parison, and the general types of curves obtained for treated cells are illustrated.

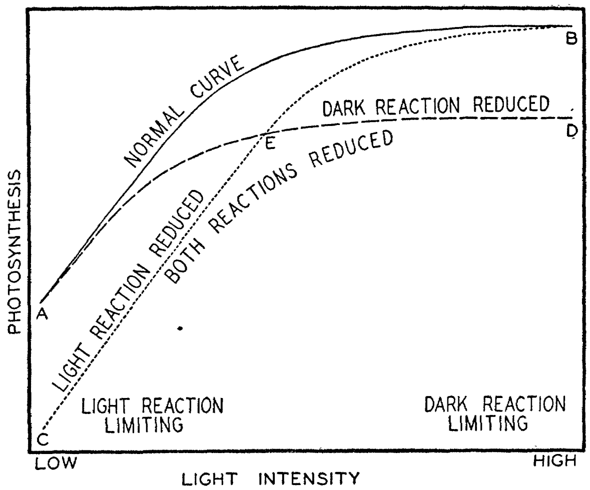

FIG. 1

The curves are interpreted according to the limiting-factor hypothesis of Blackman.[1] Thus, in the normal curve AB, the rate of photosynthesis rises with increase in light intensity over the low intensity range; here light limits or determines the rate of the whole process of photosynthesis. But at higher light intensities the curve flattens, since here light is in excess and some factor controlling the dark reaction limits the rate. In the same sense, at low light intensities the photochemical reaction is limiting and at high light intensities the dark reaction is limiting.

Treatment of cells with $ZnSO_4$, $NiSO_4$ or KCl resulted in a curve of the type AED. These substances retard only the dark reactions of photosynthesis, since their depressing effect is negligible at low light intensities, and is greatest at high light intensities, where the photochemical reaction is in excess and the dark reaction determines the rate of the whole process. In their ability to reduce the dark reaction, these salts resemble hydrocyanic acid[2] and heavy water.[3] It is known that low temperature or deficiency of carbon dioxide gives a similar curve and that cells cultivated in weak light respond in the same manner.

If a substance affected the photochemical reaction alone, greatest inhibition of photosynthesis, as shown by greatest divergence of curves for control and treated cells (as between A and C of curves AB and CEB), would be evident at low light intensity where the photochemical reaction determined the photosynthetic rate, and retardation would become negligible at the highest light intensity. None of the inorganic substances tested gave a curve like CEB, but phenylurethane in Warburg's[2] experiments approximated this type. Chlorophyll deficiency has a similar effect.

Treatment with $CuSO_4$, H_3BO_3, KI, $CoSO_4$ and $(NH_4)_2SO_4$ inhibited photosynthesis over a wide range of light intensities and resulted in curves which were variations of the composite curve CED. It is evident that these substances inhibited both the photochemical and the dark chemical reactions.

Copper sulfate, known to be highly toxic to algae, was found to depress photosynthesis in *Chlorella* exposed for 20 minutes to a 10^{-7} molar solution. In contrast, treatment with 0.4 M $MnSO_4$ had no apparent effect, and *Chlorella* withstood equally high concentrations of KNO_3 and $MgSO_4$. Solutions of higher osmotic pressure, however, depressed photosynthesis and tests with hypertonic sucrose solutions demonstrated that this effect was an inhibition of the dark reaction.

Since inorganic compounds are capable of differential inhibition of the reactions in photosynthesis, their use may provide new evidence regarding the mechanism of the process.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN IMPROVED METHOD FOR THE CONTINUOUS MEASUREMENT OF THE RATE OF OXYGEN CONSUMPTION FOR HUMAN SUBJECTS[1]

THE purpose of the following note is to describe a simple method for the conversion of an ordinary clinical basal metabolism machine to one where continuous measurement of oxygen volume consumption for a subject whether at work or at rest is possible. The accompanying figure shows schematically how this is accomplished. Considering first the apparatus to the left of the dotted line down the middle, one recognizes the usual components of the closed-circuit clinical machine, namely, the mouthpiece (A), triple valve (B), respiration spirometer (C), soda-lime bottle (D) and motor-blower (E). A heat exchanger (F) may be included in the circuit to cool the respiration system. The modification to be described could apply just as well to the clinical machine with soda-lime container and flutter valves inside the spirometer instead of an outside soda-lime bottle and motor-blower.

In our modified apparatus, as the subject metabo-

[1] F. F. Blackman, *Ann. Bot.*, 19: 281–295, 1905.

[1] Contribution No. 32 from the John B. Pierce Laboratory of Hygiene, 290 Congress Avenue, New Haven, Connecticut.

[2] O. Warburg, *Biochem. Zeitschr.*, 100: 230–270, 1919.

[3] F. N. Craig and S. F. Trelease, *Amer. Jour. Bot.*, 24: 232–242, 1937.

lizes, the carbon dioxide exhaled is absorbed and at the same time the levels of exhalation and inhalation of the spirometer (C) fall. When the level of inspiration falls below a fixed level set by a cam, attached to one of the lips of the pulley-wheel (G), a roller leaf micro-switch[2] (H) is actuated, causing a solenoid valve[3] (I) to open and allowing oxygen to flow from a second spirometer (J) acting as supply into the respiration circuit. A water pressure of 5 to 10 mm in (J) is sufficient to accomplish this. Thus, the oxygen consumed is continuously and automatically replaced during breathing. The supply spirometer (J) may be an old or second-hand (5 liter) clinical machine with flutter valves left in position. The outgoing connection acts as the supply line to the respiration system. At arbitrary fixed time intervals (1, 2, 3 or 6 minutes) a master clock sends an impulse shorting signal closing a double-pole-double-throw relay[4] which, in turn, opens the solenoid valve[5] (K) and refills the spirometer through its in-going connection from a high pressure cylinder (L) with reducing valve (M). The level of refilling is set by a cam on pulley-wheel (N), actuating another roller leaf micro-switch[6] (O), which opens the relay and thus closes the supply valve (K). One sees from the electrical circuit in Figure 1 that valve (I) is always closed when valve (K) is open.

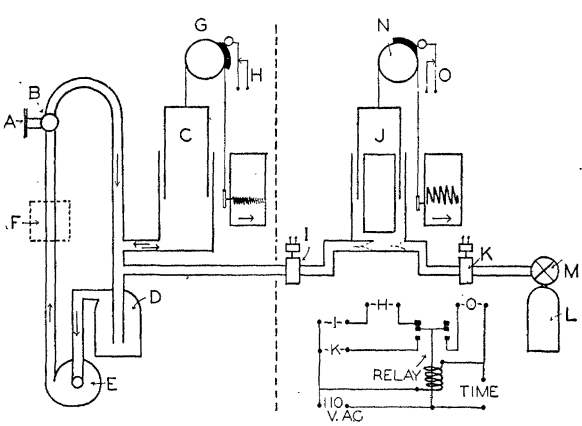

Fig. 1. Apparatus for the continuous measurement of the rate of oxygen consumption.

The motion of the respiration spirometer may describe the type of breathing. The Warren E. Collins, Inc., of Boston, supplies a pulley head for (G) which both integrates the respiration volume and closes a contact for operating an electric counter measuring respiration rates. A second record, that of the motion of the supply spirometer, shows a series of refill lines, the height of each representing the oxygen consumed in the chosen time interval. A very accurate estimation of the rate of oxygen consumption may be had by integrating over several of the refill lines.

The method described has been used at this Laboratory for the measurement of the rate of oxygen consumption during work on a bicycle ergometer. Frequent estimation and even continuous records of this measurement were necessary. At the same time, it was desirable to avoid the space-filling handicap always present when using Douglas bags or large collecting spirometers with the usual gas analysis techniques.

In concluding it may be pointed out that there are several features of the above method that are useful under odd and unusual circumstances:

(a) The apparatus to the left of the dotted line in the figure, being portable, and the size of the usual clinical machine, may be placed compactly and conveniently in the experimental room.

(b) The supply spirometer may be permanently located in another room.

(c) The temperature conditions in these two rooms, provided they are constant relative to each other, may be quite different.

(d) For those interested in metabolism in low pressure chambers, where large volumes of oxygen are used at normal basal rates, the part of the apparatus to the left may be used in the low pressure chamber, while the supply unit giving the record of consumption may remain outside the chamber. For this purpose a needle valve in the supply line would be necessary to adjust the flow from the supply spirometer.

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New Haven, Conn.

[2] Manufactured by the Micro-Switch Corp., Freeport, Illinois; Catalogue No. YZ–RL2 (normally open), $1.69 list.

[3] Manufactured by General Controls, New York, N. Y., Type K–3B, 5/16″ port, 110 v. 60 cyc., $6.40 list.

[4] Manufactured by Struthers, Dunn, Inc., Philadelphia, Pa., Catalogue No. CBTX1, 110 v. 60 cyc., $4.25 list.

[5] See Footnote 3. Type K–20, ¼″ port, 110 v. 60 cyc., $6.40 list.

[6] See Footnote 2. Catalogue No. WZ–RL2 (normally closed), $1.69 list.

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