L. A MANOMETER FOR COMPARATIVE STUDY OF PHYSIOLOGICAL PROCESSES.

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IN the course of investigations on the purification of the polyphenol oxidase of potato it was found necessary to measure the degree to which purification had been achieved in the various enzyme preparations. The degree of purification of an enzyme is generally denoted by a number which is the ratio of enzymic activity per mg. dry weight of final preparation to the enzymic activity per mg. dry weight of the original material [Haldane, 1930]. Warburg and Barcroft manometers are inconvenient for the purpose, as what is required is the ratio and not the actual amounts of the oxygen absorbed by the final preparation and the material from which it has been obtained. It was with the idea of developing an apparatus in which the scale readings could be directly compared in order to get the degree of purification of an enzyme, and which could, at the same time, be employed for quantitative determinations, that the manometer described in the present note has been constructed.

It is arranged that both the vessels (*P*) and (*Q*) of the apparatus (Fig. 1) shall have the same vessel-constant; methods of ensuring this have been described in detail by Dickens and Greville [1933]. The gas in the pipette (*C*) is maintained at the initial atmospheric pressure throughout, the tap (*E*) being closed after the Brodie solution has been adjusted to the mark (*X*) at the commencement of the experiment. When it is required to compare two reaction velocities, involving the evolution or absorption of the same gas, all that is necessary is to compare the scale readings in the two graduated limbs (*A*) and (*B*). The apparatus can also be used for quantitative determinations, in which case one of the vessels is employed for the main reaction while the other serves to indicate the fluctuations in the temperature of the water-bath. If the vertical distance between the menisci in the two manometric limbs (*A*) and (*B*) be *h*, and *x* the amount of gas evolved in μ l. at N.T.P. (dry), we have

$$x = h \cdot k'_g.$$

The calibration of the apparatus consists in finding the value of this constant k'_g , which is calculated from the following simplified formula¹:

$$k'_g = \frac{Vg \frac{273}{t+273} + Vf \cdot \alpha'_g}{P_0} + A \frac{273}{t+273},$$

where *Vg* = μ l. gas in reaction vessel and manometer tubing down to the mid-point on the scale; *Vf* = μ l. liquid in reaction vessel; α'_g = Bunsen absorption coefficient of the gas at *t*°; *P*₀ = pressure of one atmosphere expressed in mm. of manometer liquid; *A* = area of cross-section of the manometer tube in mm.²

¹ It may be noted that in this formula the coefficient of *A* is unity and not $\frac{1}{2}$ as in the differential manometer, the reason being that in this case the depression of liquid in one graduated limb is not accompanied by a corresponding rise in the other.

The manometric limbs (*A*) and (*B*) and the central pipette (*C*) are constructed out of glass tubing with an internal cross-sectional area not exceeding $\frac{1}{2}$ mm.² The central pipette (*C*) carries a circular mark (*X*) exactly corresponding with the 150 mm. marks on either side-limb, beyond which it is blown into a small bulb (*D*), capacity about 3.5 ml., and carries a tap (*E*) at its extremity. The two side-limbs carry mm. scales, 0 to 300, etched on them. To maintain it at a uniform temperature during experimentation, the bulb (*D*) is covered by a piece

Fig. 1.

Fig. 2.

of flannel, and a rectangular piece of wood (*W*), hollowed on one side so as to fit on the bulb, is screwed on to the wooden board (*M*) carrying the apparatus. Fig. 2 depicts a portion of the apparatus with the wooden piece (*W*) in position. A screw-clamp arrangement with a rubber reservoir (*R*) is provided, the manometric liquid being adjusted by means of the screw (*S*). The taps (*F*) and (*G*) are of the type used by Dixon and Elliott [1930] with the Barcroft respirometer. The vessels used are those generally employed with Warburg manometers. Each vessel possesses a side-bulb, above which an outlet is provided with a stopper of the type introduced by Warburg and Kubowitz [1929] which is so constructed as to act also as a tap and the outlet may be closed by giving it a half turn. The

vessels should be made nearly the same size; several methods [Dickens and Greville, 1933] are then available for securing equality of volumes.¹

The manipulation is easy. By means of the screw (*S*) the manometric liquid is adjusted to the mark (*X*) on the pipette (*C*) when it should also stand at the 150 mm. marks on both the side-limbs. During this adjustment all the three limbs (*A*), (*B*) and (*C*) are kept in communication with the air, the taps (*E*), (*F*) and (*G*) being subsequently closed. At the conclusion of the experiment, the Brodie solution is again adjusted to the mark (*X*) on the pipette (*C*) and the scale readings in the two graduated limbs are read and directly compared.

When used for quantitative determinations the accuracy of the apparatus, as found by liberating known amounts of CO₂ by adding measured amounts of standard acid from the side-bulbs to an excess of the bicarbonate solution contained in the main part of the vessels, is $\pm 1.25\%$.

¹ Equality of vessels is easily checked by the following method. Empty vessels are worked on to their ground joints and secured by rubber bands. After the manometric liquid has been brought to the mid-points in all the three limbs, the taps are closed as usual. Now by means of the screw (*S*) the manometric liquid is adjusted to a point much higher than the point (*X*) on the central pipette. Any difference of reading on the two side-limbs indicates an inequality of vessels and is corrected by introducing a small glass bead or by blowing the side-bulb of one of the vessels.

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