intravenous and intracutaneous inoculations into the footpads have been negative. The agent has been demonstrated in the brain as well as in the viscera of mice that have succumbed to the infection. Macroscopically the only changes noted are a nutmeg liver and slight enlargement of the spleen. A preliminary microscopic examination shows a certain degree of infiltration of the meninges, ependyma, choroid plexus and perivascular lymph spaces with round cells. In addition there is necrosis of some of the nerve cells in the cerebral cortex, cerebellum, brain stem and spinal cord. In the last the anterior horn cells are predominantly involved. In the cerebellum it is the Purkinje cells that are affected. There may be some proliferation of the ependyma and of the glia cells of the gray matter.

Guinea-pigs have proved to be very susceptible as they develop symptoms following intracerebral, subcutaneous and intranasal inoculation. The mortality has varied with different strains used but has been practically 100 per cent. after intracerebral inoculation and from 80 to 90 per cent. following subcutaneous injection. The course of the disease is more chronic than in mice, there being a remittent type of fever with emaciation, somnolence, salivation and markedly labored breathing. Death occurred in from 10 days to 3 weeks after inoculation. One of eight guinea-pigs in contact with an infected animal developed the disease. At autopsy pneumonia of the virus type is often encountered. In addition to the changes noted in the mouse brains, acidophilic intranuclear inclusions have been found in the round cells present in the meninges and choroid plexi. The infectious agent has been demonstrated in the brain, blood and suspensions of the diseased lungs. Three guinea-pigs have recovered from the disease and have resisted further injections. In a limited number of experiments attempts to infect rabbits have been negative.

Material known to be infectious has shown no organized forms when examined by the usual bacteriological procedures and no growth has occurred on a variety of media. The disease has been produced by material passed through Berkefeld "N" and "W" filters that have held back *B. prodigiosus,* and also by material that has been in 50 per cent. glycerol for at least one month. From these facts we conclude that the agent is a filterable virus.

The disease caused by this virus is definitely different from infectious ectromelia.[1] The virus of spontaneous encephalitis of mice described by Theiler[2] produces a different clinical picture and is confined to the central nervous system, whereas the virus we have been working with is distributed generally. The origin of the virus recovered by Armstrong[3] from a monkey inoculated with virus from a human case of encephalitis during the St. Louis epidemic has not been definitely established. It produces a clinical picture in mice which is strikingly like that described above, and the lesions in the central nervous system have much in common with those observed in our animals.

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[1] J. Marchal, *Jour. Path. and Bact.,* 33: 713, 1930.
[2] M. Theiler, SCIENCE, 80: 122, 1934.

THE RELATION OF STREAM DOUBLE REFRACTION TO TOBACCO MOSAIC VIRUS

IN a previous publication[1] we reported that juice expressed from tomato tissues infected with tobacco mosaic virus contains a high concentration of M.C.S.D.R. (material causing stream double refraction), whereas juice from healthy tissues contains a relatively slight concentration of material causing this phenomenon.

The high concentration of M.C.S.D.R. in mosaic plants is probably subject to one of the following three explanations. (1) The M.C.S.D.R. in mosaic plants may be the same material as that in healthy plants, but is in much higher concentration in mosaic plants. (2) The stream double refraction exhibited by juice from mosaic plants may be caused by a high concentration of virus particles, together with a very low concentration of the material which causes stream double refraction in healthy plants. (3) Most of the M.C.S.D.R. in mosaic plants may be composed of a product of the virus or of the diseased host not present in healthy plants.

Previous work[2] has shown that Vinson's purification technique removes all the detectable M.C.S.D.R. from juice of healthy plants but leaves a high concentration of M.C.S.D.R. and virus in infective juice. When different methods of juice extraction were used it was found[1] that the method which yielded the highest concentration of M.C.S.D.R. from mosaic plants yielded the lowest concentration from healthy plants and the method yielding the highest concentration from healthy plants yielded the lowest from mosaic plants. When juice from healthy plants has been stored at room temperature for from 12 to 24 hours it no longer exhibits stream double refraction, whereas juice from mosaic plants contains a concentration of M.C.S.D.R. even slightly higher than freshly extracted

[3] C. Armstrong, with pathology by R. D. Lillie, *Publ. Health Rep.,* 49: 1019, 1934.
[1] W. N. Takahashi and T. E. Rawlins, "Application of Stream Double Refraction in Identification of Streak Diseases of Tomato." *Phytopath.* In press.
[2] W. N. Takahashi and T. E. Rawlins, SCIENCE, 77: 284, 1933.

juice. All the above results indicate that the M.C.S.D.R. in healthy plants and that in mosaic plants have different properties and suggest that they may be different substances. This evidence therefore favors the second or third explanations given in the preceding paragraph.

If most of the stream double refraction produced by juice from mosaic plants is due to tobacco mosaic virus particles one should find the concentration of virus and M.C.S.D.R. to be positively correlated. In order to gain evidence on this relation juice was extracted from tissues which differed greatly in virus content. Different organs of mosaic tobacco plants, leaves of different hosts and chlorotic and dark green tissues of mosaic tobacco leaves were used as virus sources.

The critical dilution, which is the minimum amount of dilution required to cause the disappearance of stream double refraction, was used as a measure of the concentration of M.C.S.D.R. in infective juice. The virus concentration was determined by a modification of the half leaf method of Samuel and Bald.[3]

Following are typical examples of the critical dilutions found for infective juice from various tissues: Tobacco leaves, 1:768; tobacco roots, 1:256; tobacco stems, 1:96; tomato leaves, 1:256; *Martynia louisiana* leaves, 1:256; *Nicandra Physalodes* leaves, 1:224; chlorotic tissues of mosaic tobacco leaves, 1:2048; dark green tissues of mosaic tobacco leaves, 1:256. When the virus concentration in each of the above critical dilutions was determined by the half leaf method all were found to be approximately the same. (Differed by less than 12 per cent.). This work has been repeated a number of times with similar results. It is therefore evident that when samples of juice obtained from different sources and containing different concentrations of virus are diluted until the stream double refraction just disappears all the diluted samples contain approximately the same concentration of virus. The stream double refraction technique therefore provides a rapid and satisfactory method for determining virus concentration in fresh juice or that which has been preserved by freezing, the virus concentration the original undiluted sample being proportional to the dilution required to cause the disappearance of stream double refraction.

Heating mosaic juice to 100° C. for 10 minutes is known to inactivate tobacco mosaic virus and was also found to destroy the power of the M.C.S.D.R. to produce stream double refraction. A heavy precipitate was formed during the heating, and it is supposed that the M.C.S.D.R. was coagulated and was therefore unable to cause stream double refraction.

[3] G. Samuel and J. G. Bald, *Ann. Appl. Biol.*, 20: 70–90, 1933.

If the virus particles are not the colloidal particles causing most of the stream double refraction exhibited by juice from mosaic plants they may have a different size or a different isoelectric point and if so the virus should be separable from the M.C.S.D.R. by ultrafiltrations or electrophoresis. All experiments conducted have indicated that the M.C.S.D.R. in the mosaic plants behaves the same as the virus during ultrafiltration and electrophoresis and can not be separated from the virus by such treatments. All the above evidence has favored the hypothesis that the virus particles are responsible for most or all of the stream double refraction exhibited by juice from mosaic plants.

Two conditions were found in which virus concentration was not positively correlated with concentration of M.C.S.D.R. In certain samples of aged virus from mosaic tobacco plants the concentration of active virus was found to be much lower than that of M.C.S.D.R.; and in virus treated with ultrasonic radiation the virus was completely inactivated after two hours,[4] whereas the concentration of M.C.S.D.R. remained high. These two experimental results are probably subject to one of two interpretations: (1) That the virus and M.C.S.D.R. are different; (2) that the virus particles inactivated by aging or ultrasonic radiation are not changed in external form sufficiently to prevent stream double refraction.

Although much of the evidence cited above favors the supposition that the virus particles are the causal agent of most of the stream double refraction exhibited by juice from mosaic plants the evidence remains inconclusive. However, since the concentrations of virus and M.C.S.D.R. in fresh juice or that preserved by freezing have always been found to be positively correlated the stream double refraction technique provides a rapid and reliable method for determining virus concentration in such juice.

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