A TRIAL OF THE DIRECT METHOD FOR DETERMINING THE TENSIONS OF O₂ AND CO₂ IN BLOOD

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There is a demand for a quick and reliable method for determining the tensions of O₂ and CO₂ in arterial and venous blood in the investigation of pulmonary and cardiac function. The direct bubble equilibration method described by Riley, Proemmel, and Franke (1945) seems to answer this demand, for within about 30 minutes duplicate analyses may be performed, only 3 ml. of blood is required, and the equipment is simple and relatively inexpensive. But the reliability of the method is in question, for a number of modifications have been reported both from the authors' laboratories (Brinkman, Johns, Donoso, and Riley, 1954; Riley, Campbell, and Shepard, 1957), and from others (Lambertsen, Bunce, Drabkin, and Schmidt, 1952; Filley, Gay, and Wright, 1954; Björk and Hilty, 1954). The purpose of this investigation was to carry out a further trial of the technique and to assess its value for routine investigation and research.

METHOD

Blood was equilibrated in a tonometer and the tensions of O₂ and CO₂ in the gas phase, estimated by Haldane's method, were used as standards against which the tensions, as measured by the bubble equilibration method, were compared.

The tonometers were of 350-ml. capacity and could be closed by a two-way stopcock at one end and a hollowed-out rubber bung at the other; they were filled with varying gas mixtures, prepared by the use of rotameters (Cunningham, Cormack, O'Riordan, Jukes, and Lloyd, 1957). The composition of the gas mixture was known only to the investigator, who carried out all the Haldane analyses after equilibration of the blood.

Human venous blood was collected with heparin as anticoagulant, and immediately 7 ml. was introduced into the tonometer. Equilibration was carried out in a water-bath at 37.5° C.; after 10 minutes the tonometer tap was opened to allow the gas pressure to reach the atmospheric level at the new temperature.

After 40 minutes the tonometer was removed from the water-bath and inverted so that the blood collecting in the end with the hollow bung could be immediately withdrawn into a syringe and promptly transferred into Roughton-Scholander syringes for analysis by the method described by Riley *et al.* (1957). The gas remaining in the tonometer was analysed in samples transferred to Haldane's apparatus.

The gas bubble which was equilibrated with the blood contained $13.3 \pm 0.2\%$ O₂ and 6.1% CO₂ in nitrogen. The gas tensions at 37.5° C. were approximately 95 mm. Hg for O₂ and 43 mm. Hg for CO₂. The bubble volumes were measured with a small travelling telescope attached to a Vernier scale with a hair line in the ocular.*

Estimations were made in duplicate on each sample of blood and the results did not differ by more than 4 mm. Hg for both O₂ and CO₂; the mean of the two were used in the calculation of the regression line. On four occasions out of 38 experiments the bubble fractured and only a single result was obtained. The latter, suitably identified, have been included in the graphic presentation of the results; their inclusion in the calculated regression lines and standard deviations has no significant effect.

RESULTS

CARBON DIOXIDE TENSION.—There is good agreement between the tension determined in the blood by the bubble equilibration method with that obtained by the Haldane in the gas phase of the tonometer (Fig. 1); the regression of the values obtained by the bubble method, on the Haldane figures, has a slope of $0.9593 \text{ S.D.} \pm 2.3 \text{ mm. Hg}$. The regression line crosses the line of identity, that representing perfect agreement between the two methods, at 101 mm. Hg. The necessary correction of the pCO₂ determined by the bubble equilibration method is obtained by finding the difference between the regression line and the line of identity at the point concerned;

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FIG. 1.—Comparison of CO_2 tension obtained in blood by the bubble (direct) method with that of the tonometer gas phase (Haldane). Standard gas mixture used for bubble was $13.3 \pm 0.2\%$ O_2 , 6.1% CO_2 and balance nitrogen. ● = mean of duplicate estimations. ○ = single result, due to bubble fracture.

for practical purposes the subtraction of only 2 mm. Hg is needed in the 20–60 mm. Hg range.

OXYGEN TENSION.—The bubble method underestimates the pO_2 in the higher tension range and overestimates it below 60 mm. Hg (Fig. 2). The slope of the regression line, for the values obtained with the bubble technique on those in the gas phase, is $0.9013 \text{ S.D.} \pm 3.2 \text{ mm. Hg}$. This line crosses the line of identity at 69 mm. Hg, and Table I shows the necessary correction factors at different oxygen tensions.

FIG. 2.—Comparison of oxygen tension obtained in blood by the bubble (direct) method with that of the tonometer gas phase (Haldane). Standard gas mixture used for bubble was $13.3 \pm 0.2\%$ O_2 , 6.1% CO_2 and balance nitrogen. ● = mean of duplicate estimations. ○ = single result, due to bubble fracture.

TABLE I
CORRECTION FACTORS TO BE APPLIED TO OXYGEN TENSIONS OBTAINED BY BUBBLE METHOD OVER DIFFERENT TENSION RANGES*

Oxygen Tension Range (mm. Hg)	Correction Factor (mm. Hg)
40–49	–2
50–60	–1
61–79	0
80–89	+1
90–100	+2

* Standard gas mixture of bubble used in equilibration was $13.3 \pm 0.2\%$ O_2 , 6.1% CO_2 , balance nitrogen.

DISCUSSION

The results demonstrate the limits of reproducibility of the bubble equilibration technique for measuring O_2 and CO_2 tension in blood, as used by us; the standard of accuracy obtainable is sufficient for many clinical and physiological purposes. There are, however, two differences between the results obtained by Riley *et al.* (1957) and those reported here. First, Fig. 1 shows that the bubble equilibration method overestimated the pCO_2 in the 30–60 mm. Hg range and that at higher tensions the regression line and the line of identity were so close that no correction factor was required. Riley *et al.* (1957) only overestimated the pCO_2 in the 0–30 mm. Hg range and underestimated it from 30 mm. upwards. They used a progressively larger positive correction factor with increasing tension. Since the gas bubble used by both of us contained the same tension of CO_2 , these differences are difficult to explain.

Secondly, the results reported for oxygen tension have the same form as those described by Riley *et al.* (1957), that is, oxygen tension as measured by the bubble equilibration technique is overestimated in the low oxygen tension ranges and underestimated above 80 mm. Hg; however, the present correction factors differ from those of Riley *et al.*, not only because of the use of a higher oxygen tension in the gas bubble but also on account of some unexplained factor. Riley *et al.* found that their regression line crossed the line of identity at 85 mm. Hg, close to the tension of O_2 in the standard mixture used for the gas bubble, while our regression line crosses the line of identity at 69 mm. Hg, 26 mm. Hg below the oxygen tension in the gas bubble. This difference between our results and theirs was also apparent in a preliminary trial of 32 experiments in which a gas bubble composed of the standard mixture used by Riley *et al.* was employed; the regression line crossed the line of identity at 60 mm. Hg, again 26 mm. Hg below the tension of the gas

bubble. The results obtained in this trial using the gas mixture with a pO₂ of 95 mm. Hg (13.3% O₂) required no correction factors between tensions of 61 mm. and 79 mm. This is a range in which many physiological and clinical studies will be made; it is for this reason that the oxygen content of the standard gas mixture was changed from 11.65% O₂, that originally suggested by Riley *et al.* (1945).

The differences between the present results and those previously described are difficult to explain, especially as the technique described by Riley *et al.* (1957) was followed carefully. Shepard and Meier (1957) found that differences in results as between operators in the same laboratory were negligible, but suggested that significant differences between operators trained in different laboratories might occur. Our results would seem to fit into the latter category and emphasize the importance of each laboratory establishing its own correction factors.

SUMMARY

The results of another trial of Riley's bubble equilibration technique for measuring O₂ and CO₂ tensions in blood are presented; the standard

deviations of ± 2.3 mm. Hg for CO₂ and ± 3.2 mm. Hg for O₂ are sufficiently accurate for many clinical and physiological purposes.

The differences between the present results and those previously reported demonstrate once more that each laboratory should determine its own correction factors.

A new gas bubble mixture containing 13.3% O₂ and 6.1% CO₂ gave results with the present operators which needed no correction for oxygen in the 60–80 mm. Hg range in which many clinical pulmonary and cardiac studies will be made. The subtraction of 2 mm. Hg was required for carbon dioxide in the 20–60 mm. Hg range.

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