

### Minute "Organisms" isolated from the Virus of Mosaic Disease of Tomato.

THE nature of the infective principle in plants suffering from mosaic disease is obscure, although most recent workers favour the view that it is a living organism. Allard and Duggar have emphasised the minuteness of the causal agent, whilst Matz, Kunkel, Nelson, and others have described protozoan-like bodies in the cells of affected plants.

A considerable amount of work has been done on this problem at Cheshunt, and the present note records the isolation and growth in pure culture of a minute "organism" from the filtered virus of tomato mosaic.

Isolations were made from affected plants by a modification of Noguchi's method. Tubes of sterile extract of tomato stem and leaf (100 gm. fresh material to 1000 cc. distilled water) were prepared, and into each was dropped a small piece of living tomato tissue cut, under aseptic conditions, from the interior of healthy green fruits. Ten tubes were inoculated by touching the tomato tissue under the liquid with a loopful of tomato virus filtered aseptically through a sterile Doulton candle. Ten uninoculated tubes were left as controls. All tubes were incubated in a Bulloch's anaerobic jar for two months, and on removal were found to be clear. The tubes were then left under ordinary atmospheric conditions, and two months later one tube was contaminated by a fungal growth, but the liquid in the remaining nineteen was quite clear in both the inoculated and the control tubes. This liquid was examined for micro-organisms by plating and streaking upon different culture media, but no growth was observed. On the glass, however, of each of the original inoculated tubes about one centimetre above the liquid were small brown bodies, the largest of which was  $200\mu$  in diameter. No such bodies were present in the controls.

These bodies were tightly fixed to the glass, and not easily detached. They are brittle and break into fragments of a crystalline appearance. The bodies are discoid convex, and when stained with borax carmine the surface shows concentric and radiating markings. They clear in acid with evolution of gas, the cleared bodies having a fine granular appearance. When stained by Giemsa's method they resemble bacterial colonies, containing deeply stained purple granules standing out distinctly on a stained background. These granules are  $0.3$  to  $0.4\mu$  in diameter (occasionally smaller granules are seen) and appear as cocci, diplococci, polar bodies, or unstained rods. These are not merely crystals or detritus but definitely organised bodies growing in colony formation. Distinctive preparations have been made by staining with Giemsa for 24 hours and then differentiating with absolute alcohol. The granules are best seen in smears made from the cleared colonies prior to fixation by drying.

Tubes of virus kept in the laboratory for six to eighteen months under aerobic conditions revealed similar colonies on the glass in those tubes where no toluene had been added for preservative purposes or from which the toluene had disappeared. After acid clearing and staining, the minute granules were readily demonstrated. Films made from the clear liquid in the tubes bearing the colonies were also stained with Giemsa, and purple-stained granules similar to those so abundant in the colonies were regularly found in these preparations. They were not numerous, five or six only being seen in a single field, and appearing as cocci or as diplococci.

Continued cultivation of the "organism" has been maintained in tubes of tomato extract containing

cubes of sterile raw tissue. (The addition of  $0.3$  gm. calcium carbonate to  $10$  c.c. of extract hastens the production of colonies. Increased concentration of carbon dioxide in the atmosphere also seems to assist in the formation of colonies and alters their appearance; the brown colonies becoming white and chalky.) Numerous media have been inoculated with negative results, but one inoculation is especially interesting. A flask of lemco gelatine containing a high proportion of gelatine was inoculated with a drop from one of the original culture tubes. No growth was apparent for four months, but after six months the surface was covered with minute hard white bodies, which on examination proved to be similar to those described. Colonies transferred to Noguchi tubes dissolved in the liquid, and films prepared from this ten days later showed the presence of minute granules either singly as diplococci or as aggregates in alveolar plasmodium-like structures in which cocci stood out deeply stained in comparison with the faintly stained matrix.

The bodies forming on the glass of Noguchi tubes and in the liquid, and the lemco colonies, have been inoculated into healthy plants under various conditions; and while there are indications that they may be causally related to mosaic disease, no definite claim can yet be made. The presence of these "organisms" in the virus of tomato plants suffering from mosaic, and their very interesting nature, appear, however, of sufficient importance to warrant the immediate direction of the attention of workers on this difficult problem to their existence. A detailed investigation of the character and genetic relationships of the "organisms" recorded in this note and their relation to mosaic disease is being carried out at Cheshunt.

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### Globular Lightning.

YOUR correspondent, Mr. E. Kilburn Scott, suggests in NATURE of November 24, p. 760, that "the ball may be a mass of concentrated nitrogen oxides," and considers that this would "fit in well with the formation and action of such gases," and he compares the chemical activity of lightning with the well-known reactions occurring in high-tension arc flames.

Although I do not wish to be understood as expressing any opinion regarding "globular" lightning, I should like to point out that in the letter which appeared in NATURE of September 15, p. 396, I produced evidence in connexion with the extremely vivid and prolonged thunderstorm of July 10, 1923, which left no doubt that the chemical changes that occurred then resembled those of the silent electric discharge, rather than high-tension arc flames, because, although there was no increase in the proportion of the oxides of nitrogen in the air within the area of the storm, there was a very great increase in the proportion of ozone.

I may add that since the proportion of nitrogen peroxide is always much higher in London than in country air, and is considerably greater in winter than in summer, we may look, as in the case of sulphur dioxide, to combustion of coal as the probable source of most of it at least. The seasonal changes of the curves for these two variable ingredients of the atmosphere are very similar, and are not in any way related to that for ozone.

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"Wharfedale," Upminster, Essex,  
November 26.