to expect these variables to change between the two experiments.

Half elimination times based on the semilogarithmic relationship shown in Eq. (6) and (7) were 226 ± 6.5 and 148 ± 5.7 ($\bar{X} \pm S_{xy}$) for dogs with 5-16% and 20-43% HbCO levels, respectively. As might be expected, these rates are 19% and 10% greater than the values obtained with linear relationships. These discrepancies point out the importance of the linear relationship in the decline in %HbCO levels over short periods of time after CO exposure. Reliance on the long-term elimination rates of CO from blood can lead to substantial errors in estimating blood %HbCO for short periods of time after CO exposure.

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*III. Technique for Accurately Producing Desired Carboxyhemoglobin Levels During Rest and Exercise**

In the course of studying the effects of carbon monoxide (CO) on human and animal performance, various techniques have been utilized for administering and maintaining a desired body burden of CO. The body burden is conventionally represented as the percentage of carboxyhemoglobin (%HbCO), since approximately 90-95% of the administered CO remains bound to the circulating hemoglobin (12). It has been assumed that the results of the various methods of CO loading produced comparable physiological results, with the basis of comparison of results between experiments being either the final %HbCO or the average %HbCO during the experiment. This assumption may be unwarranted due to the differing patterns of change in %HbCO levels which have resulted from the widely varying methods of CO administration.

Investigators who have attempted to delineate the effects of CO on psychological and exercise performances have used several methods for attaining a desired body burden of CO. The techniques can be placed in the following general categories:

1. Administration of a quantitated bolus in a closed system, resulting in a desired level of HbCO which may or may not have been maintained by further breathing of a gas mixture of lesser CO concentration (13);
2. By exposure to a high concentration of CO for a fixed brief period of time (2, 8); and
3. By exposure to a moderate concentration of CO for the duration of the experiment (5, 10).

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* See pages 57 and 58 for literature references applicable to this section.

The end result of these methods produced pictures in which HbCO was either increasing (5, 11, 13), decreasing (2), or oscillating (8) during the exposure period, raising some concern regarding the interpretation of the results. If further work in this area is to be carried out, expecially at the lower levels of HbCO, a method of accurately adjusting %HbCO and then maintaining these levels within narrow limits would be needed.

(a) METHODS

(1) Administration of CO

The volume of CO required to elevate the subject's HbCO to a predicted level was determined by a pre-test in which the subject's whole body CO dilution space was measured. In order to measure the dilution space, total red cell mass and total Hb had to be measured. The red cell mass measurements were carried out using a standard CO rebreathing method (3, 9). The pre and post CO administration HbCO levels were measured using a gas chromatograph technique (1). The hematocrit values were determined via microhematocrit, with the values being corrected by 0.95 for trapped plasma. Hemoglobin values were measured using an IL-182 CO-Oximeter which was standardized by Van Slyke and chromatographic analysis of blood sample saturated with CO or with oxygen (7) with the hemoglobin content being determined by dividing the combined gas by 1.39. All blood samples were obtained without stasis from an antecubital vein. The following equation describes the method for calculating red cell mass:

$$VRBC = \frac{VCO \text{ inspired}}{\frac{\text{post (CO)}}{\text{post Hct}} - \frac{\text{pre (CO)}}{\text{pre Hct}}}$$

where

VRBC is in ml

VCO inspired is in ml STPD

post Hct and pre Hct are expressed as ratios

pre (CO) and post (CO) are in ml/dl blood

The CO dilution space was determined by the following set of relationships:

red cell mass ÷ whole body Hct = blood volume

blood volume × whole body Hb conc. = total body Hb

total body Hb × 1.389 = CO dilution space

The desired level of carboxyhemoglobin can then be obtained by administering a requisite amount of CO (STPD) from a closed breathing apparatus, with this amount calculated from the desired %HbCO and the CO dilution space.

(2) Maintenance of the Level of HbCO

The level of CO in the blood can be maintained constant when the partial pressure of CO in the venous blood is equal to the P_{CO} in the alveolar gas. If one assumes a steady-state situation for %HbCO, the P_{CO} would be effectively equal in the inspired gas, alveolar gas, and in the venous and arterial blood. Under these conditions, no net transfer of CO would occur. It was then necessary to determine at what concentration of inspired CO and at what corresponding %HbCO the above conditions were met.

In order to maintain a constant inspired CO concentration, a system was designed for providing any desired level of CO, as shown in Fig. 6. The compressed air entering the system was passed through an activated charcoal, silica gel, and glass wool filter to assure absence of impurities. The air was passed through a temperature regulated humidifier and the airflow monitored with a calibrated pitot tube. The carbon monoxide supply of 10% CO balance nitrogen was admitted just down stream from the pitot tube. The CO volume flow rate was monitored with a rotometer controlled with a needle valve. An 8-foot section of 3-inch-diameter pyrex tubing was used to permit mixing, and the resultant CO concentration was continuously monitored by drawing a sample (just upstream from the subject's mouthpiece) through an infrared CO analyzer (Beckman 315, Long Path). Fine adjustments of the CO level could be made with the rotometer needle valve based upon the recorded CO level reaching the subject. A shunt placed around the mouthpiece permitted the adjustment of the desired CO level prior to the subject's breathing on the system. Without the shunt, the breathing valve resistance was sufficient to direct the excess airflow to pass into the inspired capacitance reservoir (meteorological balloon). This arrangement permitted the measurement of respiration while breathing the gas mixtures from this system. The expired air bag (meteorological balloon) was used to prevent the exhaled air from contaminating room air. This system permitted a continuously variable volume flow rate while maintaining a constant CO concentration. The maintenance inspired CO concentrations were calculated initially on the basis of Peterson and Stewart's (10) equilibrium extrapolations and by use of the equilibrium relationship

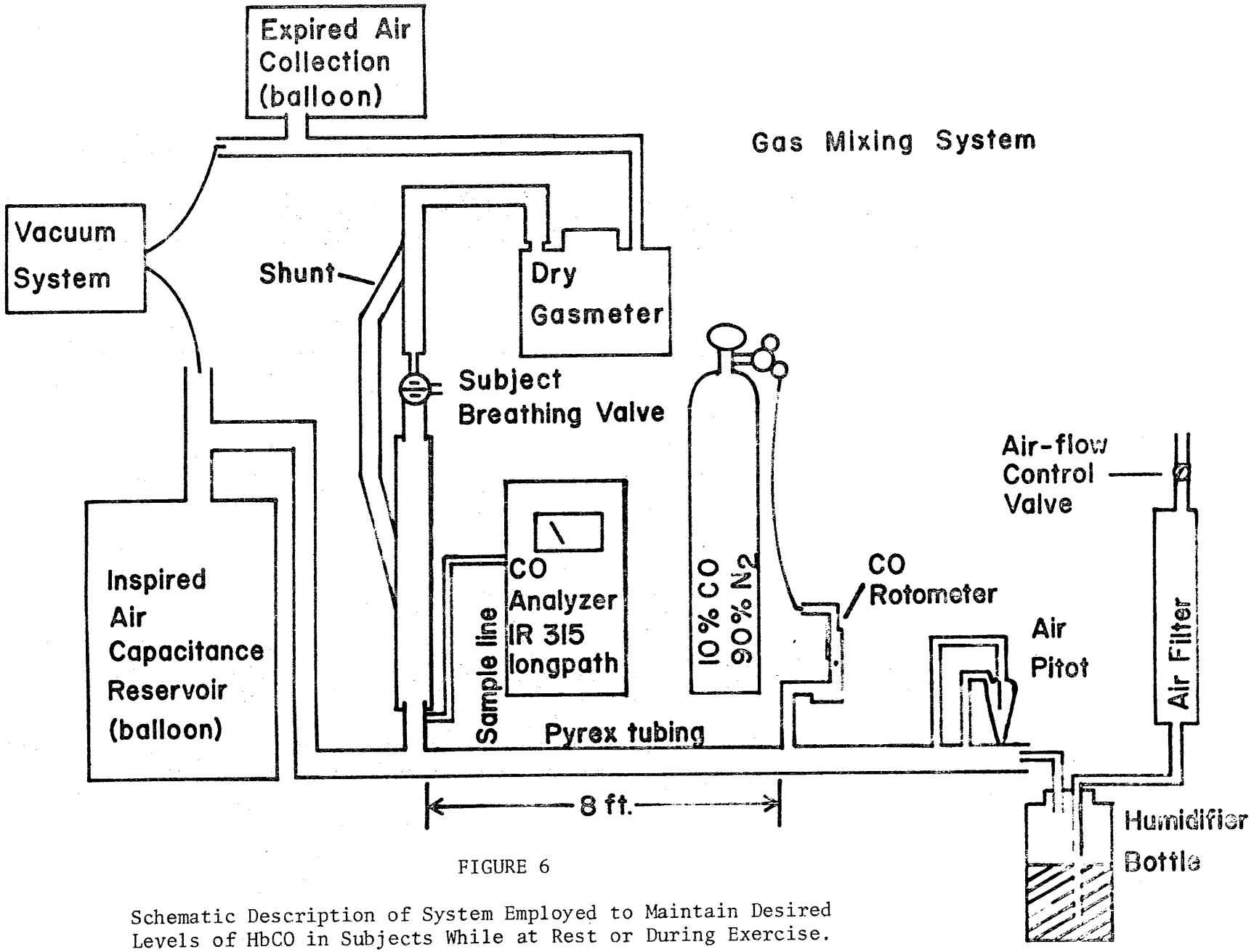


FIGURE 6

Schematic Description of System Employed to Maintain Desired Levels of HbCO in Subjects While at Rest or During Exercise.

known as Haldane's First Law (12):

$$M = \frac{P_{O_2} \quad [\%HbCO]}{P_{CO} \quad [\%HbO_2]}$$

An assumed value of 240 was used for M (12).

Four subjects were studied at two desired levels of HbCO. The subjects' descriptions are given in Table 3. The experiments consisted of drawing a pre-test blood sample, then administering the required dose of CO while the seated subject rebreathed on a closed system. Thesubject held his breath and transferred to the gas mixing system for an additional 15 minutes of resting breathing, at the end of which a second blood sample was taken. The subject then began a progressive maximal exercise test which lasted 20-25 minutes. Immediately following the exercise, a third blood sample was drawn while the subject was still breathing the maintenance level of CO. A fifth subject underwent the same protocol except that an indwelling venous catheter was utilized to draw minute by minute blood samples during the exercise segment of the test.

(b) RESULTS AND DISCUSSION

The ability of this system to maintain a constant blood carboxy-hemoglobin under a wide range of ventilations and oxygen uptakes is illustrated in Fig. 7. The slight change in HbCO from rest to mid exercise was due to inability to precisely set the HbCO level and adjust the inspired P_{CO} accordingly. However, a 0.2% HbCO error is correctable to within 0.1% HbCO when the desired HbCO level can be predicted more

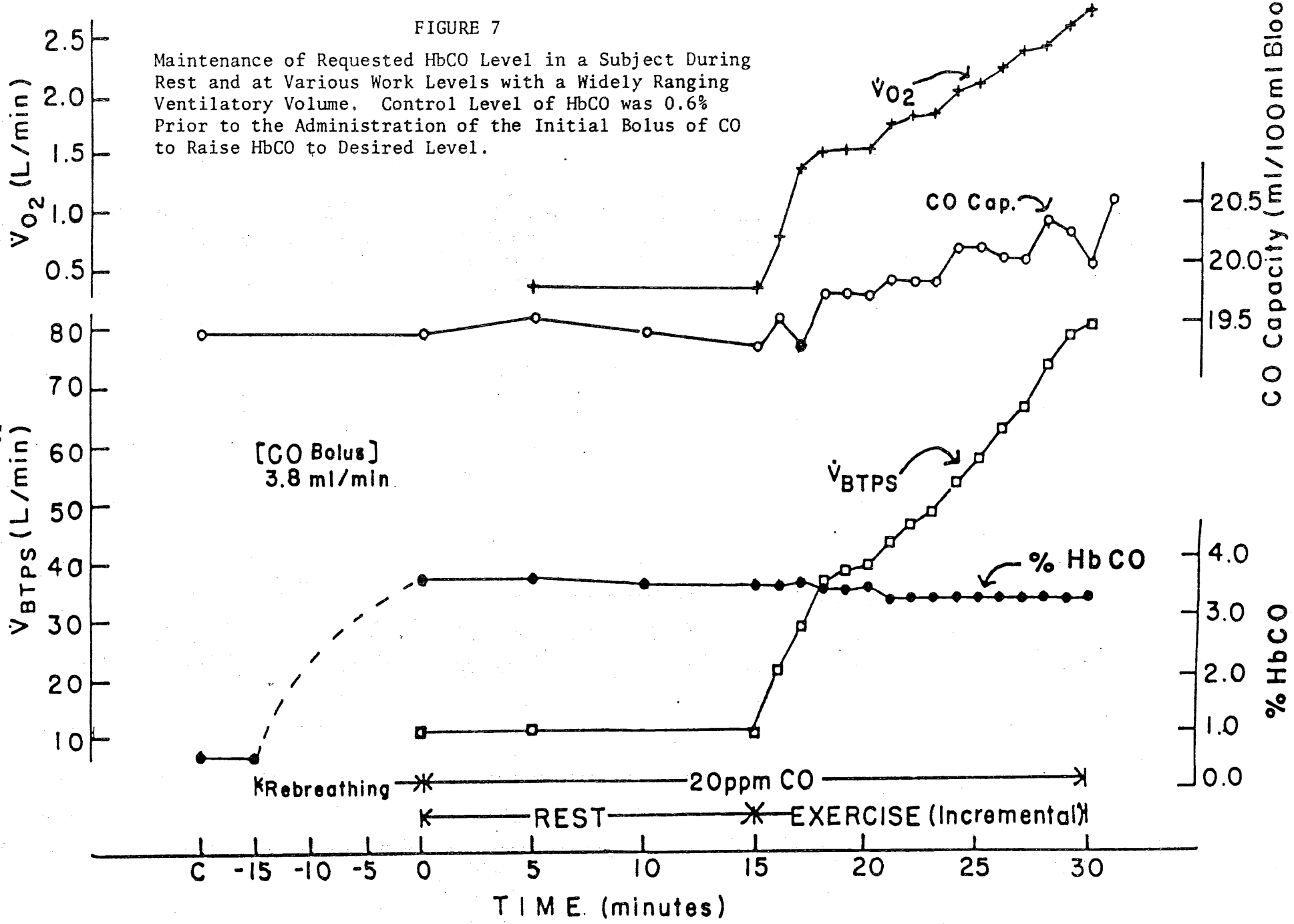
TABLE 3

SUBJECT DESCRIPTION

Subject	Weight (kg)	THb (g)	CO Dilution Volume (ml)
A	79.2	781	1085
B	77.0	747	1038
C	78.0	882	1225
D	61.0	698	970

FIGURE 7

Maintenance of Requested HbCO Level in a Subject During Rest and at Various Work Levels with a Widely Ranging Ventilatory Volume. Control Level of HbCO was 0.6% Prior to the Administration of the Initial Bolus of CO to Raise HbCO to Desired Level.



accurately, as shown in Table 4. It should be noted that the level of HbCO remained constant (Fig. 7) over a wide range of ventilation (10-80 liters/min), metabolic rate (0.35-2.6 liters/min), and heart rate increase (60-160 bpm), and presumably a similar increase of perfusion. HbCO remained at a constant level despite the hemoconcentration which accompanied severe exercise (Hb increased 13.9-14.5 g/dl). The data on the subject in Fig. 7 illustrates that the system described is capable of maintaining a desired HbCO concentration over a wide range of physiological states.

The accuracy of this method was further demonstrated by a series of maximal exercise experiments where desired levels of HbCO were set and maintained as shown in Table 4. The desired level of HbCO was reached and maintained to within 0.1% HbCO. All of the experiments carried out are reported; these results are from routine use of this method.

The relationship between inspired CO levels and final blood %HbCO is illustrated in Fig. 8. The inspired CO levels represented the average inspired CO levels during the exercise portion of the experiment, and the HbCO levels are the final HbCO levels taken immediately after exercise. The subject continued to breathe on the gas mixing system during recovery, so there was no CO lost during the post-exercise period. The slope of this relationship gives an equation such that $\%HbCO = 0.180\ (CO_I)$, where CO_I is in ppm. Lilienthal et al. (6) had proposed a simpler predictive equation based on their studies at higher levels of inspired P_{CO}. The variability in their reported attained levels does not provide for the requisite control of blood HbCO represented by the present procedure.

TABLE 4

RESULTS OF CARBON MONOXIDE ADMINISTRATION AND MAINTENANCE AT DESIRED LEVELS

	Low HbCO Trials				High HbCO Trials			
Subject	Desired %HbCO	End Rest %HbCO	Inspired CO Conc. (ppm)	Post Exercise %HbCO	Desired %HbCO	End Rest %HbCO	Inspired CO Conc. (ppm)	Post Exercise %HbCO
A	3.18	3.13	15.8	2.94	4.29	4.33	22.2	4.28
B	3.66	3.48	19.4	3.32	4.78	4.74	26.9	4.84
C	3.22	3.15	16.2	3.08	3.69	3.84	20.4	3.75
D	3.52	3.58	19.3	3.58	4.70	4.90	25.0	4.55

TABLE 5

RELATIVE AFFINITY CONSTANTS FOR CO

	Rest			Exercise		
Subject	Low CO	High CO	$\overline{X}$	Low CO	High CO	$\overline{X}$
A	266	266	266	275	272	274
B	242	241	242	254	271	263
C	256	255	256	281	274	278
D	250	269	260	276	273	275
MEAN			256			273

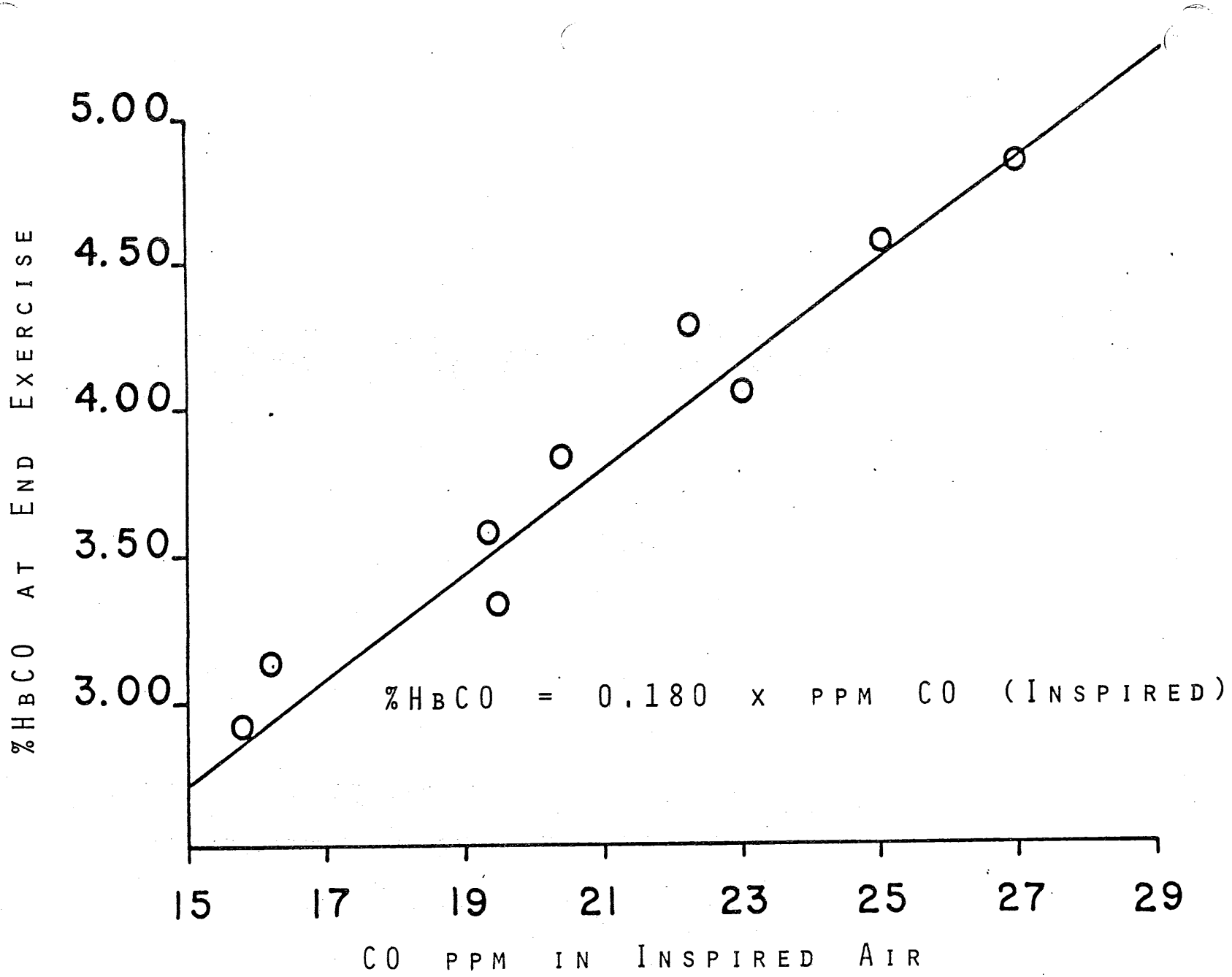


FIGURE 8

Relationship Between Inspired CO (ppm) and the Final HbCO Level After an Initial Bolus Was Given to Raise HbCO to Desired Value. Final HbCO Level Is That Measured at the End of a Maximum $\dot{V}_{O_2}$ Test.

In an attempt to carry out a similar procedure, Vogel et al. (13) administered CO to elevate his subjects to 17-18% HbCO and then attempted to maintain these subjects at this level with 225 ppm. In these subjects the %HbCO rose to 19-20% HbCO under these conditions during exercise due to the use of inspired P_{O_2} rather than arterial tension utilized by Lilienthal et al. (6) in their predictive formula. These data suggested a much lower affinity of hemoglobin for CO under exercise conditions than has been reported for resting conditions (10). If one assumes that Vogel et al.'s subjects were near equilibrium at 225 ppm, the levels of 18-20% HbCO are half the expected values at rest of 35% as predicted by Peterson and Stewart (10). The data of this present experiment suggest no change in affinity during exercise, and the equilibrium conditions during rest and exercise agree closely with those reported for resting subjects by Peterson and Stewart (10).

If one assumes that the subjects' HbCO levels in this study were in equilibrium at rest and a new equilibrium was reached during exercise, it is possible to calculate relative affinities of hemoglobin for CO in these two physiological states. Based on the data of Holmgren and McIlroy (4), the following assumptions were made: rest P_{aO_2} of 90 torr and $\%HbO_2$ plus %HbCO of 97%; and during maximal exercise P_{aO_2} of 98 torr and $\%HbO_2$ plus %HbCO of 98%. Given these conditions, the affinity constant (M) can be calculated by Haldane's First Law (12). The average affinity constant of 256 at rest was lower in all subjects than the average exercise value of 273. This increase in affinity of hemoglobin for CO with exercise may be due to the decrease in P_{aCO2} and a decrease in pH, both of which serve to increase the affinity of hemoglobin for CO (12).

The method described for adjusting and maintaining desired blood carboxyhemoglobin levels should make it possible to perform controlled studies involving other pollutants. It has been utilized in experiments on peroxyacetylnitrate and several other gaseous compounds utilizing either humans or animals as experimental subjects.

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*IV. Cardiovascular and Metabolic Adjustments to Exercise Before and After Carbon Monoxide Exposure**

Increases in cardiac output, ventilation and coronary blood flow, and decreases in myocardial oxygen, pyruvate and lactate extraction ratios have been observed in human subjects (2) under resting conditions as a result of 4-13% blood carboxyhemoglobin levels. These changes were not observed in dogs until HbCO levels greater than 20% were produced (2), however, Adams, et al. (1) observed increases in heart rate and coronary blood flow and decreases in myocardial oxygen extraction and consumption when 4-23% HbCO levels were produced in unanesthetized dogs. Carboxyhemoglobin levels lower than 5% appeared to have little effect on cardiovascular and metabolic parameters during submaximal effort in healthy human subjects (16), but 7-24% HbCO levels have caused increases in heart rates in human subjects (10, 15, 20) during submaximal exercise. Nearly maximal heart rates were observed in dogs during submaximal exercise when HbCO levels were elevated to 18% (12). These studies indicated that a better understanding of possible adverse physiological effects of 0-20% HbCO levels was needed, since these levels are of greatest concern regarding air pollution and industrial accidents. The following experiments were conducted on dogs under resting and submaximal exercise conditions in order to study this problem in greater detail.

(a) METHODS

Male mongrel dogs weighing between 20-27 kg were trained to run on a motor-driven treadmill. Each dog had two to three practice sessions per week over a 3-week period during which heart rate was

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* See pages 75-77 for literature references applicable to this section.

recorded (electrocardiograph) while oxygen consumption was measured using a Noyon's diaferometer and a hood. An attempt was made to determine the maximal aerobic capacity of the dogs by increasing the speed and grade of the treadmill during successive practice sessions until oxygen consumption no longer increased with an increased work load.

After the dogs became accustomed to these procedures, they were prepared for surgery and anesthetized by intravenous administration of pentobarbital sodium (25 ml/kg). Thick-walled silastic catheters (0.062 in. ID, 0.125 in. OD) were inserted into the jugular vein and carotid artery and positioned in the right ventricle and descending aorta under fluoroscopic guidance. The catheters were anchored in place at the back of the neck with a silastic skin button. The skin area around the button was cleansed daily and protected with a bandage and a canvas vest. The catheters were flushed with a sterile heparin solution every two days and the dogs were allowed at least four days to recover from surgery.

After recovery, cardiovascular and metabolic parameters were measured in the dogs at rest and while running either 6.4 km/hr on a 10% grade or 8.0 km/hr on a 16% grade. These parameters were studied before and after breathing ambient air (0.4% carboxyhemoglobin levels) and before and after breathing 14% carbon monoxide (CO) in air mixture for 1 min or 22% CO in air mixture for 2 min resulting in blood HbCO levels of 6-30%. The following protocol was followed for all experimental conditions: 15 min standing at rest on the inclined treadmill and breathing ambient air, 1-2 min breathing ambient air or CO in air mixtures, 15 min standing at rest on the inclined treadmill and breathing ambient air, and 25-30 min exercise. Seven dogs ran at 6.4 km/hr on a 10% grade

and five dogs exercised at 8.0 km/hr on a 16% grade with each of three levels of blood HbCO.

Cardiac outputs were measured by direct Fick method over a 2-min period during both rest periods and during the 4-6th, 15-17th, and last 2 min of exercise. Aortic systolic pressures and heart rates were obtained from blood pressure tracings using a Statham P 23 series pressure transducer and Sanborn recorder. Preliminary testing showed no differences in pressures obtained using thick-walled silastic, dacron or polythylene catheters. Aortic blood pressures and heart rates were measured before and after each resting cardiac output determination and minute by minute during the exercise period. Oxygen consumption was measured continously throughout rest and exercise using a Noyon's diaferometer with an air flow of 250 liters/min through the respiratory hood. Rectal temperatures were measured during rest and exercise with an indwelling thermistor. Arterial and mixed venous blood samples were analyzed for pH, P_{O_2}, and P_{CO_2}, using calomel, Clark and Severinghaus electrodes with a Radiometer model 27 pH meter. This instrument was calibrated hourly during analyses with precision tank gases and buffer solutions. Oxygen, CO_2, and CO contents were determined using chromatographic techniques (8). Arterial blood samples were assayed for hematocrit by micro method, hemoglobin by the cyanomethemoglobin technique, and lactate by the method of Ström (17).

The sequence in which each dog was exposed to the experimental conditions was randomized in order to minimize any effect of training of familiarization with experimental protocol. Analysis of variance and regression techniques were used to test for statistical significance

and the null hypothesis was rejected at the 5% level.

(b) RESULTS AND DISCUSSION

During the preliminary treadmill runs the apparent mean maximal oxygen uptake ($\dot{V}_{O_2}$) obtained in eight dogs was 3.21 ± 0.13 liters/min (135.0 ± 4.4 ml/kg·min) while working at 977 ± 68 kg·m/min. This was accomplished at treadmill speeds of 11.7 ± 0.3 km/hr on a 20% grade or 15.8 ± 0.2 km/hr on a 16% grade. Mean heart rate increased to a maximal level of 301 ± 12 beats/min and the mean rectal temperature increased to 108.0 ± 1.0°F. The oxygen uptakes observed in these dogs were the highest levels reported for adult dogs (3, 6, 7, 9, 12) and equal to the maximal oxygen uptakes of 50-week old beagles (22). Maximal heart rates were in agreement with other studies (3, 6, 12, 13, 19, 22). The maximal $\dot{V}_{O_2}$ values achieved by these dogs may have been limited by their high central body temperature. The hood with its high ventilation rate permitted animals to pant and did not interfere with normal thermoregulatory processes. Mean $\dot{V}_{O_2}$ levels observed in dogs running 6.4 km/hr, on a 10% grade (250 ± 11 kg·m/min) and 8.0 km/hr on a 16% grade (531 ± 17 kg·m/min) were approximately 32 and 50% respectively, of the dogs' maximal aerobic capacities.

When control dogs (0.4% HbCO) ran at 32% $\dot{V}_{O_2\ max}$ their cardiac outputs and oxygen extractions each increased to about twice the testing levels ($P < 0.01$) while oxygen uptake increased from 0.25 ± 0.02 to 1.03 ± 0.06 liters/min ($P < 0.01$) (Table 6). Control dogs running at 50% $\dot{V}_{O_2\ max}$ increased their cardiac outputs and oxygen extractions by 123 and 132% ($P < 0.01$), respectively, while oxygen uptakes increased to five times the resting levels, ($P < 0.01$) (Table 7). The relationships between $\dot{V}_{O_2}$ and cardiac output, heart rate, a-v_{O_2} difference, and

TABLE 6

METABOLIC AND CARDIOVASCULAR CHANGES DURING REST AND MODERATE TREADMILL EXERCISE (6.4 km/hr, 10% GRADE; 32% $\dot{V}_{O_2\ max}$) IN SEVEN DOGS WITH NORMAL AND ELEVATED BLOOD CARBOXYHEMOGLOBIN LEVELS*

	HbCO	HR (beats/min)	$\dot{Q}_c$ (liters/min)	$\dot{V}_{O_2}$ (liters/min)	a sat (%)	v sat (%)	a-vO_2 (ml/dl)
CONTROL							
$Rest_1$	0.5 ± 0.1	121 ± 11	4.81 ± 0.20	0.25 ± 0.02	101.2 ± 1.7	72.3 ± 3.5	5.20 ± 0.43
$Rest_2$	0.5 ± 0.1	120 ± 9	4.78 ± 0.20	0.24 ± 0.01	99.4 ± 2.3	71.1 ± 2.7	5.13 ± 0.22
Exercise:							
5 min	0.5 ± 0.0	190 ± 7	8.95 ± 0.23	0.96 ± 0.03	98.4 ± 1.9	43.8 ± 1.5	10.75 ± 0.30
16 min	0.4 ± 0.0	190 ± 8	9.41 ± 0.59	1.02 ± 0.05	99.9 ± 1.2	44.4 ± 1.1	10.89 ± 0.23
28 min	0.4 ± 0.0	190 ± 10	9.71 ± 0.60	1.03 ± 0.06	97.2 ± 2.4	44.5 ± 1.1	10.63 ± 0.30
LOW HbCO							
$Rest_1$	0.5 ± 0.0	119 ± 5	5.63 ± 0.55	0.26 ± 0.01	99.4 ± 3.0	73.9 ± 0.6	4.88 ± 0.46
$Rest_2$	8.4 ± 0.9	122 ± 6	5.05 ± 0.28	0.26 ± 0.01	93.1 ± 1.8	65.8 ± 1.1	5.13 ± 0.23
Exercise:							
5 min	6.5 ± 0.5	201 ± 6	10.45 ± 0.80	1.04 ± 0.03	96.2 ± 1.8	45.1 ± 2.2	10.34 ± 0.52
16 min	5.2 ± 0.4	201 ± 5	10.32 ± 0.60	1.09 ± 0.03	98.5 ± 2.3	43.8 ± 2.1	10.66 ± 0.41
28 min	4.2 ± 0.3	201 ± 6	10.85 ± 0.62	1.08 ± 0.03	98.2 ± 1.5	47.1 ± 1.7	10.05 ± 0.37
MODERATE HbCO							
$Rest_1$	0.5 ± 0.1	130 ± 9	4.53 ± 0.42	0.25 ± 0.02	98.7 ± 1.6	67.3 ± 1.9	5.74 ± 0.38
$Rest_2$	20.8 ± 3.1	137 ± 12	4.88 ± 0.35	0.26 ± 0.02	81.7 ± 2.1	53.4 ± 3.0	5.39 ± 0.34
Exercise:							
5 min	15.7 ± 2.1	193 ± 8	9.39 ± 0.46	0.88 ± 0.06	86.6 ± 1.4	39.3 ± 1.3	9.27 ± 0.38
16 min	12.2 ± 1.8	201 ± 9	10.25 ± 0.35	1.00 ± 0.07	90.1 ± 1.7	40.9 ± 1.4	9.66 ± 0.46
28 min	9.4 ± 1.4	202 ± 9	9.95 ± 0.39	1.02 ± 0.08	90.0 ± 1.3	39.3 ± 2.3	10.27 ± 0.78

*Values are means ± S.E. $Rest_1$ = values obtained before carbon monoxide (CO) administration; $Rest_2$ = values obtained during rest 9 min after CO administration; %HbCO = arterial blood percent carboxyhemoglobin levels; HR = heart rate; $\dot{Q}c$ = cardiac output; $\dot{V}_{O_2}$ = oxygen uptake; a sat = arterial blood percent oxygen saturation; v sat = mixed venous blood percent oxygen saturation; a-vO_2 = difference in oxygen content between arterial and mixed venous blood.

TABLE 7

METABOLIC AND CARDIOVASCULAR CHANGES DURING REST AND HEAVY TREADMILL EXERCISE (8.0 km/hr, 16% GRADE; 50% $\dot{V}_{O_2\ max}$) IN FIVE DOGS WITH NORMAL AND ELEVATED BLOOD CARBOXYHEMOGLOBIN LEVELS*

	HbCO	HR (beats/min)	$\dot{Q}_c$ (liters/min)	$\dot{V}_{O_2}$ (liters/min)	a sat (%)	v sat (%)	a-vO_2 (ml/dl)
CONTROL							
$Rest_1$	0.3 ± 0.0	121 ± 10	6.10 ± 0.55	0.32 ± 0.05	96.9 ± 5.8	71.1 ± 5.0	5.34 ± 0.67
$Rest_2$	0.3 ± 0.0	125 ± 8	6.11 ± 0.61	0.32 ± 0.03	98.4 ± 4.0	72.1 ± 3.4	5.24 ± 0.48
Exercise:							
5 min	0.3 ± 0.0	231 ± 9	13.22 ± 1.02	1.58 ± 0.12	98.5 ± 4.8	40.8 ± 3.3	12.22 ± 0.75
16 min	0.3 ± 0.0	230 ± 8	13.55 ± 0.71	1.65 ± 0.15	101.7 ± 4.1	42.6 ± 2.9	12.13 ± 0.66
24 min	0.2 ± 0.0	232 ± 9	13.03 ± 0.61	1.62 ± 0.17	101.8 ± 2.5	42.2 ± 2.8	12.31 ± 0.75
LOW HbCO							
$Rest_1$	0.4 ± 0.1	121 ± 10	6.58 ± 0.58	0.32 ± 0.04	97.0 ± 1.5	71.2 ± 2.5	4.94 ± 0.48
$Rest_2$	9.1 ± 1.7	131 ± 10	6.70 ± 0.26	0.32 ± 0.02	90.3 ± 0.8	64.9 ± 0.8	4.78 ± 0.25
Exercise:							
5 min	6.6 ± 1.1	236 ± 9	13.27 ± 0.48	1.54 ± 0.09	93.5 ± 1.2	36.8 ± 2.1	11.63 ± 0.53
16 min	4.9 ± 0.8	225 ± 12	14.39 ± 1.06	1.64 ± 0.19	94.8 ± 1.3	39.1 ± 1.7	11.32 ± 0.49
24 min	3.6 ± 0.5	219 ± 15	13.78 ± 1.01	1.56 ± 0.21	94.8 ± 1.0	40.2 ± 2.1	11.20 ± 0.74
MODERATE HbCO							
$Rest_1$	0.4 ± 0.2	118 ± 6	7.04 ± 1.61	0.34 ± 0.04	97.5 ± 2.0	70.8 ± 3.1	5.27 ± 0.61
$Rest_2$	16.7 ± 1.5	129 ± 10	6.93 ± 0.44	0.32 ± 0.01	83.1 ± 2.7	59.9 ± 3.3	4.63 ± 0.25
Exercise:							
5 min	13.0 ± 1.2	241 ± 10	13.98 ± 1.28	1.51 ± 0.12	90.0 ± 2.0	38.1 ± 3.1	10.83 ± 0.48
16 min	9.8 ± 1.0	240 ± 9	14.42 ± 1.47	1.56 ± 0.20	92.8 ± 1.9	40.2 ± 2.7	10.60 ± 0.63
24 min	7.6 ± 0.8	237 ± 14	13.90 ± 1.32	1.54 ± 0.19	93.8 ± 1.7	40.5 ± 2.6	11.04 ± 0.65

*Values are means ± S.E. $Rest_1$ = values obtained before carbon monoxide (CO) administration; $Rest_2$ = values obtained during rest 9 min after CO administration; %HbCO = arterial blood percent carboxyhemoglobin levels; HR = heart rate; $\dot{Q}_c$ = cardiac output; $\dot{V}_{O_2}$ = oxygen uptake; a sat = arterial blood percent oxygen saturation; v sat = mixed venous blood percent oxygen saturation; a-vO_2 = difference in oxygen content between arterial and mixed venous blood.

treadmill speed and grade were in agreement with those obtained in previous studies (3, 5, 6, 7). The increases in cardiac outputs during exercise were primarily due to increases in heart rates ($P < 0.01$) (Tables 6 and 7) with smaller increases in stroke volumes ($P < 0.01$).

In the present study the dogs could not complete 30 min of treadmill running at 8.0 km/hr on a 16% grade, and their cardiac outputs declined slightly during the final 15 min of heavy work. This decrement in performance was unexpected since dogs are known for remarkable stamina during exercise. The relatively limited endurance of the dogs in the present study may have been due to a lower level of physical fitness and motivation of these dogs compared to Alaskan sled dogs or to the type of work being a continuous uphill grade. Alaskan sled dogs were able to run, with heart rates of 250-300 beats/min, more than 30 miles cross-country in 2.5 hours while under a load of sled, driver and equipment (19).

Arterial systolic blood pressures increased from a mean value of 154 ± 6 torr at rest to a steady state level of 200 ± 10 torr after the dogs exercised 5 min at 32% $\dot{V}_{O_2\ max}$ ($P < 0.01$) while diastolic pressure fell from 84 ± 4 to 57 ± 4 ($P < 0.01$) and then remained constant for the remainder of the run. Arterial mean pressures remained constant at resting levels of 110 ± 4 torr. Left ventricular work increased to 29.0 ± 3.2 $kg \cdot m/min \cdot m^2$, 160% above the resting level, while stroke work and stroke power increased 70% during moderate exercise ($P < 0.01$). Dogs performing heavy work (50% max) had increases ($P < 0.01$) in mean arterial systolic blood pressures from 174 ± 13 torr at rest to 240 ± 17, 238 ± 14, and 228 ± 23 torr 5, 16, and 24 min, respectively, after the onset of work. Diastolic pressures decreased

from a resting value of 105 ± 14 to 51 ± 14 torr after 5 min of work ($P < 0.01$) but decreased further to 40 ± 15 torr by the end of work. Arterial mean pressure was 125 ± 10 throughout rest and exercise. Left ventricular work increased from 15.3 ± 1.4 kg·m/min·m^2 at rest to 45.5 ± 3.1 kg·m/min·m^2 after 5 and 16 min of the exercise bout ($P < 0.01$), and declined to 43.0 ± 5.0 kg·m/min·m^2 by the 24th min of exercise. Stroke work and stroke power increased 60 and 120%, respectively, during the first 16 min of heavy work ($P < 0.01$), but these parameters also declined by the end of exercise.

Several studies have shown that arterial diastolic (11) and mean pressures (6, 9, 11, 18) increase above resting values in the exercising dog. Others have shown decreases in diastolic pressures (19) and little or no change in mean pressures (5, 12, 19) during exercise. In the present study the mean arterial blood pressures in working dogs were maintained at resting levels by a reduction in peripheral vascular resistance. Peripheral vascular resistance was 52 and 43% of resting levels ($P < 0.01$) when the dogs worked at 32 and 50% $\dot{V}_{O_2\ max}$, respectively.

Arterial pH increased to 7.530 ± 0.017 during moderate exercise (Table 8) and 7.613 ± 0.034 during heavy exercise ($P < 0.01$) (Table 6). The alkalosis was probably a respiratory alkalosis, originating from an excess elimination of CO_2 consequent to hyperventilation. Wathen, et al. (21) reported trends toward increases in venous P_{CO_2} and CO_2 content during 6 min of light to heavy exercise. The data of the present study (Tables 8 and 9) show the same trend early during the exercise bout, followed by a decline as the exercise continued. Mixed venous P_{CO_2} levels were actually lower than the resting levels at the end of exercise ($P < 0.05$), although venous CO_2 contents were not

TABLE 8

BLOOD GAS AND HEMATOLOGIC CHANGES DURING REST AND MODERATE TREADMILL EXERCISE (6.4 km/hr, 10% GRADE; 32% $\dot{V}_{O_2\ max}$) IN SEVEN DOGS BREATHING NORMAL ROOM AIR*

	Rest	Exercise		
		5 min	16 min	28 min
ARTERIAL BLOOD:				
O_2 content (ml/dl)	18.02 ± 0.80	19.38 ± 0.52	19.64 ± 0.59	19.63 ± 0.67
O_2 tension (torr)	86.3 ± 1.9	79.6 ± 6.3	79.4 ± 6.2	81.7 ± 5.6
CO_2 content (ml/dl)	39.62 ± 2.09	34.69 ± 1.15	33.01 ± 1.84	31.49 ± 1.92
CO_2 tension (torr)	29.1 ± 2.1	22.9 ± 1.6	19.1 ± 1.6	18.3 ± 1.5
pH	7.445 ± 0.011	7.487 ± 0.010	7.518 ± 0.015	7.530 ± 0.017
Hemoglobin (g/dl)	12.6 ± 0.5	14.0 ± 0.5	14.0 ± 0.5	14.4 ± 0.7
Hematocrit (%)	39.1 ± 1.8	43.1 ± 1.7	42.9 ± 1.6	42.6 ± 1.9
Lactate (mg/dl)	8.5 ± 0.7	12.2 ± 1.0	18.3 ± 2.8	17.5 ± 2.5
MIXED VENOUS BLOOD:				
O_2 content (ml/dl)	12.82 ± 0.89	8.62 ± 0.45	8.75 ± 0.48	8.99 ± 0.51
O_2 tension (torr)	38.7 ± 1.7	26.9 ± 2.0	26.6 ± 1.8	26.2 ± 1.8
CO_2 content (ml/dl)	44.85 ± 2.21	45.26 ± 1.22	42.93 ± 1.82	41.84 ± 1.96
CO_2 tension (torr)	32.5 ± 2.1	31.6 ± 1.6	28.2 ± 2.3	26.3 ± 1.9
pH	7.406 ± 0.012	7.417 ± 0.016	7.447 ± 0.015	7.467 ± 0.015

*Values are means ± S.E.

TABLE 9

BLOOD GAS AND HEMATOLOGIC CHANGES DURING REST AND HEAVY TREADMILL EXERCISE (8.0 km/hr, 16% GRADE; 50% $\dot{V}_{O_2\ max}$) IN FIVE DOGS BREATHING NORMAL ROOM AIR*

	Rest	Exercise		
		5 min	16 min	24 min
ARTERIAL BLOOD:				
O_2 content (ml/dl)	19.39 ± 0.98	20.85 ± 1.09	20.85 ± 1.20	20.97 ± 1.32
O_2 tension (torr)	91.5 ± 1.7	84.8 ± 2.2	87.3 ± 3.5	81.3 ± 3.5
CO_2 content (ml/dl)	40.85 ± 0.70	37.63 ± 1.24	34.94 ± 1.41	30.70 ± 2.00
CO_2 tension (torr)	29.6 ± 3.0	22.3 ± 2.0	16.0 ± 1.7	12.7 ± 1.5
pH	7.440 ± 0.012	7.477 ± 0.023	7.542 ± 0.041	7.617 ± 0.034
Hemoglobin (g/dl)	14.0 ± 1.9	15.1 ± 0.8	14.6 ± 0.9	14.7 ± 1.0
Hematocrit (%)	42.2 ± 2.9	45.4 ± 2.4	43.9 ± 2.7	43.8 ± 2.9
Lactate (mg/dl)	13.6 ± 2.5	17.4 ± 1.8	21.3 ± 3.3	25.5 ± 2.0
MIXED VENOUS BLOOD:				
O_2 content (ml/dl)	14.15 ± 0.94	8.63 ± 0.76	8.73 ± 0.75	8.66 ± 0.74
O_2 tension (torr)	43.6 ± 1.5	29.6 ± 0.9	27.4 ± 0.6	25.7 ± 0.4
CO_2 content (ml/dl)	45.39 ± 1.34	49.30 ± 1.55	46.90 ± 1.12	42.44 ± 1.84
CO_2 tension (torr)	30.5 ± 2.4	32.2 ± 3.1	26.1 ± 2.9	20.8 ± 2.8
pH	7.412 ± 0.012	7.391 ± 0.021	7.468 ± 0.023	7.522 ± 0.023

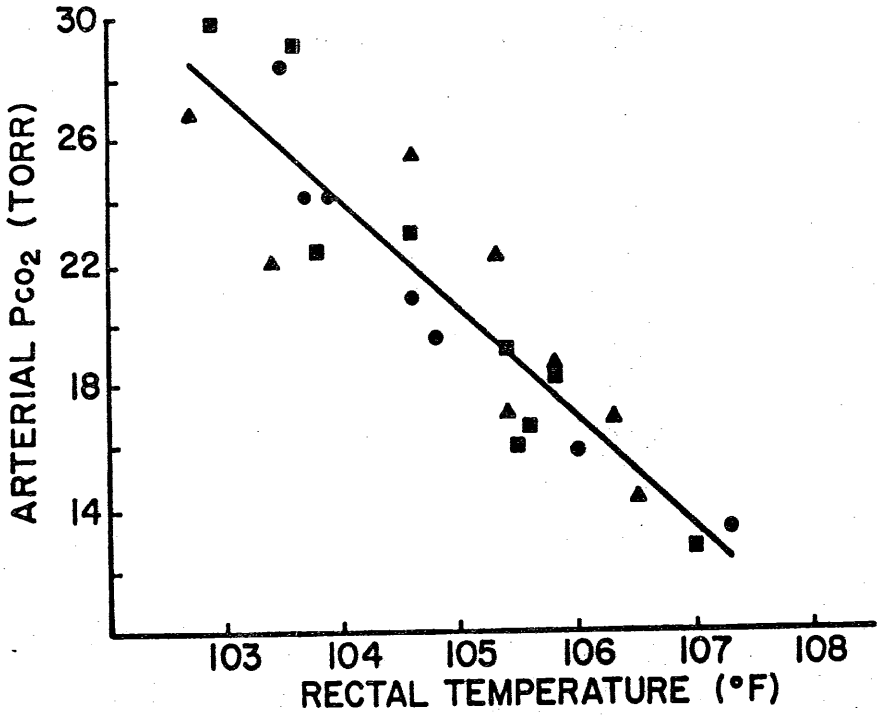
*Values are means ± S.E.

significantly decreased by the end of 30 min of exercise. The pH, P_{CO_2}, and CO_2 contents of arterial blood were altered more by exercise than those of venous blood ($P < 0.01$) (Tables 8 and 9). Arterial blood lactate levels increased during the beginning of both moderate and heavy work ($P < 0.01$) (Tables 8 and 9), tended to plateau during the latter half of moderate work but continued to rise during heavy work. The increases observed were minimal in comparison to other studies (7, 14) indicating that the work loads in the present study represent borderline conditions for an anaerobic energy requirement. At the lower work load lactate production slowed or utilization balanced production during the latter part of work. At the higher work load lactate production continued to exceed utilization.

Minute ventilation in the dog has been shown to be closely regulated during rest and exercise by changes in arterial blood P_{CO_2} (4). The precise regulation of ventilation by P_{CO_2} can be modified by pH, P_{O_2}, temperature, psychic and mechanoreceptor input. Elevated P_{CO_2} levels of venous blood during the early part of exercise may have stimulated venous chemoreceptors and contributed to an increased ventilation, but this potential stimulus diminished by the end of exercise. The elevated acid levels due to blood lactate were probably buffered by hemoglobin and other proteins or completely offset by the respiratory elimination of CO_2. Both the decreased P_{CO_2} levels and the increased pH in the arterial blood of the dogs in the present study should reduce their ventilatory drive during exercise unless there were changes in the sensitivity of the chemoreceptors to these stimuli. The moderately lower arterial P_{O_2} levels observed during exercise may have stimulated the arterial chemoreceptors and contributed to a

ventilatory drive. However, increased blood temperature in the dogs probably was a very potent ventilatory stimulus at rest and during exercise since panting is the major avenue for heat loss in the dog. The mean central body temperature (rectal temperature) increased from a resting level of 103.6 ± 0.4 to 105.8 ± 0.6°F at the end of the lighter work load and 102.9 ± 0.4 to 107.0 ± 0.5°F at the end of heavy work ($P < 0.01$). Although measurements of minute ventilation were not possible using the diaferometer, a direct relationship was observed between rectal temperatures, the arterial blood P_{CO_2} and pH levels ($P < 0.01$) (Fig. 9). These relationships indicated that central body temperatures were potent stimuli to ventilation in dogs.

Additional studies were performed on the exercising dogs to evaluate the influence of lowered available oxygen supplies on their performance, and on their adjustments to this situation. Fifteen min after administration of CO in air the dogs with 8.4 ± 0.9 and 20.8 ± 3.1% HbCO began performing the moderate work and those with 9.1 ± 1.7 and 16.7 ± 1.5% HbCO began performing the heavy work (Tables 6 and 7). Five minutes after the onset of moderate work, when the first exercise measurements were made, the mean HbCO levels were 6.5 ± 0.5 and 15.7 ± 2.1%, respectively. HbCO levels were 6.6 ± 1.1 and 13.0 ± 1.2% at the same time after the onset of heavy work. Throughout the remainder of each experiment the blood HbCO levels were further reduced because of the rapid elimination of CO by hyperventilation. Nonetheless final levels of HbCO were still between 55-65% of the levels recorded 5 min after the onset of work (Tables 6 and 7).



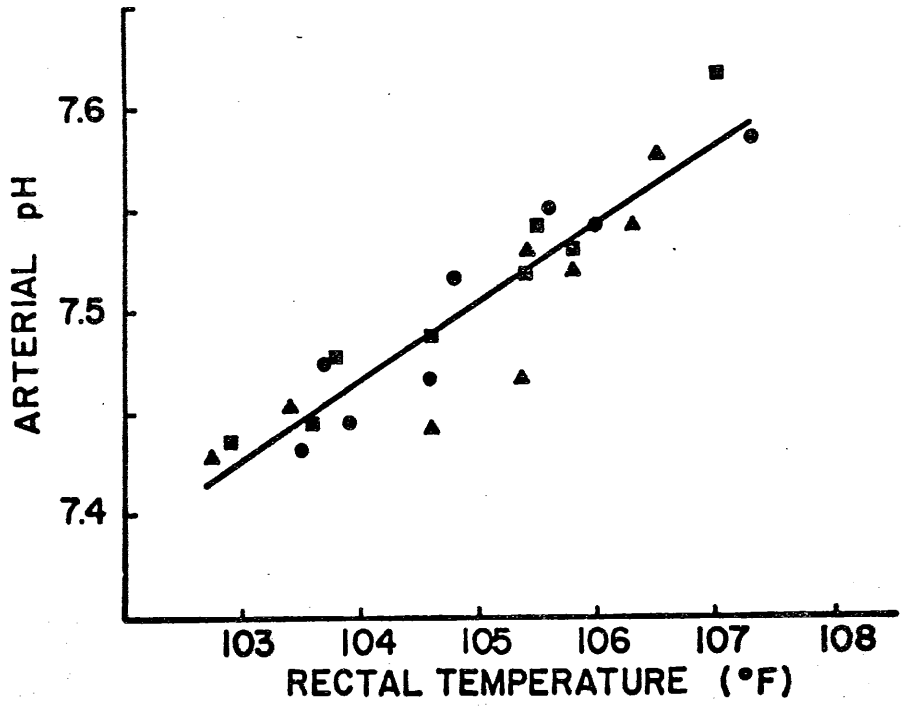


FIGURE 9

Left: Mean Rectal Temperatures Vs. Arterial Carbon Monoxide Tensions (P_{CO_2}) During Rest and Exercise in Dogs with 0.5% HbCO (●), 4-9% HbCO (▲), or 8-21% HbCO (■) Levels. $Y = -3.55X + 393.47$, $r = -0.908$, $S_{yx} = 2.09$, $P < 0.001$.
Right: Mean Rectal Temperatures Vs. Arterial pH in the Same Dogs Under the Same Conditions as Described Above. $Y = 0.387X + 3.4389$, $r = 0.916$, $S_{yx} = 0.0217$, $P < 0.001$.

The elevated blood %HbCO levels resulted in greatly reduced percentage oxygen saturation of hemoglobin in both arterial and mixed venous blood (Tables 6 and 7). The greatest reduction in O_2 saturation occurred at rest ($P < 0.01$) before the hyperventilation of exercise reduced the blood HbCO levels. Due to the reduction in percentage O_2 saturation and to a leftward shift in the oxygen dissociation curve the mixed venous P_{O_2} level was reduced in resting dogs ($P < 0.01$). This effect was almost eliminated during exercise probably because of the rapid reduction in HbCO levels. Since there was a small requirement for oxygen and a great extractable reserve supply in resting dogs, the arterial and venous O_2 saturations were decreased by the same amounts, a-vO_2 extractions were maintained constant and no significant changes occurred in cardiac outputs, heart rates and stroke volumes. A greater level of cardiovascular fitness in these dogs, due to exercise training, may have provided them with a greater resistance to low HbCO levels than the dogs used by Adams', et al. (1). Ayres, et al. (2) reported that dogs were more resistant than human subjects to HbCO levels lower than 25%.

During exercise the elevated blood HbCO levels caused larger reductions in the arterial oxygen contents and saturations than in the venous blood ($P < 0.05$), consequently, reductions in the arterial - mixed venous oxygen difference (a-vO_2) and O_2 extraction ($P < 0.01$). Cardiac outputs increased moderately in response to the decreased O_2 extraction, however the increases were significant only in the group of dogs performing the lower level of work ($P < 0.05$). Although small increases in heart rate and stroke volume occurred during moderate work consequent to CO administration, neither heart rate nor stroke

volume were significantly affected by elevated blood HbCO levels. After CO administration aortic systolic blood pressures and pulse pressures during moderate work were increased 15-20 torr above the exercise levels of control dogs. CO administration had no effect on blood pressures during heavy work. Left ventricular work, stroke work, and stroke power were also slightly increased when blood HbCO levels were elevated during moderate work.

Horstman, et al. (12) found nearly maximal heart rates in dogs doing submaximal treadmill work after their blood HbCO levels were raised to 18%. Stroke volumes were lower than in control dogs only at the lowest work load (14 km/hr, 5% grade), with no differences occurring when the treadmill grade was raised to 10% or greater. During submaximal work at 18% HbCO levels no effect on oxygen consumption, cardiac output, mean arterial pressure, or total peripheral resistance was noted. The large increases in heart rate which these investigators observed during exercise when CO was administered may be partially related to the low level of physical fitness of their dogs, as indicated by their low aerobic work capacity (86 ml/kg·min). The high heart rates also may have been partially due to the relatively higher and sustained HbCO levels which they employed compared to the present study. Whereas Horstman, et al. (12) maintained 18% HbCO levels throughout exercise, the blood HbCO levels in the present study were reduced during exercise because the dogs were breathing room air. Since Horstman, et al. (12) found little or no other physiological effects of the elevated HbCO levels, other factors must have contributed to the production of nearly maximal heart rates during light submaximal work.

While it was evident that dogs running at 32 and 50% of their apparent maximal aerobic capacity made many marked physiological adjustments to the increased metabolic requirement, lowering the oxygen availability by raising HbCO levels up to 30% produced only small changes in the normal dogs' cardiovascular and metabolic functions during rest and moderate and heavy work. The decreased oxygen-carrying capacity of the blood was not detrimental during rest because of the large reserve extractable oxygen supply, allowing the a-vO_2 difference to remain constant. The decreased a-vO_2 extraction caused by elevated HbCO levels was effectively offset during work by an increased delivery of blood to the working muscles.

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