Three of eleven animals received one 10 cc dose; the remaining 8 were given two such doses. Of the entire group, nine showed some tissue immunity, which is evidenced by the animals' resistance to direct intracerebral inoculation of active virus. Unlike those receiving 5 cc amounts, the tissue immunity of these animals was lower than that of monkeys receiving similar doses of active virus. Too, the degree of immunity was lower than that developed by the animals in the foregoing series of twelve animals. Nine of the eleven had humoral antibodies.

Comparing formalized non-infective virus with active virus, as to antigenic value, the following facts were derived:

(1) In the case of active virus, the dose and the subsequent degree of immunity were directly proportional. Using formalized virus, however, the optimum dose was found to be 5 cc.

(2) With formalized cord tissue, the humoral immunity was usually better than the tissue immunity, but decidedly lower than that developed when active virus was used, even though with 5 cc amounts of formalized antigen, the tissue immunity obtained using either antigen was quite comparable. In some instances, humoral immunity was present in the absence of demonstrable tissue resistance and *vice versa*. To be able to say definitely that there is a correlation between tissue and humoral immunity in the case of active virus, but no such correlation using formalized virus, more work must be carried out concerning the relationship between neutralizing substances in the serum (humoral immunity) and resistance to direct intracerebral inoculation (tissue immunity) of virus. Toward this end, and also to determine whether a non-specific neutralizing substance destroyed by formalin was present in the nerve substance, two monkeys received some non-virus-containing-tissue intracutaneously. Neither of the two developed any antiviral substances.

(3) Using active virus, two inoculations given 10 to 20 days apart were more efficient than one inoculation, but with inactivated virus this did not seem so.

A comparison between the immunity produced due to the injection of inactivated virus and that of convalescent monkeys showed that, using the inactivated antigen, the tissue immunity was sometimes about equal to, but that the humoral immunity was lower than, the corresponding immunities in the case of recovered animals.

The inactivated antigens used in this work produced no reaction whatsoever upon combined inoculations of large doses both intracerebrally and intraperitoneally; for neither temperature increase nor cerebro-spinal fluid pleocytosis were demonstrable. Whether immunity developed from either a killed or highly attenuated antigen can not be definitely stated. If immunity was due to a small quantity of residual virus or to virus of very low virulence, then one must suppose that the stimulation brought about by less than one intracerebral infective dose gave immunity. However, since less than 120 intracerebral infective doses of active virus produced no demonstrable immunity, it is not likely that immunity was due to a sub-infective quantity of virus.

To further investigate this possibility, a series of 8 animals were given, intracutaneously, formalized virus suspension of sufficient infectivity to produce only a mild reaction upon combined intracerebral and intraperitoneal inoculation. Though still viable, the virus failed to give better immunity than did virus rendered non-infective. This again argues against the probability that non-infective material immunized by means of a slight amount of residual virus of extremely low infectivity.

It was found during the course of this work that the majority of the immune animals showed increased erythrocytic sedimentation rates at some time during the development of immunity. There seemed to be a definite relationship between immunity and changes in erythrocytic sedimentation rate.

This work indicates that definite immunity can be developed against the virus of poliomyelitis using virus rendered non-infective by formalin. However, in the concentration used, the formalin gave considerable skin irritation. Therefore, in the present work virus suspensions are being inactivated with lower concentrations of formalin.

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COMPARING SOIL FUNGICIDES WITH SPECIAL REFERENCE TO PHYMATOTRICHUM ROOT ROT[1]

Phymatotrichum omnivorum (Shear) Duggar, the fungus that causes the highly destructive root-rot disease, attacks the roots of plants from a few inches to several feet deep in the ground. For this reason, none of the chemicals customarily used as soil fungicides have proved effective in eradicating this fungus even from small infested areas. Recent work has shown that a group of volatile chemicals,[2] relatively insoluble in water, have strongly fungicidal properties and appear of particular promise for use against this fungus and other soil organisms.

In preliminary evaluation of these and other possible fungicides, the direct toxic effect was tested as

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[2] The writers are indebted to the Roessler and Hasslacher Company (now R. and H. Chemicals Department, E. I. du Pont de Nemours and Co., Inc.) for furnishing many of the chemicals tested.

follows: Small pieces of fresh sclerotial masses of *P. omnivorum* were placed on wads of moist absorbent cotton in culture tubes, the tubes placed inside 2-quart Mason jars, and materials to be tested placed also in the jars at 0.144, 0.72 and 2.88 gm per jar. The materials could act on the sclerotial masses only after diffusing through the air and through the cotton plugs of the tubes. After various periods of exposure the sclerotial masses were transferred aseptically to agar slants and growth observed. At the lowest concentration tested, pentachlorethane, tetrachlorethane, xylene and ammonia killed the fungus masses within 24 hours. Carbon disulfide and 14 other materials were less rapidly toxic; and naphthalene and alpha naphthol were not toxic within the limits of the test.

The ability of the materials to penetrate moist soil and then inhibit growth of the root-rot fungus was tested as follows: Mason jars were filled with moist Houston black clay soil, inoculum of the fungus placed against the glass at various depths in the soil, the materials to be tested placed on the surface, and the jar lids clamped tightly. The following materials, when added on the surface at rates of only 100 ppm of the air-dry soil weight, were able to penetrate to the bottom of the jars (135 mm with 1-quart jars) and there completely prevent growth from the fungus inoculum: pentachlorethane, tetrachlorethane, xylene, carbon disulfide, perchlorethylene, trichlorethylene, dichlorethylene, turpentine and paradichlorobenzene. Some of the materials were ineffective at the highest rates tested: New Improved Ceresan and the other organic-mercury compounds when added at 2,000 ppm; formaldehyde at 4,000 ppm; and ammonia even at 10,000 ppm.

Only pentachlorethane, tetrachlorethane and xylene were completely effective against the root-rot fungus, at the lowest concentration tested, by both methods.

A preliminary field test with tetrachlorethane was started late in the summer of 1933. Tetrachlorethane was applied around cotton plants in six comparable areas at the advancing edges of root-rot spots, 4 additional areas serving as checks. The material was poured into holes (4 per square foot) pierced with a crowbar to a depth of 6 inches. Rates of application were calculated on the soil-weight basis to supply, respectively, 500 or 1,000 ppm of tetrachlorethane to a depth of 4 feet. After 15, 21 and 32 days, trenches 2 feet deep were dug next to the plants, and the roots were separated from the remaining soil, cut into sections corresponding to various depths, placed in separate jars of moist soil, and kept under observation for at least a month.

Phymatotrichum strands grew profusely from the roots from all the check areas. From the 39 plants from the treated areas, on the contrary, there was no Phymatotrichum growth, except from the 12 to 16 inches deep portion of the tap-root of a plant located in exceptionally dense clay at the edge of an area treated at 500 ppm and dug after only 15 days. With this exception, the treatments were successful in killing the root-rot fungus on or within the affected cotton roots in the treated areas, to a depth of at least 2 feet.

Excavation of plants adjoining the treated plats showed that the fungicidal effectiveness of the treatment did not extend more than a few inches horizontally beyond the areas treated. There was no evident injury from the tetrachlorethane to either the treated or neighboring cotton plants.

Summary: Pentachlorethane, tetrachlorethane and xylene were most effective in laboratory tests of ability of volatile materials to kill the root-rot fungus *Phymatotrichum omnivorum,* and to penetrate moist soil and inhibit growth of the fungus. In a preliminary field test, tetrachlorethane placed in the soil at a depth of only 6 inches killed the fungus on cotton roots to depths of at least 2 feet. These highly toxic, soil-penetrating fungicides appear promising for further trial against Phymatotrichum root rot, and are suggested for trial also against other injurious organisms found in the soil.

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