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RECORDING OXIDATION-REDUCTION POTENTIALS IN PLANT PREPARATIONS[1,2]

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The study of oxidation-reduction potentials often represents a powerful tool in biological and chemical investigations (1). This paper describes simple techniques for measuring and recording oxidation-reduction potentials in photosynthetic systems. The methods described could easily be adapted to measurements on other biological and chemical systems.

Isolated chloroplasts of higher plants suspended in solutions of suitable electron acceptors (oxidants) such as ferricyanide carry on reactions of the following type upon illumination:

(1) $4\ \text{ferricyanide} + 2\ H_2O \rightarrow 4\ \text{ferrocyanide} + 4\ H^+ + O_2$

This reaction, usually called the Hill reaction (2) apparently represents the energy-absorbing and water-splitting part of photosynthesis. A number of useful techniques have been developed for studying this reaction including the measurement of oxygen evolution (2, 3), the measurement of hydrogen-ion formation (3), and the measurement of rate of decolorization of oxidation-reduction indicators (4). The Hill reaction has been studied in this laboratory by determining the rate of reduction of added electron acceptors potentiometrically (5). Advantages of this method include (a) increased precision of measurement (± 1 %), (b) system volumes of less than 0.25 ml, (c) use of oxidant concentrations as low as 10^{-6} M, (d) automatic recording of results, and (e) use of reaction cells with simple optics.

MATERIALS AND METHODS

RECORDING OF OXIDATION-REDUCTION POTENTIALS: The output of a platinum-calomel electrode system was fed through an impedance matching device into a Brown "Electronik" Single-Record Strip Chart Recorder with a self-contained amplifier. Two models were used successfully: a Series 153X11 with a pen speed of 12 seconds for full scale travel (11 inches) and a range of 0 to 2.5 mv, and a Series 153X18 with a pen speed of 1 second and a range of 0 to 1 mv. An impedance matching circuit was necessary since the input impedance of the recorder was 1000 ohms while the electrode system required a high impedance circuit to avoid polarization effects. A typical two-tube cathode follower with a bias circuit to match the tubes was used as shown in figure 1. This seemed ideal since it (a) is extremely stable, (b) shows low sensitivity to changes in plate voltage, (c) shows very low distortion, (d) has a high input impedance (estimated at 50 megohms), and (e) has a low output impedance to match the recorder. The tubes were aged before use, and stability was further increased by using low filament and plate voltages. Unsuccessful attempts were made to use an alternating cur-

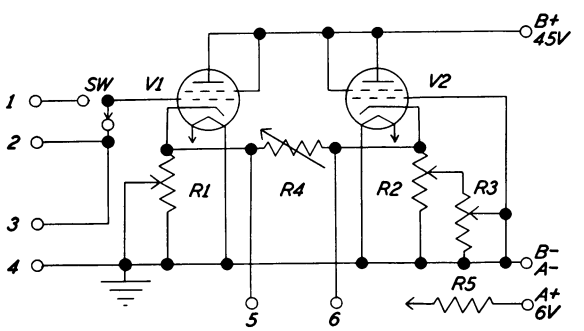

FIG. 1. Battery operated, cathode-follower, input device to match the high impedance of a platinum-calomel electrode system to the low input impedance of a Brown recorder.

V1, V2: 6K6GT radio tube.
R1, R2, R3: 10,000 ohm wire-wound potentiometer for balancing the circuit.
R4: decade resistance box to control sensitivity.
R5: 2 ohm wire-wound filament dropping resistor.
1, 2: to electrode system.
3, 4: to potentiometer for calibration and bucking voltage.
5, 6: to input of Brown recorder.
SW—SPDT: switch to connect electrode system into the input circuit.

[1] Received July 9, 1953.
[2] This work was supported by grants from the United States Atomic Energy Commission and the University of Utah Research Fund.

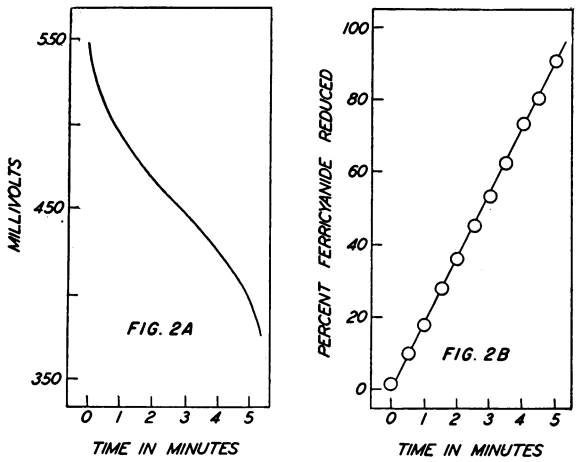

FIG. 2A. Tracing from an actual Brown recorder chart. This curve shows the results of an experiment in which red light (filter cutoff at 5800 Å) with an intensity of 8000 lux was used as a source of illumination for a Hill reaction system in a cell assembly as shown in figure 3. The reaction mixture had a total volume of 0.5 ml and contained Swiss chard chloroplast fragments equivalent to 0.1 mg of chlorophyll. The system was 0.001 M in potassium ferricyanide, 0.01 M in potassium chloride, 0.17 M in sucrose and 0.05 M in potassium phosphate buffer of pH 6.4.

FIG. 2B. Curve showing the rate of reduction of ferricyanide as derived from the potential versus time curve in figure 2A by use of a template as described in the text.

rent operated power supply. The circuit, as operated on batteries, was very stable and showed output changes of less than ± 1 % over 8-hour experimental periods. The device was connected to the Brown recorder, the electrode system, a resistance box, and a potentiometer (fig 1). The full scale sensitivity of the recorder could be adjusted from approximately 4 mv to 14 volts with the resistance box R4, while the potentiometer permitted adjusting the recorder to any part of the scale and also provided known voltages for calibration.

The potential versus time recording of a typical Hill reaction experiment is shown in figure 2A. These data may be transformed into a rate curve (percent reduction of ferricyanide versus time) by an application of the Nernst equation (5) as shown in figure 2B. Transformation was simplified by use of a slotted plastic template which, when centered on the E^o of the potential versus time recording, permitted percent reduction versus time points to be obtained directly where the recording showed through the slits.

REACTION VESSELS: The type of reaction chamber used routinely is shown in figure 3. The chamber proper consists of two pieces of transparent plastic separated by a U-shaped spacer of black opaque plastic and is clamped in an aluminum holder to permit satisfactory heat transfer. The plastic chamber is bored out at the top to hold a special calomel electrode with a pointed tip. A piece of 22 to 26 gauge wire was used for the platinum electrode since electrode area was not limiting under the conditions used. The reaction system was stirred with a piece of stainless steel wire vibrated by a small electromagnet. Stirring was essential for accurate rate measurements, especially under light-limiting conditions. All parts of the reaction system had to be illuminated to avoid non-equilibrium conditions. The principal disadvantage of the small plastic cells was the difficulty of obtaining equilibrium between the gas phase and the system because of the small surface area exposed.

To avoid this, Warburg vessels (7) were constructed of Pyrex and were provided with a calomel electrode and a platinum and/or a glass electrode as shown in figure 4. If desired, then, oxygen evolution, hydrogen ion formation, and the reduction of an oxi-

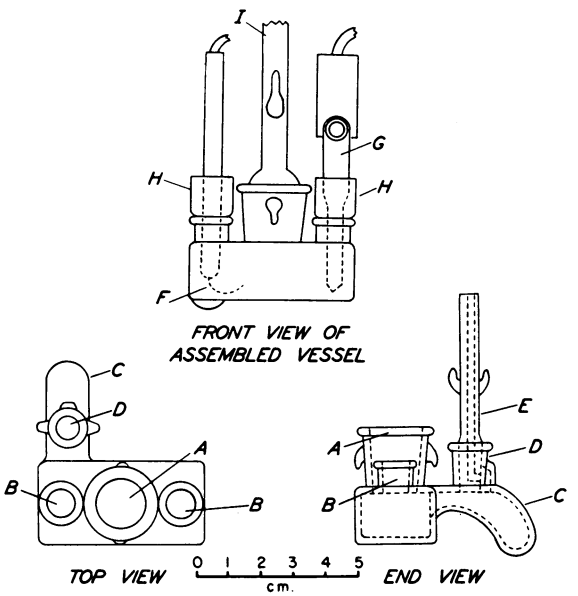

FIG. 4. Rectangular Warburg vessel modified for use in potentiometric measurements.

A —female standard-taper joint (17/20) to fit Warburg manometer or gas manifold.
B —openings for electrodes.
C —sidearm.
D—standard-taper joint for vent plug.
E —vent plug in open position.
F —platinum wire electrode.
G—calomel electrode.
H—serum bottle stopper.
I —male standard-taper joint of manometer or manifold.

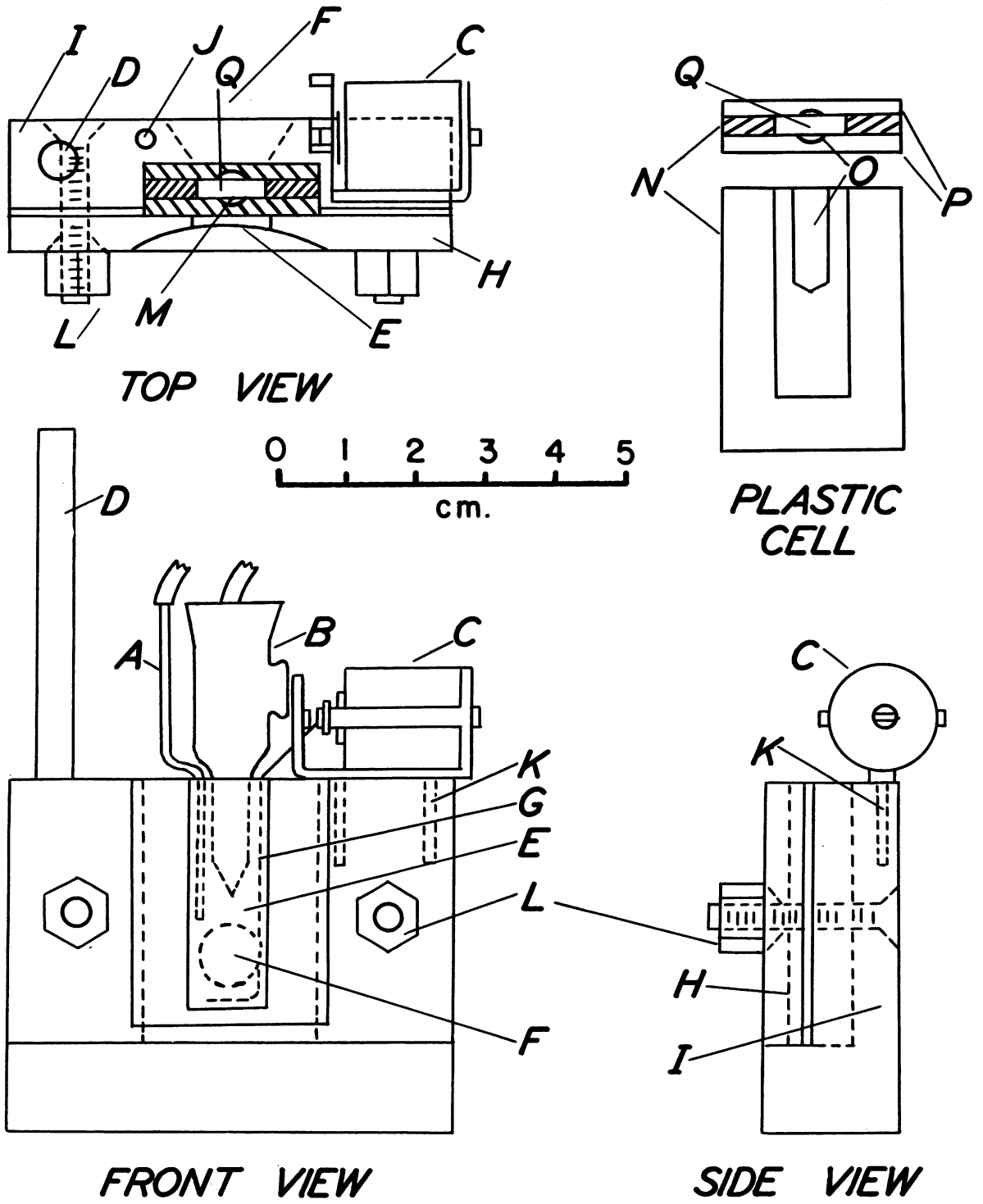

FIG. 3. Irradiation cell and associated devices for the potentiometric measurement of the photochemical activity of isolated chloroplasts.

A—platinum wire electrode.
B—calomel half-cell.
C—vibrating magnetic stirrer.
D—handle.
E—opening for light beam.
F—opening for light beam to photoelectric cell
G—wire stirrer.
H—front plate.
I—back plate.
J—well for thermocouple.
K—stirrer mounts.
L—tapered clamping nuts.
M—plastic cell assembly.
N—opaque plastic.
O—groove for calomel half-cell.
P—clear plastic.
Q—space for chloroplasts.

dant could be measured simultaneously as described earlier (6). These electrode vessels were very useful for making potentiometric measurements under different gas phases since the large surface area of the reaction mixture (3.3 cm^2 per ml with a 3.0 ml system) permitted rapid attainment of equilibrium conditions. The gassing, shaking, and temperature control procedures were carried out with the standard Warburg apparatus (6, 7). Electrodes were inserted through sleeve-type serum bottle stoppers. The sidearm was provided so that parts of the reaction system could be kept initially separated if necessary, while the vent permitted passing any desired gas mixture through the vessel.

Summary

A simple, battery-operated, impedance matching circuit for connecting a platinum-calomel electrode system to a Brown recorder for use in recording oxidation-reduction potentials from biological systems is described. Application to the study of the Hill reaction (photolysis of water by isolated chloroplasts) is discussed. Reaction cells and modified Warburg vessels for making potentiometric measurements on photosynthetic systems are discussed.

The authors wish to acknowledge the technical assistance of David H. Taysum and David C. Evans.

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STUDIES ON THE PHOTOSYNTHETIC REACTION. II. SODIUM FORMATE AND UREA FEEDING EXPERIMENTS WITH NOSTOC MUSCORUM[1, 2]

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The previous paper in this series (1) discussed the metabolism of acetate by the blue-green alga, *Nostoc muscorum*. These studies have been extended to include other low molecular weight compounds and the present paper describes the results of feeding sodium formate and urea to *Nostoc*. It will be shown that the carbon in these compounds is not assimilated to a significant degree without prior conversion to carbonate.

Materials and Methods

The growth, harvesting, and feeding procedures have been described (1). Carbon^{14}-labeled sodium formate (0.40 mc per mg obtained from the Oak Ridge National Laboratory) and C^{14}-labeled urea (61.7 μc per mg obtained from J. L. Williams and A. R. Ronzio of the Los Alamos Scientific Laboratory) were used as labeled substrates. The pre-experimental conditions and the conditions followed after addition of the tracer are described for each experiment. The light and dark portions of each experiment were carried out simultaneously on aliquots of the same algal suspension. Ten-ml aliquots were removed from the algal suspension 5, 15, 30, and 60 minutes after addition of the labeled substrate, and killed by addition to acid. In urea experiments the acid used was 4 ml of glacial acetic acid. In formate experiments, except two where 8 ml formic acid were needed as a carrier, 2 ml of concentrated H_2SO_4 were used.

The CO_2 liberated upon acidification of each aliquot was removed by subjecting the samples to vacuum (30 mm Hg) for ten minutes. In most experiments this acid-liberated CO_2 was collected in 10 % NaOH, assayed for radioactivity, and designated as the carbonate fraction. Further fractionation varied according to the type of experiment.

In most formate experiments, the samples were next subjected to high vacuum (2 mm Hg) for 48 hr to remove unchanged formic acid, the condensate being collected in dry-ice cooled receivers. The nonvolatile residues and aliquots of the condensates were oxidized and assayed for radioactivity, and designated as the assimilated and unused fractions.

In the urea feeding experiments, acidified suspensions were evaporated to dryness on aluminum planchets and assayed for total radioactivity. The urea was then hydrolyzed on the planchets by treatment with urease and the residual, non-urea C^{14} was determined after re-evaporation to dryness, suitable corrections being made for the absorption of radiation by the urease present. The loss in activity on hy-

[1] Received July 16, 1953.

[2] This work was supported by the Charles F. Kettering Foundation.