

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 was 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.

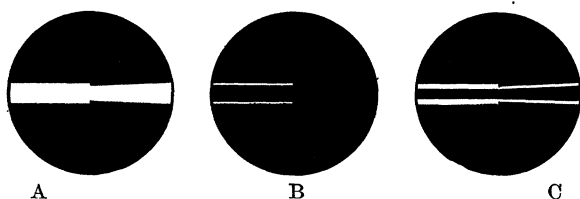


FIG. 2. Showing the appearance of the field when different liquids were expelled from the pipette.

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.

Freundlich² 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 Brandt³ 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 Winoogradsky'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.

binations, although controls inoculated with a little garden soil or even sea bottom muds have given positive results. There was no perceptible nitrification in media consisting of raw sea water to which ammonium salts were added in different concentrations. Varying the temperature from 4° to 30° C. by six-degree intervals did not give any evidence of the presence of nitrifiers in the sea water.

On the other hand, soil nitrifiers, *nitrosomonas*, failed to elaborate any nitrites when inoculated into sea water with the addition of magnesium carbonate in excess and ammonium sulfate, although they were still viable after eight weeks at 20° C. In a medium consisting of 50 per cent. sea water and 50 per cent. Winogradsky's solution inoculated with *nitrosomonas*, nitrification occurred, but not as readily as in straight Winogradsky's solution. With 75 per cent. sea water the amount of the reaction was negligible and 90 per cent. sea water stopped it. The addition of 5 to 10 per cent. sea water to Winogradsky's solution accelerated the oxidation of ammonium to nitrites by the biological agents mentioned above. Such evidence seems to warrant the conclusion that if bacterial nitrifiers are in the open seas, they are different from the soil forms, but it does not preclude the possibility of there being bacterial nitrifiers in the sea which require special methods of cultivation. Investigations are now in progress which may help to elucidate this point.

In continuing the inquiry into the mode of origin of nitrates, it was found, contrary to expectation, that sunlight favored nitrification in sea water under certain conditions. When pyrex flasks partly filled with raw sea water plus 0.005 per cent. ammonium sulfate were exposed to light on the top of a building for two weeks there was a measurable increase in nitrites as well as nitrates and a corresponding decrease in the ammonium content. The controls in the dark were unchanged. Examinations revealed that there were fewer viable bacteria in the flasks which had been exposed to sunlight than in the dark controls. The removal of bacteria by filtering the water through a Berkefeld N candle into sterile flasks did not influence the oxidation of ammonium to nitrites when thus irradiated. The reaction was accelerated by the addition of manganese dioxide. No perceptible oxidation of ammonium occurred in distilled water nor in artificial sea water under comparable conditions. Autoclave sterilization (120° C. for 30 minutes) of sea water destroyed its photochemical nitrifying properties. This is attributed to the thermal destruction of certain oxidizing substances or even organic catalysts.

Many of the above experiments were repeated, using ultra-violet light having a maximum intensity in the 2950Å region. Such irradiation of sea water mix-

tures produced results not unlike those observed under the influence of sunlight, except for the speed of the reaction. Exposure to the mercury arc at thirty inches for two hours caused the oxidation of as much ammonium as the daylight did in two weeks. Again it was found that more ammonium was oxidized to nitrites by irradiation of sea water than distilled water. Ultrafiltration of sea water did not alter it in this respect, but autoclaving it retarded the reaction. Increasing the intensity of the irradiation ten times by bringing the source to within about 9½ inches from the test solutions actually caused the destruction of more ammonium than could be accounted for as nitrites or nitrates.

In view of these observations it appears that at least part of the nitrification which occurs in the sea is photochemically activated. Zolcinski⁴ has repeatedly found that chemical nitrification in the soil is activated by sunlight in the exclusion of biological agencies. It is well known that under certain conditions ultra-violet light causes the oxidation of ammonium to nitrites. Due to the slight penetrability of water by these rays, they could play an important rôle only at or a few millimeters from the surface. Inasmuch as no light filters were used in these preliminary tests, many rays other than the ultra-violet may have been the effective ones. The mercury arc did supply visible light of great intensity. As previously stated, the solar rays responsible for the reaction will pass through pyrex glass. The point to be emphasized at this time is not which rays are active, but rather that the irradiation of sea water causes the oxidation of ammonium to nitrites and that sunlight supplies the necessary rays. In a later communication a report of the foregoing experiments will be amplified and additional observations given.

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⁴ *Int. Rev. Sci. Pract. Agric.*, 2: 838, 1924 (Abstract).