A METHOD OF STUDYING RESPIRATION.

By W. J. V. OSTERHOUT.

(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)

(Received for publication, July 2, 1918.)

In 1915 the writer suggested to Dr. Haas the desirability of experiments with indicators with a view to measuring the amount of CO₂ given off by organisms. The method finally developed by Dr. Haas in the writer's laboratory consists in adding the indicator directly to the liquid in which the organism is placed. The indicator is usually present at the start, but in some instances it is not added until after the CO₂ has been produced.

The method is simple, accurate, and extremely satisfactory but it has obvious limitations. It is restricted to the use of aquatic organisms and furthermore it does not permit us to study the effect upon respiration of reagents which have a pronounced acid or alkaline reaction. There are also difficulties in using organisms or tissues which give off alkali or acid (other than CO₂). In addition, the use of small suspended organisms which color the solution must be avoided. A further disadvantage is that some reagents cause organisms to give off coloring matters which interfere with the results. The use of toxic indicators also presents difficulties.

These difficulties may be obviated by means of an apparatus designed by the writer. The purpose of the present article is to make clear the principle employed and to describe a simple form of the apparatus without mentioning other forms or discussing the modifications of mechanical details which are of importance for special purposes.

The apparatus shown in Fig. 1 consists of a bottle, A, in which the organisms are placed; the CO₂ which they exhale passes out of A through D into the indicator solution in the Pyrex glass tube, B.

---

2 The tube which dips into the indicator solution should be of Pyrex glass. This glass is chosen because the amount of alkali given off is negligible.
3 This tube should be about ½ inch in diameter; the stopper should be covered with a paraffin which does not give off acid.
METHOD OF STUDYING RESPIRATION

returning through the rubber syringe, F, into A. A constant circulation is kept up by the motion of K, each downward movement of which compresses the syringe, F, forcing a stream of bubbles through the indicator solution in B; K is attached at one end to the hinge, L, and at the other to the connecting rod, G, which moves when H is made to revolve by means of a small motor. The syringe,

![Fig. 1. Apparatus for the measurement of respiration and photosynthesis. The organisms are placed in A; by compressing the syringe, F, the CO₂ is forced through the indicator solution in B, returning through F into A. The wheel, H, is caused to rotate by means of a motor; the resultant motion of K compresses F and keeps up a constant circulation of gas.](image)

4 This has valves at the outlet and inlet. In place of the syringe a tambour-bellows, pump, miniature fan, or other simple device may be employed.

5 It is desirable to have the gas circulate at a constant rate. The rate may be controlled in a number of ways; it may be tested by observing the rate at which the color of the indicator changes, when a stream of air, free from CO₂, runs through it.

6 If the stream of bubbles does not flow satisfactorily some of the solution may be removed so that the top of the tube is brought nearer to the surface. Inclining the tube, B, may assist in freeing the bubble as it issues from the tip of the tube. The compression of the syringe may also be increased and a syringe with stiffer walls may be used. If this does not produce the desired result the elasticity of the system must be increased by making the rubber connections longer, or by using softer rubber; or, better, a small elastic bulb may be introduced at any convenient place.
F, rests upon a board which is held in position by a support not shown in the drawing. The amount of compression of F can be varied either by shifting F or by altering the point at which G is attached to H.

A and B are provided with tightly fitting stoppers into which the tubes are fastened in air-tight fashion. All other connections should likewise be air-tight.

The carbon dioxide produced by the organism is absorbed with surprising rapidity by the indicator solution, provided a rapid circulation of air is maintained. This is evident from the fact that when known amounts of CO₂ are introduced into A the CO₂ is at once carried over into B and equilibrium is quickly established.

Ordinarily it is not important to know the absolute amount of CO₂ produced by the organism, since we are concerned only with comparative values. If for example we wish to compare the normal rate of respiration with the rate under the influence of ether, we proceed as follows: We place the organisms in A and close the clamp, D, opening C and E so that the air passes through the U-tube (containing an absorbent of CO₂, such as lumps of NaOH) into the indicator solution in B. The indicator solution may consist of distilled water (free from CO₂) to which enough NaOH has been added to make its pH value about 7.3; phenolsulfonephthalein is added, so that the solution becomes pale pinkish in color. As the gas passing into B is practically free from CO₂ little or no change will be produced by its circulation; it should, however, be allowed to circulate until the color of the indicator has become constant. The clamps, C and E, are now closed and D is opened, allowing the CO₂ given off by the organisms to pass into B. The time required to produce a clearly marked change in the color of the indicator is noted and buffer solutions (contained in Pyrex glass tubes of the same size as B and having the same concentration of indicator) are selected which match the color of the indicator at the start and finish. The clamp D is now closed; C and E are opened, thus washing the CO₂ out of B until the indicator returns to

7 In place of the U-tube a tower or potash bulb may be employed.
8 When a volatile reagent, as ether, is added, the substance in the U-tube must be one which does not absorb the reagent.
9 Unless the joints are tight CO₂ may leak in.
METHOD OF STUDYING RESPIRATION

the original color. This is repeated until the normal time of respiration is established (unless it is fairly constant the experiment should be rejected).

The reagent is now introduced into A (by means of the short tube) and the time is ascertained which is required to produce the same change in the color of the indicator. A comparison of this with the normal time gives the relative rate of respiration under the influence of the reagent.

The amount of CO₂ produced by the organisms is ascertained by comparing the color of the indicator with the colors of a series of buffer solutions having the same concentration of indicator and contained in Pyrex glass tubes of the same size as B. This determines the pH value of the solution. In order to find out how much CO₂ must be evolved to produce a given change in the color of the indicator we introduce known amounts of CO₂ into the apparatus by means of a device which has recently been described. The gas is made to circulate in the apparatus until the introduced CO₂ is thoroughly mixed with the air and with the indicator solution, so that equilibrium is established between the latter and the CO₂ in the apparatus. We know that this has occurred when continued circulation fails to produce any further change in the color of the indicator.

Instead of introducing gaseous CO₂ we may introduce a solution whose CO₂-content is known from its pH value.

The change in the color of the indicator produced by a given amount of CO₂ will vary according to the volume of air in the apparatus (the amount of indicator solution being constant). When determinations have been made for a number of volumes, intermediate values may be obtained by interpolation. As a rule there will be no changes in volume except those produced by variations in the volume of the organisms which are introduced into the apparatus.

Ordinarily the respiration of aquatic organisms may be studied by the method described by Haas but such organisms as impart a color to the solution or give off acid (other than CO₂) or alkali may be

---

10 This should be done, if possible, under a "Daylight" lamp.
12 This can be done by means of the short tube in the stopper of A.
studied by means of the apparatus here described. In this case A is partly filled\textsuperscript{14} with liquid,\textsuperscript{15} and due allowance must be made for this in calibrating the apparatus with respect to absolute amounts of CO\textsubscript{2} required to produce a given change of pH value. This calibration must be revised if any reagent is added which has a pronounced buffer action; this should also be done when A is filled with gas if any volatile substance with a pronounced buffer effect (or with an acid or alkaline reaction) is used.

In general it would seem to be desirable to have A large enough to contain a number of organisms, so as to reduce as much as possible the irregularities which may occur when small numbers are used. But if the amount of CO\textsubscript{2} given off is small, it may be necessary to reduce the size of A by substituting a U-tube for the bottle. The size of F may also be reduced by substituting a smaller bulb, or by using in place of a bulb a tambour provided with valves.

Whenever it becomes desirable to sweep out the exhaled CO\textsubscript{2} and to fill the apparatus with ordinary air, this can be easily and quickly accomplished, without disturbing the organism. It is only necessary to remove the U-tube and to close the screw clamp, D, (which is usually open) and open the screw clamps, C and E, (which are ordinarily closed) and then to start the motor and allow the gas to circulate, passing out of the apparatus at E. Air will then enter at C and will quickly wash out the excess of CO\textsubscript{2}. When this is accomplished (as shown by the color of the indicator), C and E are closed and D is opened.

Ordinarily it will be found desirable to remove the excess of CO\textsubscript{2} at frequent intervals. In this case phenolsulfonephthalein will be found useful as an indicator.\textsuperscript{1} If, however, CO\textsubscript{2} is allowed to accumulate, other indicators or mixtures of two or more indicators may be used to measure the lower pH values.

\textsuperscript{14} Except in cases where the organisms respire normally when merely moistened and kept in a saturated atmosphere. Ordinarily the air will become partly saturated with the moisture taken up from the indicator solution. If this is not desirable, in the case of non-aquatic organisms, it can be obviated by adding something to the indicator solution to lower vapor tension or by drying the gas on its way from B to A.

\textsuperscript{15} The tube by which the gas enters A is drawn to a point and brought down to the bottom of A to permit the gas to bubble through any liquid contained in A.
In case it is desired to withdraw a sample of gas in order to determine the amount of O₂ consumed, it is only necessary to intercalate a glass tube in the neighborhood of D, with stop-cocks at each end which can be closed when the tube is removed.

It is evident that this method is just as applicable to photosynthesis as to respiration. In studying photosynthesis it may be desirable to introduce known amounts of CO₂ into the apparatus at the beginning of the experiment.

In conclusion it may be added that in carrying out such investigations it is desirable to compare the times required to produce equal amounts of CO₂ rather than to compare the amounts of CO₂ produced in equal times. The former method compares reaction velocities while the latter may not.

**SUMMARY.**

An apparatus is described which makes it possible to measure rapidly and accurately small amounts of CO₂ given off by organisms of all kinds. The apparatus can also be used to measure photosynthesis.