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POSSIBLE CHEMICAL NATURE OF TOBACCO MOSAIC VIRUS¹

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UNDER the above title Barton-Wright and McBain² make the interesting announcement that virus fractions have been obtained which were free of nitrogen.

Lojkin and Vinson³ reported the inactivation of purified virus preparations by trypsin. Caldwell⁴ announced that this inactivation by trypsin is only apparent; for upon heating the trypsin digest to a suitable temperature, the activity of the virus was restored. Our results during the past year fully agree with Caldwell's announcement. Since the proteinsplitting enzymes do not readily attack this virus, it would seem possible that it might be non-nitrogenous in character; hence the announcement of Barton-Wright and McBain is in keeping with this possibility.

Barton-Wright and McBain precipitated the virus from juice of diseased plants with a solution of lead acetate; removed the virus from this precipitate with neutral phosphate solution, then precipitated the virus from the neutral phosphate solution with safranin. The safranin-virus precipitate was suspended in water and the safranin removed with normal amyl alcohol. The decolorized aqueous phase thus obtained was infectious and contained no nitrogen.

Under our conditions and using plants of Nicotiana Tabacum, var. Turkish, I still find nitrogen present in infectious preparations obtained as described above. The nitrogen content, however, is not high, especially when the diseased plants have been grown during the short gloomy days of midwinter. Also, in extracting the safranin with normal amyl alcohol, emulsions tend to form and the undecomposed safranin precipitate tends to concentrate in the surface films. As the emulsion breaks, the safranin precipitate collects at the amyl alcohol-water interface. In case this interfacial material is not allowed to remain with the aqueous phase after each extraction, the nitrogen content of the final virus fraction may be very low, indeed, and the results indicate that the

¹ Contribution from the Department of Horticulture, Missouri Agricultural Experiment Station. Journal Series No. 380.

² E. Barton-Wright and A. M. McBain, "Possible Chemical Nature of Tobacco Mosaic Virus," *Nature*, 132: 1003, December 30, 1933.

³ Mary Lojkin and C. G. Vinson, "Effect of Enzymes upon the Infectivity of the Virus of Tobacco Mosaic," Contr. Boyce Thompson Institute, 3: 147-162, 1931.

⁴ John Čaldwell. Ann. of Appl. Biol., 20: 111, February, 1933.

infective power was also low. When, however, the leaves of 10 plants were rubbed with a cloth dipped in the virus preparation, 100 per cent. infection was produced. Starting with 500 cc of juice from diseased plants, carrying through and running the Kjeldahl determination on the entire sample (except for an aliquot of 20 cc removed for inoculating plants and for pH determinations), the final virus fraction may contain only one or two milligrams of nitrogen. When, however, care was exercised to retain the interfacial layer with the aqueous phase after each extraction until decomposition was complete and there was no further interfacial layer, the nitrogen content of the final virus fraction from 500 cc of juice was found to vary from 8 to 24 milligrams in 8 experiments. The number of plants diseased out of one hundred plants inoculated with the last mentioned preparation was much greater than in the case of inoculations with those preparations obtained by discarding the interfacial layer each time.

Vinson and Petre⁵ have already reported that a suspension of the washed interfacial layer is infec-Had nitrogen determinations been made on tious. the ordinary sample derived from 25 cc or even 100 cc of juice, it would have been impossible to detect nitrogen in some of the above fractions by the ordinary Kjeldahl method. It is also interesting to note that when Lloyd's reagent⁶ was employed to decompose the safranin precipitate the nitrogen content and infective power of the final fraction were increased. This greater infective power should not be taken, however, as an absolute indication of greater virus content, since the fraction obtained by the use of normal amyl alcohol and that obtained by the use of Lloyd's reagent passed through different procedures and hence were not comparable.

Barton-Wright and McBain also state that their fractions, obtained by the procedure described above, were free of phosphate. Again, under our conditions, and starting with 500 cc of juice from diseased Turkish tobacco plants, the final infectious virus fraction obtained by the method of Barton-Wright and Mc-Bain contained phosphorus. Phosphorus was present to the extent of about one half milligram even when the safranin precipitate had been washed 3 times with a concentration of safranin (200 cc of a 1 per cent. aqueous solution added to 500 cc of redistilled water) equal to that in the mother liquor from the safranin precipitate, then washed once with redistilled water. It would seem very difficult to obtain a safranin precipitate from such a highly concentrated

⁵C. G. Vinson and A. W. Petre, "Mosaic Disease of Tobacco," Contr. Boyce Thompson Institute, 1: 479-503, 1929.

⁶C. G. Vinson, "Mosaic Disease of Tobacco. V. Decomposition of the Safranin-Virus Precipitate," Phytopathology, 22: 965-975, December, 1932.

phosphate solution without carrying along phosphate in some manner.

Barton-Wright and McBain also announce in the same article that a crystalline product has been obtained which was free of nitrogen, but contained phosphorus and active virus. In 1928, I obtained several crystalline products,⁷ one of which, for instance, was recrystallized three times. The recrystallized product contained virus, as its solutions were infectious, but 358 milligrams of the air-dry crystals contained no Kjeldahl nitrogen. Qualitative tests for reduced sulfur, sulfate, phosphate and chloride were negative. The product charred on heating and contained calcium. It was soon learned, however, that the mother liquor from which the original crystalline product was obtained seemed to contain the major portion of the infectious agent; consequently, the failure to find nitrogen in the sample taken was without particular significance. A dilution of 1 in 100 of our purified virus fractions would make the detection of nitrogen uncertain by the ordinary Kjeldahl method, using ordinary samples.

The virus fraction in most of our preparations is readily precipitated by means of a small amount of $N/_1$ aluminum sulfate solution. This did not hold true for the virus preparations obtained by the amyl alcohol procedure.

Under our conditions purified virus preparations which seemed to contain the major portion of the original virus have not yet been obtained free of nitrogen. This is not stated, however, as argument against the possibility of nitrogen-free preparations having been obtained by others under their conditions.

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SOIL MINERALS AS A CHECK ON THE LOCATION OF THE WISCONSIN-ILLINOIAN DRIFT BOUNDARY IN NORTH CENTRAL OHIO

As a part of a study of the Pleistocene geology of the region in and around the reentrant angle in the glacial boundary in northeast central Ohio, the outer limits of the Wisconsin and of the Illinoian drift sheets were mapped with considerable care. Fig. 1 shows the location of these boundaries in Knox, Coshocton, Richland and Ashland counties. The Wisconsin boundary farther west, where no older drift lies beyond the younger, has already been described in detail.¹

The problem of mapping the Wisconsin boundary,



FIG. 1. Map of a portion of north central Ohio, showing areas covered by Wisconsin and Illinoian drift and location of soil samples taken.

where Illinoian drift lies beyond, was more difficult than that of mapping the Wisconsin boundary, where no early drift lies beyond. The boundary between the Wisconsin and Illinoian drifts shown in Fig. 1,² was determined by a study of the varying amount of erosion, degree of drainage integration, depth of leaching, et cetera. Its position will be described in detail elsewhere. Incident to a study of the minerals in Wisconsin and Illinoian drifts,3 it was discovered that the minerals in the soil of the two drifts had different characters. The location of the boundary between the two drifts was then checked by a study of the minerals in the soil. It is the purpose of this note to describe the results so far attained in mapping a boundary between drifts of two ages by means of a study of the soil minerals.

Samples of surface soil from reasonably flat areas were collected for laboratory study. Samples were prepared for study as follows: About 25 grams were separated by washing and decantation into sand, silt and elay fractions. After just enough water had been added to wet the sample, it was ground for a few minutes with the ball of the thumb or with the index finger against the inside of a 150 cc beaker. The beaker was then almost filled with water and the silt and clay decanted from the sand. Decantation was repeated until the sand was free from silt and clay. The clay was decanted from the silt and the

² The names of the townships within the counties shown may be determined from the Geologic Map of Ohio, published by the Geological Survey of Ohio.

³ The writer is indebted to Professor William J. Mc-Caughey, of the Ohio State University, for advice on methods of separation and examination of soil minerals.

⁷C. G. Vinson and A. W. Petre, "Mosaic Disease of Tobacco. II. Activity of the Virus Precipitated by Lead Acetate," Contr. Boyce Thompson Institute, 3: 142, 1931. ¹G. W. White, "Glaciation of Northwestern Holmes County, Ohio," Ohio Journal Sci., 31: pp. 429-53, 1931; "An Area of Glacier Stagnation in Ohio," Jour. Geol., 40: pp. 238-258, 1932.