REDUCED OXYGEN CONTENT IN EQUILIBRATED FRESH HEPARINIZED AND ACD-STORED BLOOD FROM CIGARETTE SMOKERS

R. A. MILLAR AND I. C. GREGORY

SUMMARY

Fresh heparinized and ACD stored blood from smokers showed a reduced oxygen content when equilibrated with 95 per cent oxygen in vitro for 30–45 min. This was associated with a raised carboxyhaemoglobin level, which persisted beyond the normal 21-day period of storage of donor blood.

We have shown recently, in fresh heparinized blood drawn from smokers and equilibrated with 95 per cent oxygen in vitro for periods exceeding 30 min, that the oxygen content determined by Van Slyke analysis is significantly lower than in non-smokers’ blood. The findings, which we have noted briefly elsewhere (Gregory, Hulands and Millar, 1972), were obtained during investigation of the normal oxygen combining capacity of human haemoglobin, and are similar to those reported by Bureaux and associates (1971). We have, therefore, sought similar effects in blood donated by heavy smokers and stored in the usual way in ACD solutions.

METHOD

The data were obtained during investigation of the normal maximum oxygen combining capacity of human haemoglobin. Blood samples, 4–6 ml, were equilibrated with 95 per cent oxygen, 5 per cent carbon dioxide for 30–45 min, in water-jacketed glass tonometers maintained at 37°C (Adams and Morgan-Hughes, 1967). A continuous flow of gas was maintained through the tonometer at approximately 2.5 ml/min.

In blood which is fully saturated and in which all the haemoglobin is available for combination with oxygen, the oxygen capacity of haemoglobin (ml/g) is:

\[
\text{Total oxygen content (ml/100 ml)} = \frac{\text{PaO}_2 \times 0.0031}{\text{Haemoglobin concentration (g/100 ml)}}
\]

When some of the haemoglobin is “unavailable” for oxygen, due for example to the presence of carboxyhaemoglobin, the factor (ml/g) obtained from this equation will be reduced accordingly. From the figure measured thus, we have derived firstly the oxygen content in terms of a constant 14 g of haemoglobin. This simplifies interpretation since the haemoglobin concentration varied between different equilibrated blood samples, and there is no concern with oxygen dissolved in solution. Secondly, we have expressed the results as differences in the “oxygen content per 14 g of haemoglobin” between control (non-smoker) blood samples drawn from ourselves and samples from smokers.

Total oxygen content was measured by the Van Slyke manometric technique. The standard deviation of repeated measurements of the oxygen contained in solutions of hydrogen peroxide was ± 0.10 about a mean of 15 vols/100 ml.

\(\text{PaO}_2\) was measured with an electrode calibrated with whole blood equilibrated continuously with 100 per cent oxygen. At a mean \(\text{PaO}_2\) of 676 mm Hg, the standard deviation was ± 8.9.

Haemoglobin concentration was measured from optical density measurements at 540 nm, using a Pye Unicam SP 1800 spectrophotometer. The international cyanmethaemoglobin standard of 11.5 g/100 ml (Diagnostic Reagents Ltd.) was compared to a 1/201 dilution of the unknown blood (0.5 ml added to 100 ml Drabkin solution), or lyzed whole blood standards of 13.8 g/100 ml or 14.1 g/100 ml were used for quadruplicate comparisons with the unknown. The standard deviation was ± 0.09 around a mean of 14.6 g/100 ml.

Data were obtained from one patient and seven subjects who usually smoked 20–30 cigarettes daily; three were regular blood donors, and four were members of the hospital staff. Each of the authors supplied the control blood for the measurement in tables I and II, and tables III and IV respectively.


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In the case of fresh samples, 50 ml of venous blood was withdrawn into heparinized plastic syringes between 9.00 and 10.00 a.m., and was placed in the refrigerator. During the day's study, separate 6 ml aliquots were used for equilibration with 95 per cent oxygen/5 per cent CO₂. Two tonometers with identical characteristics were used, the control (non-smoker) samples being interspersed randomly among the unknowns, so that a total of 12 equilibrations was usually carried out on each day.

After a period of heavy smoking, two subjects gave heparinized blood samples in the morning of separate days for repeated measurements of oxygen capacity, percentage carboxyhaemoglobin (Commins and Lawther, 1965), and methaemoglobin (Evelyn and Molloy, 1938); these were compared with the values in control fresh blood samples on the same day.

Another subject smoked several cigarettes, then breathed 100 per cent oxygen for a period of 100 min, during which periodic determinations of carboxyhaemoglobin were made in order to plot the rate of elimination of carbon monoxide.

From two of the three regular blood donors, who gave the standard volume for storage in ACD solution in plastic bags, fresh heparinized samples were taken at the same time, and then again 6 days and 14 days later (i.e., during storage of the ACD donation); this permitted an in vitro comparison of the oxygen capacities of the fresh heparinized and ACD blood from each of the two donors. Carboxyhaemoglobin was measured spectrophotometrically at intervals. ACD blood in a bottle from a third donor was also studied on several occasions following the normal 21-day expiry date for transfusion.

Two staff members, who were heavy smokers, donated approximately 100 ml of venous blood which was then stored in ACD solution in plastic bags for later study. Subsequently, the same procedure was followed for blood from 3 non-smokers (including the two authors).

### RESULTS

**Oxygen content of heparinized smokers' blood.**

When heparinized venous samples were withdrawn from two smokers (blood donors) and equilibrated with 95 per cent oxygen/5 per cent CO₂ for 30-45 min (Pao₂ approximately 650 mm Hg), the oxygen content was lower than in control samples from a non-smoking subject (equilibrated at the same time). This was confirmed on three occasions at intervals of 6-7 days. Table I shows the comparative deficit in oxygen content in smokers' blood, expressed as ml oxygen/14 g haemoglobin.

**Table I.** Mean reductions in oxygen content (ml/14g) in fresh heparinized blood from 2 smokers below that measured at similar times in a non-smoking control subject. The figures are derived from samples equilibrated with oxygen, and show the mean of 4 control values and the same number from the smokers' blood (3 measurements only on day 1). The figures in brackets are the SE of mean difference.

<table>
<thead>
<tr>
<th>Reduction in oxygen content compared to control ml/14g (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Donor 1</td>
</tr>
<tr>
<td>Donor 2</td>
</tr>
</tbody>
</table>

**Table II.** Mean reductions in oxygen content (ml/14g haemoglobin) from 6 measurements on heparinized blood from 2 smokers studied on separate days, below that in non-smokers' blood equilibrated at the same time. The carboxyhaemoglobin level before in vitro equilibration with oxygen is the mean of two measurements; that after equilibration is the mean ± SD of six determinations.

<table>
<thead>
<tr>
<th>Reduction in oxygen content compared to control ml/14g (± SE)</th>
<th>Percentage carboxyhaemoglobin Before equilibration (± SD)</th>
<th>After equilibration (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject A</td>
<td>1.60 (0.167)</td>
<td>10.4</td>
</tr>
<tr>
<td>Subject B</td>
<td>1.38 (0.192)</td>
<td>10.1</td>
</tr>
</tbody>
</table>

**Table III.** Reductions in oxygen content (ml/14g) in ACD stored blood from 3 smokers below that measured in fresh heparinized blood from a non-smoker. The mean values for donors 1 and 2 are derived from 4-7 measurements and from 4 determinations in the case of donor 3 and the control blood. The figures in brackets are the SE of the mean differences. The day numbers indicate the duration of storage after donation.

<table>
<thead>
<tr>
<th>Reduction in oxygen content (ml/14g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1-2</td>
</tr>
<tr>
<td>Donor 1</td>
</tr>
<tr>
<td>Donor 2</td>
</tr>
<tr>
<td>Donor 3</td>
</tr>
</tbody>
</table>
Table IV. Mean reductions (n = 4) in oxygen content (ml/14g haemoglobin) in ACD stored blood from 2 heavy smokers, comparison being made with the values (n = 4) determined on the same day on fresh heparinized blood from a non-smoker. Measurements 1 and 2 were made after 18 and 24 days storage of ACD blood. The percentage carboxyhaemoglobin indicates the mean of 4 measurements (± SD) after equilibration of the stored smokers' blood with oxygen; the levels before equilibration are the mean of 2 measurements.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Reduction in oxygen content compared to control</th>
<th>Percentage carboxyhaemoglobin before equilibration</th>
<th>Percentage carboxyhaemoglobin after equilibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.77 (0.213)</td>
<td>4.7</td>
<td>4.2 (0.40)</td>
</tr>
<tr>
<td>2</td>
<td>0.80 (0.170)</td>
<td>5.1</td>
<td>3.8 (0.67)</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.45 (0.224)</td>
<td>11.8</td>
<td>11.1 (0.83)</td>
</tr>
<tr>
<td>2</td>
<td>2.00 (0.262)</td>
<td>12.5</td>
<td>10.3 (2.04)</td>
</tr>
</tbody>
</table>

Similar findings were obtained in two experiments when members of the hospital staff smoked heavily before giving heparinized venous samples, table II showing the reduction in oxygen content in comparison to control (non-smoker) samples. Measurements of percentage carboxyhaemoglobin in the blood of both these smokers indicated that high levels remained even after equilibration in vitro.

Oxygen content of ACD stored blood from smokers.

The ACD stored blood (in plastic bags) from the two donors who were smokers also showed lower oxygen contents in comparison with fresh heparinized non-smoker's blood, when equilibrated with oxygen on three separate occasions up to 18 days of storage (table III). Measurements of percentage carboxyhaemoglobin in the ACD blood (as sampled from the container, not equilibrated), at intervals between the 7th and 27th days of storage, showed levels averaging 7.6 per cent and 6.5 per cent for the two donors. Similar reductions in oxygen capacity were also measured when bottled blood from a third donor, also a smoker, was studied 23 days after donation (donor 3 in table III).

To confirm the preceding studies, a period of deliberate heavy smoking by two volunteers, before donating blood for storage in ACD solution in plastic bags, was found to reduce the oxygen content when this was measured 18 days and 24 days later (table IV). As shown, equilibration with oxygen failed to reduce greatly the percentage haemoglobin which was combined with carbon monoxide.

When blood was withdrawn from three non-smokers and was then stored in ACD solution in plastic bags, there was no significant reduction in oxygen capacity in comparison to fresh heparinized blood, when measured on two occasions 21 days and 24 days after donation. Also, the oxygen contents of equilibrated smoker's blood did not differ significantly according to whether it was withdrawn into ACD solution or heparinized syringes.

The carboxyhaemoglobin level in non-smokers never exceeded 1 per cent, and was commonly undetectable. Similarly, before or after equilibration of smokers' or non-smokers' blood, the amounts of methaemoglobin measured were low, in the range of 0–1 per cent only.

These studies indicate that the reduction in oxygen content in smokers' blood is attributable to the presence of carbon monoxide. This is reinforced by figure 1, which shows the linear relation, from the data used for tables II and IV, between percentage carboxyhaemoglobin and oxygen content (ml/14 g haemoglobin) in fresh heparinized and ACD stored blood.

It should be noted that variations in the oxygen content measured at different times in the same stored blood, shown in tables III and IV, reflect variability in carbon monoxide elimination during equilibration rather than an alteration in the carboxyhaemoglobin level during storage.

![Figure 1](image-url)
These experiments have also demonstrated a slow and incomplete displacement of carbon monoxide by oxygen in vitro. Figure 2, by comparison, shows the reduction in percentage carboxyhaemoglobin which was measured when a volunteer smoker breathed 100 per cent oxygen, \( P_{\text{aO}_2} \) being above 600 mm Hg. The half-time for elimination of carbon monoxide under these optimum conditions was about 45 min.

**DISCUSSION**

The findings described here probably underestimate the actual reduction in "available oxygen capacity" caused by cigarette smoking, since of necessity the measurements had to be made after equilibration of blood with 95 per cent oxygen in vitro. The nature of the experiment, therefore, created a situation most favourable for converting carboxyhaemoglobin to the oxygenated form, without the possibility of endogenous carbon monoxide production. Nevertheless, the "oxygen deficit" was readily demonstrated in fresh heparinized blood, and more unexpectedly in ACD bank blood from smokers throughout the duration of storage. We have no reason to suspect that factors other than carbon monoxide were responsible.

In spite of the slow half-time for elimination of carbon monoxide during breathing 100 per cent oxygen in vivo, which is extended to about 250 min in an air atmosphere (Forbes, Sargent and Roughton, 1945), it is surprising that so little carbon monoxide displacement occurred in vitro under conditions of full oxygenation, employing tonometers which allow a continuous steady inflow and outflow of gas. In these experiments, the half-time of carbon monoxide in vitro was considerably longer than the in vivo period of 45 min.

The present findings arose incidentally from a study of the normal oxygen capacity of haemoglobin (Factor, in ml/g), for which blood free from carboxyhaemoglobin, methaemoglobin, or other inactive compounds is required. Over the period of these investigations, the variations in the factor value from day to day was significantly greater than that occurring on a single day. It was essential, therefore, to compare the blood of smokers and of non-smokers at coincident times. Also, the haemoglobin content of each of the equilibrated blood samples varied. For these reasons, the results have been expressed as reductions in oxygen content below that of normal blood, after standardization to 14 g haemoglobin.

Including the present studies, we have analyzed blood samples from eight smokers, all obtained in the morning and showing carboxyhaemoglobin levels in the range 3.4–12.5 per cent. While the highest figure was associated with the deliberate smoking of eight cigarettes before a sample drawn at midday, it is likely that under usual conditions the blood levels will increase as smoking is continued throughout the day. It is also of interest that 8.6 per cent carboxyhaemoglobin was measured recently in a patient who reported for pulmonary function tests in our department, and had not been advised to stop smoking beforehand.

Certain pulmonary investigations may involve the indirect derivation of blood oxygen content from haemoglobin concentration, \( P_{\text{aO}_2} \), and percentage oxygen saturation, and commonly assume a normal combining capacity; in the presence of "unavailable haemoglobin" caused by carbon monoxide, substantial errors might be introduced. For example, in assessing pulmonary shunting, the calculation is usually obtained from directly measured \( P_{\text{aO}_2} \). If the oxygen combining capacity is assumed to be normal but is in fact reduced by about 10 per cent, the figure for "calculated shunt" could be overestimated by as much as 100 per cent. This error would be avoided if both values are derived, or both could be measured.
Blood carbon monoxide levels in smokers have been extensively documented (Kjeldsen, 1969). Apart from reducing oxygen content because of a haemoglobin affinity about 210 times that of oxygen, carbon monoxide also shifts to the left the dissociation curve of the remaining oxyhaemoglobin (Douglas, Haldane and Haldane, 1912), leading to impaired oxygen release to the tissues. Concurrently, there would be a higher oxygen saturation at lower Po2, an effect which might enhance oxygen equilibration in vitro.

In a recent assessment of the carbon monoxide levels in smokers about to donate blood, it was suggested that smoking should be restricted in the period before blood donation (Shields, 1971). Our data show that a high carboxyhaemoglobin level will remain virtually unchanged during storage in ACD solutions in closed containers, and suggest that it will persist in the recipient's circulation for some time after transfusion. While a reduction in oxygen capacity of the order of 10 per cent may be unimportant in many situations involving healthy patients, there could be an adverse effect if large amounts of smokers' blood must be given rapidly, as in major surgery, resuscitation after severe trauma or exchange transfusion. In anaesthetized patients, also, elimination of carbon monoxide from donated blood could be delayed as a result of respiratory depression, the use of closed circuit apparatus, or because nitrous oxide is often the main anaesthetic agent in combination with relatively lower (20-30 per cent) than higher oxygen concentrations. Given the choice, there seems little doubt that in the emergency situation the clinician would select nonsmokers' blood for transfusion.

ACKNOWLEDGEMENTS

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REFERENCES


CONCENTRATION REDUITE D'OXYGENE DANS LE SANG EQUILIBRE FRAIS HEPARINISE ET STOQUE-ACD DE FUMEURS DE CIGARETTES

SOMMAIRE

Du sang frais heparinisé et stocké-ACD de fumeurs avait une concentration réduite d'oxygené, lorsqu'il était équilibré avec 95 pour cent d'oxygené in vitro durant 35-40 minutes. Ceci s'associa avec un taux élevé de carboxihémoglobine, qui persista au dela de la période normale de 21 jours de stockage de sang-donateur.

ÜBER DEN VERMINDERTEN SÄUERSTOFFGEHALT IN AQUELLIBRIERTEM, FRISCHEN HEPARINISIERTEN UND ACD-STABILISIERTEN BLUT VON ZIGARETTEN-RAUCHERN

ZUSAMMENFASSUNG


REDUCCION DEL CONTENIDO EN OXIGENO EN SANGRE EQUILIBRADA, RECIENTE HEPARINIZADA Y EN SANGRE CONSERVADA ACD PROCEDENTES DE FUMADORES DE CIGARRILLOS

RESUMEN

Sangre hepaminisada reciente y sangre conservada ACD de fumadores mostraron una reducción en el contenido de oxígeno cuando fueron equilibradas con oxígeno la 95 por ciento in vitro durante 30-45 min. Esto estaba asociado con una elevación del nivel de carboxihemoglobina, la cual persistió más allá del período normal de 21 días de conservación de la sangre del donador.