under pressure as compared to controls, the temperature and relative humidity being constant and comparable in both pressure and control incubators. It was noticed, however, that the control eggs lost water more rapidly than the experimental, and it was therefore suggested that the retention of water might be responsible in part for the accelerated growth. Other workers had already observed that increased humidity, within rather wide limits, increased the growth rates of chick embryos at normal pressures.

To determine whether or not the decreased water loss, which in turn is dependent upon the humidity of the surrounding atmosphere, was in part responsible for the increased growth rate of the chick embryos, the humidity in the pressure incubator was lowered until the water loss of the experimental eggs was comparable to that of the controls. When these adjustments had been made, 40 eggs were placed in the pressure incubator and maintained at a pressure of 25 to 30 pounds; 33 control eggs, matched by weight with the experimentals, were incubated at normal pressure. Both lots of eggs were incubated at 100° F.

After eleven days, the embryos were removed, stripped of their membranes and weighed with the following results.

TABLE 1

	Control	Experimental
Average original weight of eggs ..	58.9 gr.	59.7 gr.
Average water loss	1.8 "	2.0 "
Average weight of embryos	3.69 "	6.07 "

Per cent. increase in weight of experimental embryos over the controls was slightly more than 60 per cent.

When compared to the 42 per cent. increase secured in the best of the earlier experiments where the relative humidity in control and experimental incubators was kept the same, it would appear that the retention of water prevents the maximum effect of the pressure, and pressure may now be definitely considered as responsible for the accelerated growth.

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CRYSTALLIZATION OF TOBACCO-MOSAIC VIRUS PROTEIN

METHODS for the crystallization of tobacco-mosaic virus protein specify a preliminary precipitation of certain proteins in amorphous form previous to obtaining the crystalline or liquid crystalline protein. Bawden and Pirie[1] reported that heating the crude infectious sap to 70 C. before clarifying it by centrifugation saved considerable time in that the virus protein could be isolated by direct precipitation with acid or ammonium sulfate, thus eliminating preliminary precipitation with alcohol. It has been found and herein reported that upon heating the macerated frozen tissue to 40 C. and adjusting the reaction to about pH 7.0 by dissolving disodium phosphate salt, and clarifying the juice by filtration, crystallization of the protein comes about during the gradual change of reaction from about pH 7.0 to 6.0 and cooling of the filtrate to room temperature. By adjusting the reaction to pH 4.5 (green to bromecresolgreen) with acetic or sulfuric acid, salting out with 0.3 saturation of ammonium sulfate and storage at 0 to 5 C. over night, further crystallization comes about.

Initial crystallization of the virus protein was obtained in the crude infectious tobacco juice by heating the frozen macerated green tissue to 40 C. for 10 minutes in presence of disodium phosphate salt (35.8 grams per 1,000 grams of green tissue); pressing the liquid through doubled cheesecloth by wringing with the hands; filtering this liquid through celite (25 grams per 1 liter of liquid); allowing the reaction of the filtrate to change without further treatment for 30 minutes (from about pH 7.0 to pH 6.0); adjusting the reaction to pH 4.5 with acetic or sulfuric acid; and dissolving ammonium sulfate to 0.3 saturation (175 gms per liter). Upon agitation the solution possessed a velvety appearance (sheen) which is considered characteristic of crystalline protein in suspension. The crystals developed rapidly and were similar to those illustrated by Stanley.[2] The material was allowed to stand over night at 0 to 5 C. before separation of the crystals from the liquid and subsequent recrystallization by three procedures:

Procedure A. Filtration and dissolving the crystals in alkaline phosphate buffer at pH 8.0 and 40 C. A 1,000 mls sample of the material (first crystallization) was filtered through 25 grams of celite by suction. The residue was dissolved in 500 mls of 0.1 molar phosphate buffer at pH 8.0 and 40 C. and the celite removed by filtration through paper. The filtrate was adjusted to a reaction of pH 4.5 and ammonium sulfate added to 0.3 saturation as in the first crystallization. The procedure was repeated for the third crystallization.

Procedure B. Filtration and Stanley's[3] calcium oxide method for dissolving the crystals. A 1,000 mls sample of the material was filtered as in procedure A,

[1] F. C. Bawden and N. W. Pirie, *Proc. Royal Soc. London*, Ser. B, 123: 274–320, 1937.
[2] W. M. Stanley, *Phytopath.*, 26: 305–320, 1936.
[3] W. M. Stanley, *Jour. Biol. Chem.*, 115: 673–678, 1936.

but the residue was dissolved at room temperature in 500 mls of distilled water by adjusting the reaction to pH 8.0 with a 0.1 per cent. aqueous suspension of calcium oxide. The celite was removed and recrystallization brought about as in procedure A. Procedure B was followed for the third crystallization.

Procedure C. Centrifugation at 1,500 times gravity for 10 minutes with treatment of sediment (supernatant liquid decanted) as in procedure A. Procedure C was followed for the third crystallization.

Assay of virus infectivity by the local-lesion method on primary leaves of Scotia beans was made by Mr. H. H. McKinney. Primary leaves of 20 bean plants at a susceptible age were inoculated with a 10^{-4} dilution (in 0.1 molar phosphate buffer at pH 7.0) of each sample previously adjusted to 1 mgm of protein per 1 ml of solution (nitrogen analysis by microkjeldahl of the material precipitated by 5 per cent. trichloroacetic acid). The average number of lesions per leaf was 4.2; 8.1; 4.4; 4.9; and 4.3 for the cheesecloth filtrate; celite filtrate of the crude juice; and the dissolved material from the third crystallization by procedures A, B and C, respectively. Infectivity of the material of the first or second crystallization was not determined.

These infectivity data based upon the protein content of each sample show no marked change in activity upon crystallizing the protein three times by either of the three procedures. The increase in infectivity of the celite filtrate, if significant, may have been due to particles of the celite in the liquid aiding infection or to filtration increasing the infectivity possibly by increasing the dispersion of the virus or removing some inhibitor. The increase is in agreement with that reported[4] from filtering crude juice in 0.1 molar phosphate buffer at pH 8.5 through Berkefeld "W" candles.

Tobacco juice from healthy plants when treated in a similar manner failed to develop any evidence of crystalline protein. The precipitate that formed was composed of amorphous material as far as could be determined microscopically.

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PHOTOPERIODIC STIMULUS TRANSFER IN PLANTS

IN experiments to determine the perceptive locus and mode of transfer of photoperiodic stimuli in plants, investigators have commonly used grafts of flowering scions upon vegetative stocks or *vice versa*.[1,2,3] Such entire grafted plants under a vegetative photoperiod have shown transfer of the residual flowering stimulus across the graft union, presumably in the form of a hormone, tentatively designated florigen.

These techniques are open to the objections that they introduce the extraneous stimulus of severe traumatism, result in considerable mortality, necessitate prolonged post-operative care, and interfere with nutrition. They are tedious, entail delay with its attendant hazards and inconvenience, and to a great degree they impose such severe numerical limitations as to preclude statistical evaluation of results. The photoperiodic responses also vary with the type of graft employed.

In order to minimize these difficulties, while at the same time differentially illuminating contiguous parts of the same plant, the writer has employed a thin, opaque panel with an adjustable, horizontal slit through which the tops of potted plants are trained from both sides as they grow under a vegetative photoperiod. At the desired time, short-day lighting is initiated on one side and long day on the other, thereby keeping the bases of one set vegetative and inducing flowering of the tops of the same plants, while the reciprocal responses are simultaneously induced in the other set on the opposite side of the panel. During the spring and summer, the daylight period can be shortened by use of a curtain suspended from a wire frame on the panel. Simpler, however, is the performance of the experiment under natural conditions of short day with extension of illumination for several hours by use of automatically timed, adjustable electric lights on the long-day side of the panel. Undesired reflection on the short-day side can readily be prevented by sufficient height and length of the panel. The slit panel technique is especially convenient when an interchange of lighting on opposite sides is desired, as it can be reversed without moving the plants.

The panel procedure yields more consistent results and hence makes interpretations simpler which, in combination with larger experimental populations, adds an important element of reliability. In an investigation on dioecious plants the writer has found the functional and structural responses of staminate parts to be distinctly different from those of pistillate parts to a given photoperiod, when top and base of the same plant are subjected to contrasted length of day. In other words, the reproductive photoperiod both in long- and short-day species exerts a different effect upon the "male" and "female" processes, a response which may permit the elucidation of many as yet ob-

[4] H. H. Thornberry, *Phytopath.*, 25: 618–627, 1935.

[1] M. C. Cajlachjan and L. M. Yarkovaja, *C. R. Acad. Sci. URSS*, 15: pp. 215–217, 1937. Also pp. 85–88; 3: pp. 443–447; and 4: pp. 77–81, 1936.

[2] B. S. Moskov, *C. R. Acad. Sci. URSS*, 15: pp. 211–213, 1937.

[3] J. Kuijper and L. K. Wiersum, *Proc. Acad. Sci. Amsterdam*, 39: pp. 1114–1122, 1936.