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Note

Measurement of mass and radioactivity of ^{14}CO using a reduction detector

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Measurement of low concentrations of carbon monoxide have been used to assess several different biological functions including hemolysis[1] and membrane permeability[2] and blood flow[3,4]. A variety of techniques of varying simplicity and sensitivity have been used in these studies to measure CO mass or ^{14}CO radioactivity. Low concentrations of CO, such as are present in biological samples, have generally been assayed by gas–liquid chromatography (GLC) techniques in which CO is catalytically converted to CH_4 which is then measured by a flame ionization detector. Analysis of ^{14}CO radioactivity has involved the passage of gas through an ionization detector or proportional counter or the combustion of ^{14}CO to $^{14}CO_2$ which is then assayed using the above detectors or by trapping the $^{14}CO_2$ in an appropriate solution which is then counted in a liquid scintillation counter. While some of the above detection systems could be linked in series or parallel, measurements of radioactivity and mass of ^{14}CO generally have been determined by independent techniques.

Seiler and Junge[5] demonstrated that low concentrations of CO can be assayed utilizing the chemical reaction between CO and HgO at 210°C in which CO is converted to CO_2. Reduction detectors have been developed which quantitate CO by measuring the resultant Hg vapor by optical means. In this paper we present a simple method utilizing a commerciably available reduction detector to measure both mass and radioactivity of ^{14}CO.

METHODS

Reagents and chemicals

CO was obtained from Matheson (East Rutherford, NJ, U.S.A.) and sodium [^{14}C]formate (specific activity: 55 mCi/mmol) from International Chemical & Nuclear Corporation (Irvine, CA, U.S.A.).

Instrumentation

A reaction chamber (to facilitate release of CO from hemoglobin), a column, a Model RG-D2 reduction gas detector (Trace Analytical, Menlo Park, CA, U.S.A.), and an effluent carbon dioxide collection tube were connected in series. The reduction detector utilizes a heated bed of solid mercuric oxide. When a reducing gas (X) passes over this bed, the following reaction takes place: $X + HgO \rightarrow XO + Hg(vapor)$.

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The Hg vapor is then measured photometrically. A reaction chamber similar to that of Collison *et al.*[6] was employed to facilitate the quantitative transfer of hemoglobin-bound CO to the column. This chamber consisted of a stainless-steel body with an inlet and outlet for carrier gas, a screw-in porthole for a rubber septum and a tapered joint to which a standard tapered flat bottomed tube (6 ml volume) was attached. The sealing of the reaction tube with the main body was accomplished with PTFE sleeves. Two reaction chambers were used in parallel and connected by four separate 4-way valves such that as the gas content of one chamber was being transferred to the gas chromatographic (GC) column, the other chamber was being flushed. The column was separated from the reaction chamber by two traps, a short glass column (15 × 8 cm O.D.) and a longer glass column (40 × 8 cm O.D.). The shorter trap contained silica gel to remove the moisture arisding from the reaction chamber. The longer trap contained anhydrous calcium chloride, soda lime, molecular sieve 13X and Ascarite in that order, each separated by wads of glass wool. Calcium chloride, soda lime and Ascarite were used to remove carbon dioxide and residual moisture whereas molecular sieve 13X removed trace amounts of hydrogen sulphide and sulphur dioxide which interfere with the CO assay. It was necessary to separate the silica gel column fraction from the other absorbents in that the moisture trapped on the silica gel eventually migrated (overnight) onto the calcium chloride absorbent. Excess moisture fused the calcium chloride absorbent resulting in altered elution pattern. Hence, fresh silica gel was placed into the shorter trap at the end of each day. A 3 ft. × 1/8 in. stainless-steel column containing Carbosieve S, 100–120 mesh (Supelco, Bellefonte, PA, U.S.A.) was operated at room temperature. Helium carrier gas (40 ml/min) was prepurified by passage through a silica gel–molecular sieve 5A trap and a catalytic combustion filter.

The effluent from the column passed through the reduction detector and the detector effluent then passed through a tube designed to trap carbon dioxide. This collection apparatus consisted of a modified vacuum trap with a central inlet tube comprised of a capillary glass tubing (0.8 mm I.D.) which conducted gas into the detachable standard tapered glass collection tube (6 in. long). A spiral glass rod encircling the inlet tubing was used to facilitate better contact of the effluent gas with the absorbent in the collection tube. The collection tube was separated from the detector outlet by a silver nitrate trap to remove traces of Hg vapor escaping from the detector system. A small vacuum pump (All Tech Assoc., Deerfield, IL, U.S.A.) was attached to the outlet of the collection tube to draw the effluent gas through the carbon dioxide absorbent. The carbon dioxide trapping solution consisted of 7 ml of ethanolamine–ethanol (2:5, v/v) with the ethanolamine containing 0.025 *M* sodium hydroxide. After effluent collection, the absorbent solution was siphoned into a counting vial and the collection tube was rinsed with 12 ml of liquid scintillation fluid which was also siphoned into the counting vial. The scintillation fluid consisted of 0.5 g/l of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and 5.0 g/l of 2,5-diphenyloxazole (PPO) in toluene–ethanol–dioxane (109:73:100) mixture. The scintillation fluid contained 0.015 *M* sodium hydroxide. Stainless-steel tubing (1/16 in.) was used to connect the various components of the system and Cajon ultra torr union fittings were used to connect the glass columns into the system.

Standardization of CO mass measurement

Pure CO was diluted with helium to appropriate concentrations of which 1.0 ml was instilled directly into the closed reaction chamber. The chamber was then opened to the GC column for a 15-min period (>99% was transfered within 10 min). CO mass was determined from peak area.

Preparation of ^{14}CO

Radioactive ^{14}CO was prepared by treating sodium [^{14}C]formate with 85% phosphoric acid in a nitrogen flushed, 35 ml septum bottle at 196°C in a sand bath for 20 min. The bottle was then cooled to −60°C in acetone and dry ice to condense several volatiles other than CO that are released during this reaction. The gas phase was then withdrawn from the septum bottle into a plastic syringe. Appropriate dilutions were made with helium gas. The possibility that radioactive volatiles other than ^{14}CO were present in this preparation was tested by incubating 20 ml of the gas phase with 1 ml of human blood. Negligible radioactivity or CO mass remained in the gas phase after this incubation indicating that all radioactivity was taken up by the blood and presumably all radioactivity was ^{14}CO.

Measurements of mass and radioactivity of ^{14}CO

Appropriate dilutions of the above ^{14}CO preparation were injection into the closed reaction chamber as above and carrier gas was allowed to flush through the reaction chamber for 15 min. The chamber was then closed to the GC column and the column effluent was collected for an additional 5 min to insure the complete transfer of $^{14}CO_2$ through the silver nitrate trap between the detector and the collection tube. Repetition of the above technique with a sample containing no radioactivity showed that negligible radioactivity of the preceding injection appeared in the analysis of the subsequent sample. Virtually no radioactivity (<1%) escaped from the collecting solution into a second trap in series.

Determination of ^{14}CO in blood

^{14}CO-labeled rat blood cells were prepared according to the method of Collison *et al.*[6]. The method for releasing CO from the blood cells was also based on the procedure of Collison *et al.* It is imperative that CO dissolved in the diluents be removed by flushing with nitrogen. The procedure is essentially as follows: 0.1–2.0-ml aliquots of the diluted red cells were injected into the closed reaction chamber, followed by 0.1 ml of 1% Flaminox solution (Fisher Scientific, Fairlawn, NJ, U.S.A.) and 0.5 ml of 20% ferricyanide solution. The reaction was allowed to proceed in this closed system for 10 min at room temperature with constant stirring. The release of bound ^{14}CO from the blood cells was essentially complete within 10 min (>98%). The chamber was then opened to the GC column and the carrier gas was allowed to flush through the chamber for 15 min. During this interval, the second chamber was being flushed with carrier gas for 15 min followed by the introduction of a second sample and the reaction initiated as above in this second chamber. In this manner, blood samples were assayed at approximately 20–25 min intervals.

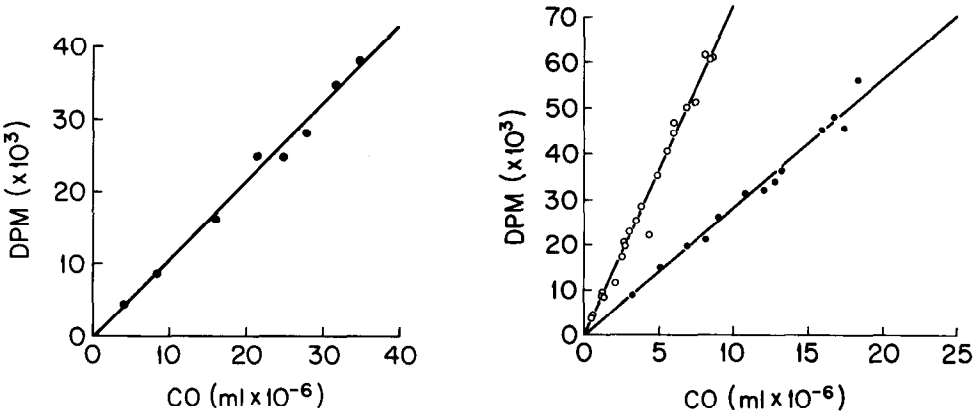

Fig. 1. Correlation between radioactivity and mass measurement of gas samples containing varying quantities of ^{14}CO.

Fig. 2. Correlation between radioactivity and mass measurements for ^{14}CO released from two different preparations of erythrocytes.

RESULTS

Hydrogen and CO are the only gases normally found in biological systems which are detected by the reduction detector. Although not shown, hydrogen and CO were well separated on the Carbosieve S column with retention times of 1.5 and 5 min, respectively.

Fig. 1 shows the good corellation between radioactivity and CO mass measurements for dilutions of a sample containing ^{14}CO.

Fig. 2 shows the good correlation between measurements of ^{14}CO and CO mass for varying dilutions of two different blood samples containing differing ratios of ^{14}CO and CO.

The reproducibility of the CO mass measurement expressed as coefficient of variation (S.D./mean) ranged from $\pm 8.8\%$ at 1 pppm of CO to $\pm 3.5\%$ at 20 ppm. The reproducibility of the radioactivity measurement averaged $\pm 3.8\%$ when approximately 3000 cpm were injected into the chromatograph. The accuracy of the mass measurement when CO was instilled into the reaction chamber averaged $\pm 7\%$. Because no good standard of ^{14}CO radioactivity is available, the accuracy of the ^{14}CO measurement could not be ascertained.

DISCUSSION

Most biological studies involving the measurement of ^{14}CO have investigated the metabolities of ^{14}CO-labeled heme[1,2]. In these studies $PdCd_2$ or hopkalite was used as a catalyst to convert ^{14}CO to $^{14}CO_2$. The method described in this paper relies on the ability of HgO in the reduction detector to convert ^{14}CO to $^{14}CO_2$. In the process, Hg vapor is evolved and serves as an indicator of CO mass. If the $^{14}CO_2$ is then trapped in ethanolamine and counted in a liquid scintillation counter, we postulated that mass and radioactivity of ^{14}CO could be determined on a single injection of ^{14}CO.

The experiments reported in this paper demonstrated that this techniques can provide accurate measurement of both mass and radioactivity of ^{14}CO over a fairly wide range of CO concentrations. As shown in Fig. 1 there was an excellent linear relation between CO and ^{14}CO measurements when varying quantities of ^{14}CO were injected into the chromatograph and Fig. 2 shows similar results for ^{14}CO released from erythocytes. As indicated in Figs. 1 and 2, the response of the CO detector was linear for quantities of CO up to $40 \cdot 10^{-6}$ ml.

The reliability of the proposed method depends on the effectiveness of the trapping of $^{14}CO_2$ in an absorbent. Numerous investigators have studied this problem and $^{14}CO_2$ has been shown to be efficiently trapped as sodium [^{14}C]carbonate when proper conditions are observed. In our experiments, when sodium hydroxide was omitted from the absorbent mixture, the radioactivity initially present in the absorbate diminished rapidly within a short period. However, the addition of small amounts of sodium hydroxide to both the absorbent mixture and the scintillation fluid, stabilized the recovery of $^{14}CO_2$ over the range of CO concentrations studied.

The method described in this paper provides a simple and rapid means of measuring both mass and radioactivity of ^{14}CO utilizing a single injection of a sample into a minimally modified, relatively inexpensive piece of standard equipment. We believe this technique offers several advantages over other methods described in the literature.

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