

THE CALIBRATION OF THE OSTERHOUT RESPIRATORY
APPARATUS FOR ABSOLUTE QUANTITIES OF CARBON
DIOXIDE.

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In the course of a piece of work on the amount of carbon dioxide produced by sea anemones it was found desirable to use the respiratory apparatus devised by Osterhout (1918), and as it was necessary in this work to determine the absolute quantity of carbon dioxide eliminated by these animals, an attempt was made to calibrate this apparatus for such determinations. The method finally adopted seems to be of enough general importance to justify a brief statement of it.

The Osterhout apparatus consists of a closed system in which the air is made to circulate by means of a pump. The air passes from a chamber, in which the organism is confined and in which consequently the carbon dioxide is produced, either directly to a glass tube containing an indicator in solution or indirectly to this tube through a U-tube filled with fragments of sodium hydroxide. In the direct course the air containing the carbon dioxide is forced to bubble through the indicator solution, thus causing it to change its tint. In the indirect course the air is relieved of its carbon dioxide by contact with the sodium hydroxide, and, after purification, it is bubbled through the indicator thus washing out the contained carbon dioxide and bringing the indicator back to its original color. From the tube containing the indicator the air returns by a direct course through the pump to the chamber in which the organism is confined. A figure of the apparatus is given by Osterhout (1918). In taking readings with this apparatus the time in seconds required for the indicator to change from one tint to another as compared with standard solutions of known pH value is recorded and the rate

of this change is assumed to be identical with that of the excretion of carbon dioxide by the organism.

In calibrating the apparatus various means were used to produce a constant and measurable flow of carbon dioxide as a substitute for that from the organism. About 100 cc. of a normal solution of sulfuric acid were poured into the chamber in which the organisms had been kept, and into this were dripped at a constant rate solutions of sodium bicarbonate of different but known concentrations. The rates of change in the indicator did not correspond to the calculated rates of liberation of carbon dioxide from the solutions, and it soon becomes apparent that all the carbon dioxide produced was not liberated but that part of it remained behind in the solution. The dripping of weak acid on dry sodium bicarbonate or on marble also failed to yield consistent results.

The method of calibration finally adopted was that of making mixtures of carbon dioxide and atmospheric air and of introducing them at a known rate into the apparatus in place of the organism. Into a narrow graduated glass receiver filled with water carbon dioxide was bubbled till a given volume at atmospheric pressure was attained, whereupon, the remainder of the receiver was quickly filled with atmospheric air and the whole closed. This operation was carried on over a water bath so quickly that the solution of the carbon dioxide in the water was reduced to a negligible amount. Mixtures of gases containing 0.4, 1, 2, and 4 per cent of carbon dioxide were used. Each of these mixtures was introduced through a connecting tube into the chamber of the apparatus in which the organism had been kept by running into the gas receiver at a known rate a minute stream of mercury and thus driving out the mixture of gas. As the glass gas receiver was graduated, the amount of gas driven out of it in any given time could be easily read off by observing the levels at which the mercury stood in it as shown by the scale on its wall. Thus a constant and measurable supply of carbon dioxide was substituted for that produced by the organism. In this form of procedure the rate of change in the indicator corresponded very closely to the rate of introduction of carbon dioxide. Since the volume of mixed gases introduced into the apparatus in any test was very small in comparison with the total volume of the apparatus,

no attention was given to the slight increase of density that must have ensued in the course of a test by introducing gas into an apparatus already filled with air at atmospheric pressure.

The indicator used in these tests was an aqueous solution of phenolsulfonephthalein (Hynson, Westcott, and Dunning), and the times in seconds necessary to change its tint from that characteristic for pH 7.78 to 7.36 (Osterhout and Haas, 1918), at the four concentrations of carbon dioxide used, are given in Table I.

The steps necessary to determine the amount of pure carbon dioxide in ten-thousandths of a milligram delivered per second to

TABLE I.

Times in Seconds Needed to Change a Standard Solution of Indicator (Phenolsulfonephthalein) from pH 7.78 to 7.36 by the Introduction into the Osterhout Respiratory Apparatus of Four Mixtures of Gases Containing Respectively 0.4, 1, 2, and 4 Per Cent of Carbon Dioxide.

Concentration of carbon dioxide, per cent.....	0.4	1	2	4
Time to change indicator from pH 7.78 to 7.36, sec.....	569	218	106	58
	559	228	104	58
	563	214	106	56
	558	221	108	58
	564	229	106	57
Average time.....	562.6	222.0	106.0	57.4
Rate $\left(\frac{1}{\text{average time}}\right)$	0.00178-	0.00450+	0.00943+	0.0174+

the apparatus are shown in Table II. In the table are given: (A) the percentage concentration of the carbon dioxide mixture, followed by the time (B) required to deliver 10 cc. of this mixture to the apparatus. By dividing 10 cc. by the number of seconds needed to deliver that amount of gas to the apparatus, the volume of gas delivered per second was found (C). By multiplying this volume by the appropriate per cent indicating the proportion of the impure carbon dioxide contained in the given mixture, the several volumes of impure carbon dioxide delivered were determined (D). By absorbing with sodium hydroxide in a graduated tube a sample of

the carbon dioxide used, it was found that this gas was pure to the extent of 97.2 per cent and on introducing this correction into the calculation, the volume of pure carbon dioxide delivered per second

TABLE II.

Observed Times in Seconds for the Delivery of 10 Cc. of the Four Gas Mixtures Containing Respectively 0.4, 1, 2, and 4 Per Cent of Impure Carbon Dioxide (B), Calculated Amounts in Hundred-Thousandths of a Milligram of Pure Carbon Dioxide Delivered per Second to the Apparatus (F), and Calculated Constants for the Apparatus (G).

A. Impure carbon dioxide, per cent.....	0.4	1	2	4
B. Observed times for delivery of 10 cc. of mixed gas, sec.....	297	296	293	298
C. Calculated volume of gas delivered per sec. ($= \frac{10}{297}$ etc.), cc.....	0.003367+	0.003378+	0.003413-	0.003356-
D. Calculated volume of impure carbon dioxide delivered per sec. ($= C \times A$), cc.....	0.00001347-	0.00003378+	0.00006826-	0.00013424-
E. Calculated volume of pure carbon dioxide delivered per sec. ($= D \times 0.972$), cc.....	0.00001309+	0.00003283+	0.00006635-	0.00013048+
F. Calculated weight (in hundred-thousandths of a milligram) of pure carbon dioxide delivered per sec. ($= E \times 1.75984 \times 100,000$).....	2.304-	5.777+	11.676+	22.962+
G. Constants for apparatus ($=$ Average time, Table I, \times F.).....	1,296+	1,282+	1,238-	1,318+

in each of the four tests was calculated (E). To change the quantitative determinations of carbon dioxide from volumes to weights, the volumes of this gas in cubic centimeters were multiplied by 1.75984,

the weight in milligrams of 1 cc. of carbon dioxide at 24°C. and 762 mm. of barometric pressure, the conditions of the test. The result of this operation was then multiplied by 100,000 to permit the final number to be expressed in hundred-thousandths of a milligram (F). In this way the weight of carbon dioxide delivered per second and expressed in hundred-thousandths of a milligram was arrived at.

In the use of this apparatus it is assumed that equal concentrations of carbon dioxide produced the same color tints in the indicator. Since in all four tests the readings were always begun at the same tint and ended at another tint always the same, it follows that the amount of carbon dioxide delivered per second in each test multiplied by the number of seconds over which the test extended ought to yield a constant. And such seems to be the case, as is shown in the last line of Table II in which the product of the average times (Table I) by the weights of carbon dioxide delivered per second are given. These constants vary from 1,238- to 1,318+ and average 1,283.5; they are a measure of the amount of carbon dioxide necessary in this particular piece of apparatus to change the indicator from the tint characteristic of pH 7.78 to that for pH 7.36.

Another way of expressing these relations is shown in the graph (Fig. 1) in which the weights in hundred-thousandths of a milligram of carbon dioxide delivered are plotted as abscissæ, and the rates at which the indicator changed as ordinates. As the plotting shows, the relations are clearly linear.

In using these results to determine the absolute amount of carbon dioxide produced by an organism, it is convenient to express them in the form of an equation thus:

$$K = T \times W$$

where K is the constant already determined for the apparatus, T the time in seconds for the change in the indicator from one pH value to the other, and W the weight of carbon dioxide in hundred-thousandths of a milligram delivered per second. As the constant and the time in the operation described are the known factors and the weight the desired one, the most convenient statement of the equation is

$$\frac{K}{T} = W$$

and it is in this form that I have used it in calculating the weights of carbon dioxide produced by sea anemones. Thus in one instance a sea anemone weighing 0.5 gm. brought about the necessary color change in the indicator in 424.8 seconds. This animal must, therefore, have produced $1,283.5/424.8$ or $3.0+$ hundred-thousandths of a milligram of carbon dioxide per second. Another one also weighing 0.5 gm. brought about the same change in 420.4 seconds and by a similar calculation can be shown to have produced 3.1 hundred-

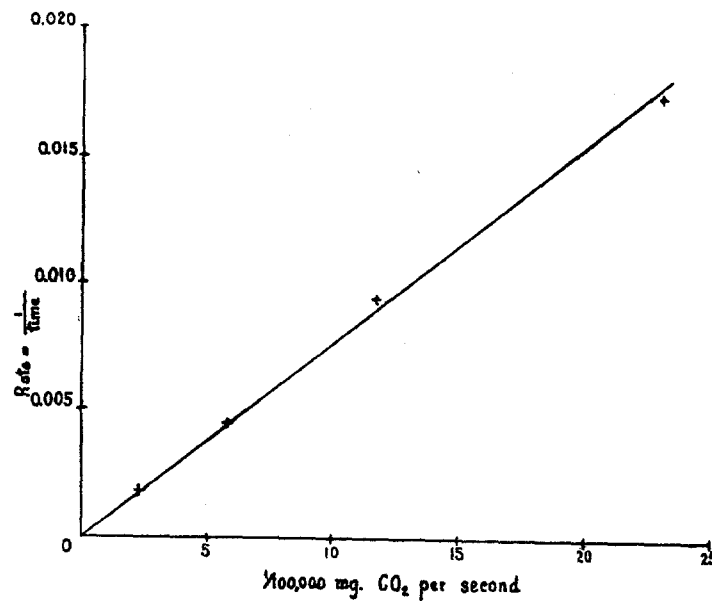


FIG. 1.

thousandths of a milligram of carbon dioxide per second. The two animals together changed the indicator over the requisite range in 213 seconds which when used as a basis of calculation yield $6.0+$ hundred-thousandths of a milligram of carbon dioxide per second or almost exactly twice that of the sea anemones taken separately. Thus the proposed formula affords an easy means of calculating the absolute amount of carbon dioxide excreted when the time of the indicator change and the apparatus constant are known.

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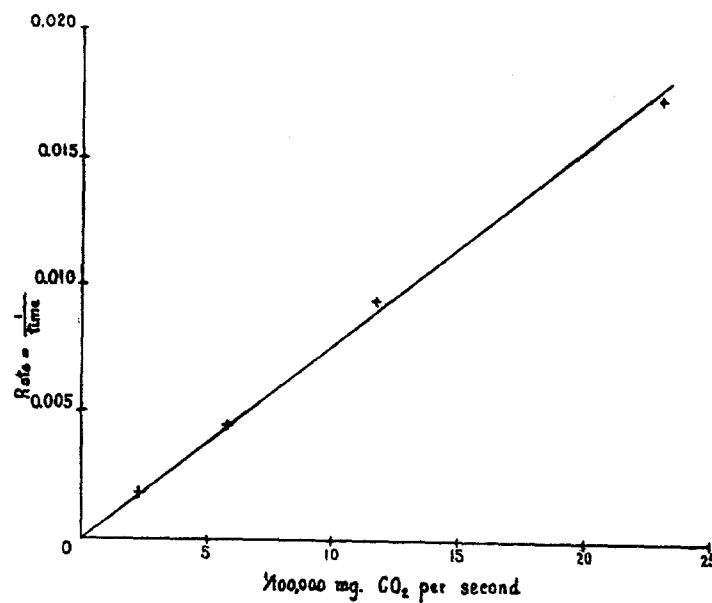


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It must be clear from the foregoing account that each apparatus will have its own constant, and that anyone who wishes to calibrate such an apparatus for the absolute amount of carbon dioxide produced will first have to determine this constant by some such method as that already described. Once, however, having made this determination, the formula already given may be used with perfect accuracy for the calculation of the absolute amount of carbon dioxide produced. Since the constant is a measure of the amount of carbon dioxide necessarily present in a given piece of apparatus, in order that a standard change in the indicator shall take place, and since this amount is spread through the space contained within the apparatus, it is probable that the constant is directly related to the volume of the apparatus and will be small in an outfit of small volume and large in one of large volume, but such relations have not as yet been worked out.

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