A METHOD FOR THE DETERMINATION OF DISSOLVED CARBON DIOXIDE.

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The determination of carbon dioxide, liberated in respiration experiments by aquatic animals, is especially difficult and uncertain. This is due partly to the fact that the quantity liberated is only a small fraction of the total quantity dissolved in the water in the form of carbonates, bicarbonates, and free carbon dioxide, and partly because the animals experimented on are often likely to give off substances which may interfere with the results obtained in many of the methods for the determination of carbon dioxide. It is known that urine, feces, and mucus will yield some carbon dioxide when the water in which they have been excreted is boiled after acidification. Some observers have also noted that acids may be given off by some aquatic animals, fish for example, in such amounts as to interfere with the results obtained by titration methods (Krogh, 1916).

Morgulis and Fuller (1916) have shown that the titration methods for the determination of carbon dioxide, especially by using phenolphthalein as an indicator, give a wide range of error. They conclude that the results obtained by such methods are of questionable value and no accurate conclusions can be derived from them. Johnston (1916) states similar objections.

The vacuum extraction principle has certain advantages over that of many of the other methods employed for the determinations of dissolved gases. (1) The relatively short time within which an equilibrium is attained between the gas content of water and the atmosphere; (2) the gases are obtained and may be directly measured volumetrically; (3) there is a non-interference of metabolic products.
Determinations of Dissolved CO₂

Apparatus.

The apparatus used in the determination of free and combined carbon dioxide in water consists in general of three parts: an evacuation tube, a measuring burette, and an absorption pipette (Fig. 1). The evacuation tube is constructed on the principle of the well known Van Slyke blood gas apparatus (Van Slyke, 1917; Van Slyke and Stadie, 1921). Because of the large amount of the sample necessary to yield sufficient carbon dioxide for accurate determination the Van Slyke apparatus cannot be used for dissolved carbon dioxide in water satisfactorily. As in the Van Slyke apparatus a Torricellian vacuum is produced by a column of mercury. The apparatus is constructed of strong glass in order to withstand the weight of the mercury without danger of breaking. A cup, A, to which is attached a three-way stop-cock, I, is calibrated into 10 cc. divisions, the total capacity being approximately 100 cc. Below this cup is the evacuation pipette, B, with a capacity of 250 cc. The mercury is drawn from the pipette through a three-way stop-cock, 2, by way of C' or C. D serves as a receptacle for the mercury having a capacity of about 500 cc. A fine bore burette, F, is calibrated into 0.01 cc. divisions. The total capacity is about 5 cc. This burette is surrounded by a large glass tube, G, for the purpose of maintaining a constant temperature during the analysis. A mercury receptacle, L, is used for levelling the height of the mercury in the burette. Between the evacuation pipette and the burette is inserted a three-way stop-cock, 3. The outlet, K, serves as a means of emptying the evacuation pipette and also in enabling the operator to fill the tubes N and N' with greater ease. An absorption pipette, P, filled with 10 per cent potassium hydroxide is used for the absorption of the carbon dioxide. A fine bore tube, H, enables the operator to level accurately the mercury in the measuring burette.

Method.

The procedure for the determination of carbon dioxide is as follows: The air in the tube, H, is made carbon dioxide-free by absorption in the alkali, by methods that are commonly used in gas analysis apparatus. The potassium hydroxide is brought to
Fig. 1.
Determination of Dissolved CO$_2$

the mark, $M$. Stop-cock 4 is then turned so as to allow the mercury to rise to stop-cock 3. The evacuation pipette, $B$, is then filled with mercury, stop-cock 3 is turned so as to allow a small amount of mercury to rise in outlet tube $K$. The apparatus is then ready for the introduction of the sample.

25 or 50 cc. of the water to be analyzed are introduced into the cup, $A$, by using the calibration or by pipette. A pipette is usually found the more practicable. Where the free carbon dioxide is high in amount a few drops of paraffin oil are first placed in the cup which forms a thin film on the surface of water, preventing to a large extent the escape of gas. The sample is slowly drawn into the evacuation pipette by lowering the mercury receptacle $D$, the oil remaining in the cup. Stop-cock 1 is tightly closed and the evacuation begun. The mercury is lowered in the evacuation pipette to the 250 cc. mark. After standing for about a minute the water is trapped in $C'$ and the mercury allowed to rise in the pipette through $C$. The gas liberated is allowed to displace the mercury in the tubes $N$ and $N'$. When the total carbon dioxide determinations are to be made 10 cc. of 10 N sulfuric acid are introduced, following the sample, and evacuation proceeds in a similar manner to that described.

Several evacuations are usually necessary to liberate completely all of the carbon dioxide present; ten will suffice in most cases. Shaking is not practical with such a large apparatus. Repeated evacuations appear, however, to be as efficacious, especially when the sample is trapped in $C'$ and the pipette $B$ filled from $C$. After the gas liberated has been expelled the mercury is drawn back through $C'$ in a fine spray. This greatly increases the surface of the liquid exposed, thus hastening the liberation of the gases.

When all the gas has been expelled and collected in the burette the mercury is raised so as to flow into the tube $N$, forcing the gas into the burette $F$, none remaining in the tube. The stop-cock 4 is then turned, connecting the burette with the absorption pipette $P$, care being taken that the mercury in the levelling tube $L$ is very nearly the same level as that in the burette. The potassium hydroxide is brought exactly to the point $M$. A reading is then made on the burette. The gas is then forced over into the absorption pipette several times. When a constant
reading is obtained the amount absorbed is determined. This represents the volume of carbon dioxide liberated from the sample at a given temperature, pressure, and vapor tension. The air is kept saturated with water vapor. The volume is reduced to standard conditions by the formula:

\[ V_0 = \frac{(B - p) V}{760 (1 + 0.003667 t)} \]

\( V_0 \) is the volume of dry gas at 0°C. and 760 mm., \( B \) being the barometric pressure, \( p \) the pressure of aqueous vapor at \( t^\circ \mathrm{C} \), \( V \) the volume of the gas at \( t^\circ \) and the observed pressure, and 0.003667 the coefficient of cubic expansion of gases.

The apparatus described serves as a convenient and an accurate means of determining the amount of carbon dioxide dissolved in water. It is especially useful in the analysis of water during respiration experiments and in other analyses where titration methods appear inadequate.

BIBLIOGRAPHY.

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