LXIII. A MODIFICATION OF THE BARCROFT AND WINTERSTEIN MICRORESPIROMETERS.

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THE apparatus described in the present paper was designed for taking observations on the rate of absorption of oxygen by an isolated frog's muscle in any desired atmosphere, provision being made for simultaneous measurements of tension. The principle of the gas measurements is the same as that commonly employed in microrespirometric work, namely, the observation of the contraction in volume in a chamber containing the tissue, together with a little alkali, whose function is to keep the carbon dioxide tension negligible. To record the tension, a fine wire is led from the upper end of the muscle through a fine capillary plugged with vaseline. This vaseline seal appears to be quite gastight and to cause no measurable error in the gas measurements.

The apparatus consists essentially of two suitably shaped glass vessels (*A* the respiration, and *B* the compensation, chamber) in communication with a capillary manometer showing the pressure difference between the chambers: together with a calibrated gas burette by means of which the necessary measured volume of gas may be forced into the respiration chamber to bring the manometer to equality on the two sides. Readings of the burette give directly the volume of gas absorbed by the tissue, the correction for pressure and temperature being that for the conditions prevailing at the moment of closing the apparatus.

The instrument thus consists essentially of a Barcroft differential manometer with the addition of the burette of the Winterstein microrespirometer [Winterstein, 1912]. The use of the burette has several advantages; that calibration of the chamber volume is unnecessary, that the pressure may be maintained constant in the respiration chamber during a prolonged experiment, and that the range of the instrument is increased, so that greater changes of volume may be measured than with a simple differential manometer of equal sensitiveness. The manometer was adopted instead of the oil drop in a horizontal tube used by Winterstein, Thunberg and others, since it possesses a much greater range for equal compactness, and it should not be subject to the curious spontaneous movements described by some who have used the latter arrangement [Winterstein, 1912; Haberlandt, 1911].

¹ This work was carried out during the tenure of the Benn W. Levy studentship.

Details of construction.

The scale of centimetres in *D* gives the scale of the drawing (Fig. 1). The respiration chamber (*A*) has an opening at the bottom, closed with a ground stopper, through which the tissue is introduced. One platinum electrode passes through the stopper, which is continued in the form of a bent tube reaching above the level to which the instrument is immersed in the water-bath. This tube is filled with salt solution and serves to conduct the stimulating current to the muscle without short-circuiting through the water-bath. There is space at the bottom of each chamber for about 1 cc. of the CO_2 absorbent. Each chamber is provided with a tube and tap, which serves as an exit for the gas when filling. The compensation chamber (*B*) is made of similar dimensions to the other, but is not provided with the opening at the bottom nor with the capillary at the top. Exact correspondence in volume is unnecessary, but the chamber walls should be of the same thickness.

The chambers are connected with the manometer and burette by accurately ground joints, and are held in place by elastic bands passing over the hooks shown.

The manometer and burette, *C* and *D* (Fig. 1), with the necessary connections for filling, are made in one piece of glass and are clamped upon a glass scale mounted on a board: for this purpose ordinary spring wooden clothes-pegs have been used. The manometer is of about $\frac{1}{2}$ mm. bore and is fused at each end to the three-way stopcocks 1 and 2. The burette and its connections have the form shown in the drawing. The portion 4-5-6 is calibrated before being built into the instrument: it is provided with one mark at 5 on the glass which gives a means of fixing the burette during an experiment over the same part of the scale as at the time of calibration: a precaution which is only necessary if the calibration shows that the inequalities are great enough to affect the accuracy of an experiment. Below 6, the calibrated tube is fused to a bent tube ending in a thick walled rubber tube of suitable length (three to five inches according to bore), closed with a glass stopper at the top and provided with a number of ordinary spring pinch clips for adjusting the mercury level. A stopcock, 3, is fused on to the burette at the top for filling purposes.

The manometer with its taps, and the burette with its connections, may be made and fitted to the board separately, then clamped to the board in their correct positions over the scale and the final joint made by fusing the glass at 7 when the parts are on the board. It is well to strengthen the apparatus by securing the mercury tube to the manometer at the point 8 where the tubes cross.

Cleaning and filling.

All parts of the apparatus are filled with a chromic acid solution and the whole is either immersed in, or heated over (if a sufficiently large basin is not available), a basin of the same fluid. Frequently many hours' heating at or near the boiling point is necessary. The acid is washed out, finally using

distilled water, and at this stage insufficient cleaning may be detected by the presence of greasy unwetted places on the glass. Drying is simply effected by warming on or over a sand-bath while air is drawn through all parts of the apparatus. Care must be taken that all parts are thoroughly dried in turn.

Fig. 1.

The tap grease employed was a mixture of white vaseline and paraffin wax (2 : 1) and was used as sparingly as possible: this was selected in preference to the usual lubricants on account of its smaller power of absorbing oxygen.

Filling the manometer.

Paraffin oil boiling between 160° and 190° approximately is suitable, having a low vapour pressure and high mobility, so that equilibrium is reached at once. Clove oil, which is occasionally recommended for capillary manometers, is much too viscous. The barrel of the tap, 1 (or 2), is greased and inserted: the apparatus is held in a stand with the tap, 2 (or 1), from which the barrel has been removed, above the other, and a small amount of oil is introduced in a pool above the opening of the manometer: by cautiously opening the lower tap the correct quantity is allowed to run in, avoiding air bubbles. The excess in the tap seating is cleaned away with filter paper, and the column allowed to run down into place. It should fill the two limbs slightly more than half full. No difficulty is found in adjusting the length of the oil column if it is run back again to the empty tap and excess removed with filter paper or more added with a capillary pipette as may be required. Taps 2 (or 1) and 3 may now be inserted. Except when the instrument is in use it is well to leave both taps 1 and 2 closing the ends of the manometer (as in the diagram), since if the oil column is broken or allowed to come into contact with the tap grease the apparatus will probably require cleaning again.

The mercury burette is filled from the rubber tube: it is well to fill it only to within a few centimetres of the zero mark, since the mercury in the long vertical column may descend slightly, owing to the contraction of the rubber or to leaks.

Details of manipulation.

The compensation chamber, containing a little potash ($2\frac{1}{2}\%$), is attached. The excised tissue is tied to the platinum loop on the stopper of the respiration chamber and the other end to the fine wire: the wire is threaded through the capillary, and the capillary dipped into molten vaseline till the grease runs up to within about 2 mm. of the chamber proper. It must not be allowed to spread over the inside of the chamber or errors due to the grease absorbing oxygen will occur. Finally about 0.5 cc. of the potash is introduced by means of a capillary pipette and the respiration chamber attached to the remainder of the apparatus, which is placed in a well-stirred water-bath, preferably provided with a thermostat. The taps on the chambers are opened and the gas mixture required is passed in through taps 2 and 3 and out through the chambers. If the mercury in the burette is raised to the level of the T-piece when the gas is first passed, and is quickly lowered to the zero before discontinuing the stream, the whole of the apparatus is washed out except the manometer, which is a negligible volume. The incoming gas stream is then shut off at 2 and 3, tap 2 turned so as to connect the compensation chamber with the manometer (through 90° clockwise from the position of the diagram), tap 1 to connect the respiration chamber with the manometer and burette

(through 180°), and the taps on the chambers closed. The barometer and temperature are read.

Reliable readings can usually be taken 15 minutes after closing the taps. To take a reading, the screw clips are opened or closed so as to alter the mercury level until the manometer shows equal pressures in the respiration and compensation chambers. The height of the mercury is then read, parallax being avoided by the silvering on the back of the glass scale. When the mercury reaches the top of the calibrated tube the gas must be renewed, and the mercury adjusted to zero during the passage of the gas; this need not interrupt the readings for more than 20 minutes. With my two instruments the volume of one centimetre on the burette is 0.02266 and 0.01231 cc. respectively, so that the length of 20 cm. suffices for several hours' continuous readings at a rate of respiration of the order 0.03 cc. per hour.

Limits of accuracy.

The stability of the zero of this instrument is the most important factor determining the accuracy with which rates of oxygen absorption can be measured. In blank experiments without any tissue but with the potash in the bulbs, it has been found that, neglecting the first 20 or 30 minutes after passing the gas, the variations in zero are appreciable though small, not usually exceeding 0.5 cubic millimetres per hour for the first hour, and falling considerably later. The cause of these variations has not been definitely ascertained; they may be due to absorption of gas by the grease used for the joints and taps, or possibly to very slight movements at the ground joints. In any case the minimum possible amount of tap grease should be employed and this should consist of substances as saturated chemically as possible, consistent with proper lubrication of the surfaces. The figures are given for the approximate amount of zero change I have observed with the grease made of paraffin wax and white vaseline: mixtures containing rubber, and ordinary vaseline, appear to give a much larger spontaneous change. An apparatus similar in principle to this, but with joints of rubber pressure tubing instead of ground joints, was found to have a very much larger zero change, comparable in amount with the actual consumption of small muscles, and since this tendency to change zero was either abolished or much reduced when the apparatus was filled with hydrogen instead of oxygen, it appears to be due to the absorption of oxygen by the rubber connections. The errors with the present apparatus are of the order 1 in 20,000 of the volume of the chambers: it may be of interest to note that I have observed similar slow changes of the zero of an ordinary Barcroft blood gas apparatus.

The oxygen consumption of a single resting sartorius of the frog is of the order 20 cubic millimetres per hour, so that this quantity may be determined to an accuracy of 2 or 3 %.

Errors due to a possible imperfect absorption of CO_2 appear to be very small. During a period of constant respiration the apparatus will reach a

steady state, and any small amount of CO_2 unabsorbed will remain constant so that no error is introduced if the volume diminution is taken as the oxygen absorption. When a change of rate of respiration, say an increase, occurs, there will be a slight accumulation of unabsorbed CO_2 , so that the diminution in volume will be less than the true absorption of oxygen. The amount of the increase in the unabsorbed CO_2 will be proportional to the increase in the rate of production of CO_2 , and may be calculated approximately from the dimensions of the apparatus and the constant of diffusion of CO_2 into air. The increase in the quantity of CO_2 escaping absorption which must be present in order to raise the concentration gradient of CO_2 between tissue and absorbent till the steady state is again reached at the increased rate of production, for an increase in rate of CO_2 production of 1 cubic millimetre per minute, may be calculated to be as follows. It is assumed for simplicity that the chambers are cylindrical and that the CO_2 is all evolved at the same distance from the absorbent (column 1). Column 2 gives the amount of the increase in the volume of unabsorbed CO_2 in cubic millimetres.

Distance cm.	Total increase of unabsorbed CO_2 cmm.
1	0.05
2	0.2
2.5	0.3
3	0.45
3.5	0.6
4	0.8

These amounts are several times larger than any at all likely to be met with, since the assumed rate of increase in the CO_2 production is several times larger than is probable. It is clear that the difference between the volume diminution and the oxygen absorption is so small as to be usually within the error of reading the scale with the naked eye, and is negligible unless extremely rapid changes of rate are to be followed with great accuracy.

A third condition, the effect of which must be considered, is the progressive change in the composition of the gas in the respiration chamber, for this will have an effect on the rate of respiration of the tissue, for which allowance may have to be made. The chambers used have a volume of about 10 cc., so that the absorption of 0.05 cc. of oxygen (a probable amount for work with large muscles in one hour) will cause a diminution of the oxygen concentration of about 0.5 %. A diminution in the volume corresponding to 20 cm. of the larger burette mentioned will reduce the oxygen tension by 4.5 % of an atmosphere. In cases where this alteration might have an important effect, larger chambers would be required, as recommended by Krogh [1915, p. 526].

SUMMARY.

An instrument, modified from Barcroft's blood gas apparatus and Winterstein's microrespirometer, is described, for determining the oxygen consumption of frog's muscle or other small amounts of tissue in any desired atmosphere. The instrument permits of simultaneous observations on the tension of contraction.

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