which we knew to be very badly infested with larvae, we closed the box with gummed paper except for one corner where a small hole about 2 mm wide was left. When this box was opened up a month later, hundreds of dead larvae were found in the immediate vicinity of this hole in the box and still more hundreds were in the plants in the sheets from which they had been unable to escape.

The substance paradichlorobenzene kills not only the larvae and pupae, but also the eggs. It, of course, takes longer exposure to kill the eggs, but entomologists feel that the presence of a highly saturated atmosphere for two weeks will do this. It is our experience that this is undoubtedly the case.

In summary, paradichlorobenzene has been found to be an inexpensive, very proficient means of carrying on herbarium fumigation *in situ*, as it kills all forms of the pests with a minimum of caretaker's discomfort and is not a fire hazard.

FRANK C. GATES

MANHATTAN, KANSAS

## AN IMPROVED METHOD FOR SEED GERMINATION

IN germinating seeds on either wet blotting paper or towelling paper, certain difficulties are often encountered. Chief of these is the development of root hairs into the surface of the substrata and subsequent difficulty in removing the germinated seeds without injury. A second problem is met with in keeping the seeds in a humid atmosphere most conducive to germination. The method described was designed to eliminate these problems.

Number 300 Cellophane (thin wrapping Cellophane), previously soaked in distilled water for a period of an hour or more to remove the glycerine, is placed wet upon a saturated pad of several sheets of blotting paper, or paper or cloth towelling. The Cellophane presents a very smooth surface to which the root hairs do not adhere.

The seeds are placed on the Cellophane and the blotting pad is placed in the bottom of a miniature greenhouse or other suitable enclosure, such as a bell jar. Thus a moist atmosphere is provided and excessive evaporation from the blotting pad is prevented.

In case seeds are to be germinated for planting purposes, sterilized wet fine sand may be used in place of the Cellophane and blotting pad. In this case, the seeds should be oriented for planting, the radicle in a vertical position. Seeds as large as those of pea, bean and corn may be germinated successfully in this manner.

A particular advantage of using glass-topped boxes or bell jars lies in the fact that the process of germination may be observed without disturbing the seeds. This procedure is good for preparing germinated seeds for the demonstration of root hairs.

ROBERT B. WITHROW
HARRIS M. BENEDICT

PURDUE UNIVERSITY
AGRICULTURAL EXPERIMENT
STATION

# SPECIAL ARTICLES

## MENINGITIS IN MAN CAUSED BY A FILTERABLE VIRUS

DURING December of 1934 two adult males, W.E. and R.E.S., developed an illness characterized by headache, vomiting, stiff neck and a high cell count, 1,700 and 720 per c.mm., respectively, in the spinal fluid. The cells in the fluid were practically all mononuclear elements. Both patients made a slow uneventful recovery and are now well.

The clinical pictures presented by the patients were almost identical and suggested a virus meningitis. Consequently, spinal fluid from each of them was inoculated intranasally, intraperitoneally and intracerebrally into 6 Swiss albino mice.

Of the 6 mice that received W.E.'s spinal fluid, one died on the third day of a streptococcal infection and was discarded. The remaining 5 mice became sick 6 or 7 days after inoculation. One of them died and was discarded, 2 were allowed to recover and 2 were killed in order to prepare a brain emulsion for intracerebral inoculation of other mice. The second lot of mice became sick about a week after inoculation and some of them were sacrificed for passage. By means of emulsions of bacteriologically sterile brain material injected intracerebrally into mice the active agent has been passed serially through 10 lots of mice and at present small amounts of a 10 per cent. emulsion of infectious brain material kill practically all the mice in 7 days.

In a similar manner an active agent free from bacteria was obtained by the inoculation of R.E.S.'s spinal fluid into Swiss mice. The virus has been passed through 9 sets of mice and reinoculation experiments clearly show that the W.E. and R.E.S. strains are immunologically identical.

Mice inoculated intracerebrally with either strain of virus become sick within 5 to 7 days and lose weight rapidly. Their fur is ruffled. Only a few of them develop signs referable to the central nervous system which consist of irritability and convulsions. No paralyses have been noted. The virus is in the brain, liver, lungs and blood. Mice inoculated intraperi-

toneally become sick, but only a few of them die. Intranasal inoculations of the virus cause no visible illness in mice but immunize them against the virus injected intracerebrally. The chief lesions found in mice are a mononuclear cell meningitis, a hyperplasia of Kupffer cells in the liver, and a pneumonia similar to that caused by other filterable viruses.

The virus causes no lesions in the skin of rabbits, and when it is inoculated intracerebrally the animals only exhibit a fever of short duration. We have no definite evidence, therefore, that rabbits are susceptible to this active agent.

Guinea pigs are susceptible to both strains of the virus inoculated either intracerebrally or subcutaneously. A high continued fever (104–106° F.) and loss of weight are the striking features of the infection in this host. Death usually occurs 10 to 14 days after intracerebral inoculation and many of the pigs die after subcutaneous injections. A slight meningitis or a virus pneumonia is all that our pathological studies have revealed so far.

Monkeys (*M. rhesus*) are susceptible to the active agent introduced intracerebrally, as evidenced by high fever, loss of weight and irritability. No paralyses have been noted. From the small number of monkeys injected we judge that the disease will not as a rule be fatal for this host.

From tissues containing the active agent no ordinary bacteria that might be of etiological significance have been cultivated. Furthermore, the virus passes through Seitz pads, Berkefeld V, N and W candles, and collodion membranes possessing an average pore diameter of 210 μμ. Additional work is under way to determine the size of the virus.

The results of our experiments seem to indicate clearly that the virus was obtained from the spinal fluid collected from the two patients and that it is pathogenic for man. Two mice that received W.E.'s spinal fluid and recovered and 5 mice that received R.E.S.'s spinal fluid and did not become sick were later found to be solidly immune to virus introduced intracerebrally. We have not encountered any immune animals among our stock mice. Consequently, we believe that the immune mice mentioned above realized that state through having received an immunizing dose of virus in the spinal fluid. Furthermore, neutralization tests conducted in mice and guinea pigs show that serum collected from the patients at the beginning of their illness fails to neutralize the virus, while serum collected late in convalescence does inhibit its activity. In passing it should be noted that neutralizing antibodies appear very slowly in the sera of guinea pigs, monkeys and human beings convalescing from an infection with the virus.

Our virus is not similar to any active agent heretofore described, with the exception of those of Armstrong and Lillie[1] and Traub.[2] The former workers speak of the source of their virus in the following manner: "It is not apparent whether this virus came from the case C.G. or from one of the monkeys used in the transfer of virus from this case. In either event the virus was apparently in a latent state and was activated during successive transfers." Traub has clearly shown that he recovered his virus from stock mice. We are confident that our strains of the virus were obtained from the spinal fluids of two patients and that it is pathogenic for man. Through the cooperation of Dr. Armstrong and Dr. Traub it has been possible for us to show that the viruses recovered from the three sources mentioned above are either immunologically identical or at least very closely related.

Many filterable viruses naturally attack the central nervous system of man and lower animals, causing an encephalitis and can be recovered from the brain or spinal cord. So far no virus has been shown to produce a clean-cut picture of meningitis in man. The new agent with which we are working seems to be able to produce such a picture and to appear in appreciable amounts in the spinal fluids of affected individuals. Whether this virus produces only a picture of meningitis in man and how great a rôle it plays in diseases of the central nervous system remains to be determined.

THOMAS M. RIVERS
T. F. MCNAIR SCOTT

HOSPITAL OF THE ROCKEFELLER INSTITUTE
NEW YORK, N. Y.

## ON CYMAROSE

CYMAROSE is a methyl ether of a 2-desoxy hexomethylose (2-desoxymethylpentose) which occurs in the cardiac glycosides cymarin and periplocymarin. It was first obtained by Windaus and Hermanns[1] from cymarin. They noted that it gave no phenylosazone, but that it yielded acetic acid on oxidation with silver oxide and exhibited the color reactions of digitoxose. They therefore suggested that it may be a methyl ether of this desoxysugar. Attempts to demethylate it to digitoxose were unsuccessful and the position occupied by the methoxyl group as well as its precise configuration remained undetermined.

It is now possible definitely to allocate the methyl ether group at the third carbon atom of the desoxyhexose chain. When cymarose was oxidized with 50 per cent. nitric acid, a hydroxymethoxyglutaric acid

[1] C. Armstrong and R. D. Lillie, *Pub. Health Rep.*, 49: 1019, 1934.
[2] E. Traub, SCIENCE, 81: 298, 1935.
[1] A. Windaus and L. Hermanns, *Ber. chem. Ges.*, 48: 979, 1915.