SPECIAL ARTICLES

THE INACTIVATION OF MOSAIC DISEASE VIRUS BY PULVERIZING INFECTED TISSUE

DUGGAR and Armstrong,[1] employing a motor-driven pestle in an agate mortar, found that the virus of tobacco mosaic disease is resistant to 9 hours' grinding. Using a different method, we have also been impressed with the difficulty of inactivating the virus. Complete resistance to grinding would indicate a property not usually associated with animate material; hence the importance of studying this question. We have now found that prolonged comminution of infected plant tissue results in loss of its infectivity. The possibility that the inactivation may be due to oxidation or adsorption has been investigated.

METHODS

The top leaves of several hundred tomato plants with mosaic disease were dried, and powdered by grinding for five minutes with mortar and pestle. This procedure caused no appreciable loss in virus potency.

Varying but measured amounts of the powder were placed with four polished steel balls, 1.1 cm in diameter, in Pyrex bottles[2] specially made to withstand breakage. The vessel was actively agitated in a shaking machine. Virus powder could escape comminution only by clinging to the sides of the bottle above the moving balls. Hence samples were taken only from the bottom of the container. For anaerobic pulverization, a sealed rubber stopper was used, through which passed a glass tube for attachment to the Boëz apparatus.[3] The indicator of relative anaerobiosis was 0.01 per cent. methylene blue in dextrose broth. 0.1 cc was placed in an unsealed ampoule with a double constriction of its neck to prevent leakage. It was protected by a wire cage which was attached to the lower surface of the stopper. A separate anaerobic container was used for each test material. Ten experiments were made and samples pulverized for periods up to twelve hours, were injected into 335 tomato plants.

In three adsorption tests virus powder was mixed with normal plant powder which had been pulverized 4 and 10½ hours, respectively. This mixture was either shaken for 3 hours and its aqueous suspension immediately filtered, or it was allowed to stand as a suspension for 2 hours before filtration. In addition, the relative amount of virus filtrable from suspensions of large and small particles was studied. Virus particles of three sizes were obtained by winnowing, as follows:—A glass tube, 1.5 m long and 2.8 cm inside diameter, was fixed vertically. To the bottom was attached a glass vessel containing virus powder through which a stream of air was forced, blowing the material into the vertical tubing. The top of the apparatus was fitted with an air escape and a trap for the collection of the finest particles. After removing the latter, the remaining powder was divided into coarse and medium sized particles by increasing the air current. In the three tests just described 190 plants were inoculated to determine virus potency.

All filtrations were made through Berkefeld "N" candles. Plants were inoculated by the method described by McKinney,[4] usually with a series of tenfold dilutions beginning with 1 per cent.

RESULTS AND DISCUSSION

With the method of pulverizing employed, partial or complete inactivation of the virus in the dilutions used occurred in 12 hours. The degree of inactivation varied with the time and intensity of pulverization. The following table shows the results of the inoculation of 18 plants with material obtained in two typical experiments.

		5 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	12 hrs.
Test 1	No of positives	15	10	5	No test	0	0	0
Test 2	of 18 plants	11	No test	No test	14	No test	8	2

While the procedure of pulverization did not raise the temperature of the container to a perceptible degree, nevertheless the development of local heat should be considered, for the heat may destroy the virus or act as an oxidizing agent. In this connection it is of interest that the plant tissue turns from green almost to white after 12 hours' aerobic comminution but from green to still darker green after similar anaerobic procedure. An attempt was made to minimize any local heat by packing the container in carbon dioxide snow. But in this instance no change in the degree of inactivation was observed.

It was thought that if inactivation took place under aerobic and not under anaerobic conditions, it might be ascribed to oxidation. But the inactivation of the virus took place more quickly under anaerobic conditions. For example, in two typical tests, 30 of 32 plants inoculated with material before comminution

[1] B. M. Duggar and J. K. Armstrong, *Ann. Missouri Botanical Garden*, 10, 191, 1923.

[2] Similar in size and shape to centrifuge bottle No. 3139-A of the Arthur H. Thomas catalogue, and made by the Corning Glass Works, Corning, New York.

[3] L. J. Boëz, *J. Bact.*, 13, 227, 1927.

[4] H. H. McKinney, *J. Agric. Res.*, 35, 13, 1927.

were positive and 20 of 32 plants injected with virus pulverized for 12 hours under aerobic conditions showed mosaic disease, whereas none of 29 plants inoculated with material pulverized anaerobically for 12 hours was affected. Perhaps under lowered oxygen tension the virus is reduced at the expense of tissue oxidation. However this may be, virus previously comminuted anaerobically was not reactivated when pulverized later aerobically.

The possibility of the loss of infectivity being due to adsorption of virus by powdered tissue must be considered. In the three adsorption experiments above described, 75 per cent. of 104 plants were positive after the inoculation of comminuted virus mixed with finely pulverized normal plant tissue, as compared with 77 per cent. of 86 control plants injected with virus powder alone. It should be pointed out, however, that under the conditions of the experiments forceful impact of particles as in the ordinary procedure did not occur. In the winnowing experiment, in which particles of three degrees of magnitude were obtained, no differences in virus potency were found. It follows that there was no greater tendency of the smaller particles to adsorb virus. Adsorption, if it occurs, is therefore probably not the main cause of the inactivation.

CONCLUSION

Tomato mosaic virus loses its infectivity when tissues containing it are comminuted by the method described.

PETER K. OLITSKY
FILIP C. FORSBECK

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH,
NEW YORK, NEW YORK

STUDIES ON THE ETIOLOGY OF POLIOMYELITIS: ISOLATION AND CULTIVATION OF AN ORGANISM AND TRANSMISSION OF THE DISEASE IN MONKEYS

POLIOMYELITIS (infantile paralysis) has been experimentally reproduced in monkeys with the third, fourth, sixth, eighth, ninth, eleventh and thirteenth "generations" of an organism isolated and grown artificially from the nervous tissues of monkeys known to be infected with the virus responsible for the disease. The organism was cultivated from Berkefeld filtrates prepared from such poliomyelitic materials. It is extremely small, measuring approximately 1/500,000 to 1/250,000 of an inch, and has been grown in a special food medium containing minced sheep brain. In the thirteenth subplant the dilution of the original inoculum cultivated was approximately 2×10^{-27}.

Certain requirements must be met in order to show that a microbe may have something to do with a disease. These are known as the Koch postulates, and demand first, the isolation of the organism in pure culture from the animal harboring the disease; second, the reproduction of the identical disease by the inoculation of these germs into healthy susceptible animals; third, the recovery from such experimentally infected animals of the identical original microbe; fourth, the reproduction once again of the typical disease with these "recaptured" germs.

All these conditions have been fulfilled by the organism that has been reported and described. The disease, furthermore, has been carried on through a series of monkeys in which the infection was produced by means of Berkefeld filtrates or suspensions of nervous tissues prepared from the animals originally inoculated with the microbe. From such "passage" monkeys it was also possible to recover the same germ.

The experiments proved that the "recaptured" virus had the same properties of the original substance known to cause poliomyelitis. These characteristics are its ability to pass through fine filters, to induce typical infection with the characteristic clinical and pathological changes, and lastly, to appear again in a pure culture identical with the original organism.

The most recent studies in our laboratory have shown that the blood serum from a series of monkeys infected with the organism and now convalescent from poliomyelitis, possessed the power to neutralize and combat the ordinary filterable virus of infantile paralysis. This has been demonstrated as follows: Mixtures of a minute amount of each serum with a large amount of active virus were inoculated into the brain of a series of healthy monkeys. These animals did not develop the disease whereas those that received the virus alone succumbed to poliomyelitic infection. To verify the fact further, the animals that furnished these protective serums were likewise inoculated into the brain with active virus alone. These animals resisted the inoculation whereas another set of monkeys that had never had the disease became paralyzed in the typical manner. Thus the convalescent monkeys proved to be immune to the infection and showed immune substances in the blood serum capable of neutralizing the virus of the disease. The resistance to poliomyelitis, therefore, was the same as that which results when the animal recovers from an infection caused by the ordinary poliomyelitis virus filtrate. These observations suggest that the microorganism behaved exactly like the virus.

It must be understood that there are certain established methods for the experimental proof of the