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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[4] W. N. Takahashi and Ralph J. Christensen, SCIENCE, 79: 415–416, 1934.

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