contained 0.13 mg/cc of protein, and the cytoplasmic extract contained 2.36 mg/cc of protein.

The vacuolar sap, the cytoplasmic extract and a control preparation of highly purified virus protein were each diluted to a uniform protein content of 10^{-4} mg of protein/ml in a phosphate buffer at pH 7. Each diluted sample was assayed on the primary leaves of *Phaseolus vulgaris* Var. Scotia. The vacuolar sap, the cytoplasmic extract and the control induced 1; 240; and 222 necrotic lesions respectively on 32 leaves each.

The results of this assay indicate that the protein in the vacuolar sap is chiefly a non-infectious form, and it seems likely that part or possibly all of the trace of virus in this fraction represents contamination from the cytoplasm during the pressing process.

The paracrystalline control virus has been tested many times, and there is reason to believe that its activity in this test represents very nearly the maximum for purified preparations on an equivalent protein basis. The virus activities of the cytoplasmic fraction and the control are so close it is believed that a very large part of the protein extracted in the cytoplasmic fraction is virus protein. An investigation of the total protein in tobacco leaves[6] warrants the assumption that the protein extracted in this fraction is probably less than half of the total protein in the leaf.

Livingston and Duggar,[5] working with tobacco mosaics, obtained cytoplasmic extract and vacuolar sap by means of Chibnall's method and also by means of micro-pipettes inserted directly into individual infected cells. The fractions were not adjusted to a uniform protein content, but when tested on tobacco the cytoplasmic fraction obtained by both methods was found to contain more virus than the vacuolar sap. Our assays were made after adjusting the extracts to equal protein concentrations, and the results indicate even more definitely the localization of virus protein in cytoplasm.

Since the virus is in the cytoplasm it would seem to be a comparatively simple matter to explain its passage from cell to cell through the plasmodesmata, as suggested by Livingston.[7] Many of these cytoplasmic strands which connect the cytoplasm of adjacent cells are visible under the microscope, when suitable methods are used, and therefore, they should be sufficiently large to allow the passage of the ultra-microscopic particles of virus. Eames and MacDaniels[8] consider that plasmodesmata are common in the living tissues of the seed plants, and Livingston's[7] studies indicate their presence in great number throughout the living tissues of the tobacco plant. Therefore, it seems likely that the plasmodesmata supplement the vascular system in the transport of virus.

The observations and experiments leading to the interpretations presented are not in agreement with ideas[9] that the walls of living cells are impervious to the virus and that the virus which is introduced as inoculum generates directly or indirectly some unknown physical force which is transmitted through the cell wall into the adjacent cells where this force initiates the synthesis of new virus.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TIMER FOR USE WITH A WESTINGHOUSE MOVING COIL OSCILLOGRAPH

IN using a Westinghouse four-unit moving coil oscillograph in conjunction with amplifiers for recording muscle action currents and brain potentials, some time-recording device is essential.

The methods we have seen employed have one or both of two disadvantages, the first being the necessity for use of one oscillograph element for recording the timing and the second being the limitation of the time markings to one border of the paper. Use of an oscillograph element for recording time is expensive and eliminates the element from availability for recording physiological phenomena. A time line which is limited to one border and is not projected across the entire surface of the paper is difficult and time-consuming to read.

We have devised for use on a Westinghouse four-unit oscillograph, recording on sensitized paper, a timer which does not require the use of an oscillograph unit and can be produced for substantially less money than can that type. It produces time lines which are superimposed upon the physiological records and traverse the entire width of the sensitized paper record. It is capable, with an interchange of one part, of recording time intervals of from one-half second to a fortieth of a second or less. By means of a motor geared higher or lower than the one we have used, its range can be widened considerably.

The device consists of a slotted brass cylinder mounted on bearings within a slightly larger cylinder,

[6] L. F. Martin, A. K. Balls and H. H. McKinney, SCIENCE, 87: 2258, 329, 1938.

[7] L. G. Livingston, *Am. Jour. Bot.*, 22: 75, 1935.

[8] A. J. Eames and L. H. MacDaniels, "An Introduction to Plant Anatomy." New York, 1925.

[9] W. M. Stanley, *Phytopathology*, 26: 305, 1936.

the ensemble being mounted on the top of the cover for the oscillograph about midway between the ends. The larger cylinder has cut in it two slots opposite each other on the upper and lower sides. Above the cylinder ensemble is mounted a small tubular light bulb. Below the cylinder in the top of the oscillograph box is cut a small transverse slit, five inches long. The small inner cylinder in which is cut anywhere from two to forty opposing slots is turned by a geared synchronous motor which revolves the cylinder at the rate of one revolution per second. Enlargement of one of these pairs of slots will provide heavier time lines at half-second intervals in addition to the regular markings at one-fourth, tenth or twentieth second intervals. Light from the bulb passes through the pairs of slots in the two cylinders whenever these slots coincide, and through the slotted box cover to a 90 degree reflecting prism or mirror where it is directed forward through the oscillograph condensing lens to a second mirror and onto the paper. Fig. 1 presents

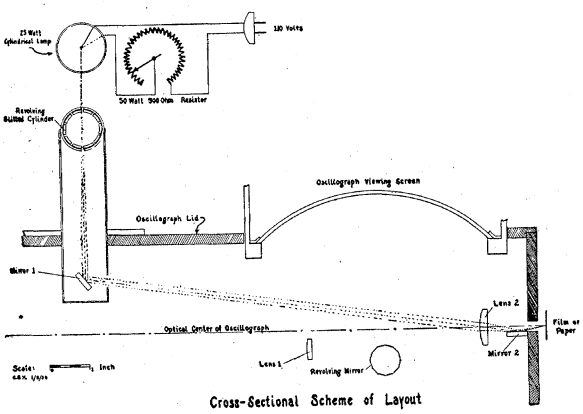

FIG. 1.

a diagrammatic sketch of the arrangement. The light used is a 25-watt, 120-volt cylindrical house bulb which may be purchased for 40 cents. The motor is a synchronous one of one-one hundred and fiftieth horse power geared to turn the drive shaft one revolution per second. It is a product of the Bodine Electric Company of Chicago. The 90 degree reflecting prism is a standard Bausch and Lomb product, while mirror number 2 is a chrome-plated first surface one produced by the Evaporated Metal Film Corporation of Ithaca, New York. A rheostat of 500 ohms permits adequate adjustment of light intensity for any sensitization of paper or film and for the different speeds at which it may be run. A sheet brass housing encases the lower portion of the cylinders and extends down through the top of the oscillograph box so as to prevent extraneous light from entering the box. The lower end of this housing serves also as the mounting for the reflecting prism. Fig. 1 shows a diagrammatic

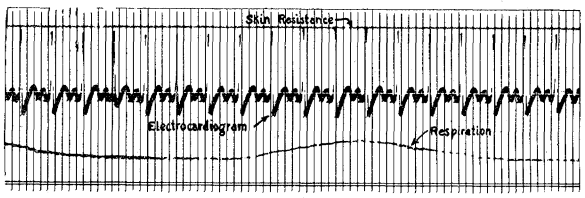

FIG. 2.

sketch of the timer and mirror arrangement, and Fig. 2 a sample of time lines at one tenth second intervals as they appear on a skin resistance, respiration and electrocardiographic record made with the apparatus. The apparatus was built by A. E. Berdon, Yellow Springs, Ohio.

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