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ENDOGENOUS CARBON MONOXIDE PRODUCTION IN MAN *

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Since the late 19th century, carbon monoxide (CO) has been known to be present in the blood of normal man and animals. The origin of the gas in blood, however, has not been entirely explained. Although it might be assumed that all the blood CO is absorbed from the environment, many authors have thought that some, at least, is formed endogenously. The difficulties in determining whether blood CO arises endogenously or not have been the lack of knowledge of the variables that govern CO uptake and loss from the body, the uncertainty of the degree of exogenous exposure to CO, and the lack of accurate and specific CO analytical methods.

In 1894, Gréhan (1) found that normal dog blood contained a small amount of a combustible gas and assumed it was CO. Nicloux (2), Leoper (3), Lépine and Boulud (4), and Jongbloed (5) later demonstrated small amounts of CO in human blood, but did not differentiate between an exoge-

nous and endogenous origin of the gas. In recent years, the endogenous formation of CO has been studied by Sjöstrand and his associates in Sweden (6, 7).

These investigators, however, have not measured the actual rate at which CO is formed. The instrument¹ used in their experiments to analyze CO actually measures the temperature increase during its catalytic combustion. In our hands, it is relatively nonspecific for CO, and it seemed possible that some of their findings might have been produced by the presence of gases other than CO. Their conclusion that CO is produced in normal man (7) depended on the demonstration of higher CO concentrations in expired than in inspired air. This, however, could be possible in the absence of endogenous CO formation if an unsteady state existed between blood CO hemoglobin (COHb) and inspired CO concentration; this could have occurred if their subjects had been exposed to higher CO concentrations in the environment as long as several hours before the experiments. We have developed an analytical method that appears to be specific for CO and can detect the addition of 0.3 ml of CO to the total adult human CO store by the analysis of a 2-ml blood sample (8). We have overcome some of the other criticisms by measuring the increase in blood COHb during extended periods of rebreathing in a closed system.

METHODS

CO excretion that normally occurs via the lungs was eliminated by having the subject breathe in a closed system as shown in Figure 1. The subject breathed through the mouthpiece in and out of the inflatable 5-L rubber bag. A one-way valve caused the gas in the system to circulate through a CO₂ absorber.² Oxygen was

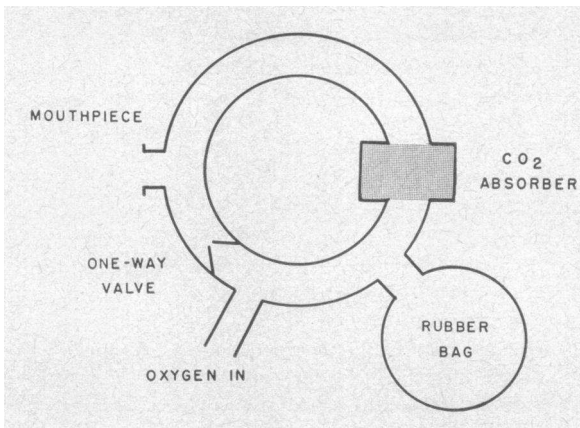


FIG. 1. REBREATHING APPARATUS.

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[†] Postdoctoral research fellow of the National Institutes of Health.

¹ Hopcalite CO meter, Stalex Corp., Stockholm, Sweden.

² Barlyme granules, National Cylinder Gas Co., Chicago, Ill.

automatically added during inspiration by a demand valve in amounts sufficient to keep the gas volume of the circuit constant. This system was designed to have a small deadspace, small total volume, and small resistance to air flow. The apparatus was constructed so that the subject could lie supine or sit up during the course of the experiment, and rebreathing in it was tolerated well for many hours. The O₂ added to the system contained less than 0.00001% CO,¹ and the components of the system do not produce or absorb significant amounts of CO.

The experimental procedure was as follows. The subject began breathing in the system. The O₂ tension was then adjusted to 20 to 30% of total barometric pressure by adding air or removing gas from the rubber bag (it was replaced by O₂). The O₂ tension was monitored³ frequently throughout the experiments and readjusted if necessary. The first blood specimen was drawn anaerobically from the antecubital vein 15 minutes following the start of rebreathing, after equilibrium between the pulmonary capillary blood and the air in the rebreathing system had presumably occurred (9). In the early experiments, a second blood sample was drawn at the end of the experiment. In later experiments, samples were also taken at hourly intervals throughout the experiment. The duration of the experiments varied from 2 to 5 hours.

The blood specimens were analyzed for CO with the method described elsewhere (8), which is based on the liberation of bound CO with potassium ferricyanide, extraction by an oxygen washout technique, and measurement of the extracted gas in an infrared CO meter. The percentage of blood COHb was calculated from the measured CO content in milliliters per 100 milliliters, and the CO capacity of the blood was determined by multiplying the hemoglobin content in grams per 100 ml of the sample by 1.34 (10). The hemoglobin present in the sample was measured as cyanmethemoglobin (11). All analyses were performed in duplicate. SD of the analysis is $\pm 0.03\%$ COHb.

The increase in body CO stores, or the CO produced in any given time, is calculated as the product of the increase in percentage of blood COHb in that time and the dilution factor. This factor was determined at the end of each experiment by measuring the increase in percentage of blood COHb occurring as a result of adding 18.2 ml STPD (standard temperature, pressure, dry -0° C, 760 mm Hg) of 100% CO to the body CO stores.⁴ The equation used in calculating the rate of endogenous CO production is

$$\dot{V}_{CO} = \Delta \text{COHb}\% \times \frac{\text{CO}_D}{\Delta \text{COHb}\%_D} \quad [1]$$

\dot{V}_{CO} is endogenous CO production in milliliters per hour; ΔCOHb , the average hourly increase in blood COHb in per cent. The second term in the equation is the dilution factor, where CO_D is the known amount of CO in milliliters added to body CO stores, and $\Delta \text{COHb}\%_D$ is the

³ Beckman oxygen meter, model B, Beckman Instruments Inc., Fullerton, Calif.

⁴ CO, 99.8%, obtained from Matheson Corp., East Rutherford, N. J.

resultant increase in percentage of blood COHb. This dilution factor is approximately equal to the total body capacity.

The subjects used in these experiments were healthy male students, research fellows, or hospitalized patients who either did not smoke or who had ceased smoking 24 hours before the experiments. None of the subjects gave a history of anemia or hematological disease. Each had normal routine blood studies: leukocyte and differential counts, hemoglobin concentration, and reticulocyte count. Of the hospitalized subjects, P.T. was being treated for essential hypertension, J.R. for an uncomplicated inguinal hernia, and W.H. for an uncomplicated duodenal ulcer.

RESULTS

The percentage of blood COHb increased in all of the experiments during the rebreathing procedure. The results in three of the experiments are shown in Figure 2, where the increase in percentage of blood COHb is plotted against time. Data from the complete series of experiments is tabulated in Table I. The total CO produced, the CO produced per hour, and CO produced per gram of body hemoglobin have been calculated. The total body hemoglobin was calculated by dividing the dilution factor, which is the total body CO capacity, by 1.34 (9).

In the first five experiments listed in Table I, the increase in percentage of blood COHb was calculated from determinations on blood samples drawn at the beginning and end of the experiment. In the latter experiments, the error of the measurement of \dot{V}_{CO} was decreased by plotting multiple percentages blood COHb versus time and constructing a regression line as in Figure 2. The average \dot{V}_{CO} measured in the complete series of experiments was $0.42 \pm \text{SD } 0.07$ ml per hour.

DISCUSSION

The percentage of blood COHb increased during the rebreathing procedure in all of the experiments and the increase was highly significant (4 to 10 times the standard error of the blood analytical method). Since we are dealing with very small amounts of CO in blood samples, the specificity of the analysis is of prime importance. This was studied by Coburn, Danielson, Blakemore, and Forster (8), who measured the sensitivity of the infrared CO meter⁵ to other gases that might

⁵ Model 15A, Beckman Instruments Inc., Fullerton, Calif.

TABLE I
 CO production in normal man

| Subject | Age | Wt | Ht | Hemo- globin | Dilution factor | COHb* | Δ COHb† | \dot{V}_{CO} total‡ | Dura- tion | \dot{V}_{CO} | $\dot{V}_{CO}/THb§$ |
|------------------|------------|------------|-----------|-----------------|--------------------|----------|-------------------|--------------------------|---------------|----------------|--------------------------------------|
| | <i>yrs</i> | <i>lbs</i> | <i>cm</i> | <i>g/100 ml</i> | <i>ml</i> | <i>%</i> | <i>%</i> | <i>ml</i> | <i>hrs</i> | <i>ml/hour</i> | <i>ml/(g × 10⁻⁴ × hr)</i> |
| RFC | 29 | 145 | 66 | 16.5 | 945 | 1.06 | 0.15 | 1.42 | 4 | 0.35 | 5.0 |
| PBK | 23 | 141 | 67 | 14.7 | 889 | 1.69 | 0.16 | 1.42 | 4 | 0.35 | 5.3 |
| JH | 22 | 153 | 68 | 14.6 | 1,025 | 0.87 | 0.15 | 1.61 | 4 | 0.40 | 3.9 |
| CS | 23 | 170 | 69 | 15.3 | 1,060 | 0.61 | 0.18 | 1.98 | 5 | 0.39 | 3.7 |
| PT | 29 | 170 | 69 | 13.9 | 934 | 1.51 | 0.14 | 1.31 | 3 | 0.43 | 4.6 |
| BO | 22 | 155 | 67 | 14.7 | 1,050 | 1.31 | 0.10 | 1.10 | 3 | 0.35 | 4.6 |
| JL | 18 | 125 | 69 | 13.9 | 814 | 0.90 | 0.19 | 1.54 | 3 | 0.51 | 8.4 |
| PBK | 23 | 141 | 67 | 14.2 | 982 | 1.42 | 0.13 | 1.26 | 3 | 0.42 | 3.9 |
| JR | 70 | 160 | 70 | 15.8 | 996 | 2.12 | 0.11 | 1.14 | 2 | 0.57 | 7.7 |
| WH | 53 | 170 | 68 | 13.7 | 900 | 0.68 | 0.10 | 0.90 | 2 | 0.45 | 6.1 |
| Mean values ± SD | | | | 14.73 | 960 | 1.22 | | | | 0.42 ± 0.07 | 5.5 ± 0.9 |

* The blood CO hemoglobin saturation at the beginning of the experiment.

† The total increase in blood COHb.

‡ Total CO produced during the experiment.

§ The \dot{V}_{CO} per hour divided by total body hemoglobin in grams.

have been dissolved in blood, such as ammonia, acetone, ethyl alcohol, methane, and acetylene. This instrument was found to be 100 to 1,000 times more sensitive to CO than to equivalent amounts of any of these gases that presumably are present in the blood in only trace amounts (12), and therefore could not be a source of error in the analysis. CO₂ and water vapor do af-

fect the infrared meter, but are removed from the extracted gas before it is analyzed. Although CO can be produced as a result of oxidation of blood *in vitro*, the method employed here produces no significant amount of CO in comparison with a photochemical method (8). From these studies, we conclude that the analytical method used for CO in the present study is highly specific, and that it is very unlikely that the measured increase in blood CO was actually caused by some other interfering compound in the blood. Nor could the increase in blood CO during these experiments have been due to exogenous CO entering the body via the lungs, since the subjects were breathing in a closed system.

Early findings of studies now in progress in our laboratory suggest that the increase in blood CO could not have resulted from CO uptake through the skin, since the CO gradient across the dermis is probably less than 0.1 mm Hg, and the diffusing capacity of the skin for CO is small. Furthermore, the measured \dot{V}_{CO} in the subject studied in this investigation appeared to be unrelated to the initial percentage of blood COHb level. If transdermal CO exchange was influencing or determining the measured \dot{V}_{CO} , then the subjects with higher percentage of blood COHb should have had lower measured values of \dot{V}_{CO} .

The possibility that the increase in blood CO resulted from transfer of CO from slowly equilibrating CO stores into the blood cannot be completely excluded. This would require that an unsteady state exist where the CO tension is higher in this hypothetical slowly equilibrating space

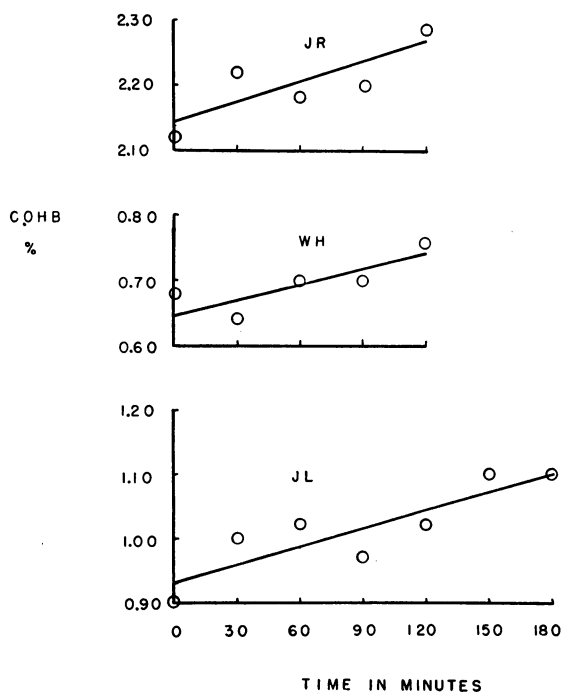


FIG. 2. INCREASE IN PERCENTAGE OF BLOOD CO HEMOGLOBIN SATURATION DURING REBREATHING IN THREE NORMAL SUBJECTS. Each point is the average of duplicate analyses.

than in the circulating blood. It is improbable that this would occur in all of the subjects. It is well recognized, however, that there is a pool for CO in the body that does not equilibrate with the circulatory blood within 15 minutes. This "slowly equilibrating CO space" could consist of CO bound to hemoglobin in an intravascular stagnant blood pool, or CO bound to cytochrome or other compounds in slowly perfused body tissues. Myoglobin is known to bind CO; however, all or most of the myoglobin CO has been thought to be in equilibrium with blood CO (13). In fact, the presence of rapidly equilibrating myoglobin CO binding sites is thought to explain the higher blood volume measurements obtained with the CO method than found with isotope (14, 15) or dye (16) methods. The size of this space has been studied by Sjöstrand (9), who administered CO to subjects rebreathing in a closed system and followed the loss of CO from the fast equilibrating space (blood) by measuring the changes in blood COHb. After the initial increase in percentage of blood COHb that reached a maximum within 15 minutes, there was a slow decrease for about 1 hour after which it appeared to level off. This drop in blood COHb was about 5% of the initial maximal value, suggesting that the slowly equilibrating space in resting man is about 5% of the total CO space. The amount of CO administered to these subjects was 50 to 100 ml. Therefore, the relatively small CO production rate could not have caused a significant error in this estimate. The CO analytical method used was not sufficiently precise and the experiment not continued long enough to detect endogenous production of CO. We have repeated the experiments of Sjöstrand and have been able to confirm his findings. Roughton and Root (13), however, using a different technique, obtained a higher estimate of the size of the slowly equilibrating CO space. These investigators administered CO to two subjects breathing in an open circuit and performed a CO balance study. Approximately 30% of the administered CO was not found present in the blood or expired air at the end of 1 hour. However, there was almost complete recovery of the administered CO following four hours of O₂ breathing. Presumably about 15% of the CO lost from the blood was bound to rapidly equilibrating extravascular compounds such as myo-

globin, leaving 15% lost to the slowly equilibrating space. On the basis of these studies, it seems likely that the slowly equilibrating space in resting man ranges in size from 5 to 15% of the total CO space. If we assume a value of 10%, then in our subjects, who had an average total CO store of about 10 ml, the slowly equilibrating space would contain approximately 1 ml CO. In most of our experiments, this is not enough to account for the increase in blood COHb even if all of it were shifted into the blood during the experiment, an extremely unlikely possibility. On the basis of Sjöstrand's experiments, it appears that approximate equilibration between blood and the slowly equilibrating CO space occurs within an hour following administration of CO. Since the blood CO continued to increase after 1 hour of rebreathing in every experiment, we conclude that the rise in blood COHb with time in our experiments could not all be a result of shifts of CO within the body.

Since the increase in blood CO could not have originated from exogenous CO and from shifts in body CO, endogenous CO production must have occurred.

Sources of error in the measurement of \dot{V}_{CO} . The blood analysis probably represents the largest source of error in the measurement of \dot{V}_{CO} . The errors in this analysis have been discussed elsewhere (8). When the percentage of blood COHb is plotted against time as in Figure 2, the standard error of estimate of the points about the regression line is 0.038% saturation, a figure similar to the standard error of the blood analysis, indicating that the scatter of points is mainly due to error in this analysis. The error in the calculated slope of the regression line from which the \dot{V}_{CO} is in turn derived can be calculated (17) from the standard error of estimate, when $N = 5$, to be 0.004% saturation per hour, which is equivalent to an error in the \dot{V}_{CO} measurement of approximately 0.04 ml per hour, or 10% of the normal hourly rate.

We have assumed, in interpreting our results, that CO is not oxidized or catabolized. If this does occur, then the measured \dot{V}_{CO} underestimates the true value and actually represents only "net" CO production. It has been amply confirmed that CO can be oxidized to CO₂ in tissues *in vitro* (18, 19), and in intact turtles and mice

(20) when high concentrations of CO are present. In man, however, evidence is lacking that this can occur in significant amounts under physiological conditions. Tobias and associates (21), using isotope techniques, demonstrated that less than 0.1% of inspired $C^{11}O$ was oxidized to $C^{11}O_2$ in 1 hour in normal subjects, and on this basis, it seems reasonable to assume that *in vivo* oxidation of CO does not significantly affect the accuracy of our measured CO production rates.

The rebreathing technique used in these experiments effectively prevents pulmonary CO exchange with the environment. As the blood COHb increases during the experiment, however, the CO tension in the pulmonary capillaries also increases, and small amounts of CO are transferred from the body into the rebreathing system. The error in the V_{CO} measurement resulting thereby was calculated from the Haldane equation (10) and the volume of the system (approximately 8 L), and is insignificant (less than 0.02 ml for an increase in blood COHb of 0.20%).

Elevation of the O_2 tension of the air in the system can also result in loss of small amounts of body CO into the rebreathing system. The greatest increase of pO_2 tension during any of the experiments was 50 mm Hg, which would result in a loss of body CO of less than 0.02 ml.

Loss of body CO via urine or sweat does not occur in significant amounts because of the low solubility coefficient of CO in water (0.018 ml per ml per atmosphere, 37° C (22)). The body CO stores are present almost entirely in "bound" forms. We have not been able to find significant amounts of CO in feces, suggesting that CO loss does not occur normally in this manner.

Error in the determination of the dilution factor could result in error in the measurement of V_{CO} . Error would result if the blood CO and tissue CO stores were not equilibrated at the beginning of the experiment. As discussed above, equilibration between the blood and slowly equilibrated tissues should occur in the first hour of rebreathing. The technique of taking periodical blood specimens throughout the period of rebreathing tends to minimize error occurring from this cause during the first hour. It is assumed that administered CO is diluted in the same "space" as endogenously produced CO. As

pointed out above, error resulting from CO transport in or out via the skin should be insignificant.

Previous investigations of V_{CO} . Sjöstrand and his associates have published a series of interesting papers in which they concluded that CO is endogenously produced in normal man. As discussed earlier, these investigators measured inspired and expired air CO concentrations and noted that the latter contained slightly more CO than inspired air (7). In another report (6), human subjects were required to breathe 100% oxygen for several hours, which resulted in a decrease of body CO stores. After this, the subjects breathed room air that had been filtered through Hopcalite (which oxidizes CO to CO_2) at room temperature. The percentage of blood COHb rose even while the subjects breathed the "filtered" air. The conclusions of these very interesting experiments can be criticized on several points. The results of the first experiment, as mentioned above, could be explained without assuming CO production if exposure to a higher inspired CO concentration had occurred even as long as several hours before the measurements were taken. The Hopcalite filter used in this experiment does not filter CO efficiently, unless kept at a temperature in excess of 100° C (23); therefore the increase in CO found could have been due to unfiltered exogenous CO. As noted above, the analyses made in these experiments were performed with a catalytic combustion type CO meter that is sensitive to other gaseous hydrocarbon compounds, so the changes observed in these experiments could have been caused by gases other than CO. Their evidence, discussed later in the paper, that CO is endogenously produced in pathological states is extremely impressive; however, this is not proof that CO is endogenously produced in normal man.

Significance of endogenous production of CO in man. Our findings confirm the conclusions of Nicloux (2), Leoper (3), Lépine and Boulud (4), Jongbloed (5), and Sjöstrand (6, 7). CO production in biological organisms appears to be common in nature. Pugh described high COHb levels occurring in the blood of seals living in the Antarctic where, presumably, very little exposure to exogenous CO occurs (24). Wilks has demonstrated that CO can be produced by plant life (25). CO originating from biological sources

and as a result of combustion may be always present in the atmosphere in variable amounts. The blood COHb in man probably originates both from endogenous and exogenous CO. The factors that determine these blood COHb levels are discussed in a subsequent publication (26).

Endogenous production of CO in man could have great importance in space travel where man confined in a small volume could asphyxiate himself in 100 to 200 days, if he continued to produce CO at a normal rate and it was not removed from the inspired gas.

There is considerable evidence reported in the literature that CO can be formed from oxidation of hemoglobin and heme *in vitro* and that it may be a catabolic by-product of hemoglobin *in vivo* in abnormal states. Sjöstrand (27) and Ludwig, Blakemore, and Drabkin (28) have demonstrated that CO can be produced from blood and heme under oxidative conditions *in vitro*. In the latter paper, CO was shown to originate from the α -methene bridge carbon atom of the heme porphyrin ring. We have demonstrated CO production occurring as a result of treating blood with ferricyanide, heat, or alkali (8). Sjöstrand (6) and Engstedt (29) have shown that the blood COHb is elevated in patients with increased rates of erythrocyte destruction. Gydell (30) has shown that the blood COHb increases after iv injection of nicotinic acid or damaged erythrocytes. With this data and the experiments reported in this paper, it seems likely that at least part of the normal CO production arises from hemoglobin catabolism in normal man. If CO is produced during hemoglobin catabolism from the α -methene carbon atom, then the expected rate calculated from the average total body hemoglobin of the subjects, assuming an erythrocyte survival time of 120 days, would be 0.30 ml per hour. This value is in the same range as the measured V_{CO} 0.42 ml per hour. Data concerning other possible *in vivo* precursors of CO is lacking. It seems likely that the measurement of V_{CO} may be of use in the study of erythrocyte destruction or hemoglobin catabolism.

SUMMARY

A method has been described for the estimation of CO production in man in which the subject breathes in a closed system, and the rate of rise

of blood CO hemoglobin is determined. In ten normal males at rest, the average measured rate was $0.42 \pm SD 0.07$ ml per hour.

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