Carbon monoxide in alveolar air as an index of exposure to cigarette smoke

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(Received 29 March 1976)

Summary

1. A rapid method for the analysis of CO in expired air has been developed, which is suitable for use in studies of smoking.
2. The Bohr equation has been used to calculate the mean alveolar CO partial pressure \((P_{a,co})\).
3. The values of \(P_{a,co}\) obtained are highly correlated with direct measurements of venous carboxyhaemoglobin \((r = 0.96)\).
4. The method will distinguish between populations of smokers and non-smokers, and can allow the changes of CO in a smoker throughout a 12 h period to be followed. It provides a measure of the dose of cigarette smoke (vapour phase) that results from smoking a single cigarette.

Key words: carbon monoxide, cigarette smoking, epidemiological methods.

Introduction

Tobacco smoke contains carbon monoxide (CO), and Castleden & Cole (1975) have shown that the venous carboxyhaemoglobin saturation (HbCO\%) of smokers who inhale cigarette smoke is significantly higher than that of non-smokers. Venous HbCO\% has been used to study smoking habits. It can be measured directly after venepuncture, by the I.L. 182 CO Oximeter (Instrumentation Laboratory Inc.), but Cole (1974) has questioned the calibration and stability of this instrument. Other techniques allow measurement to be made on finger-prick blood (Commins & Lawther, 1965), but these are more difficult to perform. These technical problems and the need for blood samples prohibit the measurement of HbCO\% in any large-scale epidemiological study, and this led to the question whether a simple analysis of CO in expired air would permit the deduction of venous HbCO\%. The partial pressure of CO in alveolar air \((P_{a,co})\) was first shown to correlate with HbCO\% by Jones, Ellicott, Cadigan & Gaensler (1958). If a rapid and simple measurement of \(P_{a,co}\) could be made, this might prove an acceptable substitute for the well-established measurement of venous HbCO\% as an index of exposure to CO.

Analysis of alveolar carbon monoxide partial pressure

The direct analysis of \(P_{a,co}\) would require either the collection of end-tidal gas samples or an analyser with a sufficiently rapid response for end-tidal measurement. Alternatively the mixed expired CO concentration could be measured during tidal breathing, and the alveolar concentration calculated from the Bohr equation (Comroe, Forster, Dubois, Briscoe & Carlsen, 1962). The fractional concentration of CO in alveolar air \((F_{a,co})\) is given by eqn. (1),

\[
F_{a,co} = \frac{V_T F_{c,co} - V_D F_{i,co}}{V_T - V_D}
\]

where \(F_{c,co}\) is the fractional concentration of CO in mixed expired air, \(F_{i,co}\) is the fractional concentration of CO in inspired air, \(V_T\) is the tidal volume and \(V_D\) is the dead space.
Expired CO was analysed with an infrared gas analyser (Uras 2T, Hartmann and Braun), set to measure from 0 to 100 p.p.m. Oxygen (100%) was used as a zero gas (<1 p.p.m. of CO), and mixtures containing 0, 30, 60 and 90 p.p.m. of CO (concentrations measured by gas chromatography) were used to establish analyser linearity, which was found to be within 1%. Routine calibration, before each study, was made with the 0 and 90 p.p.m. concentrations only. The analyser had a 90% response time greater than 1 min. Both carbon dioxide and water vapour were found to affect measurements variably, these compounds were therefore removed. Subjects sat and breathed quietly through a valve, expired air being passed through a Watters canister containing soda lime (to remove carbon dioxide) and dried calcium chloride (to remove water vapour). From the canister (which also ensured gas mixing) the expired air passed to atmosphere through a length (1 m) of wide-bore tubing. At the proximal end of this tube, just distal to the canister, the analyser continuously sampled mixed expired gas at a rate of 1 l/min. Stable readings to an accuracy of 1 p.p.m. were obtained 60-90 s after commencement of quiet breathing. Analysis of the calibration gases (dry gas, CO in nitrogen) both before and after passage through the Watters canister indicated that this did not affect the measurement of CO.

The adequacy of mixing in the canister was established by noting that an identical measurement was obtained when the expired air was collected in a Douglas bag and then analysed. Analyser reproducibility was within 2 p.p.m.

Inspired CO concentration (ambient air) varied between 1 and 4 p.p.m. Respiratory rate, minute volume and thus mean tidal volume were measured by a dry-gas meter placed on the inspiratory side of the valve. The subject's anatomical dead space was estimated (Radford, 1955) from the ideal body weight by using the factor of 1 ml/lb. The fractional concentration of CO in alveolar air was then calculated from eqn. (1) and converted into \( P_A,CO \) by eqn. (2).

\[
P_A,CO = F_A,CO (P_n - P_A,H_2O - P_A,CO_2) \tag{2}\]

where \( P_n \) is barometric pressure and \( P_A,H_2O \) is alveolar partial pressure of water vapour. \( P_A,CO_2 \) was assumed to be 5.32 kPa (40 mmHg). \( P_A,CO \) was expressed to the nearest 0.133 Pa (0.001 mmHg).

Analysis of venous carboxyhaemoglobin

Venous HbCO\% was estimated by a spectrophotometric method (Tietz & Fiereck, 1973). A blood haemolysate was prepared by dilution with ammonium hydroxide and haemoglobin deoxygenated by the addition of sodium dithionite. The HbCO\% was determined by comparing the 541/555 nm extinction ratio for the haemolysate with the ratio obtained for solutions of known HbCO\%, a narrow-band pass scanning spectrophotometer (Pye Unicam SP. 1700) being used. The technique was found to be reproducible to within 0.2%.

Measurement of \( P_A,CO \) in smokers and non-smokers

\( P_A,CO \) was measured at random times throughout the day in thirty-five non-smokers and thirty-five smokers, each subject being studied on only one occasion. The smokers had not smoked a cigarette during the preceding 20 min, but there was no selection on the basis of cigarette consumption or the tar yield of cigarettes smoked.

The overall reproducibility of the method was studied in a series of fifteen non-smokers with measurement of \( P_A,CO \) on two occasions separated by a time-interval of at least 30 min. Comparable reproducibility has not been examined in smokers because of the natural decay in CO concentrations after smoking.

All subjects, both smokers and non-smokers, had values for forced expiratory volume in 1 s (FEV\(_1\)) and vital capacity (FVC) which lay within the predicted normal ranges.

Comparison of venous HbCO\% with \( P_A,CO \)

In fourteen volunteers venous blood was drawn simultaneously with the measurement of \( P_A,CO \). The blood sample was taken into a heparinized syringe and immediately placed in a refrigerator; analysis of HbCO\% was carried out within 1 h.

Variations of \( P_A,CO \) during a day of smoking

Two volunteers, one male and one female, spent a 12 h period sitting at rest in the laboratory. Both were regular smokers of ten to twenty middle-tar cigarettes per day, but neither had smoked for 12 h beforehand. After an initial venepuncture for HbCO\% analysis and measurement of \( P_A,CO \), they
smoked at intervals throughout the day, without restriction. Both smoked the same brand of cigarettes, which yielded 20.5 mg of CO/cigarette under standard conditions. Before smoking each cigarette, and exactly 15 min after, measurements of $P_A,CO$ were obtained. At random intervals throughout the day further venous blood samples were drawn simultaneously with the measurement of $P_A,CO$.

**Effect of varying depth of inhalation on $P_A,CO$**

Three volunteers smoked a series of cigarettes of an identical brand with deliberate differences in the depth of inhalation on each occasion. The depth of inhalation was monitored from movements of the chest wall by a chest-abdominal pneumogram. Measurements of $P_A,CO$ were made before and 15 min after smoking each cigarette.

**Comparison of arterial and venous HbCO%**

Arterial and venous blood were sampled simultaneously in seven subjects for measurement of HbCO%.

**Results**

**Measurement of $P_A,CO$ in smokers and non-smokers**

The comparison of $P_A,CO$ in smokers and non-smokers (Fig. 1) shows that values for non-smokers fell within a relatively narrow range [0.266-1.197 Pa (0.002-0.009 mmHg)], with a mean value 0.532 Pa (0.004 mmHg), sd 0.21 Pa (0.002 mmHg), whereas the range for smokers was much greater [0.532-4.788 Pa (0.004-0.036 mmHg), with a mean value 2.075 Pa (0.016 mmHg), sd 1.064 Pa (0.008 mmHg)]. The two populations are significantly different (unpaired t-test, $P<0.001$), although there is an overlap, one non-smoker having $P_A,CO$ greater than 1.064 Pa (0.008 mmHg) and three smokers having $P_A,CO$ below this value.

Repeated measurements of $P_A,CO$ in fifteen non-smokers revealed a mean difference between pairs of measurements of 0.009 Pa (0.00067 mmHg), sd 0.133 Pa (0.001 mmHg), with no statistical difference between the pairs of measurements (paired t-test $P>0.5$).

**Comparison of venous HbCO% with $P_A,CO$**

Fig. 2 shows the comparison of $P_A,CO$ with HbCO%. In addition to the fourteen results in different subjects the results from the two subjects in the third study have been included. The regression equation is: $\text{HbCO\%} = 1.80 \times P_A,CO\ (Pa) - 0.26; r = 0.96; P<0.001$. The 95% confidence limits for the prediction of venous carboxyhaemoglobin from $P_A,CO$ is ±1% HbCO.
FIG. 3. Variations in alveolar carbon monoxide partial pressure with cigarette smoking throughout a 12 h period in two subjects (A and B). The vertical bars indicate periods of cigarette smoking.

FIG. 4. Relation between 'exposure index' and carbon monoxide increment in three subjects. The inset shows the pneumogram trace against time with an inhalation from a cigarette indicated. The exposure index is derived from the area contained within this curve during the period of inhalation.
Variation of $PA_{\text{CO}}$ during a day of smoking (Fig. 3)

In the male subject A the initial $PA_{\text{CO}}$ was 1.06 Pa (0.008 mmHg), and increased with each cigarette smoked except the last, the mean increase being 0.479 Pa (0.0036 mmHg) with a range 0-0.798 Pa (0-0.006 mmHg). After each cigarette the $PA_{\text{CO}}$ fell, with a mean extrapolated half-life of 124 min. In the female subject B the initial $PA_{\text{CO}}$ was 0.466 Pa (0.0035 mmHg), and again increased with each cigarette smoked, the mean increase being 0.359 Pa (0.0027 mmHg) with a range 0.133-0.931 Pa (0.001-0.007 mmHg). After each cigarette, except the third, the $PA_{\text{CO}}$ fell, with a mean extrapolated half-life of 136 min. At the end of the test period, chain-smoking four cigarettes caused an increase in $PA_{\text{CO}}$ which was linear with time.

Effect of varying depth of inhalation on $PA_{\text{CO}}$

Movements of the chest wall were recorded by a chest-abdominal pneumogram. The recorded deflection was approximately proportional to the inspired volume, and could be calibrated for each subject by a spirometer. The exposure of the lungs to cigarette smoke, taking into account both the inspired volume and the time which this remained in the lungs, was estimated by summing the area under the curve of the pneumogram trace for each inhalation through the cigarette (Fig. 4, inset). This 'exposure index' (I.s) was then plotted against the 'increment' of $PA_{\text{CO}}$ from the smoking of that cigarette (Fig. 4). Subjects either smoked normally, deliberately did not inhale or, in one subject, inhaled maximally. There was no increment in $PA_{\text{CO}}$ without inhaling, but with inhalation there is apparently a linear relation between the exposure index and the increment in $PA_{\text{CO}}$. This relation was quantitatively similar in the three subjects studied.

Comparison of arterial and venous HbCO%.

There were no systematic differences between arterial and venous carboxyhaemoglobin saturations in seven subjects when samples were taken simultaneously (arterial, venous: 3.5, 3.5; 1.7, 1.8; 0.0, 0.2; 1.7, 0.7; 2.0, 1.7; 0.6, 0.2; 1.2, 0.7). The mean arteriovenous HbCO% difference was +0.28, and by paired t-test this was not significantly different from zero ($P < 0.01$).

Discussion

The analysis of CO in alveolar air to indicate blood HbCO% is not new. Initially, measurements of $PA_{\text{CO}}$ were made to derive the blood HbCO% from the equation of Haldane (Douglas, Haldane & Haldane, 1912; Sjostrand, 1948). $PA_{\text{CO}}$ was measured by using the lungs as aerotonometers until the gas pressures of pulmonary blood and alveoli were in equilibrium. Early methods involved the rebreathing 100% oxygen (Sjostrand, 1948), but it was necessary to measure the equilibrium oxygen tension in addition to that of CO. Later techniques (Jones et al., 1958) have required the collection of end-tidal gas samples after periods of breath-holding for the measurement of CO. This technique has been employed in population studies (Rea, Tyrer, Kasap & Beresford, 1973), but requires equipment for the collection of gas samples and a degree of subject co-operation. The method described in the present study is simpler, more rapid and requires little subject co-operation. The major assumption lies in the derivation of dead space, where any error will be maximal in subjects with a high expired CO. Recalculation of our results with an error of 20% in

![Fig. 5. The classical dissociation curve for carboxyhaemoglobin measured at $P_{\text{CO}_2}$ 5-59 kPa (42 mmHg) and $P_{\text{O}_2}$ 0 kPa, with extrapolation to the origin (broken line). Two points (●) were measured after the $P_{\text{O}_2}$ had been raised to 19-95 kPa (150 mmHg). The straight line, passing from the origin, indicates the observed regression line of $PA_{\text{CO}}$ on venous HbCO% (see Fig. 2).](image-url)
dead space resulted in changes of up to 0.266 Pa (0.002 mmHg) for PA,CO in the heaviest smokers, whereas no change arose in non-smokers and light smokers. The assumption of a PA,CO2 of 5.32 kPa (40 mmHg) is not critical, as re-calculation of the results with a PA,CO2 range from 4/65 to 5.99 kPa (35/45 mmHg) made no difference to the derived value of PA,CO.

The method readily distinguishes between populations of smokers and non-smokers, but there was an overlap in individual cases. Within this group the smoker with the lowest PA,CO (0.532 Pa), who admitted to smoking fifteen to twenty cigarettes a day, was studied on another occasion after chain-smoking five cigarettes, when no detectable rise in PA,CO was demonstrated, and he would thus appear to be a true 'non-inhaler'. The other two low results were in light smokers where the measurements had been made early in the day before the first cigarette. The one non-smoker with PA,CO 1.197 Pa (0.009 mmHg) denied smoking or exposure to a high degree of 'passive smoking' at work. He may therefore have a high level of endogenous CO production (Goldsmith & Landaw, 1968). These results must qualify the use of this method to identify inhaling smokers and non-smokers from a single random measurement.

The very high ability of PA,CO to predict venous HbCO% calls for some explanation. Since alveolar gas is in equilibrium with pulmonary capillary blood, the absence of any systematic difference between arterial and venous HbCO% helps to explain why the PA,CO is such a good predictor of the venous HbCO%. The relation between Pco and HbCO% saturation is given by the classical curve for the dissociation of carboxyhaemoglobin (Douglas et al., 1912; Joels & Pugh, 1958). In Fig. 5 the curve was measured in the absence of oxygen and with PCO2 5.95 kPa (42 mmHg). The lower part of the curve below Pco 5.5 Pa (0.04 mmHg), which is relevant to the present work, is poorly defined. Oxygen unloads CO from the haemoglobin and the data of Douglas et al. (1912) indicate that if the P02 is raised to 19.95 kPa (150 mmHg) the carboxyhaemoglobin saturation at PA,CO 47.0 Pa (0.35 mmHg) falls from 94% to 40%, whereas at PA,CO 6.7 Pa (0.05 mmHg) the saturation remains at about 9% (Fig. 5). Differences in oxygen tension between arterial and venous blood do not therefore appear to affect the dissociation curve at the levels of Pco seen in smokers. The relationship that we have obtained between PA,CO and venous HbCO% corresponds to the probable position of the lower part of the classical dissociation curve (Fig. 5).

The variations of PA,CO and HbCO% during the day in two smokers is similar to that found by Castleden & Cole (1974) with venous blood alone, although in both our subjects there was a definite rise in PA,CO during the early part of the day. Our study also suggests that the increment in PA,CO with each cigarette is variable, but calculation of the mean increment gives a value similar to that derived by Wald, Howard, Smith & Bailey (1975). The half-life of PA,CO and venous HbCO% measured over the short periods of time after each cigarette also varied, but the mean half-lives closely agreed with previous values of approximately 150 min for resting subjects (Russell, Wilson, Patel, Cole & Feyerabend, 1973).

Carbon monoxide is present in the vapour phase of tobacco smoke and the increment in PA,CO reflects the dose presented to the lungs and absorbed. In subjects without ventilatory impairment this dose will depend upon the puff volume (or more correctly the proportion of the puff volume inhaled into the lungs), the total volume of gas inhaled into the lungs, and the time for which this inhaled gas remains in the lungs. Ideally these three variables should be measured. We have considered only the inspired volume and the time which this remains in the lungs when calculating our exposure index. The apparently linear relationship between our exposure index and the increment of PA,CO suggests that the puff size is relatively consistent in any individual.

This close relationship between the increment in PA,CO from smoking a single cigarette and exposure index allows objective evaluation of a subject's statement that he does or does not inhale smoke when smoking. When smoke is taken into the mouth but deliberately not inhaled into the lungs there is no 'increment' in CO and there would thus appear to be no measurable absorption of CO across the buccal, pharyngeal and nasal mucosae.

The measurement and calculation of PA,CO can be performed in less than 5 min. The technique is both simple and reproducible and this makes it ideal for epidemiological studies.

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