EQUALITY OF THE IN VIVO AND IN VITRO OXYGEN-BINDING CAPACITY OF HAEMOGLOBIN IN PATIENTS WITH SEVERE RESPIRATORY DISEASE

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SUMMARY
The in vivo and in vitro oxygen-binding capacity of haemoglobin was determined on 10 occasions in nine patients who required mechanical ventilation. The in vitro sample was tonometered with 97% oxygen for 10 min and then with air, while the in vivo sample was obtained after 20 min of lung ventilation with pure oxygen. Subsequent laboratory procedures were identical for both samples. The mean oxygen-binding capacity of haemoglobin in vitro and in vivo samples were almost equal (1.365 ± 0.010 and 1.366 ± 0.007 ml per g Hb). When the measured inactive fractions of haemoglobin (carboxy- and methaemoglobin) were taken into account, these values increased to 1.392 ± 0.005 and 1.392 ± 0.007 ml per g Hb respectively.

The oxygen-binding capacity of haemoglobin is more frequently determined in vitro (Foex et al., 1970; Theye, 1970; Bursaux, Dubos and Poyart, 1971; Gregory and Millar, 1973; Gregory, 1974; Domínguez de Villota et al., 1976, 1979; Dijkhuizen et al., 1977; Guillot et al., 1979) than in vivo (Gregory, Hulands and Millar, 1971, 1972; Gregory and Millar, 1973; Scherrer and Bachofen, 1972). Simultaneous in vivo and in vitro determinations have only been reported twice and from the same laboratory in healthy subjects (Gregory, Hulands and Millar, 1972; Gregory and Millar, 1973). In the present study, we investigated the oxygen-binding capacity of haemoglobin in seriously ill patients using both methods.

PATIENTS AND METHODS
Ten blood samples were obtained from nine patients, seven men and two women (mean age 66.5 ± 7.3 yr). Samples 4 and 8 were from the same patient, but were taken 3 weeks apart. In all patients the lungs were ventilated mechanically because of acute or chronic respiratory insufficiency.

Ten millilitre of arterial blood was withdrawn from an indwelling catheter and refrigerated immediately (in vitro sample), while the lungs were ventilated with variable concentrations of inspired oxygen; the mean PaO₂ was 8.59 ± 1.49 kPa. The ventilator was then set to deliver FIO₂ 1.0 for 20 min before the withdrawal of another 10 ml of blood (in vivo sampling). This FIO₂ produced a mean Pvo₂ of 26.73 ± 7.78 kPa measured at 37 °C in a Comby-Analysator (Eschweiler & Co., Kiel).

Both samples were divided into two aliquots. The in vitro aliquots were tonometered (Eschweiler & Co, Type II, 40-ml capacity) at 37 °C from the same laboratory in healthy subjects (Gregory, Hulands and Millar, 1972; Gregory and Millar, 1973). In the present study, we investigated the oxygen-binding capacity of haemoglobin in seriously ill patients using both methods.

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Laboratories Inc., Mass., U.S.A.) was used to measure the fraction of carboxyhaemoglobin (COHb). The co-oximeter was adjusted to read 99% of oxyhaemoglobin when blood from a non-smoker and tonometered with 97% oxygen was measured (Dominguez de Villota et al., 1976).

The oxygen-binding capacity of Hb was derived from the equation:

\[
\text{Oxygen-binding capacity of Hb (ml g}^{-1}\) = \frac{\text{maximal oxygen content (ml dl}^{-1}\)}{\text{Hb concentration (g dl}^{-1}\)}
\]

The value of Hb obtained by the cyanmethaemoglobin method was corrected according to the concentrations of COHb and MeHb which were calculated from their measured fractions. The reduced value of Hb represented the amount of "functional Hb" and was used to calculate the corrected oxygen-binding capacity of Hb as follows:

\[
\text{Corrected oxygen-binding capacity of Hb (ml g}^{-1}\) = \frac{\text{maximal oxygen content (ml dl}^{-1}\)}{\text{functional Hb (g dl}^{-1}\)}
\]

The results of the two determinations performed in each of the in vivo and in vitro samples were averaged and the mean used for statistical comparison (Student's t test for paired data).

**RESULTS**

The differences between duplicate measurements in this study were similar to those reported previously (Dominguez de Villota et al., 1976, 1979) (table I). The sum of the fractions of COHb and oxyhaemoglobin in the 80 individual measurements performed in the co-oximeter had a mean value of 99.8 ±0.8%.

**DISCUSSION**

There are few reported values of the oxygen-binding capacity of Hb calculated in vivo. In two non-smokers the mean values of nine measurements were 1.403 and 1.394 ml per g Hb (Gregory, Hulands and Millar, 1971). The same investigators reported, for their own blood, values close to 1.39 ml per g Hb in two, but significantly less in the other (Gregory, Hulands and Millar, 1972). Later they analysed blood from the first two subjects again and the values found were 1.316 and 1.331 in the same subject on separate days and 1.304 ml per g Hb for the second subject (Gregory and Millar, 1973). The values of COHb and MeHb in these studies were less than 1%. In 88 non-smokers, an oxygen-binding capacity of 1.39 ml per Hb was found; 0.15 g of Hb per dl of blood was bound to CO and 0.20 g of Hb per dl of blood was considered inactive (Scherrer and Bachofen, 1972).

In the present report, nine critically ill patients requiring mechanical ventilation of the lungs were studied. The maximum oxygen tensions attained were less than those reported in other in vivo work.
were also reduced compared with normal and with other studies (Scherrer, Kung and Moshi, 1971). However, our results for oxygen-binding capacity are similar to some of the in vivo values given above.

The in vitro measurements of the oxygen-binding capacity of Hb in non-smokers reported vary considerably with values of 1.30 (Theye, 1970), 1.31 (Bursaux, Dubos and Poyart, 1971; Gregory, 1974), 1.32 (Gregory and Millar, 1973), 1.33 (Gregory and Millar, 1973; Gregory, 1974), 1.35 (Guillot et al., 1979), 1.36 (Dominguez de Villota et al., 1976, 1979) and 1.37 (Dijkhuizen et al., 1977). These values increased to 1.39 ml per g Hb when the fraction of inactive Hb was considered (Dominguez de Villota et al., 1976; Guillot et al., 1979). In smokers, values were less: 1.26 and 1.20 (Bursaux, Dubos and Poyart, 1971), 1.30 (Dominguez de Villota et al., 1976, 1979) and 1.33 (Guillot et al., 1979), but again the corrected figures increased to 1.39 (Dominguez de Villota et al., 1976, 1979) or exceeded it (Guillot et al., 1979).

This variability of values for oxygen-binding capacity exists not only between different investigators but even between reports from the same laboratory (Gregory, Hulands and Millar, 1971, 1972; Gregory and Millar, 1973; Dijkhuizen et al., 1977) and it has prompted discussion about the most physiological value (Theye, 1971; Prys-Roberts, Foëx and Hahn, 1971; Scherrer and Bachofen, 1972; Gregory, 1974; Dijkhuizen et al., 1977).

The possible influence of different methods of determining the in vivo and in vitro values in the variability of the oxygen-binding capacity of Hb has been mentioned (Scherrer and Bachofen, 1972). However, a comparison of the two methods of measurement in our study showed no significant difference. True variability of the oxygen-binding capacity of Hb has been suggested to explain the values obtained (Foëx et al., 1970; Prys-Roberts, Foëx and Hahn, 1971; Dominguez de Villota et al., 1976). The consistency of our results obtained from ambulatory patients in vitro (Dominguez de Villota et al., 1976), from healthy smokers in vitro (Dominguez de Villota et al., 1979) and from critically ill patients in vivo and in vitro support the concept of a constant value of the oxygen-binding capacity of Hb despite variability of individual measurements.

The accuracy of the Van Slyke technique in measuring the oxygen content is not debated (Theye, 1971; Gregory, 1973), but inaccurate measurement of Hb (Theye, 1971; Gregory, 1974) or the existence of a fraction of Hb not available to combine with oxygen (Scherrer and Bachofen, 1972; Dijkhuizen et al., 1977) but measurable by spectrophotometry (Dijkhuizen et al., 1977) have been suggested to explain values of oxygen-binding capacity less than 1.39 ml per g Hb. Approximately 1% of the total Hb was found unable to combine with oxygen even after tonometry for 150 min with pure oxygen (Dijkhuizen et al., 1977). The fact that our binding capacity of Hb, once corrected for the fractions of COHb and MeHb, reached the value of 1.39 ml per g Hb might be explained by calibration of the co-oximeter, with an offset of 1%. This 1% might have been an excessive allowance for patients who had been ill for some time and compensated for this small fraction of inactive Hb (Dijkhuizen et al., 1977), thus increasing our value of the oxygen-binding capacity of Hb to the theoretical maximum of 1.39 ml per g Hb.

REFERENCES


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Egalité de la capacité *in vivo* et *in vitro* qu’a l’hémoglobine de lier l’oxygène chez les patients souffrant d’une grave maladie respiratoire

**RESUME**

Il a été déterminé dans 10 cas la capacité *in vivo* et *in vitro* qu’a l’hémoglobine de lier l’oxygène, sur neuf patients qui avaient besoin de ventilation mécanique. L’échantillon *in vitro* a été soumis à une sphygmoportométrie à l’aide de 97% d’oxygène pendant 10 min puis à l’aide d’air, tandis que l’échantillon *in vivo* a été obtenu après une ventilation des poumons à l’oxygène pur pendant 20 min. Les processus suivis ultérieurement en laboratoire ont été les mêmes pour les deux échantillons. La capacité moyenne de lier l’oxygène qu’avait l’hémoglobine des échantillons *in vivo* et *in vitro* a été presque la même (1,365 ± 0,010 et 1,366 ± 0,007 ml par g Hb). Lorsqu’on a tenu compte des fractions inactives mesurées de l’hémoglobine (carboxy et méthémoglobine), ces valeurs sont passées à 1,392 ± 0,005 et 1,392 ± 0,007 ml par g Hb respectivement.

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Gleichheit der sauerstoffbindenden Fähigkeit von Hämoglobin *in vivo* und *in vitro* bei Patienten mit schweren Atmungsstörungen

**ZUSAMMENFASSUNG**

Die sauerstoffbindende Fähigkeit von Hämoglobin *in vivo* und *in vitro* wurde in 10 Fällen bei 9 Patienten festgestellt, die mechanische Ventilation benötigten. Das *in vitro* Muster wurde 10 Minuten lang mit 97%-igem Sauerstoff und anschließend mit Luft equilibriert, während das *in vivo* Muster durch 20 Minuten lange Lungenventilation mit reinem Sauerstoff gewonnen wurde. Die anschließenden Laborverfahren waren für beide Muster gleich. Die mittlere sauerstoffbindende Fähigkeit von Hämoglobin *in vivo* und *in vitro* war fast gleich (1,365 ± 0,010 und 1,366 ± 0,007 ml per g Hb). Als die gemessenen inaktiven Fraktionen von Hämoglobin (Kohlenmonoxyd-hämoglobin und Methämoglobin) in Betracht gezogen wurden, stiegen diese Werte auf 1,392 ± 0,005 bzw. 1,392 ± 0,007 ml per g Hb.

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*IGUALDAD DE LA CAPACIDAD DE LA HEMOglobina, *IN VIVO* E IN VITRO, PARA UNIRSE AL OXIGENO, EN PACIENTES CON GRAVES ENFERMEDADES RESPIRATORIAS*

**RESUMO**

Se determinó la capacidad de la hemoglobina, *in vivo* e *in vitro*, para unirse al oxígeno, en 10 ocasiones, efectuándose las pruebas en 9 pacientes que necesitaron ventilación mecánica. La muestra *in vitro* se sometió al esfigmomanómetro con un 97% de oxígeno por espacio de 10 min y, seguidamente, con aire, mientras que la muestra *in vivo* se obtuvo después de 20 min de ventilar los pulmones con oxígeno puro. Los subsiguentes procedimientos de laboratorio fueron idénticos para ambas muestras. La capacidad media de unión con el oxígeno, por parte de la hemoglobina *in vivo* e *in vitro*, fue casi igual para ambas muestras (1,365 ± 0,010 y 1,366 ± 0,007 ml por Hb). Cuando las fracciones de hemoglobina inactiva medidas (carboxihemoglobina y metahemoglobina) se tuvieron en cuenta, estos valores aumentaron hasta 1,392 ± 0,005 y 1,392 ± 0,007 ml por g Hb, respectivamente.