A VOLUMETER FOR RESPIRATION OF AQUATIC ANIMALS[1]

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The methods for measurement of respiration of small aquatic animals are rather limited. The Wrinkler titrimetric method (Birge and Juday, 1911; Allee and Oesting, 1934) has proved not only laborious but also time-consuming for long-term routine determinations. The respirometric method described here has many advantages over it: (1) continuous oxygen consumption determinations can be made for several hours without disturbing the experimental animals, (2) the respirometric technique involving less manipulations is accordingly subject to less experimental error, (3) the technique gives more linear rates of oxygen consumptions than does the Wrinkler technique, especially when these rates are of low order (fig. 2).

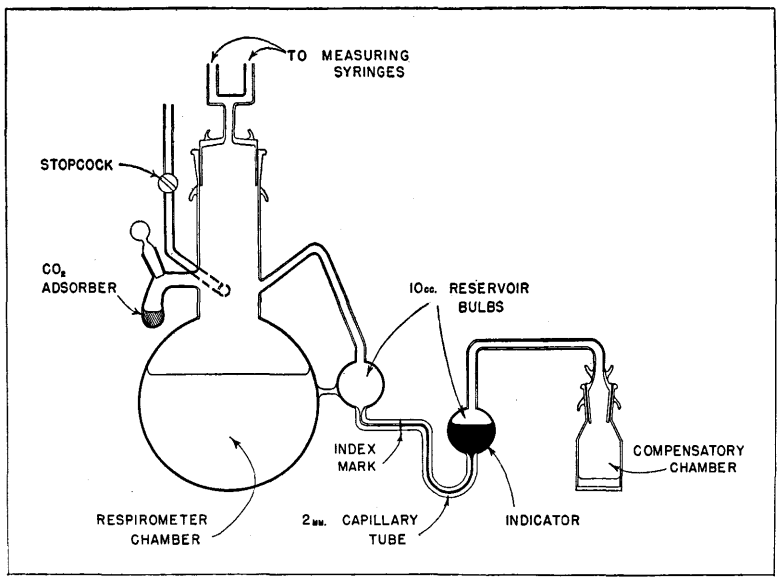

FIGURE 1. The volumeter used for respirometric studies (see text for discussion).

The method to be described here (Calhoon and Angerer, 1949) is based on the same general principle as that employed by Peiss and Vennesland (1949) and Vennesland (1951). It consists in making determinations of volume changes for a closed system maintained at constant pressure. Any decrease in gaseous pressure (oxygen consumed) is compensated by a decrease in total volume. The decrease in volume is measured by an appropriate movement of pistons having known displacements.

The main flask, the respiratory chamber (fig. 1), is of *ca* 500 cc capacity and contains the animal whose oxygen consumption is to be determined. When a

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fish is placed in the chamber, 300 to 400 cc of water are added; when a frog, 25 to 30 cc; and when a small terrestrial animal, no water is required. The upper wall of the respiratory chamber converges to form a neck which is closed by a ground-glass stopper held in place by means of helical springs. Through the neck of the chamber, the animal is placed into the respirometer. From the neck of this chamber emerge 3 side-arms: one wide-mouth arm contains the CO_2 absorber (10% KOH solution); a second side-arm is a vent with stopcock for changing the gaseous armosphere within the respiratory chamber; a third side-arm consists of a 4 mm glass tube which connects to a 10 cc reservoir bulb. On the lower surface of this bulb is attached a 2 mm capillary tube which leads to another 10 cc reservoir bulb connected, in turn, to a compensatory flask. The capillary tube has an index mark etched on it. The compensatory flask contains a few cc of water to assure equal vapor pressure in both the respiratory and compensatory chambers when at the temperature of the water bath. The sensitivity of the system is

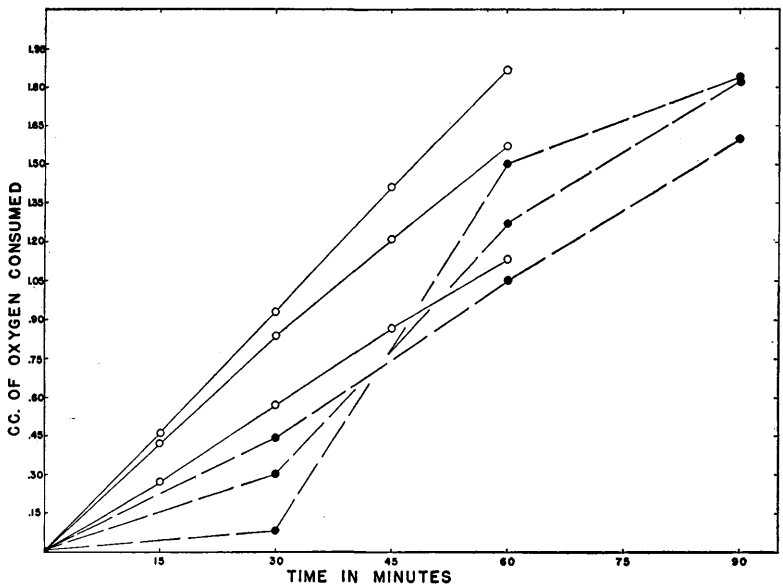

FIGURE 2. The rates in minutes (abscissa) of the oxygen consumption in cc of oxygen consumed/gm wet weight/hr, (ordinate) for the same goldfish as studied by the volumetric (O) and by the Winkler (●) techniques. Temperatures of 30.00 ± 0.01°C and 25.4°C, respectively.

directly related to the gaseous capacity of the compensatory chamber. There is placed inside of the index-marked capillary tube a colored fluid. The proximal (relative to the respiratory chamber) air-fluid interface of this column of fluid is made to coincide with the index mark at the time of obtaining each reading of the volume of oxygen consumed. This assures the maintenance of a constant gaseous pressure in the respiratory chamber. For compactness the extended third side-arm which carries the index fluid and compensatory flask encompasses the respiratory chamber, and is annealed to it in several places in order to gain rigidity.

Inserted into the ground-glass stopper are 3 small-bore outlets arising from a common vent. To each outlet is attached a syringe of any capacity dependent on the volume of oxygen to be measured during the course of the experiment. For studies on frogs and goldfish, syringes of 1 cc (100 division/cc) and 2 of either

5 or 10 cc capacity were most satisfactory. For more precise readings a microsyringe designed by Woodrow (1949) may be conceivably incorporated. These syringes are connected by pressure tubing. As the O_2 and CO_2 are removed by the organism and KOH solution, respectively, the difference in pressure between the respiratory and compensatory chambers forces movement of the index fluid towards the proximal reservior bulb. By appropriate movements of the pistons of the syringes, the proximal air-fluid interface is returned to the index mark and the volume of oxygen lost to the system is read directly.

The respirometers were immersed in a thermostatically controlled water-bath (30.00 ± 0.01° C), and were held in position by a rack which was originally adapted to fit into the shaft designed to hold Warburg manometers. The respirometers may be oscillated for varying distances and for varying frequencies characteristic of the shaking apparatus in question.

Although the volumeters have been used successfully on aquatic animals, goldfish and frogs (Calhoon and Angerer, 1949, 1950a, 1950b, 1951; Murray and Angerer, 1950), there is evidence that they may be adapted to small terrestrial animals providing that they are not cramped in the respiratory chamber. Studies were made upon the effect of shaking the apparatus at various frequencies. It was found that when the volumeters were not shaken they gave identical rates for oxygen consumption as when they were shaken 40 oscillations/minute. However, the values obtained with 40 oscillations/minute were sometimes variable. Further-

TABLE 1

Comparison of the respirometric and the Winkler methods

Methods	No. of Fish	°C. Temp.	Mean M.M.	Std. Dev.	Std. Error
Winkler	18	25.40	0.211	0.070	0.016
Respirometer	18	30.00*	0.282*	0.083	0.019

*The difference in mean metabolic rate (M.M.) is due to the difference in temperature, assuming a Q_{10} value of 2.7.

more, shaking at 80 oscillations/minute caused a three-fold increase in oxygen consumption over that obtained without shaking. This was due to the high oscillatory rate stimulating the animal to excessive movements and thus causing an increase in oxygen consumption. That the rate of oxygen consumption without shaking is indentical to that obtained with the Winkler technique is probably due to the movements of the animal being sufficient to mechanically mix the contained gas. Therefore, the respirometers were not shaken during the course of these experiments.

To compare the accuracy of the volumetric to the Winkler technique, oxygen consumption determinations were made by both methods on a group of 18 goldfish, *Carassius auratus*. All fish, weighing between 5 and 10 grams, were starved 12 to 18 hours prior to use in order to render them into, as near as possible, a post-absorptive state. The results are presented in Table 1 as cc of O_2 consumed/gm wet weight/hr. The mean value for oxygen consumption is substantially higher for the volumetric than for the Winkler technique (0.282 ± 0.019 and 0.211 ± 0.016, respectively). However, when correction is made on the basis of their Q_{10} values for the difference in temperature (30.00 ± 0.01° C and 25.4° C, respectively) at which the two sets of data were obtained, it is found that the means of the two sets of data are identical. Despite the difference in temperature at which the two techniques were run, the standard error (S.E., table 1) of the mean values for oxygen consumption is almost the same (± 0.003) in both cases.

SUMMARY

A direct reading volumeter, requiring no previous calibration has been devised for study of the oxygen consumption of small aquatic and amphibious animals. It is conceivably possible to extend its use to small terrestrial animals. A true mean value of 0.282 ± 0.019 cc O_2 consumed/gm weight/hr, at 30.00 ± 0.01° C was obtained for the common goldfish, *Carassius auratus*, by the volumetric respirometer. This value compares favorably with a true mean value of 0.211 ± 0.016 for the same fish at 25.4° C using the Winkler technique. The volunteers, therefore, show degrees of accuracy similar to the Winkler technique. An advantage in favor of the volumetric over the Winkler technique is found in the greater linearity of the points in the graph on plotting the cc of oxygen consumed as a function of time (fig. 2). Finally the volumetric respirometer reduces the laborious and time-consuming manipulations inherent in the Winkler technique.

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