APPARATUS FOR THE STUDY OF REDOX POTENTIAL IN BIOLOGICAL SYSTEMS*

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The four pieces of apparatus[1] described in this paper have been used in several investigations on biological oxidation-reduction systems. Other apparatus for similar purposes have been described by Ahlgren (5), Lehmann (4), Clark (1), Michaelis (2), Borsook and Schott (3), and Baumberger, Jürgensen, and Bardwell (7).

1. The Modified Thunberg Tube.—Ahlgren (5) has described in detail the use of the Thunberg tube in the study of the reduction of dyes by biological systems. The apparatus shown in Fig. 1 has the advantage that it is cheaper, has no ground glass stopper to be greased and thus involves no special difficulty in cleaning, and will hold a vacuum suitable for the development of extremely low redox potentials such as the complete reduction of rosinduline. The experimental procedure with this tube is to introduce the substrate, buffer, and dye and finally the enzyme, then to apply de Khotinsky cement to the pyrex vacuum tube at point *DeK*, warm the tip of the soft glass adapter *b*, and introduce it into the cement so that it rises in the interstices between *b* and the neck. Cool the cement, attach *b* to water pump, and evacuate to boiling for a minute. It is essential to keep the tube warmer than the tap water and to wet the walls with the contents, as thereby the oxygen is more readily displaced. Heat *b* at the constriction while still evacuating and finally seal off the attached lower half of *b* and anneal with care. (The introduction

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[1] This apparatus was skillfully made by Macalaster-Bicknell Co., Cambridge, Massachusetts.

of glass capillaries through *b* should be avoided, as scratching of the inner surface invariably follows and leads to cracking while heating.) After observing reduction time, or final color if a multiple dye system has been used, the de Khotinsky cement may be heated, the tip pulled off, and the tube washed. The remaining cement can be repeatedly used or removed with alcohol.

2. The Modified Borsook and Schott Tube.—Borsook and Schott (3) describe a vacuum electrode tube which can rock in a vertical position in such a way as to be in contact with a reference half-cell through a capillary tube (*a*, Fig. 2) filled with KCl agar-agar which will stand an atmosphere of pressure. A battery of such tubes may be made to rock with the salt bridge capillaries dipping in a large dish of KCl to which a calomel half-cell is connected. The changing oxidation-reduction potential of a system consisting of substrate, enzyme, and dye may be followed conveniently by measuring the P.D. between the two half-cells. When the P.D. no longer changes with time, equilibrium has been reached and the redox potential observed is a function of the free energy of the system. Borsook and Schott's tube has been modified by substituting the evacuation sealing system for ground glass stoppers as in (1) by adding a boot, *b*, at the tip so that satisfactory circulation of the contents may be obtained, which is particularly advantageous in work with slices of tissue; and by introducing the platinum electrode, *Pt*, into the boot instead of having it borne by the glass stopper where it would be exposed to breakage and contamination.

3. Reduction Burette.—The usual apparatus (Clark (1)) for the reduction and transfer of reduced dyes is very intricate, expensive, fragile, and immobile. The reduction burette here described is much less so in each of the four respects mentioned and is adequate for its purpose. It (Fig. 3) consists of a 25 cc. burette surmounted by a 200 cc. bulb from which it is separated by a perforated glass plate, *P*. A special stop-cock, *S*, at the top of the burette and a ground glass stopper at the top of the bulb complete the reduction burette. In operation the dye solution and wet[2] platinized asbestos are introduced into the bulb, the ground-in stopper is fastened in with a rubber band, the

[2] There is danger of explosion when dry platinized asbestos is introduced from air into a hydrogen atmosphere.

burette inverted, and the dissolved oxygen removed by water vacuum pump through side stop-cock *S*, which at the same time is connected

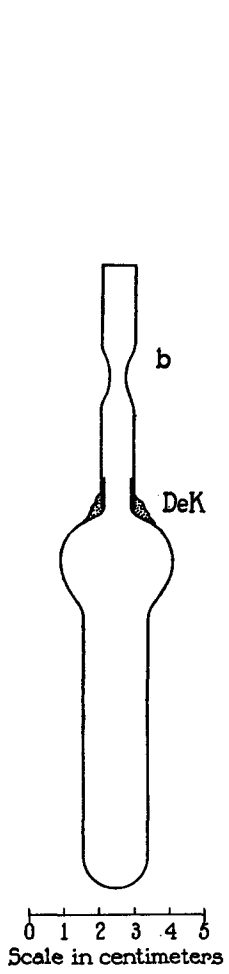

FIG. 1

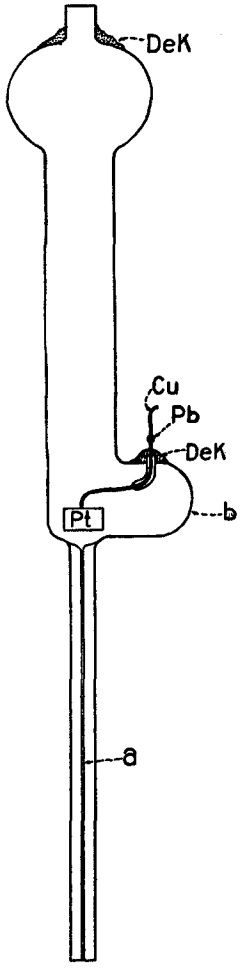

FIG. 2

FIG. 1. In all the text-figures the scale is the same as that given in this figure.

by means of a *Y* tube to a Kendall (6) furnace through which oxygen-free hydrogen and nitrogen can be obtained. When the solution has boiled, the burette is allowed to fill with hydrogen, the stop-cock is

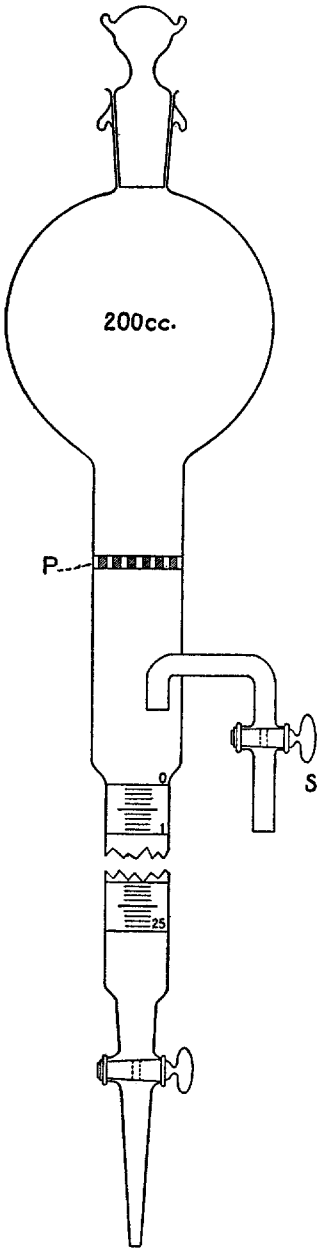

FIG. 3

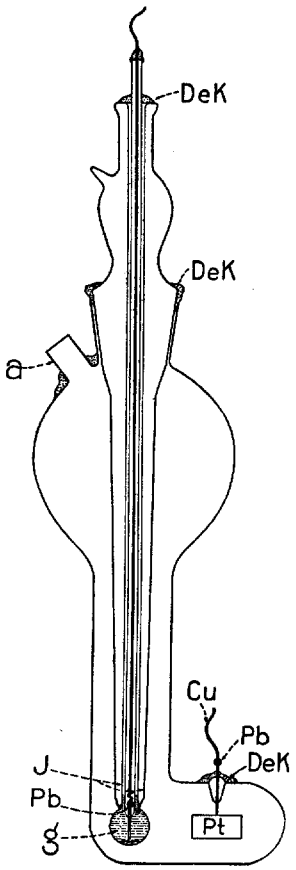

FIG. 4

closed, and the burette is detached from the vacuum pump. With the burette still inverted, the fluid is gently rotated to bring it into contact with the suspended platinized asbestos. When the dye is fully reduced, the reduction burette is again attached to the *Y* tube through *S*, the hydrogen is removed by evacuation, the burette is filled with pure nitrogen and evacuated a second time, and then finally filled with nitrogen. The next step is to slowly turn the burette right-side up and to allow the asbestos to settle on the glass filter. Create a vacuum up to the side stop-cock, open this stop-cock for an instant so that the gas pressure in the burette will become less than in the bulb, and the fluid will be driven down into the burette. When the burette is filled and the bulb dry, raise the nitrogen pressure to about 1.20 atmospheres so that the superpressure will expel the contents of the burette as needed.

The same form of burette is convenient for handling oxidized dyes from which dissolved oxygen may be removed by vacuum and the dye put under nitrogen pressure.

4. Vacuum Oxidation-Reduction Cell.—The cell shown in Fig. 4 is suitable for the study of the oxidation-reduction processes described under (2) above, but it has special features which adapt it particularly to the study of these processes in cell suspensions and in the fluid surrounding slices of tissue. This cell has the features of vacuum seal, boot, and platinum electrode mentioned in (1) and (2), and in addition contains a glass electrode as reference half-cell. The cell has the advantage that it does away with the salt bridge and thus removes the contact potential error resulting from long contact of a salt bridge with a physiological solution. Progressive pH changes, resulting from cell metabolism, are cancelled out in those systems in which the redox potential is affected by pH in the same degree as the hydrogen electrode. Any changes in ionic strength or in temperature are effective on both half-cells and thus such changes tend to cancel out rather than to augment potential differences. The potential difference due to changes in the redox potential on the platinum electrode is thus made the chief variable. The actual pH of the solution may be determined before and after the experiment by connecting the solution to a calomel half-cell by a salt bridge introduced through *a*, and measuring the P.D. between the glass electrode and the calomel half-cell. If the

particular glass electrode gives a P.D. of 0.100 in a buffer of pH 3.97 at 30°C. and a P.D. of 0.160 in the unknown, the pH of the unknown will be $0.160 - 0.100/0.060 + 3.97 = 4.97$. Perfect glass electrodes behave in this manner, within certain pH limits (Dole (9)), if no "deviation film" effects are present (Kahler and DeEds (8)). "Deviation film" effects are avoided by supporting the thin bulb of Corning 0.015 glass by a double walled tube of soft glass blown so as to fit a ground glass joint at the top. The air jacket (*J*) thus formed limits the effective contact of the outside solution to the bulb itself. The glass electrode bulb *g* contains KH phthalate buffer to which quinhydrone has been added in excess; a platinum electrode sealed into a glass tube projects into this solution.

The P.D.'s between the glass electrode and the platinum electrode are measured by means of a thermionic electrometer designed by R. K. Skow in my Laboratory. Any form of electrometer suitable for determinations with the glass electrode may be used with the vacuum oxidation-reduction cell.

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