in the Lyophile apparatus; and third, chick embryo virus, which was frozen while fresh and dried *in vacuo*. In no instance had preservatives been added. The frozen material was thawed and ground to a fine pulp by passage through a meat grinder. It was then placed in tubes, frozen with carbon dioxide snow and desiccated in the Flosdorf-Mudd Lyophile apparatus. In the first experiment carbon tetrachloride was used as a solvent; in the second, petroleum ether; and in the third, the solvent consisted of equal parts of petroleum ether and chloroform, as experience with chicken sarcoma has shown this mixture to be a better extractive. However, with the technique described, all three solvents have extracted the active principle from the sarcoma. In each instance the dried, finely ground material was put in a flask and extracted 3½ hours with four changes of the solvent in a water bath kept at 37° C. During extraction nitrogen was bubbled through the mixture with the double purpose of keeping it agitated and driving out the air. The solvent was filtered until clear and evaporated to dryness in a stream of nitrogen at 37° C. under negative pressure. The dried lipid extract was divided into three portions, one of which was emulsified in distilled water; the second, in a 10 per cent. saline extract of rabbit skin, as this was to be the test animal; and the third, in a 0.5 per cent. casein solution. The casein solution was added because it has proved capable of preserving or augmenting the activity of the lipid from the Rous sarcoma, which was inactive when injected alone, and the skin extract because of the frequency with which lesions occur in this tissue. Each preparation was tested on the skin and cornea of rabbits.

To test the activity of the original material, some of the dried but unextracted tissue was suspended in water at 37° C. for two hours. It was then centrifuged and the supernatant fluid tested on the cornea and skin of rabbits. After removal of the solvent, an extract of the treated pulp was prepared in a similar manner to learn if the virus had been destroyed by the solvent.

Briefly, the extract of the untreated virus was found to be quite active on both the cornea and the skin of rabbits, while the lipid extracts failed to produce any lesion. The water extract prepared from the lipid extracted residue was also active. Guarnieri bodies were present in the corneas of the rabbits inoculated with extracts of the untreated dried virus, and of the extracted tissue residue, but not in those inoculated with the lipid preparations. Identical results were obtained in three experiments.

The air-dried tobacco mosaic virus No. 1[11] and the plants inoculated in our tests, the *Nicotiana glutinosa L.*, were obtained from the Boyce Thompson Institute through the kindness of Dr. Helen Beale.[12] The local-lesion method[13] was used in inoculating the plants. In these experiments, the technique of preparing the extracts was similar to that used with vaccine virus and Rous chicken sarcoma. In the first experiment the solvent used was carbon tetrachloride and in the second, petroleum ether. In both experiments the disease was produced with water extracts of the unextracted and the extracted dried leaves, but there was no evidence of it in the plants inoculated with the lipid extracts.

If we accept the rather generally held view that a disease-producing agent which retains its activity after being passed through a Berkefeld filter should be termed a virus, then we must believe that viruses should be classified according to their chemical properties—as proteins, lipids, etc.—and in the lipid group place the Rous chicken sarcoma. However, it would seem more logical to look upon the agent causing chicken sarcoma not as a virus but as a product of abnormal cell metabolism. It is most unlikely that the lipid can reproduce itself, and therefore it seems probable that it possesses the ability when injected into normal animals under proper conditions to stimulate normal cells to produce a similar substance and thus perpetuate the disease. Certainly our failure to produce disease with the lipid extracts of the two viruses examined indicates that they are of a different chemical nature.

J. W. JOBLING
E. E. SPROUL

INFLUENCE OF DEUTERIUM OXIDE ON PHOTOCHEMICAL AND DARK REACTIONS OF PHOTOSYNTHESIS

IN experiments previously reported,[1] cells of the alga *Chlorella* liberated O_2 about 0.41 as rapidly in buffers prepared with 99.9 per cent. D_2O (deuterium oxide or heavy water) as in those prepared with H_2O (0.02 per cent. D_2O); CO_2 was supplied in excess and illumination intensity was 6,500 lux (correction from 2,000). To study the influence of D_2O on different stages of the photosynthetic process, additional experiments have been made with the manometric technique previously described. Warburg's[2] No. 9 buffer, prepared with potassium salts, was used in the light intensity experiments, and 0.1 M $KHCO_3$ alone was used in the experiments with intermittent light.

[11] James Johnson, *Wisconsin Agric. Exp. Sta. Res. Bull.*, 76, 1927.

[12] We wish to express our great appreciation to Dr. Helen Beale, not only for supplying us with material, but also for teaching us the technique used in this type of experiment. Without her assistance the work would have been much more difficult.

[13] Francis O. Holmes, *Bot. Gaz.*, 87: 39, 1929.

[1] J. Curry and S. F. Trelease, SCIENCE, 82: 18, 1935.

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

Preparations were exposed to continuous light for 15 minutes before observations were begun, and all rates were corrected for respiration by means of tests in darkness. Light for the first series of experiments was furnished by a bank of Mazda lamps placed under the manometer vessels. Intensity of illumination was measured at the bottoms of the vessels by means of a Macbeth illuminometer. Different intensities were obtained by varying the wattage of the lamps and the distance between them and the vessels.

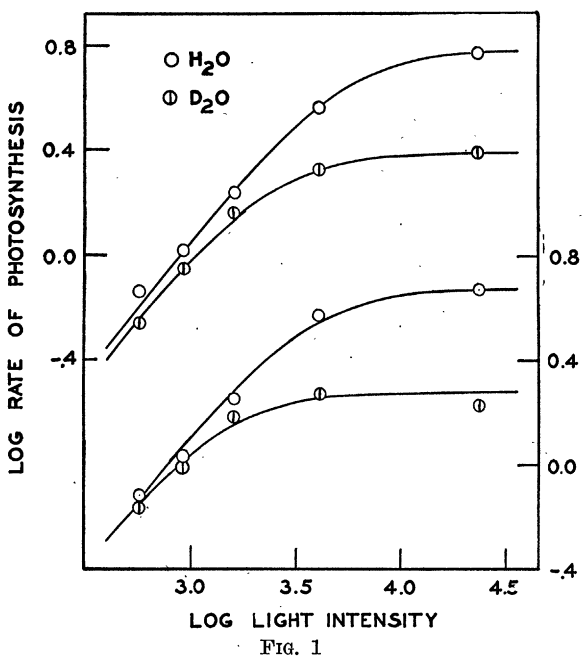

FIG. 1

Data from two typical experiments[3] are plotted in Fig. 1. (The temperature for the upper pair of curves was 23.0° C.; for the lower, 24.9° C.) The smoothed curves were drawn according to the critical equation proposed by Smith,[4] $KI = p/(p^2_{max.} - p^2)^{1/4}$, in which p is the rate of photosynthesis at light intensity I, K is a constant that locates the curve on the I axis, and $p_{max.}$ is the asymptotic maximum rate of photosynthesis.

It may be seen that with high light intensity, when the velocity of the dark chemical reaction determined the rate of the total reaction, D_2O strongly depressed the rate of photosynthesis. But with low light intensity, when the speed of the photochemical reaction determined the rate of the whole process, D_2O exerted but relatively little influence. These results indicate that D_2O retarded the rate of the dark reaction of photosynthesis, but had little, if any, effect on the photochemical stage of the process.

[3] F. N. Craig and S. F. Trelease, *Amer. Jour. Bot.*, 24: in press, 1937.

[4] E. L. Smith, *Proc. Nat. Acad. Sci.*, 22: 504–511, 1936.

Subsequent experiments with intermittent illumination furnished additional direct evidence for this conclusion. Light intensity of 41,400 lux at the bottoms of the manometer vessels was obtained by means of a system comprising a 500-watt projection lamp, a series of lenses and a mirror. For obtaining intermittent illumination, a solid disk with a suitable opening was rotated vertically in front of the light source at 900 r.p.m., so that the cells were exposed to 15 flashes of light per second—each flash being followed by a relatively long dark period. Light was available to 50 per cent. or more of the cells in each vessel about 6.8 per cent. of the time. The duration of a single flash was about 4.5×10^{-3} seconds, and the length of the dark interval was about 62.2×10^{-3} seconds. The temperature was 23.9° C.

Representative results from one of these experiments are plotted in Fig. 2, which shows evolution of

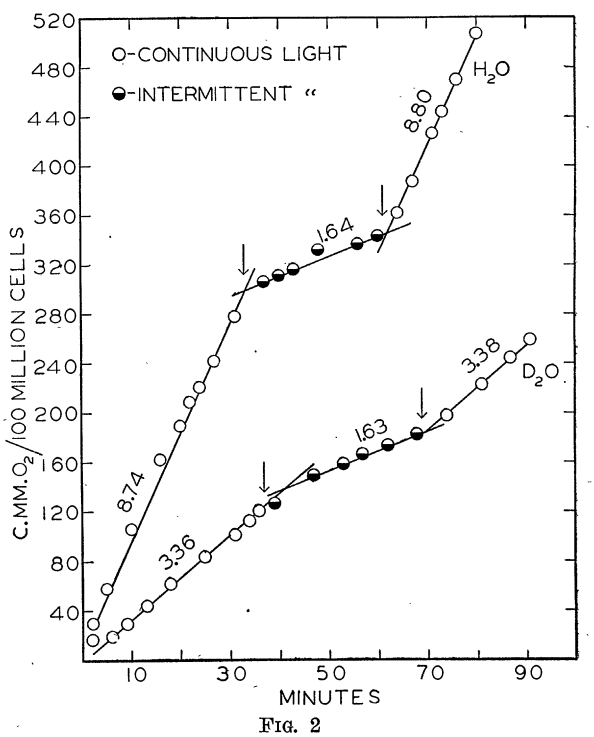

FIG. 2

O_2 from 100 million cells as a function of total time. The number near each curve gives the slope or rate of photosynthesis. Since the portions of the curves representing O_2 evolution in intermittent light are essentially parallel, it is evident that when the dark period after each light exposure was long enough to allow the dark reaction to proceed to completion in D_2O, the rate of photosynthesis was as great in D_2O as in H_2O. Apparently the dark reaction proceeded as far in D_2O as in H_2O, although its velocity was lower in the former. Additional experiments of the

same type have shown that these results are reproducible. It therefore appears that the principal influence of D_2O on photosynthesis is to retard the dark reaction, and that D_2O affects the photochemical stage of the process very little, if at all.

Through the use of the rotating disk the amount of light received by the cells per minute was reduced approximately 93 per cent., but in neither D_2O nor H_2O was photosynthesis reduced commensurately with the amount of illumination. Computations based on the data plotted in Fig. 2 show that per unit amount of light supplied, the amount of photosynthesis was increased in intermittent illumination approximately 175 and 615 per cent. in H_2O and D_2O, respectively. Since the methods employed to obtain intermittent illumination differed somewhat from those of other investigators,[5] the values given here can not be compared with the data published by these writers for photosynthesis in H_2O.

Perhaps the most important implication of our experimental results is that H_2O as well as D_2O enters into the dark stage rather than into the photochemical stage of photosynthesis.

We hope to present elsewhere a detailed account of these and other experiments in which the D_2O concentration, CO_2 concentration, temperature and ratio of dark interval to light interval were varied.

ROBERTSON PRATT
FRANCIS N. CRAIG
SAM F. TRELEASE

LABORATORY OF PLANT PHYSIOLOGY
COLUMBIA UNIVERSITY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD OF MEASURING ROTATIONAL SPEEDS

THE development of the ultracentrifuge has made it necessary to measure rotational speeds over wide ranges. In some of our previous experiments the speed was varied from about a hundred to over twenty thousand revolutions per second. Usually the working speeds are between 500 r.p.s. and 3,000 r.p.s.[1] Several different methods of measuring speeds of rotation in this range may, of course, be used, but in practice they are comparatively complicated and require special or expensive equipment. Also, as in the case of some forms of the stroboscopic method, care must be taken by the observer to distinguish between the fundamental and its harmonics.

A method is described in this paper which is practically free from the above objections and is almost ideally suited to the measurement of rotational speeds over much wider ranges than necessary at present. Fig. 1 shows a schematic diagram of the apparatus.

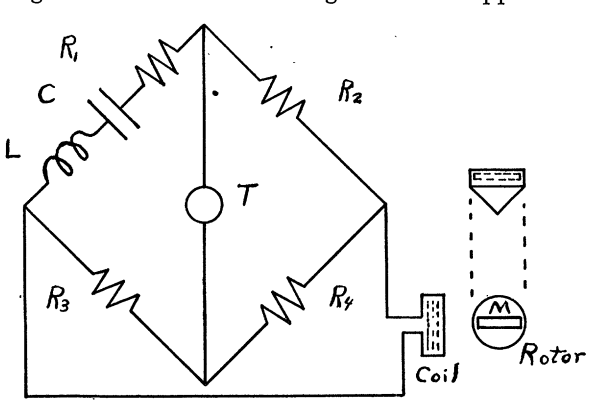

FIG. 1

A small magnet M fastened to the high speed rotor or driving turbine induces an alternating current in the coil, which is connected across a bridge that can be balanced at only one frequency. An inspection of the circuit shows that the bridge will balance when

$$\frac{R_1}{R_2} = \frac{R_3}{R_4} \text{ and } Lw = \frac{1}{Cw} \text{ or } N = \frac{1}{2\pi\sqrt{LC}}$$

where R_1, R_2, R_3 and R_4 are non-inductive resistances; L inductance, C capacity and N the frequency of the alternating current in the bridge. Therefore, the procedure in measuring the rotational speed of the rotor is simply to vary either C or L until T indicates that the bridge is balanced. Then from the known value of L and C the number of revolutions per second N is computed. The indicating instrument T may be a loud speaker, telephone receiver, thermocouple galvanometer, etc., used either with or without an amplifier, depending upon the sizes of the rotating magnet and coil as well as their distance apart. In our experiments the magnet was either a piece of sewing needle (1 to 2 cm in length) or a small cobalt steel magnet. These small magnets when properly mounted do not disturb the balance of the high-speed rotors or turbines. The resistances $R_2 = R_4 = 300$ ohms, and $R_3 = 100$ ohms. They were ordinary non-inductive wire-wound resistances. $R_1 = 100$ ohms and included the resistance of the inductance L. Three fixed mica condensers were used in various combinations as the capacity C, while L was a variable inductance (5 to 25 millihenries). The capacity could thus be varied in large steps, while L could be varied continuously. Table I shows values for the rotational speed com-

[1] Beams and Pickels, *Rev. Sci. Instruments*, 6: 299, 1935.

[5] R. Emerson and W. Arnold, *Jour. Gen. Physiol.*, 15: 391–420, 1932; 16: 191–205, 1932. Warburg, *loc. cit.*