Effects of metabolism and distribution of carbon monoxide on blood and body stores

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LUOMANMAKI, KIMMO, AND RONALD F. COBURN. Effects of metabolism and distribution of carbon monoxide on blood and body stores. Am. J. Physiol. 217(2): 354-363. 1969.—14CO was administered to 31 anesthetized dogs and 5 normal subjects who were breathing in a closed system. 14CO was oxidized to 14CO2 at average rates of 0.30 ± 0.08 and 0.18 ± 0.03%/hr of the total administered in dog and man, respectively. 14CO oxidation rates did not change significantly as a result of increasing the 14CO body stores 2-30 times normal or altering arterial oxygen tension from 30 to over 600 mm Hg. Rates of metabolic consumption of body 14CO appear to be insignificant compared to rates of 14CO production at normal levels of blood carboxyhemoglobin in man; in dog the rate of 14CO consumption was approximately 10% of the rate of production. Evidence is presented that the distribution of CO between blood and other tissues remains constant with changes in arterial oxygen tension from 40 to 650 mm Hg, arterial CO2 tension from 28 to 65 mm Hg, and carboxyhemoglobin from 0.8 to approximately 55% saturation. With more severe arterial hypoxemia up to 50% of the blood 14CO shifted into extravascular tissue.

carbon monoxide oxidation; carbon monoxide production; blood carboxyhemoglobin; carbon monoxide toxicity

In a previous publication (11) we have considered the processes that influence the blood carboxyhemoglobin percent saturation ([COHb]) and the total body CO stores ("CO" refers to the naturally occurring isotope 12CO unless otherwise stipulated. "CO metabolism" refers to metabolic consumption of CO rather than metabolic processes that result in production of this gas.) In that study we assumed a) that the body CO stores are a function of the rate of endogenous CO production (Vprod), CO concentration in inspired air and the rate of exchange via the lungs; b) that the partition of CO between blood and extravascular CO stores is a constant; and c) that extravascular CO stores are always equilibrated with the blood CO stores. The first assumption implies that CO is not metabolized, lost from the body stores except via the lung, or irreversibly bound in the body stores. We have made the same assumptions in developing our method of measuring Vprod, the rebreathing method (7), in which excretion of CO via the lung is prevented by having the subject or experimental animal breathe in a closed rebreathing system. The rate of increase in body CO stores (ΔCO) under these conditions is considered to be equal to Vprod.

We have thought these assumptions are valid for the following reasons: a) The rebreathing method precisely measures a simulated Vprod both in normal man (15) and in anesthetized dogs (16). b) Oxygen breathing by normal human subjects results in a washout of the body CO stores and a new steady state [COHb] very similar to that predicted by equations in which these same assumptions were made (11). c) 12CO and 14CO production resulting from catabolism of hemoglobin labeled with 14C occur in the expected ratio and yields are nearly 100% of that predicted (16). It is, nevertheless, possible that these assumptions are not rigorously correct.

Previous in vitro (2, 5, 19, 30, 35) as well as in vivo (4) studies have indicated that CO can be oxidized to CO2. In 1932 Fenn and Cobb (20), while looking for CO inhibition of oxygen uptake in frog skeletal muscle in a Warburg apparatus, found instead that the rate of O2 uptake increased over twofold. This increase in the rate of O2 uptake was shown to be, at least in part, a result of oxidation of CO to CO2 (19). Subsequent studies using cardiac muscle as well as skeletal muscle confirmed this finding when the muscle was exposed to high tensions of CO and at least 10% O2 (2, 30). Other studies have indicated that the reaction will take place in a phosphate buffer extract of skeletal muscle (2) and in a mixture of cytochromes a, a3, and C and an electron donor (2, 35). Clark (4) was able to show that CO is oxidized to CO2 in vivo in turtles and mice, but despite this finding the issue remains in doubt as to whether the reaction occurs in normal man or other animals. Clark’s experiments may not be equatable to the “normal” since his experimental animals required 0.07% CO which may have resulted in carboxyhemoglobin percent saturations of approximately 50-60% and his animals were probably hypoxic. In addition, Tobias (33) looked for oxidation of 11CO in normal man without success. It does not seem to be known if metabolic consumption of CO occurs at rates significant to influence body CO stores in any species.

There also do not seem to be data published in the literature regarding variables that may influence the partition of CO between blood and extravascular tissues or the processes that may influence transport between blood and these other body CO pools.

In the present investigation our approach to the study of the body CO stores has been to administer 14CO into the pulmonary capillary blood of anesthetized dogs or awake human subjects, breathing in a rebreathing system (RBS).
The RBS effectively prevents excretion of CO via the lungs, and since skin and mucosal membrane (CO) transport apparently does not occur to a significant extent (9), ΔCO could occur only as a result of endogenous CO production or metabolism or if we added CO to the body stores. Changes in body ¹⁴CO should occur only as a result of metabolic consumption of the isotope. Therefore, the use of two isotopes of CO allowed us to study the relationships of production, metabolism, and excretion as determinants of the body CO stores. It also was possible to make estimates of rates of exchange between blood CO and extravascular stores from changes in blood ¹⁴CO following administration of the isotope and to study the possibility that the partition of CO between blood and the extravascular stores might not be constant under all conditions.

METHODS

These experiments were performed on 31 dogs varying in weight from 10.6 to 17.5 kg and 5 normal human subjects. The animal experiments were performed under light pentobarbital anesthesia.

Animal experiments. The animal preparation used in these experiments has been described previously (16). A balloon cannula is placed in the trachea, inflated, and attached to a RBS which includes a CO absorber (Baralyme granules, National Cylinder Gas Co., Chicago, Ill.) and oxygen demand valve. In some experiments the RBS included a volume respirator (model 607, Harvard Apparatus Co., Dover, Mass.); in other experiments the dogs breathed spontaneously. The oxygen tension in gas in the RBS (inspired air) was determined with a paramagnetic O₂ analyzer (model D, Beckman Instrument Corp., Fullerton, Calif.). This was altered in many of our experiments by adding N₂ or removing gas and replacing it with 100% O₂ in the constant volume system. The PO₂ of inspired gas in the RBS was approximately 150 mm Hg in all of the experiments and minute ventilation adjusted to give a PAcO₂ of 35–45 mm Hg unless otherwise stated. A cannula was placed via the external jugular vein into the right atrium from which blood samples were drawn and analyzed for ¹⁴CO, [COHb], and in some experiments PO₂, Arterial PO₂, pH, and Pco₂ were determined in blood taken from the femoral or carotid artery in many of the experiments. The following animal experiments were performed.

a) In all experiments we injected ¹⁴CO into the rebreathing system and measured changes in right atrial blood ¹⁴CO for time periods of 4–11 hr. In 4 of these experiments 10–100 ml ¹³CO were injected into the RBS at the same time as the ¹⁴CO injection and changes in [COHb] compared with changes in blood ¹⁴CO. In 11 experiments erythrocytes labeled with radiochromate (27) were injected into the right atrium simultaneously with the uptake of ¹⁴CO into pulmonary capillary blood so that comparisons could be made between changes in blood ¹⁴CO and rates of mixing of the injected labeled erythrocytes. The blood volume was determined in these experiments (27), allowing calculation of the partition of the injected CO isotope between blood and extravascular CO stores from measurements of the portion of injected isotopes accounted for in circulating blood.

b) In 22 experiments where ¹⁴CO was added to the body CO stores, rates of oxidation to ¹⁴CO₂ were determined by collecting expired CO₂ in Baralyme and counting it. In 6 experiments we serially collected expired CO₂ during 1-hr measurement periods for 1.5 hr; in the remainder of the experiments we collected one or two 2-hr samples, starting 1 hr following the injection of ¹⁴CO at the beginning of each experiment. In 5 of these experiments 5–65 ml ¹³CO were also added to the body stores so that the effect on ¹⁴CO oxidation could be determined. In 4 additional experiments inspired gas PO₂ was varied in a stepwise manner over the range 40–686 mm Hg and rates of oxidation of ¹⁴CO to ¹⁴CO₂ were measured.

c) In four experiments ¹³CO was administered into the RBS at constant rates over a 3 to 4 hr period and increases in blood [COHb] measured. These experiments were performed in order to determine if the partition of CO between blood and extravascular CO stores is a function of [COHb]. In these experiments the PO₂ of blood taken from the right atrium as well as arterial PO₂ was measured serially.

d) In 3 experiments the effects of changes in arterial PO₂, Pco₂, and pH on the partition of CO between blood and extravascular stores were investigated. Under steady-state conditions the distribution of ¹⁴CO should be the same as that of ¹³CO (i.e., the CO specific activity should be approximately the same in all of the CO pools of the body).

In these experiments ¹³CO was given and after approximately 2 hr, when the isotope should have been completely mixed in the body stores, control measurements of blood ¹⁴CO were made. The animal was breathing gas containing 150 mm Hg PO₂ at this time and the respirator set so that arterial Pco₂ was 35–45 mm Hg. The inspired PO₂ was then altered over the range of 40–600 mm Hg, 30–45 min were allowed to reach a steady state, and venous blood was then taken again and analyzed for ¹⁴CO. Three to four changes in PO₂ were made in a stepwise manner in each experiment. In two similar experiments alveolar gas PO₂ was kept constant and minute ventilation varied to give arterial Pco₂ of 18–78 mm Hg.

In the experiments where the blood volume was determined, the partition of CO isotopes between the vascular compartment and extravascular tissues was calculated from the quantity of isotope injected (COinj), the blood volume (BV), and the quantity of injected isotope found in 1 ml blood ([CO]) as follows:

\[
\text{% in blood} = \frac{[\text{CO}] \times \text{BV}}{\text{COinj}} \times 100
\]  

(1)

The quantity of injected CO in extravascular tissue was computed as the portion not explained by the increase in [CO] in the vascular compartment.

The rate of change in body CO stores (ΔCO) was determined with serial measurements of blood [COHb] over at least a 2-hr period using the following equation:

\[
\Delta \text{CO} = \Delta \text{[COHb]} \times \text{CO capacity}
\]

(2)

where ΔCO is in ml/1/hr STPD, Δ[COHb] is rate of change in %/hr, and the CO capacity is a dilution factor which gives the relationships between blood [COHb] and the total body stores and is obtained by adding a known quantity of CO to the body stores and measuring the increase in blood concentration of the isotope (7).
The rate of loss of $^{14}$CO from blood could be determined from the blood measurements and was expressed as percent of total $^{14}$CO administered to each animal. The rate of oxidation of $^{14}$CO$_2$ was expressed in the same unit.

**Experiments on human subjects.** These experiments were performed on seated male subjects breathing in a hood rebreathing system which has been described previously (15). Pertinent data regarding these subjects are given in Table 1. There were no smokers among the subjects studied. In five experiments $^{14}$CO was administered via the RBS. In three of these experiments the rate of oxidation of $^{14}$CO$_2$ was determined as in the animal experiments; in three experiments serial venous blood specimens were taken from the antecubital vein over a 2 to 3 hr period and analyzed for $^{14}$CO$_2$ radioactivity. In two of these experiments 25 and 30 ml $^{14}$CO were also administered at the beginning of the experiments. Venous blood was analyzed for changes in [COHb] in all of the experiments. Two subjects (JS and AA) were studied on 2 different days. AC0 was determined the 1st day, at which time [COHb] was normal. On the 2nd day $^{14}$CO was given, resulting in [COHb] of 3.61 and 2.35 % saturation, and DCO again determined.

**Preparation of $^{14}$CO.** The $^{14}$CO used in these experiments was prepared from $^{14}$C-labeled sodium formate (New England Nuclear Corp., Boston, Mass.) by acid liberation (34). The isotope was drawn through a CO$_2$ absorber into an evacuated chamber. The method is a modification of the infrared blood CO technique developed in our laboratory (8). $^{14}$CO is extracted from a 2-ml blood sample by adding ferricyanide to lysed blood and washing out the solution with air into an evacuated ionization chamber. The equipment necessary for this technique includes a reaction chamber, burette, outflow tube containing anhydrous calcium sulfate to remove water vapor, and a 1,000-ml ionization chamber. This equipment is illustrated in Fig. 1. The burette is designed for pipetting blood into the reaction chamber and is also the inlet for room air which is used to wash out $^{14}$CO into the ionization chamber. A magnet is placed in the reaction flask for stirring the mixture. A 2-ml blood sample is pipetted into the reaction flask, followed by three drops of Triton X-100 (Rohm and Haas, Philadelphia, Pa.) and three drops of octanol. Triton X-100 is a detergent which lyases the cells; octanol is used to prevent foaming during extraction. Following complete lysis of red blood cells, 1 ml of acid ferricyanide solution is added to the reaction flask via the burette. An evacuated ionization chamber is attached to the outflow line, both stopcocks opened, and gas drawn over a period of 10 min through the burette and the solution and into the chamber. The volume of the reaction flask and outflow tube is approximately 50 ml. Residual washouts indicated complete transferred into an evacuated ionization chamber (Tolbert-Carey chamber and vibrating reed electrometer, model 31, Applied Physics Corp., Monrovia, Calif.) and the radioactivity determined. A number of procedures were performed to prove that the radioactivity found in the gas samples obtained above actually originated in CO$_2$. It was demonstrated that the gas radioactivity could be repeatedly bound to and removed from CO$_2$ absorbers. In order to exclude the possibility that the CO$_2$ absorber in the RBS was contaminated by $^{14}$CO, we passed this isotope through the absorber but detected no radioactivity in gas liberated by acid using our usual technique. The efficiency of $^{14}$CO$_2$ extraction was determined by adding known quantities of $^{14}$CO$_2$ to the CO$_2$ absorber, followed by the usual extraction procedure. The efficiency varied from 92 to 98 %. In expressing $^{14}$CO data we did not correct for efficiency of extraction. Measurements of duplicate aliquots of Baralyme revealed that the error in determining $^{14}$CO$_2$ was 4% total radioactivity. This error is thought to be secondary to variation in extraction of CO$_2$ from Baralyme and cannot be explained by error in counting in the ionization chamber.

**Determination of $^{14}$CO in blood.** A method was developed for determination of this isotope in a 2-ml blood sample. The method is a modification of the infrared blood CO technique developed in our laboratory (8). $^{14}$CO is extracted from a 2-ml blood sample by adding ferricyanide to lysed blood and washing out the solution with air into an evacuated ionization chamber. The equipment necessary for this technique includes a reaction chamber, burette, outflow tube containing anhydrous calcium sulfate to remove water vapor, and a 1,000-ml ionization chamber. This equipment is illustrated in Fig. 1. The burette is designed for pipetting blood into the reaction chamber and is also the inlet for room air which is used to wash out $^{14}$CO into the ionization chamber. A magnet is placed in the reaction flask for stirring the mixture. A 2-ml blood sample is pipetted into the reaction flask, followed by three drops of Triton X-100 (Rohm and Haas, Philadelphia, Pa.) and three drops of octanol. Triton X-100 is a detergent which lyases the cells; octanol is used to prevent foaming during extraction. Following complete lysis of red blood cells, 1 ml of acid ferricyanide solution is added to the reaction flask via the burette. An evacuated ionization chamber is attached to the outflow line, both stopcocks opened, and gas drawn over a period of 10 min through the burette and the solution and into the chamber. The volume of the reaction flask and outflow tube is approximately 50 ml. Residual washouts indicated complete

### Table 1. Data from experiments on human subjects

<table>
<thead>
<tr>
<th>Subj</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>[COHb] (% Sat)</th>
<th>Rate Loss $^{14}$CO From Venous Blood,%/hr</th>
<th>$^{14}$CO - $^{12}$CO$_2$ Rate (%/hr)</th>
<th>Rate Change Body $^{14}$CO, ml/hr, STPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>KL</td>
<td>28</td>
<td>64.0</td>
<td>0.92</td>
<td>0.16</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>LL</td>
<td>38</td>
<td>72.0</td>
<td>0.80</td>
<td>0.12</td>
<td>0.42</td>
<td>0.42</td>
</tr>
<tr>
<td>AA</td>
<td>28</td>
<td>58.2</td>
<td>0.80</td>
<td>2.35</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>JS</td>
<td>19</td>
<td>68.1</td>
<td>0.91</td>
<td>3.61</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>RFC</td>
<td>34</td>
<td>69.8</td>
<td>0.88</td>
<td>0.23</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
<td></td>
<td>0.26±0.03</td>
<td>0.16±0.03</td>
<td>0.30±0.30</td>
</tr>
</tbody>
</table>
in the dog experiments averaged 12.5 ± (sd) 3.7 min in 11 animal experiments. In 5 of these experiments 51Cr-labeled erythrocytes were injected simultaneously with the administration of 14CO. 51Cr radioactivity in these experiments decreased exponentially in central venous blood with an average half-time of 14.5 ± (sd) 3.5 min, a value not significantly different from that obtained for the "early" decrease in 14CO radioactivity (P > 0.1).

Figure 3 shows blood 14CO measurements starting 120 min after injection of the isotope into the RBS in nine animal experiments. In two experiments we took two blood specimens, one 2 hr after 14CO administration and the other after an additional 2 hr and analyzed each of them 8 times so that the precision of the mean value obtained was ± 0.5 %. Data from these experiments are shown in open circles, the first sample being expressed as 100 %. The remaining data from seven experiments were analyzed in duplicate. These data are shown as closed circles. 14CO radioactivity decreased at rates of 0.40 and 0.65 %/hr in the two experiments measured over a time period 2-4 hr after isotope administration. A least-squares regression line drawn through the remainder of the data gave a slope of 0.66 %/hr. This value is significantly different from a slope of zero (P < 0.01) and from 0.3 %/hr (P < 0.05). This loss of 14CO from central venous blood appeared to be constant since a) the decrease during the time period 2-6 hr was not significantly different from that during 6 10 hr following injection, and b) rates of decrease in two experiments depicted with open circles in Fig. 3 and measured during hours 2-4 were not significantly different from the slope obtained with all data obtained from 2 as long as 10 hr following 14CO injection.

Oxidation of 14CO to 14CO2. Data from the animal experiments where we measured the conversion of 14CO to 14CO2 are shown in Fig. 4. Radioactivity was found in expired CO2 in every experiment. Excreted 14CO2 varied only ±10 % in the hourly collections after the 1st hour which had consistently smaller radioactivity. Rates of conversion averaged 0.30 ± (sd) 0.18 %/hr varying from 0.11 to 0.66 %/hr. Adding 14CO to the body stores and thereby changing [COHb] did not significantly influence the rate of oxidation of 14CO (P > 0.05). Varying inspired Po2 over the range 700-40 mm Hg did not significantly in-

FIG. 2. 14CO and 14CO2 measurements in right atrial blood following administration of 14CO into pulmonary capillary blood of an anesthetized dog. The animal was breathing in a closed system containing a CO2 absorber and O2 demand valve. 14CO is expressed as counts/min per g hemoglobin and 14CO2 as carboxyhemoglobin % saturation.

FIG. 3. 14CO radioactivity in right atrial blood starting 2 hr after isotope was administered into rebreathing system of anesthetized dogs. Open circles represent mean values of 8 measurements on same blood sample from two experiments where measurements were made over a 2-hr period. Closed circles are pooled data taken from 7 other experiments where blood samples were analyzed in duplicate. Solid line is a least-squares regression line drawn through pooled data. Average rate at which 14CO was oxidized to 14CO2 in anesthetized dogs (0.30%/hr) is also shown here. Note expanded scale.

FIG. 4. Rates of oxidation 14CO to 14CO2 in experiments where the quantity of body CO stores was varied by adding 14CO. Measurement of the rate of excretion of 14CO2 was performed during a 1- to 2-hr period approximately 2 hr following administration of 14CO and in some cases 15CO. Least-squares regression line (solid line) has a slope of 1.4 X 10^-3 %/hr per COHb % sat, a value which is not significantly different from zero (P > 0.3). O2 tension in inspired gas was normal in these studies. These data suggest that rates of oxidation of 14CO to 14CO2 are proportional to [COHb] and body CO stores. If 14CO oxidation was independent of [COHb] expected rates of 14CO oxidation are shown by interrupted line.

RESULTS

14CO injection into anesthetized dogs. A typical plot of blood 14CO measurements following administration of the isotope into the rebreathing system is shown in Fig. 2. 14CO measurements are plotted in terms of radioactivity per gram hemoglobin, thereby correcting for small variation of hemoglobin concentration in different blood samples. 14CO was found in right atrial blood in all of the experiments at the time of the first sample, 5 min after injection into the RBS. In the animal experiments blood radioactivity decreased in an apparently exponential manner for 20-60 min. Following this 14CO continued to decrease slowly at an apparently constant rate for the duration of the study.

The average half-time of the initial exponential decrease

extraction. CO2 was not absorbed since 14CO in the blood sample could not have exceeded 0.1 % of blood 14CO radioactively. The error of this method determined from 30 paired samples was ± (sd) 2.1 %.

Additional analytical techniques. Blood [COHb] was determined using the infrared method (8) which has an error of ±(sd) 0.03 % saturation. Arterial bloods were analyzed with Po2, Pco2, and pH electrodes (26, 31). Gas concentrations were determined with a gas chromatograph or paramagnetic oxygen analyzer. Blood 51Cr radioactivity was determined in a well scintillation counter.
fluence the rate of oxidation of the isotope to $^{14}$CO$_2$ (four experiments).

Partition of CO between vascular and extravascular tissues. Calculations of the partition of CO between blood and extravascular tissue in animal experiments where $^{129}$Xe-labeled erythrocytes were injected and blood volume determined indicated that the extravascular CO stores averaged 77.1 ± (sd) 7.4 % of total body CO stores. There was not a significant difference between partition of $^{129}$CO and $^{14}$CO ($P > 0.5$) nor did the partition vary with the quantity of CO administered to the animal over the range 10–100 ml giving blood [COHb] 2–35 % saturation.

The most sensitive approach to investigation of variables that influence the partition of CO between the vascular and extravascular CO stores was the measurements of blood $^{14}$CO radioactivity per gram hemoglobin at least 1 hr after it was administered to the animal and apparently completely mixed in the body stores as described above. It was observed in three of the experiments that blood $^{14}$CO radioactivity did not change within 30 min after arterial oxygen tension was altered in a stepwise manner over the range 50–500 mm Hg. In three additional experiments where inspired gas $P_{O_2}$ was decreased to levels giving arterial $P_{O_2}$ less than 40 mm Hg, $^{14}$CO radioactivity in blood did decrease markedly giving values as small as 50 % of control. Data obtained in a typical experiment arc plotted in Fig. 5. In this experiment $^{14}$CO is seen to reenter the circulating blood following an increase in arterial $P_{O_2}$ back to near normal. The possibility that $^{14}$CO was being sequestered in splenic blood during severe hypoxemia was excluded by making measurements of splenic blood $^{14}$CO (three experiments); there was not a significant difference between splenic and central venous $^{14}$CO radioactivity expressed per gram hemoglobin, either before or after the shift in blood CO.

In three experiments where minute ventilation was varied (giving arterial $P_{O_2}$ of 18–79 mm Hg) at constant alveolar oxygen tensions there was no change in central venous blood $^{14}$CO per gram hemoglobin within 30–40 min, suggesting that changes in these variables do not influence the partition of CO between blood and extravascular pools.

Another approach to the study of partition of CO was to administer CO at a constant rate to the animal and measure rates of increase in blood [COHb]. If the partition of CO between vascular and extravascular stores remains constant, the increase in blood [COHb] should be proportional to the quantity of CO administered. Figure 6 shows data obtained in one of these experiments. [COHb] increased at a constant rate during constant CO administration resulting in [COHb] from normal to approximately 50 % saturation; however, as further CO was given the slope of the increase in [COHb] decreased, suggesting that proportionately

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**FIG. 5.** Effect of acute changes of arterial oxygen tension on partition of $^{14}$CO between blood and extravascular stores. Oxygen tension in gas in rebreathing system was altered in a stepwise manner giving arterial $P_{O_2}$ of 230, 60, 35, and 84 mm Hg, and the effect of this change on blood $^{14}$CO radioactivity was determined. There was no change in blood radioactivity when arterial $P_{O_2}$ decreased from 230 to 60, but a shift of isotope out of circulating blood occurred when arterial blood $P_{O_2}$ was decreased to 35 mm Hg and a shift back into circulating blood was observed when arterial $P_{O_2}$ was increased to a normal value.

**FIG. 6.** Constant administration of $^{14}$CO into rebreathing system of an anesthetized dog. $^{14}$CO was given in this experiment at a rate of 80 ml/hr STPD. Increase in blood $[^{14}COHb]$ was proportional to quantity of CO given over range 2.0 to 35–55 % saturation; rate of increase in [COHb] decreased at [COHb] greater than 55 % saturation. Interrupted line indicates increase in [COHb] that would occur if change in [COHb] remained proportional to quantity of gas given. Upper graph shows decrease in mixed venous $P_{O_2}$ that occurred with progressive increase in [COHb].

**FIG. 7.** Rate change of body CO stores in dogs breathing in a closed system. This measurement was made over a 9 hr period and computed from rate of change in venous blood [COHb] and CO capacity of the animal. In six experiments body CO stores were increased by adding $^{14}$CO to rebreathing system at least 2 hr before measurements of rate of changes in [COHb] were performed. Solid line is a least-squares regression line. These data suggest that $V_{pred}$ is greater than $V_{max}$ at [COHb] less than 10 % saturation and $V_{max}$ > $V_{pred}$ at higher [COHb].
larger quantities of the administered CO were entering extravascular CO stores. This figure also shows measurements of PO₂ in right atrial blood and illustrates a decrease in mixed venous blood PO₂ with increasing blood [COHb]. Arterial blood PO₂ was measured in two experiments and decreased a maximum of 10 mm Hg with increasing [COHb] at constant alveolar PO₂.

Changes in body CO stores. Serial measurements of [COHb] were made in every experiment allowing calculation of ΔCO in all of the experiments where the partition of CO between the vascular and extravascular compartments remained constant during the experiment and equation 2 remained valid. In 21 experiments where no ¹²CO was added to the body stores blood [COHb] averaged 1.03 ± (SD) 0.33 % saturation; the rate of change in [COHb] averaged +0.06%/hr which corresponded to a ΔCO of +0.21 ± 0.09 ml/hr STPD. ΔCO appeared to be constant within the error of our measurements for as long as 8 hr following anesthetization of the animal. An example of changes of [COHb] is shown in Fig. 2.

Data obtained in experiments where ¹³CO was given at the beginning of each study are plotted in Fig. 7. ¹³CO was given 2 hr before measurements of ΔCO were started so that the administered ¹³CO presumably was completely mixed and changes in ΔCO were due to relative rates of VO₂ and metabolism (V₀₂measured). At [COHb] greater than 10 % saturation the body stores appeared to slowly decrease rather than increase, as occurred in the experiments at normal [COHb].

In two experiments performed at normal [COHb] ΔCO did not change significantly when inspired PO₂ was raised to 600-700 mm Hg for an additional 2 hr.

Measurements in normal man. Data from experiments performed on human subjects are listed in Table 1. It was found that CO is oxidized to CO₂ in man as well as dogs; in three experiments this process occurred at 0.19, 0.16, and 0.20%/hr (at normal inspired oxygen tensions).

Blood ¹⁴CO curves following administration of the isotope into the RBS of normal human subjects were different from those found in dogs in that the initial exponential loss of ¹⁴CO from blood was not seen (the first sample was taken 15 min after administration). There was an apparent constant decrease in ¹⁴CO radioactivity in venous blood over a 2-hr period of 0.23, 0.25, and 0.30%/hr in the three studies where this parameter was measured.

Two experiments were performed in which the effect of increasing [COHb] and the body CO stores on ΔCO were studied. ΔCO in the control studies, where [COHb] were 0.81 and 0.91 % saturation, were 0.42 and 0.33 ml/hr. Repeat measurements after ¹³CO was given, resulting in [COHb] of 3.24 and 3.71 % saturation, gave values of 0.40 and 0.56 ml/hr which were not statistically different from the control data (P > 0.5). Figure 8 shows measurements of ΔCO in these two experiments. Measurements of ΔCO (and the initial [COHb] of each study performed previously (7)) are also plotted in Fig. 8. As with the new experiments there is not a significant correlation between [COHb] and ΔCO (P > 0.5).

Discussion

It has been suggested (1, 11, 21, 25) that the principal parameters that influence the body CO stores are production, uptake via the lungs, dilution in the body stores, and excretion via the lung. Of these, CO production has probably been studied in most detail (7, 10-12, 14-16, 37). The average normal rate of CO production in man measured with the rebreathing method as equal to ΔCO is 0.42 ml/hr (7). Approximately 70 % of this originates as a catabolic byproduct of hemoglobin in senescent erythrocytes (7, 11), the remainder apparently originates primarily as a result of degradation of hepatic heme in ineffective erythropoiesis (10, 37). CO is also added to the body stores as a result of uptake from the environment depending on Pco in alveolar gas and pulmonary capillary blood (1, 11, 21, 25). CO that is added to the body stores is diluted principally in the blood, bound to hemoglobin, but it has been generally recognized (3, 6, 24, 28, 29, 36) that CO is also bound in extravascular tissue; however, the exact distribution of this CO has not been clearly defined. It is likely that most extravascular CO is bound to myoglobin (36), but CO must also be bound to cytochrome oxidase and other iron-containing heme compounds. Due to the very small solubility coefficient of CO in tissue water (11) only insignificant quantities are to be found physically dissolved in the body. Estimates based on simultaneous determinations of blood volume and CO dilution in normal men have given values for the extravascular CO stores of 12 % (28) and 16 % (24) of the total stores. The larger extravascular CO pool of 30 % of the total stores found in the present experiments with anesthetized dogs may be explained by the larger muscle mass of dogs (18) compared to man. The relationship of [COHb] in blood to the total CO stores depends on the quantity of blood hemoglobin as well as extravascular CO binding compounds and variables that influence binding. CO excretion occurs when the Pco in mean pulmonary capillary blood is greater than in alveolar gas; this in turn depends on the level of [COHb] and PO₂ in mean capillary blood according to the Haldane equation (17). Excretion is also dependent on the CO-diffusing capacity and alveolar ventilation (11).

These variables and their relationships to each other in a steady state are illustrated in equation 3 which is taken from a previous publication (11).
[COHb] = \frac{P_{CO} M [O_2 Hb]}{P_{CO_2}} + \frac{V_{prog} M [O_2 Hb]}{P_{CO_2}} \left[ \frac{1}{D_L} + \frac{P_D - P_{H_2}O}{V_A} \right] \quad (3)

$P_{CO}$ is the partial pressure of CO in inspired gas, $M$ is the equilibrium constant of the reaction of CO with oxyhemoglobin, $P_{CO_2}$ is mean pulmonary capillary oxygen tension, $D_L$ is the CO diffusing capacity of the lung, $P_D$ is barometric pressure, and $V_A$ is rate of alveolar ventilation.

As indicated above, we were interested in the present study in determining how metabolism of CO might influence the CO body stores, whether the distribution of the stores could be altered, and if mixing in body CO pools was sufficiently rapid so that extravascular CO could be considered to be in equilibrium with blood CO.

**Metabolism of CO.** The finding of radioactivity in expired CO$_2$ following administration of $^{14}$CO indicates that CO is metabolized in dogs and in man. These data appear to represent the first evidence that CO metabolism occurs in these species. Tobias (33) has previously introduced $^{14}$CO into several human subjects but failed to find $^{14}$CO in expired gas presumably due to the fact that his technique could barely have detected an oxidation rate of 0.1–0.2%./hr.

The $^{14}$CO measurements in right atrial blood of anesthetized dogs following administration of the isotope into pulmonary capillary blood also give evidence of metabolic consumption of $^{14}$CO. These curves would be expected to initially decrease as a result of mixing in the vascular and extravascular CO compartments and then, if CO is not consumed in the body, remain constant (in our experiments where excretion from the body was prevented). The blood $^{14}$CO curves in anesthetized dog did initially decrease exponentially at a rate that was shown to parallel rates of mixing of tagged erythrocytes in blood, but instead of remaining constant continued to decrease at what appeared to be a constant rate for as long as 4–10 hr after administration. In the experiments performed on normal man no initial exponential curve was observed in the $^{14}$CO measurements, but an apparently constant decrease in blood $^{14}$CO did occur as in the latter part of the dog $^{14}$CO curves. It is most likely that these decreases in blood $^{14}$CO resulted from metabolic consumption of the isotope; however, the average rates of decrease, in both dog and in man, were approximately 2 times greater than could be explained by measured rates of oxidation of the isotope to CO$_2$ so it appears that an additional process influenced these curves. This discrepancy appears not to be an artifact resulting from error in measurement of CO$_2$ production. Other investigators (23) have infused H$^{14}$CO$_4$ intravenously at a constant rate into experimental animals and noted that rates of expiration of CO$_2$ were over 95% of that administered. We observed that rates of expiration of CO$_2$ were constant during our experiments and our methods of recovery of CO$_2$ from Baralyme gave yields of approximately 90%. There are alternate explanations for the unexplained loss of CO from the blood as follows: a) possible prolonged mixing of the isotope in a very slowly equilibrating CO pool or pools, b) slow rates of binding of CO in tissue, or c) metabolic consumption of CO in addition to oxidation to CO$_2$.

In evaluating these possibilities, we shall first consider the curves obtained in the human experiments. The early portions of these curves are similar to data previously published (using $^{14}$CO) (3) which were interpreted as indicating that mixing in the body stores occurs completely within 20 min following administration of the isotope into pulmonary capillary blood. Further evidence that mixing is complete within this time period was obtained in our experiments where it was observed that changes in blood [COHb] were not significantly different during a 2-hr time period beginning 20 min following administration of $^{14}$CO than in experiments where no $^{12}$CO was given. If any of the administered $^{14}$CO had not completely mixed in all of the body stores within 20 min there would have been a loss of this CO from blood during the subsequent 2-hr measurement period which would have opposed the normal increase in blood [COHb] resulting from production. For example, if 1% of the injected $^{14}$CO diffused out of blood during the measurement period this would have caused a 25% decrease in the rate of increase in [COHb].

The finding that giving $^{14}$CO did not influence these measurements suggests that the administered CO was in equilibrium with the extravascular CO stores within 20 min postinjection. Therefore, the prolonged decrease in blood $^{14}$CO cannot be explained by mixing.

As indicated above, the initial exponential decrease in $^{14}$CO in the animal experiments, which was not seen in the measurements on man, was explained by delay in mixing in the blood compartment. This may have resulted from slow mixing in dilated spleens of dogs anesthetized with pentobarbital. The later apparently constant loss of CO from venous blood probably resulted from metabolic consumption analogous to the finding in the human experiments. It was not possible to obtain direct evidence in the animal experiments in support of this concept or to positively exclude very slow mixing of $^{14}$CO in extravascular tissue as the cause of the prolonged decrease in blood $^{14}$CO. The finding that the rate of loss appeared to be constant rather than exponential, as expected for a first-order reaction, is probably due to the very small rate of metabolism of the isotope and that we were not able to differentiate a constant from an exponential process. Loss of isotope from blood due to mixing in extravascular stores would also give an exponential blood $^{14}$CO curve, and if mixing were influencing our data it is suggested that equilibrium between blood and extravascular stores is also a very slow process, a very unlikely situation.

We conclude that the decrease in blood $^{14}$CO starting 20 min after injection of the isotope in man and 2 hr postinjection in dog is probably not influenced significantly by mixing but reflects the total rates of metabolism of the isotope, and that there is probably another metabolic process that is consuming CO in addition to oxidation to CO$_2$. We cannot exclude the possibility that this process may be very slow reversible or irreversible CO binding in tissue. Loss of CO via the skin and hydration to formic acid apparently do not significantly affect the body CO stores (9) and could not explain the differences in rates of loss from blood and oxidation to CO$_2$. 
Whether or not metabolism of CO is a significant determinant of the body CO stores depends on the ratio of $V_{\text{met}}$ to $V_{\text{prod}}$. We shall first consider the rates of these processes in dog and in man. In dogs the average total rate of metabolism of $^{14}$CO, as determined from the loss from blood, averaged 0.66 %/hr. Since it has been shown that rates of oxidation to $^{12}$CO did not change significantly at different $^{13}$CO stores, it is suggested that the rates of oxidation of $^{13}$CO are proportional to the body $^{13}$CO stores and can be calculated by multiplying percent $^{13}$CO oxidation per hour by the total body stores. We assume that this is also true in considering other CO metabolic processes that may have contributed to the loss of $^{14}$CO from blood in our experiments. The average CO stores in dogs used in our experiments not given $^{12}$CO were approximately 3 ml, and 0.66 % of 3 ml gives a normal $V_{\text{met}}$ of 0.020 ml/hr. We can compute $V_{\text{prod}}$ from $\Delta CO$ (measured with a RBS) and $V_{\text{met}}$, where $V_{\text{prod}} = \Delta CO + V_{\text{met}}$. The average $\Delta CO$ in our anesthetized dogs was 0.21 ml/hr. Average $V_{\text{prod}}$ is found to be 0.23 ml/hr. $V_{\text{met}}$ is therefore calculated to be approximately 8 % of $V_{\text{prod}}$ in this species. Metabolism therefore does significantly influence $\Delta CO$ measured in a RBS in this species, so that $\Delta CO$ will underestimate $V_{\text{prod}}$ by about 9 %.

The finding shown in Fig. 7 that $\Delta CO$ decreases with increasing body CO stores is consistent with the above estimation of $V_{\text{met}}$ and the concept that $V_{\text{met}}$ is proportional to the body stores. For example, it was observed that $\Delta CO$ at $[COHb]$ of 10 % was zero, suggesting that $V_{\text{met}}$ equals $V_{\text{met}}$ at this $[COHb]$. $V_{\text{met}}$ of $^{13}$CO should be equal to 0.66 %/hr of body CO stores, which are approximately 30 ml at this $[COHb]$, giving a value of 0.20 ml/hr, which is very close to our estimate of $V_{\text{prod}}$ in these animals of 0.23 ml/hr.

In normal man where $V_{\text{met}}$ was much smaller than in dogs, similar calculations give $V_{\text{met}}$ equal to 0.015 ml/hr. Average $\Delta CO$ in normal man in a RBS has been found to be 0.42 ± (sd) 0.07 ml/hr (7) and average $V_{\text{met}}$ is computed to be 0.435 ml/hr. Therefore, $V_{\text{met}}$ can be considered to be insignificant in normal man amounting to only 3 % of $V_{\text{prod}}$. Data obtained in the two experiments where $\Delta CO$ was measured before and after changing $[COHb]$ and the body CO stores also give evidence of the small effect of metabolism on body CO stores in man. Body stores increased 2 and 3 times and $V_{\text{met}}$ would be expected to increase; but there was no measurable effect on $\Delta CO$. These data suggest that for the case of a normal man at normal $[COHb]$, $\Delta CO$ determined in a RBS is a close approximation to $V_{\text{prod}}$. This also would be true in a subject with an elevated $V_{\text{prod}}$ since, in such a subject, the body stores are increased approximately proportionately to $V_{\text{prod}}$. and $V_{\text{met}}$ would still be only 3 % of $V_{\text{prod}}$. However with an elevated $[COHb]$ and a normal $V_{\text{prod}}$, the ratio $V_{\text{met}}/V_{\text{prod}}$ would increase; for example, at $[COHb]$ of 10 % $V_{\text{met}}$ should be 30 % of $V_{\text{prod}}$ if data obtained in the animal experiments showing that CO metabolism is a first-order reaction are applicable to man and if $V_{\text{prod}}$ does not change as a function of the body CO stores. Metabolism would influence the $[COHb]$ of a subject not breathing in a RBS, but in a steady state, about the same extent as in a RBS since the $V_{\text{prod}}$ in equation 3 should actually be net production or ($V_{\text{prod}} - V_{\text{met}}$); metabolism would have a larger effect at higher $[COHb]$ just as in the case where the subject breathed in a RBS. If we consider a case where the CO stores are not in a steady state in regard to net production or CO uptake via the lung, and excretion, such as would follow an acute exposure to a high concentration of CO in inspired air, it can be shown that CO excretion is at least 20 times faster than is metabolism in removing CO from the body stores (11).

We conclude that metabolism has only a small effect on the body stores in normal man. Significant error in the rebreathing method of determining $V_{\text{prod}}$ should not occur as a result of CO metabolism at normal blood $[COHb]$. At high levels of $[COHb]$, metabolism may become more significant as a determinant of the body CO stores.

Our studies do not give information about the exact chemical processes involved in CO oxidation. There are data previously published in the literature which suggest that CO is oxidized in muscle (2, 19, 20, 35) and, if this is correct, the larger rate found here in dogs may be explained by the larger muscle mass per kilogram in this species compared to man. Our studies suggest that the reaction may be first order, in addition it is suggested that oxidation may be augmented during hypoxia since the measured rate during hypoxia did not change even though tissue levels of $^{14}$CO must have decreased according to the Haldane equation (17).

Distribution of body CO stores. We did not study distribution of CO in different tissues but could only measure the partition of the CO stores between circulating blood and the remaining body CO "pools." It would, of course, be of considerable interest to obtain more precise data on distribution of the extravascular stores, although it is likely that almost all of the body CO stores not found in blood are to be found in muscle as carboxymyoglobin (36). The distribution of CO within blood should be uniform in a steady state where fluxes are not occurring between blood and tissue except in the liver and spleen and perhaps other organs where CO is being produced. We determined in this study that $[COHb]$ in the spleen is the same as in venous blood in a steady-state situation. With the subject not breathing in a RBS and constantly excreting produced CO arterial blood $[COHb]$ should be slightly less than pulmonary arterial blood. As indicated above the present data suggest that mixing of CO in all body stores is sufficiently rapid in man so that extravascular body CO stores can be assumed to be equilibrated with blood.

The physiological variables that determine the partition of CO between blood and extravascular pools should include relative affinities of hemoglobin and tissue CO-binding compounds; also, all of the factors that influence this affinity such as the $P_O_2$ and pH in capillary blood, $P_O_2$ at the site of the tissue-binding compound, and of course, relative quantities of hemoglobin and extracellular compounds. In view of the complexity of processes that determine the distribution of CO, the finding in the present study that the partition between vascular and extravascular CO did not change, even though arterial $P_O_2$ was varied from 600 to 40 mm Hg and $P_CO_2$ from 28 to 68 mm Hg requires discussion. The conclusion that the partition remained constant in these experiments is based on the find
ing that venous blood $^{14}$CO radioactivity, expressed per gram hemoglobin, did not change with changes in the above variables. $P_{O_2}$ or $P_{CO_2}$ were altered at a time in the experiments when $^{14}$CO should have completely mixed in the body CO pools. Blood $^{14}$CO was decreasing slowly in these experiments, presumably due to metabolism; however, each "run" in these experiments was performed in 30–15 min and changes due to "metabolism" should not have influenced our results in this short time period. $^{14}$CO was measured in these experiments rather than $^{14}$CO since it cannot be influenced by $V_{\text{prod}}$. Since the total quantity of blood hemoglobin remains constant, a change in total $^{14}$CO in blood would cause a change in radioactivity expressed as counts per minute per gram of hemoglobin. The lack of a change in $^{14}$CO radioactivity as $P_{O_2}$ was varied from 40 to 600 mm Hg strongly suggests that no change in the overall distribution of CO between the vascular compartment and extravascular CO stores occurred. There also was no change with severe respiratory acidosis and alkalosis. From the practical point of view this indicates that we do not have to consider these variables over the range studied in considering the processes that influence relationships of blood [COHb] to the total body CO stores.

The experiments where CO was constantly administered to the RBS of anesthetized dogs give evidence that the partition of this CO between blood and extravascular CO stores remained constant over the range of [COHb] 1 to approximately 55 % saturation, indicating that the relationships of [COHb] to the total body CO stores also remains constant over this range of blood [COHb].

We were able to demonstrate a decrease in $^{14}$CO radioactivity in right atrial blood in experiments where severe arterial hypoxemia with $P_{O_2}$ less than 40 mm Hg was produced. The possibility that this occurred as a result of shifting of CO into splenic blood or expulsion of blood containing less $^{14}$CO counts per minute per gram out of the spleen into the circulation was excluded. A similar finding was seen in the experiments where CO was constantly administered into the stores where blood [COHb] exceeded 50–55 % saturation and a greater portion of the administered CO went into stores other than the circulating blood. This shift out of circulating blood occurred in these experiments at a time when right atrial blood $P_{O_2}$ had decreased below 20 mm Hg and presumably resulted from a low oxygen tension in muscle and perhaps other organs. These findings have several implications regarding the relationships of capillary $P_{O_2}$ and intracellular $P_{O_2}$ in muscle (since the partition of CO between blood and muscle is a function of these variables) which have been discussed in a separate manuscript (13).

A further implication is that the shift of CO out of blood at very high blood [COHb], or during hypoxia, might enhance survival during CO poisoning. If the "shift" occurred only from blood into muscle this would effectively lower blood [COHb] as much as 50 % and improve oxygenation to brain and other tissues. If CO was also concentrated in heart and brain giving increased binding to cytochrome oxidase, this would be detrimental to the metabolism of these tissues. A final implication of the CO shift with hypoxia relates to the use of CO to measure blood volume (3, 24). It is possible that this method may greatly overestimate the blood volume under conditions of hypoxia or shock.

CO body stores and oxygen. Finally a mention should be made about the relationships of oxygen and the body CO stores since carbon monoxide physiology is so intimately related to oxygen in several respects. Of course, carbon monoxide competes with $O_2$ for binding sites on hemoglobin, myoglobin, and other heme compounds. Also the rate of excretion via the lung is related to the mean pulmonary capillary $P_{O_2}$ (11). The diffusion coefficients of CO and $O_2$ in tissue should be similar, so mixing rates of $O_2$ and CO in the body should be similar. We have mentioned the possibility that metabolism of CO to $CO_2$ may be influenced by the $O_2$ tension at the site of the chemical reaction. Furthermore it is possible that the rate of CO production may be influenced by oxygen tensions since hyperbaric oxygenation has been associated with an increase rate of erythrocyte destruction (22). Exposure to slightly less than 1 atm oxygen tension in the anesthetized animals in the present study, however, was not associated with an increase in $V_{\text{prod}}$.

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