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.

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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

microbial cause of any disease. These methods have been followed and have fulfilled desired requirements designed to show the relationship of a microbe to the disease in question. That hidden factors may still exist is admitted frankly, only because our present methods of attack upon a problem of this type may be inadequate for bringing to light such concealed factors. However, only those facts which have come out of this research have been utilized in drawing justifiable conclusions warranted by the facts, and these must be based upon the only available methods of approach that are known at the present time.

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THE RELATIONSHIP BETWEEN THE WATER CONTENT AND OXYGEN CONSUMPTION OF THE ORGANISM

THE rôle of water as a medium of reaction within the cell has not yet been fully comprehended. This is not surprising in view of the fact that the conditions that control the distribution of water from free to bound states have been elusive. At the suggestion of Dr. J. W. Buchanan, the experiments[1] summarized here were undertaken as an attempt to disclose a possible relation between free water, *i.e.*, water that may be added or abstracted by changed osmotic conditions, and the rate of oxidative reactions in the organism. Such a relation would rest fundamentally on the law of mass action, that is, the relative concentrations of oxidizable substrates and oxidative enzymes.

Previous observations are apparently conflicting. Certain investigators[2] have measured increases in respiratory metabolism in dehydrated tissues, while, conversely, others[3] have showed water uptake to be associated with decreased metabolism. On the other hand, there is some evidence that the opposite relationship may hold true.[4]

The writer used *Planaria dorotocephala* and early embryos of *Amblystoma punctatum*. Water content was controlled by immersing the animals in solutions of differing osmotic pressures, and oxygen consumption was measured in two ways, with the Winkler method and by a microrespirometer.

As far as possible the same individuals were tested under different conditions over three-hour periods. Each period in a test solution was preceded by one in tap water to establish a norm and a control for successive measurements.

The averaged results of the Winkler tests are grouped into the following table.

PER CENT. CHANGES IN RATE OF OXYGEN CONSUMPTION

	Planaria dorotocephala	*Amblystoma punctatum*
Distilled water	−54	−21
Ringer's solution	+43	+62

These observations were checked by numerous experiments with the respirometer. It was found excellent for use with amphibian embryos but unsatisfactory for Planaria due to necessary shaking which prevented the animals from coming to rest. In the case of the former dehydration yielded an average increase of 39 per cent. above the normal in tap water, while treatment with distilled water lowered the rate of oxygen consumption about 42 per cent.

Early *Amblystoma* embryos, when immersed in distilled water, half tap- half distilled water, half Ringer's, and full Ringer's, reached and maintained their maximum swelling or shrinkage within sixty minutes, while planarians show water content changes over a period of one half to six hours in Ringer's and one half to more than sixteen hours in distilled water.[3] Sections of animals so treated indicate that water changes are both inter- and intracellular. At present no rules generally applicable to all animals can be drawn.

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[1] The work was carried out at the Osborn Zoological Laboratory, Yale University.

[2] E. Kreps, Pfluger's Arch., 222, 215–233, 1929; G. T. Caldwell, *Physiol. Zool.*, IV, 2, 324–359, 1931; J. W. Buchanan, *Jour. Exp. Zool.*, 57, 3, 455–472, 1930.

[3] J. W. Buchanan, *Jour. Exp. Zool.*, 57, 2, 307–330, 1930; *Biol. Bul.* LX, 3, 309–326, 1931.

[4] L. C. Beadle, *Jour. Exp. Biol.*, VIII, 3, 211–227, 1931.

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