patches. Leaves having white dots or patches were evident in all species studied, with the exception of the cacti. One plant of *Antirrhinum* showed a remarkable condition in its cotyledons in that a white band about 5 mm wide crossed each cotyledon in exactly the same place.

In *Myosotis,* deeply cleft cotyledons occurred in about one fifth of the seedlings. This condition was not observed in any of the other species studied.

Leaves which were slightly notched or deeply cleft were observed frequently in *Myosotis* and *Antirrhinum,* but only twice in *Oenothera franciscana*—no variation of any kind being observed in *Oenothera blandina.* Each of the two parts of a leaf resulting from a deep cleft often showed a separate, well-developed midvein.

In a large number of cases a considerable part of a leaf was deleted so that there was no tissue on one side of the midrib, only a small irregular mass with or without veinlets going into it from the midrib; or the leaf was perfectly normal except for a small deleted area. A number of rather twisted and grotesque forms resulted from the radiations.

In summary, the bombardment of dry seeds of certain species by stray neutrons had no effect on germination, whereas in other species it caused a decrease in germination directly proportional to the duration of exposure. Seedlings and mature plants grown from the neutron-bombarded dry seeds showed a number of morphological variations from the normal condition.

ROY MILTON CHATTERS
DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE SEPARATION OF PLANT VIRUSES BY CHEMICAL INACTIVATION[1]

SOME virus complexes, which may occur in nature or in accidental mixtures in experimental work, are often difficult to separate by known or convenient means. An investigation of the possibility of the use of chemicals for this purpose was therefore undertaken, with the expectation that some additional light might be thrown on the nature of the viruses themselves by their reaction toward chemical substances.

The separation of certain combined viruses has been accomplished by treatment of the plant extracts containing the viruses with chemicals which have proved to be specific inactivators for certain viruses. Water solutions of the chemicals were added to the extracts and allowed to act at 20° C. for one hour. These preparations were then diluted to one part in fifty parts of water in order to reduce any possible chemical injury when inoculated to the host (*Nicotiana tabacum* Havana variety). If symptoms caused by only one virus were apparent, extracts from such plants were tested for purity by further inoculations to Havana tobacco. Repeated trials were made with such chemicals as showed promise, and a wide variety of chemicals in various concentrations have been tested.

The separation of a mixture of the viruses of cucumber mosaic and potato ring spot may serve for illustration in this preliminary note. Tests were made to determine the minimum concentrations of chemicals necessary to inactivate each of these viruses, and it was found that cucumber mosaic virus could withstand higher concentrations of silver nitrate and mercuric chloride than could the potato ring spot virus. Conversely, it was found that the potato ring spot virus could withstand higher concentrations of potassium permanganate, lithium carbonate and copper sulfate. Mixtures of these two viruses were treated with concentrations of potassium permanganate ranging from 0.1 to 0.9 per cent. in ten separate experiments, and only the potato ring spot virus remained infective, except in one trial where both viruses were inactivated by the same concentration of the chemical. In three trials 1 per cent. lithium carbonate and 2 per cent. copper sulfate gave similar results. However, using the same extracts as above the potato ring spot virus could be inactivated, leaving the cucumber mosaic virus infective. This result was secured in four trials by treatment with silver nitrate ranging in concentration from 0.1 per cent. to 0.5 per cent., and eleven times by treatment with 0.1 per cent. to 0.9 per cent. mercuric chloride in as many trials. The exact chemical concentrations necessary for a definite separation can not always be accurately determined since fairly wide variations in behavior have been observed.

The reasons for the differential action of the chemicals used are obscure. In preliminary determinations, hydrogen-ion concentration did not seem to be correlated with the inactivation of the viruses in these experiments.

WM. B. ALLINGTON
WISCONSIN AGRICULTURAL EXPERIMENT STATION

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A METHOD FOR FINDING THE FREE WATER IN PLANT TISSUE

ABOUT a month ago I was approached by the scientists at the Northern Rocky Mountain Forest Experi-

mentation Station, who wanted to know if I could suggest a method whereby the amount of free water in samples of forest vegetation could be determined with reasonable accuracy. It occurred to me that by determining the heat capacity of a sample of a plant tissue, then driving off the moisture and then redetermining the heat capacity, a measure of the amount of free water, which has a specific heat of approximately one calorie per gram per degree Centigrade, could easily be obtained.

A sample experiment convinced me that the method could be used to good advantage. For my test experiment, I selected a sample of potato tissue, and in order not to destroy any of the cell structure in the process, I cooled the sample to a temperature near the freezing point of water, then placed it in a calorimeter, the water content of which had a temperature slightly above that of the room and thus determined from the temperature fall of the water the heat capacity of my weighed sample of potato tissue.

After two days and nights of gentle drying on a moderately warm radiator, I determined the heat capacity of the dry residue, subtracting this heat capacity, which was quite small, from the original heat capacity. I found that the water originally in the potato had in that state an average specific heat of .70 calories per gram per degree, indicating that only a part of this water could have been present in the form of free water. The rest must then have been present in a chemically bound form.

Since the determination of free water in plant tissue is a somewhat cumbersome process, and at times yields dubious results due to the effects of maceration, I hope this description of a calorimetric method will be found useful by horticulturists, plant pathologists and others.

G. W. HAMMAR

UNIVERSITY OF IDAHO

AN IMPROVED TISSUE CULTURE CHAMBER[1]

THE observation of tissue cultures, made in the hanging drop on a cover glass, is often difficult because of the excessive and uncontrollable thickness of the drop, and also because of the curvature of its free surface. In addition, the spherical surface of the depression in the slide increases the optical difficulties. Although there are on the market hollow slides with a plane-parallel bottom of the chamber, all of them are so thick that even the employment of a long focused condenser can not render proper illumination.

To correct these imperfections, a chamber was devised which consists primarily of an ordinary slide, about 0.75 mm thick, with a round hole of about 1.5 cm in diameter, drilled through its center. This hole is bridged over by a cover glass which is cemented to the lower surface of the slide along the edge of the hole, thus forming a shallow container with a plane and thin bottom (Fig. 1) which obviates the optical difficulties mentioned above.

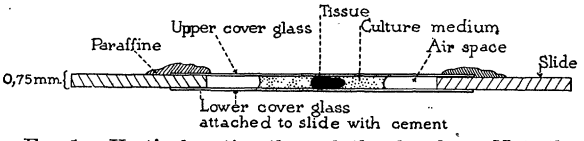

FIG. 1. Vertical section through the chamber. Note the plane-parallel surfaces of the chamber and the even thickness of the culture medium.

In preparing the tissue culture, one places the drop of the medium and the tissue particle in the center of the chamber so that the peak of the drop reaches slightly above the level of the upper surface of the slide; then the drop flattens out to a plane-parallel layer between the two cover slips. Care must be taken that the drop does not fill the entire chamber so that enough air space is left for the respiration of the tissue. The upper cover glass is sealed with paraffin in the usual manner.

The advantages of this chamber are: simplicity of construction, use of standard material (ordinary slide and cover glass), easy replacement of bottom when broken, elimination of surfaces causing optical disturbance and close proximity of culture to condensor.

GUSTAV ZECHEL

MEDICAL COLLEGE
UNIVERSITY OF ILLINOIS

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