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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* (Foëx et al., 1970; Theye, 1970; Bursaux, Dubos and Poyart, 1971; Gregory and Millar, 1973; Gregory, 1974; Dominguez 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Ten millilitre of arterial blood was withdrawn from an indwelling catheter and refrigerated immediately (*in vitro* sample), while the lungs were ventilated with variable concentrations of inspired oxygen;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 Pa_{O_2} was 8.59 ± 1.49 kPa. The ventilator was then set to deliver FI_{O_2} 1.0 for 20 min before the withdrawal of another 10 ml of blood (*in vivo* sampling). This FI_{O_2} produced a mean Pa_{O_2} 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 with a saturated water vapour gas mixture of 97% oxygen and 3% carbon dioxide 10 min and subsequently with air for an equal period of time. The *in vivo* aliquots were processed immediately.

The oxygen content was determined in the four aliquots by the Van Slyke micromanometric technique (Van Slyke and Plazin, 1961). The dissolved oxygen ($Pa_{O_2} \times 0.0031$) was subtracted from the measured value to obtain the amount of oxygen combined with haemoglobin. This we refer to as "oxygen content". The concentration of haemoglobin (Hb) was measured by the cyanmethaemoglobin technique in a spectrophotometer (Beckman DU-2, Beckman Instruments Inc., Ill., U.S.A.) according to the International Standards (International Committee for Standardization in Haematology, 1978). Similarly, the fraction of methaemoglobin (MeHb) was measured in the same apparatus according to the technique of Hegesh and others (1970). A Co-Oximeter (IL 182, Instrumentations

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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 (Domínguez de Villota et al., 1976).

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

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

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:

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

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 (Domínguez 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 $99.8 \pm 0.8\%$.

TABLE I. *Mean of differences between duplicate measurements with the techniques used*

	Number	Mean	SD	Range
Haemoglobin (g dl^{-1})	20	0.07	0.09	0.00–0.3
Carboxyhaemoglobin (%)	20	0.07	0.07	0.00–0.2
Oxygen content (ml dl^{-1})	20	0.18	0.12	0.00–0.45
Methaemoglobin (%)	10	0.007	0.009	0.00–0.02

The mean value of the oxygen-binding capacity of Hb, uncorrected for the inactive fractions of Hb, was 1.366 for the *in vivo* and 1.365 ml per g Hb for the *in vitro* samples. When the inactive fractions of Hb were taken into account, both values increased to 1.392 ml per g Hb. No significant differences were found in the paired comparison of the *in vivo* and *in vitro* determinations (table II).

TABLE II. *Mean values of the measurements performed. No significant differences* ($P > 0.05$) *were found by Student's test for paired data*

	In vivo	*In vitro*
Haemoglobin (g dl^{-1})	10.79 (1.46)	10.66 (1.41)
Carboxyhaemoglobin (%)	1.23 (0.52)	1.31 (0.60)
Oxygen content (ml dl^{-1})	14.75 (2.04)	14.55 (1.95)
Methaemoglobin (%)	—	0.62 (0.10)
"Functional" haemoglobin (g dl^{-1})	10.59 (1.45)	10.45 (1.39)
Oxygen-binding capacity (uncorrected) (ml per g Hb)	1.366 (0.007) 1.354–1.376	1.365 (0.010) 1.352–1.379
Oxygen-binding capacity (corrected) (ml per g Hb)	1.392 (0.007) 1.384–1.405	1.392 (0.005) 1.384–1.399

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

(Gregory, Hulands and Millar, 1971, 1972; Scherrer and Bachofen, 1972) because of respiratory impairment. The Hb concentrations 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), 1.34 (Foëx et al., 1970), 1.35 (Guillot et al., 1979), 1.36 (Domínguez 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 (Domínguez 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 (Domínguez de Villota et al., 1976, 1979) and 1.33 (Guillot et al., 1979), but again the corrected figures increased to 1.39 (Domínguez 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; Dijkhuisen 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; Domínguez de Villota et al., 1976). The consistency of our results obtained from ambulatory patients *in vitro* (Domínguez de Villota et al., 1976), from healthy smokers *in vitro* (Domínguez 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; Dijkhuisen 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.

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EGALITE DE LA CAPACITE *IN VIVO* ET *IN VITRO* QU'A L'HEMOGLOBINE DE LIER L'OXYGENE 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 sphygmotonométrie 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 ($1{,}365 \pm 0{,}010$ et $1{,}366 \pm 0{,}007$ ml par g Hb). Lorsqu'on a tenu compte des fractions inactives mesurées de l'hémoglobine (carboxy et méthémoglobine), ces valeurs sont passées à $1{,}392 \pm 0{,}005$ et $1{,}392 \pm 0{,}007$ ml par g Hb respectivement.

GLEICHHEIT DER SAUERSTOFFBINDENDEN FÄHIGKEIT VON HÄMOGLOBIN *IN VIVO* UND *IN VITRO* BEI PATIENTEN MIT SCHWEREN ATMUNGSKRANKHEITEN

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 ($1{,}365 \pm 0{,}010$ und $1{,}366 \pm 0{,}007$ ml per g Hb). Als die gemessenen inaktiven Fraktionen von Hämoglobin (Kohlenmonoxydhämoglobin und Methämoglobin) in Betracht gezogen wurden, stiegen diese Werte auf $1{,}392 \pm 0{,}005$ bzw $1{,}392 \pm 0{,}007$ ml per g Hb.

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

SUMARIO

Se determinó la capacidad de la hemoglobina, *in vivo* e *in vitro*, para unirse al oxigeno, 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 oxigeno 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 subsiguientes procedimientos de laboratorio fueron idénticos para ambas muestras. La capacidad media de unión con el oxigeno, por parte de la hemoglobina *in vivo* e *in vitro*, fue casi igual para ambas muestras ($1{,}365 \pm 0{,}010$ y $1{,}366 \pm 0{,}007$ ml por Hb. Cuando las fracciones de hemoglobina inactiva medidas (carboxihemoglobina y metahemoglobina) se tuvieron en cuenta, estos valores aumentaron hasta $1{,}392 \pm 0{,}005$ y $1{,}392 \pm 0{,}007$ ml por g Hb, respectivamente.