SPECIAL ARTICLES

ACETYL AND PHENYLUREIDO DERIVATIVES OF TOBACCO MOSAIC VIRUS

A STUDY of the relationship of chemical structure to biological activity possesses a unique interest in the case of viruses since, when a virus derivative is active, the nature of the virus produced in the host cells on inoculation of the derivative may be determined. If, subsequent to inoculation and multiplication, the virus derivative could be reisolated from the host, evidence would be afforded that the infecting molecules served as more or less exact patterns for the building up of other virus molecules. Furthermore, it might be expected that there would be a difference in the disease produced by such an altered virus, for it is well known that the nature of the disease varies with the strain of the virus. It has been shown that strains of a virus differ in their chemical properties,[1] and recently definite information concerning some of the structural differences that exist between strains of tobacco mosaic virus was obtained.[2] The possibility exists, therefore, that one might cause structural changes *in vitro* which would, in effect, correspond to the mutation of a virus. If, on the other hand, the inoculation of the virus derivative resulted in the production of normal or unaltered virus, it might be concluded that the structural change was reversed within the cells of the host or that that portion of the molecule involved in the structural change was unimportant and played a subordinate role in the reaction of virus reproduction.

It was shown recently that the sulfhydryl groups of tobacco mosaic virus could be oxidized with iodine without changing the specific virus activity, but that inoculation of the oxidized virus was followed by the production of normal virus.[3] Schramm and Müller[4] reported that the amino groups of tobacco mosaic virus could be completely covered by treatment with ketene or phenyl isocyanate without a decrease in specific virus activity, but it was not determined whether these derivatives could be propagated as such in susceptible hosts. In the present study, which was begun before this work came to our notice, it was not possible to secure complete coverage of the amino groups without a decrease in virus activity, but it was established that derivatives of unchanged specific activity, yet containing about 3,000 substituted groups per molecule of virus, could be prepared. In addition, the nature of the virus produced on inoculation of these derivatives was determined.

In the first experiments, samples of tobacco mosaic virus in 1 M acetate buffer at pH 5.5 were treated with ketene for varying periods of time up to 4 hours. The procedure was adapted from the directions of Herriott and Northrop[5] and of Li.[6] In later experiments, it was found that more complete acetylation could be obtained by carrying out the reaction in 0.5 M phosphate buffer at pH 8.1 for separate 1-hour intervals. After each period of acetylation, the virus was dialyzed free of phosphate, isolated by ultracentrifugation, and dissolved in phosphate buffer at pH 8.1 for further acetylation.

The changes in amino nitrogen were followed by the ninhydrin method as applied by Ross and Stanley[7] and by the Van Slyke manometric method.[8] It was found that most of the amino groups were acetylated during the first 10 minutes of reaction but that further acetylation proceeded very slowly. According to the ninhydrin test, 50 per cent. of the amino groups were covered during this period. Analyses by the Van Slyke method, however, revealed 70 per cent. acetylation, indicating some lack of specificity in the former method. When the acetylation reaction was carried out at pH 8.1 for periods of 2 and 4 hours, the extent of acetylation of amino groups as determined by the Van Slyke method was increased to 75 and 83 per cent., respectively. The amino nitrogen content of 9 different samples of untreated virus was 0.13 ± 0.01 per cent. The analyses were carried out at 24° C. and digestion with nitrous acid was allowed to proceed for 20 minutes. Under similar conditions, Schramm and Müller obtained the value of 0.25 per cent. amino nitrogen. In addition, they reported negative ninhydrin and Van Slyke tests for their acetylated virus. Since the authors stated that their virus stock was obtained from this laboratory, it is unlikely that the discrepancies can be explained on the basis of different strains of virus.

The decrease in tyrosine plus tryptophane groups due to acetylation was determined colorimetrically by means of Folin's phenol reagent, applied to the unhydrolyzed protein according to the directions of Herriott.[9] The color changed more slowly with time of acetylation than did the amino nitrogen values, but similarly it also reached a plateau which in this case amounted to a decrease of around 20 per cent. Moreover, the extent of substitution could not be appreci-

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[5] R. M. Herriott and J. H. Northrop, *Jour. Gen. Physiol.*, 18: 35, 1934.
[6] C. H. Li, SCIENCE, 90: 143, 1939.
[7] A. F. Ross and W. M. Stanley, *Jour. Gen. Physiol.*, 22: 165, 1938.
[8] J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry," Vol. II. Williams and Wilkins Company, Baltimore, 1932.
[9] R. M. Herriott, *Jour. Gen. Physiol.*, 19: 283, 1935.

ably increased by repeated acetylation of the virus. After treatment with NaOH at pH 11, the acetylated virus gave with the Folin reagent 97 per cent. of the color given by a control of untreated virus. In view of Herriott's findings that the chromogenic power of acetylated tyrosine derivatives can be recovered by treatment with alkali, whereas that of acetyl tryptophane can not, it seems probable that a portion of the tyrosine in the virus, but little or none of the tryptophane, was affected by the acetylation.

Tests for biological activity carried out on both *Nicotiana glutinosa* and *Phaseolus vulgaris* plants showed that the specific virus activity of preparations in which 70 per cent. or less of the amino groups were covered was the same as that of the untreated virus. Samples in which the amino groups were 75 to 83 per cent. acetylated showed 25 to 50 per cent. inactivation. A preparation in which the amino groups were covered to the extent of 70 per cent. and the tyrosine plus tryptophane groups to the extent of 20 per cent. was inoculated into a number of young Turkish tobacco plants. The disease produced in these plants was indistinguishable from that in a group of control plants. After a period of 4 to 5 weeks, the viruses were isolated by differential centrifugation. The yields were comparable in the test and control plants. The virus obtained from the plants inoculated with the acetylated virus possessed the normal amino nitrogen content and showed the same chromogenic power towards the Folin reagent as did the virus from the plants infected with normal virus. Further evidence was thus obtained that infecting virus molecules may not necessarily function as exact patterns for reproduction. However, as in the case of the iodine oxidized virus, the objection might be raised that the plant cells had transformed the derivative into the normal form before reproduction occurred. In an effort to obtain a virus derivative less likely to be affected by the plant cells, samples of tobacco mosaic virus in 0.1 M phosphate buffer were treated with an excess of phenyl isocyanate at pH 8 and preparations of phenylureido virus were obtained. The amino groups were 43 to 63 per cent. covered, depending on the time of reaction. The virus derivative showed no significant change in specific virus activity and the disease produced in Turkish tobacco plants was indistinguishable from that caused by ordinary virus. The virus reisolated from the plants possessed the normal amino nitrogen content, in agreement with the results obtained with the acetylated virus.

In order to determine whether the treated preparations were chemically uniform or consisted of molecules altered to widely different degrees, tests were made with the ultracentrifuge and the Tiselius electrophoresis apparatus. The homogeneity of the preparations as determined in the ultracentrifuge was not measurably altered by the two types of chemical treatment. However, because of the nature of the chemical changes involved, a more sensitive test was provided by the electrophoretic mobility. The electrophoresis experiments were carried out at pH 7.3 in 0.1 ionic K_2HPO_4-KH_2PO_4-KCl buffer in which 80 per cent. of the ionic strength was provided by the KCl. The acetyl and phenylureido derivatives each possessed mobilities close to -9.3×10^{-5} cm.^{2}/volt sec. as compared with a mobility of -8.3×10^{-5} cm.^{2}/volt sec. for normal virus under the same conditions. It may be seen from the

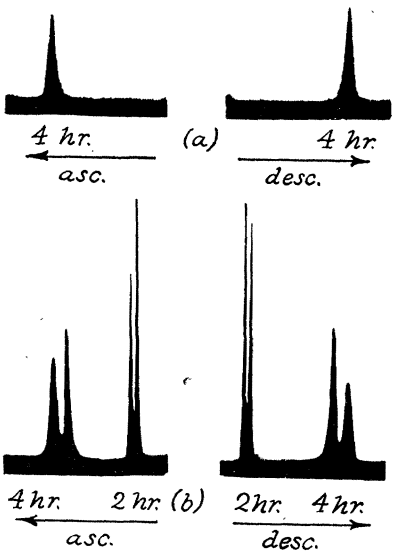

FIG. 1. (a) Acetyl virus. (b) Mixture of normal virus and phenylureido virus.

electrophoresis diagrams presented in Fig. 1 that the derivatives were very homogeneous with respect to electrophoretic mobility and that no detectable amount of the altered proteins migrated with the boundary representing the unchanged virus. In runs made with mixtures prepared with each of the virus derivatives and untreated virus, it was shown that the latter could be separated readily from the derivatives. The results as a whole indicate that the propagation of normal virus did not arise from unchanged virus present in the preparations of the derivatives and demonstrate that a large portion of certain functional groups of the virus molecule may be altered without interfering with the basic reaction of virus reproduction.

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LEUCOCYTE LEVEL AND LONGEVITY IN RATS

PRELIMINARY to a study of the effects of various carcinogenic agents and the growth of induced and