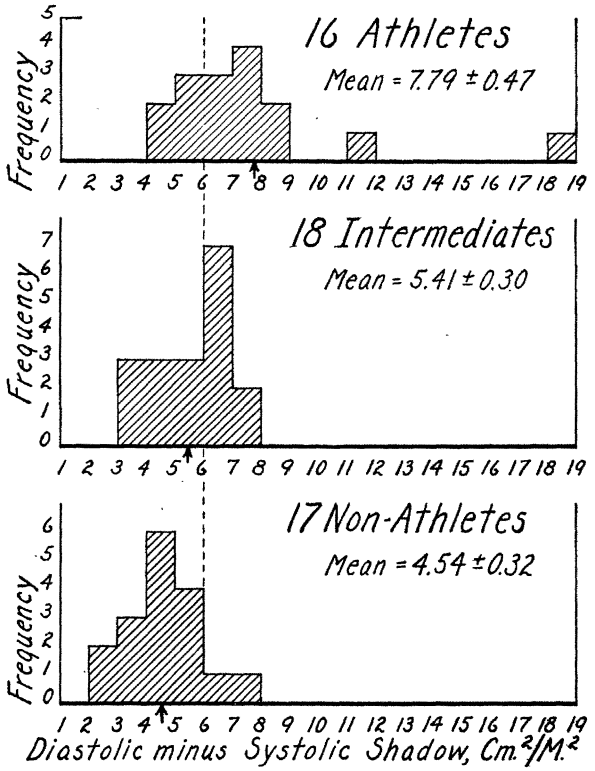

FIG. 2. Size of the stroke of the heart in posterior-anterior projection. Means, probable errors and numbers of individuals in groups separated by intervals of 1 square cm per square meter of body surface.

unit of body weight. We found, however, that the variation between individuals was 12 per cent. greater when body size alone was used.

In our series bradycardia was well marked in the athletes, and the slowness of pulse was, on the whole, proportional to the extent of participation in strenuous sports. It would be tempting to estimate rates of circulation from these data, but special experimental studies are needed to provide the numerical factors and to evaluate the accuracy of such a procedure. These experiments, on which we are now engaged, include:

(1) Simultaneous Roentgen kymography and direct cardiac output studies on the heart-lung preparation of the dog.

(2) Simultaneous Roentgen kymography and cardiac output measurement by the acetylene method in man.

(3) Roentgenograms on the heart in the intact cadaver followed by direct measurements on the heart removed from the chest.

(4) Calculations from mean cardiac outputs and mean cardiac shadows in normals.

The results thus far indicate that the estimation of cardiac output and total heart volume is perfectly feasible from the Roentgen kymograms. Since completion of this note we have partially completed an analysis on a larger series of subjects with the results in full agreement with those given here.

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TOBACCO-MOSAIC VIRUS CONCENTRATED IN THE CYTOPLASM[1]

INVESTIGATIONS carried out by Chibnall[2,3] and by Phillis and Mason[4] indicate that the vacuolar sap and the cytoplasmic extract can be obtained as essentially separate fractions when the leaves are subjected to suitable treatments. Phillis and Mason separated the vacuolar sap from the cytoplasm of cotton leaves by simple application of 6,000 to 16,000 pounds per square inch pressure to the whole leaves. It was found that this method is unsuitable for tobacco leaves, the texture of the leaf being destroyed at pressures much less than those to which cotton leaves were subjected. We were successful in using the method of Chibnall,[2] which was also used by Livingston and Duggar[5] in similar studies.

Fresh leaves were harvested from mature *Nicotiana tabacum* L., variety Maryland Medium, systemically infected with tobacco common mosaic. Midribs were removed, and 175 grams of leaf tissue completely immersed in 100 grams of ether for 10 minutes. Surplus ether was poured off and the leaves placed flat in a pile of cheese cloth, between flat plates of a hydraulic press. Pressure was applied slowly, up to 5,000 pounds per square inch, and the vacuolar sap obtained as a clear brown liquid, amounting to 45 cc. The pressed tissue was frozen over night at zero degrees F. and thoroughly pulped in a meat grinder. The ground pulp was diluted with 50 cc of water and 20 cc of 0.5 M disodium phosphate solution added. After extracting at two to four degrees C. over night the whole juice was pressed out. This extract was dark green and contained much pigment and cell debris. After filtering through Celite, 78 cc of clear brown solution was obtained, which was regarded as cytoplasm sap.

In agreement with the results of Chibnall[2] on spinach, the vacuole sap contained a very small amount of protein. It was partially insoluble in 0.5 saturated ammonium sulfate and precipitated by trichloro-acetic acid. The 45 cc of vacuolar sap contained 0.317 mg/cc of total N, and only 0.022 mg/cc of protein N. Most of the protein was in the 78 cc of cytoplasmic extract, which contained 0.583 mg/cc of total N, and 0.389 mg/cc of protein N. On this basis the vacuolar sap

[1] Studies conducted under Bankhead-Jones Project S.R.F. 2–17, U. S. Department of Agriculture, Bureaus of Plant Industry and Chemistry and Soils cooperating. Food Research Division Contribution No. 387.

[2] A. C. Chibnall, *Jour. Biol. Chem.*, 55: 333, 1923.

[3] *Ibid.*, *Jour. Biol. Chem.*, 61: 303, 1924.

[4] E. Phillis and T. G. Mason, *Nature* (London) 140: 370, 1937, No. 3539.

[5] L. G. Livingston and B. M. Duggar, *Biol. Bull.*, 67: 504, 1934.

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.