

month there were approximately 45 per cent. more animals in the test than in the control jars. With triphenyl benzene, whose carcinogenic activity has been recently questioned, the whole animals were similarly stimulated while the segments failed to react.

With glutathione, a known tissue growth stimulant, the results were similar to those obtained with dibenzanthracene. Derivatives of glutathione, as glutamic acid, glycine and cysteine, produced no evident stimulation of growth in the segments or increase in the number of planaria.

Allantoin and preserved larval extract did not stimulate growth or reproduction of the cut or uncut animals.

Complete details of the technique, histology of the treated specimens and further results will be presented when the study is completed.

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THE EFFECT OF FAST NEUTRONS ON DRY SEEDS

THE experiments discussed in this paper had a two-fold purpose: (a) to determine whether dry seeds left on the outside of the cyclotron "tank" would receive enough stray neutrons to produce a cumulative harmful effect preventing their subsequent germination; and (b) to determine whether changes in external morphology, similar to those produced by x-rays,^{1, 2} radium and radium salts, would occur in plants grown from the neutron-bombarded seeds.

Plants with very small seeds were chosen for these experiments in order that a large number might be used at one time in the limited space available for bombardment by the cyclotron.

Seeds from each of the selected species and varieties were put into small (2.3 × 0.8 cm) gelatin, medical capsules which proved to be most satisfactory for handling the tiny seeds. More than 500 seeds of the *Oenotheras*, for example, were placed in a single capsule without crowding.

The capsules were in turn placed in a small (8 × 8 × 6 cm) lead box whose 1 cm. thick walls were lined inside with a 2 mm layer of paraffin. The purpose of the lead was to filter out all emanations from the cyclotron except the neutrons, and that of the paraffin to increase the effect of the neutron bombardment within the seeds. A lead cover 1 cm thick was

anchored to the box after the capsules bearing the seeds had been placed within.

The whole was then laid on a small metal shelf attached to the outside of the cyclotron "tank" so as to be close to the bombarding chamber, but at a distance of about 60 cm from the target. In this way the seeds could absorb only stray emanations from the cyclotron, whenever it was in operation, during the three months in which these experiments were conducted.

Seeds of the various kinds were removed from the capsules from time to time and put on wet filter paper in Petri dishes to obtain germination counts. Then the germinating seeds were planted in soil in order to obtain seedlings and mature plants upon which to observe possible morphological changes due to the neutron bombardment.

All exposures were made on the University of Michigan cyclotron through the cooperation of Professor J. M. Cork and Dr. R. L. Thornton, of the physics department.

Normal germination for all plants used in these experiments is above 90 per cent.

Table 1 shows three things: (a) that as exposure time increases the percentage of germination in some plants decreases, whereas (b) in others there is little or no appreciable effect, and (c) that there is a wide range of susceptibility to neutrons as has already been found for a large number of x-rayed plants by Johnson.

TABLE 1

Species studied	Weeks subjected to emanations	Percentage germination
<i>Oenothera franciscana</i>	1	95.0
<i>Oenothera franciscana</i>	2	82.5
<i>Oenothera franciscana</i>	9	15.4
<i>Oenothera blanda</i>	2	94.8
<i>Oenothera blanda</i>	9	51.9
<i>Echinocereus papillosus</i>	1	16.8
<i>Rhipsalis rhombica</i>	2	29.2
<i>Neomammillaria multiceps</i> ..	2	99.5
<i>Antirrhinum</i> sp. (1)	6	5.0
<i>Antirrhinum</i> sp. (2)	6	75.0
<i>Antirrhinum</i> sp. (3)	6	23.0
<i>Myosotis</i> sp.	6	18.7

The morphological variations in the plants grown from treated dry seeds were numerous, and it is noteworthy that often they are found to be similar in more than one species. This same phenomenon occurs in x-rayed plants. A brief summary of the variations and their relative frequencies are discussed in the following paragraphs.

A condition which was observed to be most common was the decrease in size of plants grown from rayed seeds as compared with normal plants. They were not only shorter, but the stems and leaves were smaller and weaker.

In *Antirrhinum* the cotyledons were invariably found to be covered with numerous white dots or

¹ J. H. Lawrence and E. O. Lawrence, *Proc. Nat. Acad. Sci.*, 22(2): 124-133, 1936.

² R. E. Zirkle and P. C. Aebersold, *Proc. Nat. Acad. Sci.*, 22(2): 134-138, 1936.

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.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE SEPARATION OF PLANT VIRUSES BY CHEMICAL INACTIVATION¹

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. Con-

versely, 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.

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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-

¹ Supported by Wisconsin Alumni Research Foundation.