A METHOD TO MEASURE THE TENSION OF CARBON DIOXIDE IN SMALL AMOUNTS OF BLOOD

By J. K. W. FERGUSON

(From the Department of Physiology, University of Toronto, Toronto, Canada)

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This method was designed to measure directly the CO₂ tension of venous blood from isolated gastrocnemius muscles in small dogs. It has been used by Irving, Foster, and Ferguson (1) in the determination of the CO₂ capacity of living mammalian muscle. The principle is that, employed by Krogh and others (2–4), of equilibrating a small volume of air with a relatively large volume of blood, with subsequent analysis of the air. The methods by which this principle had been applied previously were all, for one reason or another, unsuited to our purpose. Moreover, from the viewpoint of general utility, they seemed to leave room for improvement. These considerations led us to attempt the elaboration of this method, which is simple, and easy to apply, and which may be capable of refinement to obtain greater accuracy than we have achieved.

The apparatus consists of two parts (Fig. 1). The first is a combined microtonometer and micro gas analyzer which will be referred to in future as the pipette. It consists of a bulb of 2 cc. capacity blown in glass capillary tubing whose outside diameter is about 5 mm. On one side of the bulb the tubing is 8 to 10 cm. long with an inside diameter of 1 mm. The end is beveled to slide easily into rubber tubing. On the other side of the bulb the tubing is 30 cm. long with an inside diameter of 0.4 to 0.5 mm. At a distance of about 1.5 cm. from the free end, this tube bends at right angles to the axis of the pipette and ends as a small cup, 5 mm. in diameter and depth. The second part is a reservoir for mercury, the capacity of which can be varied at will. It consists of a piece of thick walled rubber tubing about 10 cm. long, into one end of which the short arm of the pipette will fit snugly. The
other end is plugged and the tube is mounted on a metal block in such a manner that it can be compressed by a pair of set screws, one for fine, and the other for coarse adjustments of the volume of the reservoir. Regulation of temperature is obtained by supporting the apparatus horizontally in a large, open water bath equipped with a thermostat. If we had been able to work at room temperature it would have been more convenient to surround the pipette with a water jacket. We had hoped to determine the pCO₂ at room temperature and calculate the pCO₂ at 38°, at constant CO₂ content. A little investigation showed that such a procedure would give sufficiently accurate results only over a range of 4 to 5°.

**FIG. 1.** The pipette is shown connected to the mercury reservoir as it would be when lying in the water bath. P, the pipette, comprises micrometer and micro gas analyzer. A is the cup, which is 5 mm. broad and deep; B, the fine capillary tube of 0.4 to 0.5 mm. inside diameter, 5 mm. outside diameter, and 30 cm. long; C, the bulb of 2 cc. capacity; and D, the coarse capillary tube of 1 mm. inside diameter, 5 mm. outside diameter, and 6 to 8 cm. long. R, the mercury reservoir is essentially a rubber tube of about 4 cc. capacity, plugged at one end, set on a brass block, and compressible by two set screws. F is a brass block; E, a rubber tube of 7 mm. outside diameter and 4 mm. inside diameter; H, a glass reducing tube to join the narrower rubber tube to a wider one of about 1 cm. outside diameter; J, a fine adjustment set screw; K, a coarse adjustment set screw; and L, a glass plug in the end of the rubber tube.

**Manipulation**—Heparin is used as an anticoagulant. When the ordinary dose of about 14 mg. per kilo is injected into a dog, clotting is prevented for several hours, to all gross appearances, but the formation of small white particles of fibrin which tend to clog the capillary still occurs. About three times the stated dose was found to prevent the formation of these particles. As this is an expensive practice, we have tried recently, with good results, injecting about 20 mg. per kilo into the animal, and in addition drying about 0.003 cc. of 10 per cent heparin solution in the pipette.
A small drop of caprylic alcohol is drawn a few cm. up the short arm of the pipette and blown out again as completely as possible. This is sufficient to prevent the blood from frothing in the bulb. The short arm is now connected to a cannula inserted into a branch of the vein from which blood is to be drawn. Intermittent pressure is applied to the main vein proximal to its point of junction with the branch in which the cannula is inserted, to divert blood into the pipette. When all the air except for a small bubble (the size of which may be gauged by the eye or by a mark on the bulb), has been displaced from the bulb, the pipette is brought nearer to the horizontal, allowing the blood to flow past the bubble which is now trapped in the bulb. When blood has filled the fine capillary the pipette is disconnected and laid in a horizontal position in the water bath. By a little adjustment before the immersion of the pipette, a short column of air can be made to occupy the open end of the coarse capillary, preventing blood and water from mingling. 10 minutes seem to be ample time for equilibrium to become established. During this time the pipette is rolled between the fingers at frequent intervals, to insure mixing.

With the bulb wrapped in gauze soaked in bath water, the pipette is momentarily removed from the bath to be connected with the mercury reservoir. The short arm is dried quickly, and held at such an angle that the blood flows down and fills it completely. The beveled end is slipped into the open end of the reservoir, which has been filled to the brim with mercury. The mercury, which is forced up the coarse capillary, displaces blood into the fine capillary and of necessity disturbs the pressure in the bulb. The pipette and reservoir are returned to the water bath. The blood which has been forced into the cup is mopped out of it. 5 minutes more are allowed for equilibrium to become reestablished before the analysis is begun. When this time has elapsed the bulb is wrapped as before, in wet gauze, and the pipette is brought to a vertical position causing the bubble to lie under the opening of the fine capillary. By turning the coarse adjustment screw the bubble is pushed into the capillary till it occupies a length of 15 to 20 cm. When the pipette is brought again to the horizontal the part of the bubble in the capillary will remain there, confined by two columns of blood. It must be mentioned here that the position in which the pipette reelines in the bath is not
truly horizontal, but slightly tilted to allow the brim of the cup to be well clear of the water while the capillary and bulb are immersed. By turning one or the other of the set screws on the mercury reservoir, the bubble is moved slowly along the capillary till the column of blood separating it from the cup is about 2 cm. long. Now the cup is wiped dry and the bubble moved slowly back into the middle of the capillary. Since the column of blood limiting the bubble distally is only 2 cm. long and since it lies in a practically horizontal, uniform capillary, it has little inertia, and exerts on the bubble practically no pressure above that of the atmosphere. When the total length of the capillary is 30 cm. it is of some advantage to use a bubble not longer than 15 cm.; for then, by moving it slowly up and down the full length of the capillary, all fluid adherent to the walls in excessive amount, because of the somewhat hasty introduction of the bubble, may be removed.

The length \( l_1 \) of the bubble is now measured. For this purpose the capillary tubing may be graduated in mm. We have found, however, that the measurement can be made very satisfactorily by laying a steel rule under the tubing. Errors due to parallax can be avoided by looking down into the reflection of your own eye in the water bath. In this way we used home-made pipettes after expensive manufactured ones had been broken. A correction curve was made for each pipette to allow for lack of uniformity in the bore of the capillary.

To absorb the CO\(_2\) from the bubble, a 3 to 4 per cent solution of sodium hydroxide is used. 1 drop of caprylic alcohol is shaken up with about 100 cc. of the solution. This overcomes the tendency of the bubble to break up in the absorber. The bubble is moved along the capillary towards the cup till all but 0.5 cm. of the distal column of blood is in the cup. The latter is wiped out and filled with alkali by means of a medicine dropper. The alkali is drawn into the capillary tube where it mixes with the blood. Since dilute alkali is used, coagulation of the blood with consequent plugging of the capillary need not be feared. The cup, filled to the brim, is inverted. Capillarity keeps the alkali from running out. The pipette must be supported now at an angle of about 15\(^\circ\), that the inverted cup may be clear of the surface of the water. This necessitates a large part of the capillary being out of
water, but that does not matter during the absorption process. The bubble is moved slowly out till it lies almost wholly in the inverted cup. After a pause, it is drawn slowly back into the capillary. Two or three such manipulations suffice to remove all the CO₂. With the bubble drawn back into the capillary the cup is rotated to an upright position and the tube is reimmersed in the water bath. The alkali is removed from the cup, leaving a column about 2 cm. long in the capillary to confine the bubble. The latter is moved slowly back and forth along the tube till repeated measurements show that it has attained a constant length \( l_2 \). 

\[
pCO₂ \text{ (in mm. of Hg)} = \frac{l_1 - l_2}{l_1} \times B \text{ where } B = \text{ barometric pressure.}
\]

The analysis may be repeated on the part of the bubble remaining in the bulb. Two analyses can be made with all precautions in 30 minutes or less.

Sources of Error—As Krogh (2) has pointed out, it would be impossible for the partial pressure of CO₂ in the bubble to equal that in the blood if the sum of the partial pressures of the gases in the blood did not equal the sum of the partial pressures in the bubble. In the pipette the total pressure of the latter must always be practically atmospheric. The total pressure of gases in the venous blood, however, is nearly always below atmospheric. Its total pressure must be increased by the absorption of nitrogen and oxygen from the bubble. Nitrogen, because of its low solubility coefficient can be absorbed in sufficient quantities to equalize the pressures without diminishing perceptibly the volume of the bubble and presumably, without affecting the CO₂ equilibria in the blood. The absorption of oxygen, however, tends to raise the CO₂ tension. With a bubble whose volume is not greater than 0.06 cc., the available oxygen is about 0.012 cc. If this were all absorbed (which could never quite happen) by 2 cc. of blood, the oxygen content of the blood would rise by 0.6 volumes per cent. From a consideration of the CO₂ dissociation curves of oxygenated and reduced blood it is seen that such an increase in oxygen content could not produce a rise in \( pCO₂ \) greater than 1 mm. of Hg. On the other hand, the loss of CO₂ to the bubble, even when the tension is as high as 150 mm. does not diminish the tension by more than 1 mm. of Hg. The larger the bubble which can be used,
the larger may be the capillary in which it is analyzed. In pre-
liminary experiments we found that the use of a capillary of 0.25
mm. bore was unsatisfactory because it frequently became plugged
and often could not be cleaned out. A capillary of 0.4 to 0.5 mm.
bore rarely becomes plugged, and in all respects is handier to
work with. In a capillary of 0.4 mm. bore a bubble of 0.06 cc.
occupies a length of about 47 cm., while in a capillary of 0.5 mm.
bore a bubble of that volume occupies a length of 30 cm. A
length of 15 to 20 cm. is desirable for each analysis. If it is any
shorter, the quantity \((l_1 - l_2)\) becomes so small that an error of
0.1 mm. in both \(l_1\) and \(l_2\) will make an error of 2 to 3 per cent
in \((l_1 - l_2)\) when the CO₂ content of the bubble is about 6 per cent.

In addition to the difficulty of temperature regulation, working
at 38° presents another disadvantage in that glycolysis becomes a
factor of importance. In experiments conducted over a period of
4 or 5 hours it was found that the tension might increase at a rate
of 15 to 20 mm. of Hg per hour. However, it does not always in-
crease so rapidly and sometimes does not increase at all.

Two methods of combatting this source of error may be adopted.
First, the time of equilibrating and analysis may be cut down to a
minimum so that only 20 to 30 minutes elapse between the drawing
of the blood and the beginning of the second analysis. This may
be done by making the first equilibration last only 5 to 10 minutes.
In a large number of cases with dog blood, this procedure seemed
to be fairly efficacious, for in less than half the experiments did
the second analysis give a higher result than the first. The second
method is safer when a speedy determination cannot be made, or
when the CO₂ tension is low. It consists of drying enough sodium
fluoride solution in the pipette to make a 0.05 per cent solution in
the blood. This procedure was effective in all cases where it was
tried. Typical experiments are illustrated in Fig. 2, which rep-
resents graphically the results of successive determinations made
over a period of 4 hours, with and without sodium fluoride.

Loss of CO₂ may occur from the surface of the blood as it is
filling the bulb of the pipette. To evaluate this source of error
and others which may be considered as systematic, such as the
possible effect of allowing the alkali to moisten the capillary, the
method was compared with a macrotonometric method. 5 cc. of
blood (usually human) containing sufficient heparin (about 2
mg.) and 1 drop of caprylic alcohol were placed in a tonometer of 400 cc. capacity. The latter was flushed with a 6 to 8 per cent mixture of CO₂ in air. Half an hour was allowed for equilibrium to become established, during which time the tonometer was kept rotating. The blood was then collected in a well at the bottom of the tonometer whence it was drawn by suction through a tap into the pipette. From a tap at the other end of the tonometer a sample of gas (about 30 cc.) was taken into a Brodie sampling pipette and later analyzed for CO₂. The equilibration was done in the macrotonometer at room temperature in a gas analysis room where changes in temperature were relatively slight (0.5° per hour). The pipette, however, was surrounded by a water jacket.

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**Fig. 2.** This diagram illustrates how CO₂ tension may rise (presumably because of lactic acid formation by glycolysis) in blood kept at constant CO₂ content at 38°. It shows too the efficacy of adding sodium fluoride (0.05 per cent) to prevent rise in CO₂ tension.
CO₂ Tension of Blood

containing water at room temperature. The results are given in Table I. The average of the positive and negative discrepancies in fourteen consecutive experiments shows that the micro-

TABLE I

The CO₂ tension of blood which had been equilibrated with a CO₂ mixture in a large tonometer was determined in a microtonometer and compared with the tension of CO₂ in the large tonometer as measured in a Haldane gas analyzer. Fourteen consecutive experiments are reported below.

<table>
<thead>
<tr>
<th>Date</th>
<th>CO₂ tension in large tonometer (gas analysis in Haldane apparatus)</th>
<th>CO₂ tension in small tonometer</th>
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Average...... 54.6 52.8 53.2 54.5

Though the figures in each column are not measurements of the same quantity, the averages for each column are calculated because they show at a glance whether the microtonometric determinations tend to be higher or lower than those from the large tonometer. (The difference between the mean of each of the three right-hand columns and that of the first is numerically equal to the mean of the positive and negative deviations of the measurements in each of the small tonometer columns from those in the large tonometer column.)

tonometer gives a result 1.6 mm. lower than the macrotonometer. This can hardly be regarded as a significant difference.

To evaluate the probable error of a single determination on the microtonometer, under the conditions in which it is being used in practice, two methods were employed. In the first way several
pipettes were filled with blood from a glass syringe, in which the blood had been well mixed and protected against glycolysis by addition of sodium fluoride. Two determinations were made on each pipette so that a series of about eight figures, which should have the same value, were obtained and from which a mean, average deviation, and probable error could be calculated. The probable error was taken to be 0.845 × (the average deviation of a single determination). A series of twenty such determinations gave a probable error of 1.2 mm.

An idea of the probable error may be obtained too from the results of the animal experiments in which a large number of tensions varying from 18 mm. to 120 mm. have been measured with duplicates on each sample. In this series of 72 consecutive determinations, fluoride was not used, glycolysis being left as an added source of error.

(The average discrepancy, in mm. of Hg, of duplicate measurements) ÷ 2 is taken as the average deviation of a single determination. Probable error = 0.845 (average deviation). The probable error of a single determination calculated in this manner = 1.98 mm. of Hg.

When two pipettes were filled with venous blood from the same muscle, another source of error was included; viz., the possibility of change in the CO₂ tension of the venous blood in the few minutes which necessarily elapsed between filling of the first and second pipette. For this reason these figures were kept separate and constitute another series of thirty-two consecutive experiments. The probable error calculated from this series is 1.7 mm. of Hg, indicating that the added source of error could not have been very important. From the foregoing we may conclude that the probable error of a single determination is less than 2 mm. of Hg in these experiments.

**SUMMARY**

A method to measure directly the tension of carbon dioxide in 2 cc. of blood is described. The principle is that of equilibrating a bubble of air with the blood and analyzing the bubble in a capillary tube. One instrument, the pipette, includes both analyzer and microtonometer. In the analyzer the bubble is confined by blood. CO₂ is absorbed with 3 to 4 per cent NaOH. The prob-
able error of a single determination made as it was in a large number of experiments, is less than 2 mm. of Hg. With certain precautions it may be reduced to about 1.2 mm. of Hg.

In conclusion I wish to express my gratitude to Dr. Laurence Irving for his advice and encouragement, and to thank Mr. H. C. Foster, who made many of the determinations, of which the results are used in this paper.

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