An Apparatus for Determining the Respiratory Volume of Large Aquatic Birds

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The apparatus shown in Fig. 1 is designed to adapt the oxygen dilution method of clinical lung-volume determination to the measurement of the total respiratory volume of various aquatic birds. In this method, the respiratory gases of the subject are mixed, through a mouthpiece, with a known quantity of oxygen, and the lung volume is calculated by the equation:

(1) Lung Volume (Respiratory Volume) =

$$VO_2 \times \frac{\%N_2 - a}{79.1 - \%N_2}$$

where VO_2 is the volume of oxygen in the apparatus used, a is a correction for impurities in this oxygen, 79.1 is the approximate percentage of nitrogen in the respiratory gases, and $\%N_2$ is the percentage of nitrogen found in the mixed gases (*3*).

The spirometer, S (Fig. 1), has a bell adequate to the tidal air of the bird being tested. Two interchangeable bells, with capacities of 90 and 140 cc.,

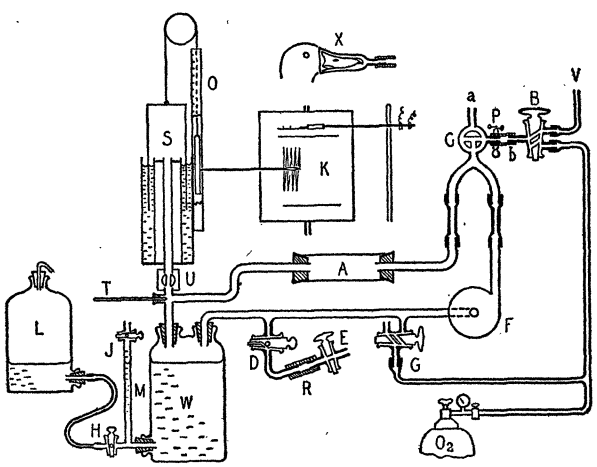

FIG. 1

and calibrated to equal 1.33 and 2 cc./mm., respectively, on the scale, O, and the record, K, have been used. The technique of Herrald and McMichael (*2*) is employed by keeping constant during a test the volume of the bell, as read on K, through the addition of oxygen at stopcock G. Tidal air and rate of ventilation as well as spirometer volume are recorded on K. The centrifugal fan, F, 5 cm. in diameter, circulates the gases in the apparatus. The water level in the 500-cc. Woulff bottle, W, calibrated to the scale, M, is adjusted with the leveling bottle, L. The chamber, A, contains 16 grams of dry, No. 4 mesh sodium calcium hydrate for absorbing carbon dioxide. The bore of the three-way stopcock, C, is 5 mm. and that of the rubber and glass tubing in the circuit is 8 mm. Negative pressure is exerted through the tubing, V. The temperature of the apparatus is read on the thermometer, T.

The apparatus is rinsed and filled with oxygen, as follows: The water level in the bottle, W, is adjusted to zero on the scale, M, and the spirometer bell, usually removed between tests, is replaced in the water seal, while stopcocks C and B are in the position shown. Then, with the fan, F, running, enough oxygen (90–140 cc.) to fill the bell is alternately admitted and withdrawn, about 25 times, by manipulating stopcock B.

The dead space (here 180.0 cc.) may be determined by filling the apparatus with oxygen as above, setting the spirometer bell at zero, and mixing the oxygen with a measured volume of atmospheric air from an aspirator bottle attached at a of stopcock C. The mixture is analyzed for percentage of nitrogen, as below, and the dead space calculated by applying equation (1) inversely.

In preparing for a test, sufficient oxygen is added to the dead space to make the total VO_2 of the apparatus equal to the estimated volume of the bird's respiratory system. This is done by opening stopcock B, raising the spirometer bell from zero to the desired point or points on scale O and allowing the measured oxygen to flow into the bottle, W, by opening stopcock H. The bell is set about one-third above zero on scale O, stopcock C is given a half-turn from the position shown, and stopcock B is removed from the coupling, b.

A bird is prepared by first tying the tibiotarsal joints and resting the animal on the operator's lap. A glass hood, X, is slipped over the bill and sealed to the bird's head with a rubber flange, prepared from a No. 7 latex balloon. The hood is then attached to the apparatus at a on stopcock C, so that the bird breathes air at b. The bird is cut into the apparatus at the inspiratory phase, by giving C a quarter-turn counterclockwise. When the fan, F, is started, the spirometer bell falls, and the points on the record rise regularly as oxygen and carbon dioxide are absorbed. Stopcock G is then opened to admit just enough oxygen to restore the points on the record to the original level for the remainder of the test.

A volumetric test, lasting 4 to 8 minutes, is terminated at the inspiratory position by turning stopcock C to the starting position. The bird is now prevented from breathing by the pinchcock, P, which has been clamped on the coupling, b, during the mixing process, and while in this condition, samples are quickly drawn

from the interclavicular and right abdominal air sacs, through No. 23 Yale needles into 2-cc. glass syringes, oiled and freed of air. Samples of the spirometer mixture are drawn similarly through the rubber tubing at R, after first rinsing some of the mixture through stopcocks D and E while valve U is closed and water flows from the bottle, L, into W. All samples are analyzed immediately for percentage of nitrogen, using Bayer's technique (*1*) with Krogh's pipette.

The respiratory volume (lungs, air sacs, and trachea) of the bird is calculated using equation (1). The figure 79.0 for the normal percentage of nitrogen in the air sacs has been found suitable for this calculation. The final VO_2 of the apparatus is measured accordingly if the points on the record, K, are slightly above or below their starting place.

Corrections for difference in temperature and the vapor pressure of water are added to the calculated respiratory volume. The dead space of the hood, X, is subtracted from the corrected volume. No correction has been made for the error, estimated at less than 2 per cent, due to the diffusion of nitrogen from the blood and tissues of the bird during a test.

Although normally the ventilation of the various air sacs differs greatly, samples taken from the air sacs during a test have been found to check, as a rule, within .5 per cent with those from the spirometer. The apparatus has been used with gulls, and with mallard, black, redhead, and wood ducks. Using mallards and wood ducks, we have tested the duplicability of observations with the apparatus and found the coefficient of variability of test runs made on the same duck, the same day, to be less than 6 per cent and that of runs made on the same duck on different days to be less than 8 per cent.

References

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A New Sterile Technic for Preparing Agar Cup-plates

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The agar cup-plate method has found considerable application as a test for bacterial inhibitory properties of many substances. Examples of substances which have been tested by this method are liquid products, ointments, dusting powders, catgut, creams, and suppositories (*1*). The test, however, has been greatly limited by the difficulty encountered in preparing, in a sterile manner, suitable uniform cups in the agar. Two standard methods have been used: (1) Before the agar cools, a depression or cup is made in the medium by standing a sterile, flat-bottomed, glass tube in the liquified agar. When the agar is hard, the glass tube is removed by slightly twisting and pulling, and at the same time inserting a sterile wire down the side of the tube for the introduction of air in an attempt to prevent the cracking of the agar. (2) The agar is allowed to harden and then a disk is cut out of the agar by means of a suitably sized sterile cork borer. Both these methods are very cumbersome, and a uniform cup without cracked edges is difficult to obtain. Contamination of the plate is also very common in these procedures, since the lid of the plate must be removed. Still another operation is necessary in these methods—that of placing one or two drops of melted agar in the cup to seal cracks of crevices formed.

A simple new technic is proposed for the preparation of uniform agar cups in a sterile manner. Sterile, flat-bottomed, pyrex glass rods, the diameter of which determines the size of the agar cup, are placed in the liquid agar in the Petri dish, as is shown in Fig. 1. The pyrex glass rods are 10 mm. in length. The agar is allowed to harden, after which each glass rod is heated by means of a small heating element (see Fig. 1). The heating element is slipped over the end of the rod and is held in place by a small glass plunger operated with the forefinger. The agar melts evenly around the rod, which is then easily removed, leaving a uniform agar cup. The small amount of agar which melts adjacent to the rod flows down to the bottom of the cup and solidifies, forming an agar seal at that point. The agar cup-plate is then ready for use.

The heating element is simple in construction and operation. It consists of a 22-gauge chromel resistance wire, wound in a four-turn spiral, the ends of which are sealed with pyrex glass onto the outside of a glass tube with a 6-mm. O.D. and 14 cm. long. The ends of the resistance wire extending beyond the glass seal are attached to an ordinary extension cord held in place on the glass tubing by a tight wrapping of asbestos cord. The temperature of the wire spiral is controlled by plugging the extension cord into any variable resistance transformer; 8 to 10 volts produce a suitable temperature. The plunger consists of a 4-mm. pyrex glass rod which will slip inside the glass tubing. By manipulation with the forefinger the plunger is pressed against the side near the top of the glass rod standing in the agar. At the proper moment the glass rod may be lifted from the agar to form a uniform cup.