Finally, cultures of *Bact. granulosis* were isolated from monkeys of the first and second passages of the series of transmissions initiated by inoculation of trachomatous tissue, and from animals of the third and sixth passages of the *Bact. granulosis* series.

In conclusion, we have found that the experimental disease induced by human trachomatous tissue is as definitely transmissible as that produced by cultures of *Bact. granulosis,* and that from different animals of both series the microorganism can be recovered. We also record the fixed character of the incitant in consecutive animal passages and the anatomical changes of early experimental lesions.

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ROD-SHAPED PARTICLES IN TOBACCO MOSAIC VIRUS DEMONSTRATED BY STREAM DOUBLE REFRACTION

FOR some years pathologists have been attempting to obtain evidence regarding the nature of the filterable viruses which cause numerous diseases of man, lower animals and plants. Although much information has been gained regarding the properties of viruses the available evidence is still insufficient to prove whether the virus particles are inanimate colloids or organisms which are too small to be seen through the microscope.

We have recently attempted to obtain evidence regarding the shape of virus particles by the use of polarized light. Methods somewhat similar to those described by Ambronn and Frey[1] and Freundlich[2] for determining the shape of colloid particles have been used in this work. According to these workers minute isotropic rods, disks or leaf-shaped particles contained in a flowing liquid tend to become oriented with their long axis parallel to the direction of flow. Under these conditions a liquid containing rods is doubly refractive when the direction of transmission of the incident light is perpendicular to the direction of flow; a liquid containing disks or leaf-shaped particles is doubly refractive when the incident light is perpendicular to the direction of flow and parallel to the faces of the particles. This so-called form double refraction only occurs when the dispersed phase has a refractive index which differs from that of the continuous phase and when the shortest axis of the particle is small in relation to the wave-length of the light used. Liquids containing anisotropic rods, disks or leaf-shaped particles also show form double refraction under the above conditions, and in addition to this may show an intrinsic double refraction due to the arrangement of atoms in the particles. This intrinsic double refraction occurs when the anisotropic particles are so oriented that their long axis is parallel to the direction of flow, and the direction of transmission of the incident light is not parallel to the optic axis of the particles. Since most of the studies which have been made on virus particles indicate that they are smaller than the wave-lengths of visible light it appears that if virus particles were to have the form of rods, disks or leaves, and the proper refractive index, and were in sufficient concentration, the flowing virus suspension should show double refraction.

In order to test this idea the apparatus shown in Fig. 1 was set up. It consists of an ordinary microscope lamp L and a microscope stand S, from which

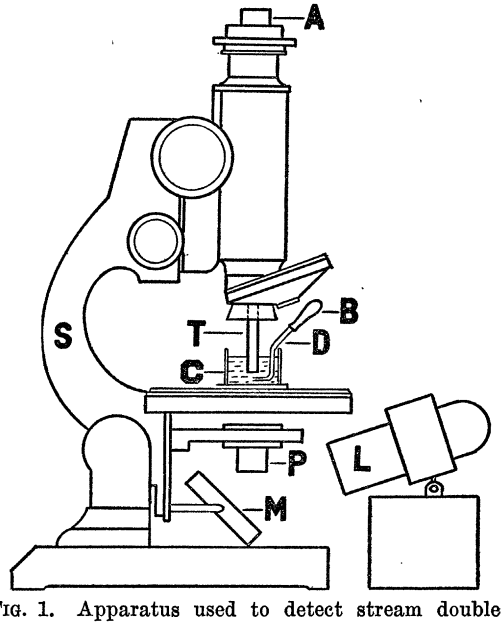

FIG. 1. Apparatus used to detect stream double refraction in virus-containing plant juices and other sols.

the ocular, objectives and condenser have been removed. A polarizer P was placed in the diaphragm carrier and an analyzer A was attached to the microscope tube. A curved glass pipette D, having an inside diameter of 0.5 mm at the orifice, was prepared. This was cemented to the inside of a cylindrical glass chamber C, with its orifice so directed that pressure on the bulb caused a stream to flow across the middle of the chamber. A small round cover glass was cemented to the lower end of the glass tube T, and the upper end of the tube was pushed through a hole in a rubber stopper. The stopper was held in one of the

[1] H. Ambronn and A. Frey, "Das Polarisationsmikroskop." Leipzig. 1926.

[2] H. Freundlich, "Colloid and Capillary Chemistry." E. P. Dutton and Co., New York.

sockets in the nose piece. The glass tube T, the peripheral portion of the cover glass and the lateral surface of the vessel C were painted with black paint so that only light from the polarizer passed up through tube T. The polarizer was turned on its vertical axis until its vibration direction made an angle of 45° with the direction of flow of the liquid expelled from the pipette. The analyzer was then turned until the field was dark as a result of the nicol prisms being crossed.

Leaves of tobacco plants affected with typical mosaic (Johnson's tobacco virus 1) were frozen at 0° F. for 15 hours. The leaves were then thawed at room temperature for about 1 hour and the juice was pressed out of the leaves by means of a screw press. The juice was centrifuged and about 5 cc of the supernatant liquid were poured into the chamber C. A small volume of the liquid was then drawn up into the pipette by means of the rubber bulb B. Upon pressing the bulb while looking through the analyzer the liquid flowing from the pipette was seen to be distinctly doubly refractive, and appeared as a bright streak across the dark field, as seen at A in Fig. 2.

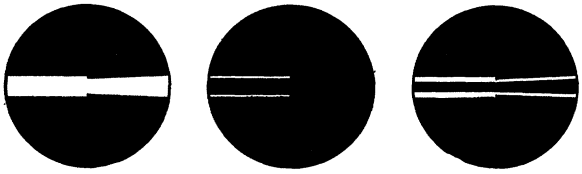

FIG. 2. Showing the appearance of the field when different liquids were expelled from the pipette.

A = Field produced by solutions known to contain rod-shaped particles and by virus-infected plant juice.

B = Field produced by juice from healthy plants.

C = Field produced by ferric oxide sol containing disk-shaped particles.

Juice from healthy leaves prepared in the same way showed no detectable double refraction, the field appearing dark as shown at B in Fig. 2. This experiment was repeated 6 times, with leaves from 6 different healthy and mosaic tobacco plants, once with juice from the roots of a healthy and mosaic tobacco plant, and once with juice from the leaves of a healthy and mosaic tomato plant. In each case double refraction was shown only by juice from the mosaic plants.

Freundlich[2] has reported that sols of vanadium pentoxide, aniline blue and benzopurpurin have rod-shaped particles, while old ferric oxide sols have disk-shaped particles. In order to gain evidence regarding the shape of the particles in the virus-bearing juice which are responsible for the double refraction, sols of vanadium pentoxide, aniline blue, benzopurpurin and ferric oxide were tested in our apparatus. It was found that, like the virus-bearing juice, the sols containing rod-shaped particles produced a stream which showed uniform light intensity throughout the width of the stream as shown at A in Fig. 2. On the other hand, the ferric oxide sol containing disk-shaped particles produced a stream which showed double refraction only along its edges as shown at C in Fig. 2. This behavior is presumably due to the fact that it is only in the edges of the stream that a large proportion of the disk-shaped particles are oriented with their faces parallel to the direction of transmission of the incident light.

It has not been determined whether the double refraction observed in the infective juice is a form double refraction, an intrinsic double refraction or a combination of both types. In any case the occurrence of stream double refraction in the juice of mosaic tobacco plants, its absence in the juice of healthy plants and its similarity to the stream double refraction shown by sols containing rod-shaped particles, indicate that the virus of tobacco mosaic or some substance regularly associated with it, is composed of rod-shaped particles.

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PHOTOCHEMICAL NITRIFICATION IN SEA WATER

SINCE nitrates frequently are considered to be a limiting factor in the productivity of the sea, phenomena which increase the available nitrogen for plant growth are of great importance. It has been tacitly assumed by many authors that the same biological agencies which are operative in the transfer of nitrogen from one form to another in the soil similarly activate these processes in the sea. However, Lipman,[1] Atkins,[2] Brandt[3] and other investigators have reported their failure to demonstrate nitrifying bacteria in the open seas, although nitrifiers are frequently found near the shore and in bottom muds. Similar observations in the Pacific Ocean off the coast of California in the vicinity of San Diego have been made repeatedly by the writer. Brandt has mentioned the possibility of nitrites being formed from ammonium near the surface, due to the presence of inorganic catalysts in the sea water.

It has been impossible to demonstrate nitrifying bacteria by the conventional soil methods when from 0.1 to 100 cc of sea water was inoculated into Winogradsky's solution and other similar nutrient com-

[1] SCIENCE, 56: 501, 1922.
[2] *Jour. du Conseil*, 1: 197, 1926.
[3] *Wissensch. Meeresuntersuchungen*, 20: 203, 1927.