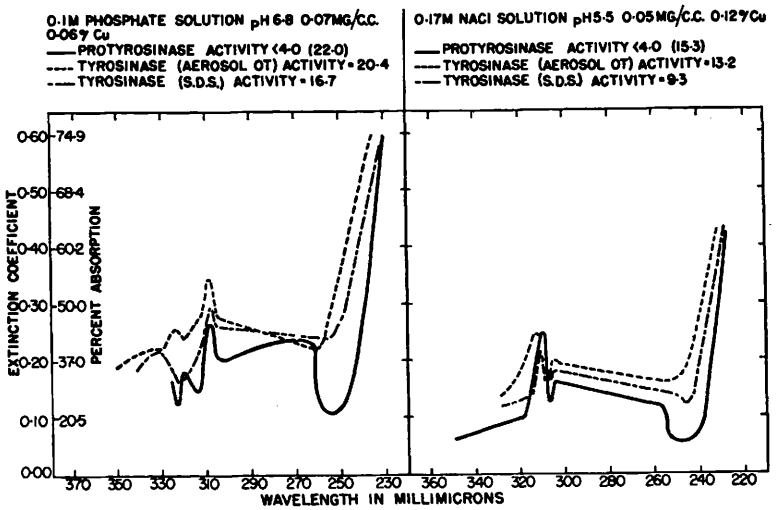

FIG. 1. Shows ultraviolet absorption curves for preparation, B-18a, both as protyrosinase and tyrosinase. Rates expressed as 4 for protyrosinase samples are impossible to accurately measure and can be assumed to represent zero activity. All other rates shown are for tyrosinase or activated protyrosinase. For further description see text.

spectrum in the ultraviolet accompanying the activation of the protyrosinase molecule to the active tyrosinase form.

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Effect of Iodine on Serological Specificity of Tobacco Mosaic Virus.* (20817)

JOHN J. BOLTRALIK AND W. C. PRICE.

From the Department of Biological Sciences and Plant Virus Laboratory, University of Pittsburgh.

In complement fixation tests, Weaver and Price(14) found the Type strain of tobacco mosaic virus (TMV) to react optimally with its homologous antiserum, or with antiserum to closely related strains, when a specific weight of virus, designated as the optimum antigenic unit (OAU), was present; TMV strains were differentiated from one another by differences in their OAU as well as by differences in antiserum dilution end points (ADE) in homologous and heterologous reactions, thus indicating a high degree of specificity for the serological reaction used. In precipitin tests, iodinated globulin was found to react weakly or not at all with antiserum to native protein(6,11,16); serum globulin iodinated at pH 7.3 reacted with antiserum to native globulin whereas that iodinated at pH 8.5 did not(6). In the work here reported, an attempt was made to iodinate specific amino acid groups on the TMV particle at neutral (7.0) and alkaline (7.65) pH values and to correlate the changes thus pro-

* Based on thesis by J. J. B. in partial fulfillment of requirements for Master of Science degree.

duced with the changes in serological activity.

Materials and methods. Antigens and antigen preparation. The Type strain of TMV was grown in Turkish tobacco plants and then concentrated and purified by alternating cycles of low (2950 x G for ½ hour) and high (25000 x G for 2 hours) speed centrifugation. The pellets obtained were suspended in 0.85% saline and stored at −20°C. *Antiserum to native and iodinated TMV.* Anti-TMV serum was prepared by injecting rabbits intraperitoneally with 2-ml portions of purified virus solutions containing about 26.5 mg/ml on the first and third day of initial immunization. Thereafter, one ml portions were administered intraperitoneally or intravenously every fourth day for 35 days. Preparation of antiserum to diiodotyrosine virus differed from this only in the volumes administered initially and the route of injection, 1.0 ml and 0.25 ml portions containing 4 mg/ml being injected exclusively by the intraperitoneal route. All sera were inactivated at 56°C for ½ hour. *Iodine-treated virus.* Abolition of sulfhydryl (SH) groups was accomplished with various volumes of 0.05 N iodine in 0.18 N potassium iodide. The preparation was maintained at pH 7.0 with phosphate buffer and the reaction allowed to proceed for 10 minutes at 35°C. The reaction was usually complete in 10 minutes as indicated by a negative nitroprusside test and the disappearance of iodine by a negative starch test(2). The reaction was reversible when 2 N KCN in the presence of 27% ammonium hydroxide was added. To accomplish the oxidation of SH groups past the S-S stage, quantities ranging from 0.05 to 0.08 ml of 0.05 N I-KI were used. Treatment for 15 to 25 minutes at 35°C was required to oxidize completely all SH groups past the S-S stage at pH 7.0. pH changes were no greater than 0.2 pH unit on the acid side of neutrality after reaction. Negative cyanide nitroprusside tests(1) indicated the absence of the S-S linkage. Insoluble protein was removed by centrifugation. Diiodotyrosine virus was prepared in the manner described by Anson and Stanley(2). Iodination was carried out at pH 7.65 in the presence of 0.09 N I-KI. The reaction was allowed to proceed for one hour at 56°C. All resulting preparations were tyrosine negative. Insoluble protein was removed by centrifugation. Excess iodine was neutralized with N/10 sodium thiosulfate. Millon tests performed on the supernatant indicated that tyrosine had been converted to diiodotyrosine. The preparation was dialyzed for 27 hours against flowing distilled water to remove tetrathionate and to readjust the pH to approximately 6.9. The preparation was centrifuged and the concentration of remaining protein determined by the micro-Kjeldahl method of Ma and Zuazaga(9). *Complement fixation technic.* The complement fixation technic used has been described in detail by Wertman(15) and by Tall *et al.*(12). ADE and OAU were determined from grid titrations involving 2-fold serial dilutions of antigen and 2-fold serial dilutions of antiserum(14). The OAU has been defined as the weight of virus per ml in the highest dilution of antigen giving a 4+ reaction with the highest dilution of antibody, the latter being defined as the ADE.

TABLE I. Optimum Antigenic Units (OAU) and Antiserum Dilution End Points (ADE) Obtained in Titrating SH Abolished, S-S Abolished, and Diiodotyrosine Virus against Antiserum to Native TMV and Antiserum to Diiodotyrosine Virus.

Antigen	Antiserum to Native TMV* OAU	ADE‡	Antiserum to Diiodotyrosine TMV† OAU	ADE‡
	μg		μg	
Native virus	2.8	320	2.8	40
SH abolished virus	2.8	320	2.8	40
S-S " "	5.6	160	5.6	40
Diiodotyrosine "	5.6	10	5.6	20

* Results of 4 titrations.
† " " 3 " .
‡ Reciprocals.

Results. On the basis of past experience, 2-fold differences in OAU are significant but 2-fold differences in ADE have doubtful validity unless based on a large number of titrations(14). The 2-fold differences in ADE (Table I) are therefore questionable, whereas 4-fold differences are undoubtedly significant.

The OAU for native TMV, 2.8 μg, was the same as that found by Weaver and Price(14). Virus with SH groups abolished gave exactly the same reactions with both antisera as the native virus, indicating that neither cysteine

nor cystine, as such, plays a major role in serological specificity. Abolition of disulfide groups, however, resulted in increasing the OAU by 2-fold and, in the reaction with antiserum to the native virus, decreasing the ADE by 2-fold. The latter is questionable but there is a possibility that it may have resulted from an effect of iodination on tyrosine groups; although Millon tests on S-S abolished virus were strongly positive, this does not exclude the formation of monoiodotyrosine.

The most significant changes occurred with the diiodotyrosine virus; in reactions of antiserum to native TMV with diiodotyrosine virus the OAU was doubled and the ADE reduced 32-fold as compared to the homologous reaction with native virus. This suggests the importance of aromatic groups for combination of antigen and antibody and agrees in principle with observations of others(6,16). Increase in OAU can probably be explained on the basis of a shift in redox potential of iodine, since a shift of 1.53 pH units on the acid side of neutrality was measured(3).

Reactions between the various antigens and antiserum to diiodotyrosine virus are of particular importance because they show that the treated virus was still capable of stimulating antibody formation in rabbits, despite the comparatively low titer. Because of the low titer it is virtually certain that completely negative results would have been obtained by the precipitin technic, as has indeed been the experience of others(6,11,16). The sensitive complement fixation test thus demonstrated serological activity of chemically altered virus not demonstrable by the relatively insensitive precipitin test.

Discussion. Although many postulations for the intermediate reaction between cysteine and cystine and oxidants are found in the literature(4,5,13) none offers proof of the exact intermediate with chemically treated proteins. Evidence that RSO_3H could not be formed in the reaction between iodine and cysteine was given by Anson and Stanley(2).

It is theorized that TMV contains sub-antigenic groupings, as well as the known aromatic determinants; change in sub-antigenic groupings, such as oxidation past the S-S stage, leads to a change in the OAU, whereas change in antigenic groupings, of which tyrosine is representative, leads to drastic change in the ADE.

Moorhead and Price(10) found that antiserum to the Type strain of TMV reacted with the Rosette strain of TMV with an ADE 8-fold less than that in the homologous reaction, whereas antiserum to the Rosette strain reacted with the Type strain antigen at the same ADE as in the homologous reaction. Kleczkowski(7) reported a similar relationship between the Type and Aucuba strains of TMV and their respective antisera when the quantitative precipitin test was used. A similar result can be seen in Table I with normal and diiodotyrosine virus. Knight(8) found no difference in tyrosine content between the Type and Aucuba strains of TMV which could account for the serological differences observed.

Summary. The sensitive complement fixation reaction was used to detect antigenic differences between normal tobacco mosaic virus (TMV) and TMV treated with iodine in KI in such a manner as to oxidize SH groups or to form diiodotyrosine. No change in antigenicity was detected in TMV in which the SH groups had been oxidized to S-S. Oxidation past the S-S stage resulted in change in the weight of virus reacting optimally with antiserum. Virus in which tyrosine was converted to diiodotyrosine was only weakly effective in stimulating formation of antibodies in rabbits and reacted with antiserum to normal virus with a 32-fold drop in antiserum dilution end point. These serological differences are comparable to the serological differences occurring between strains of TMV.

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Partially Depolymerized Hyaluronic Acid (PDHA) as a Spreading Agent. (20818)

JOSEPH SEIFTER AND DAVID H. BAEDER.
Wyeth Institute of Applied Biochemistry, Philadelphia, Pa.

The spreading action of testicular hyaluronidase is usually attributed to the decrease in viscosity of the ground substance which results from depolymerization of the hyaluronate by the enzyme. The effect is to decrease the normal resistance of the barrier. A more recent view suggests that the enzyme releases PDHA which as a protective colloid and peptizing agent participates actively in the dispersion and transfer of materials through the ground substance and in the urine(1). It has also been suggested that hyaluronic acid is capable of acting as an ion exchange resin(2). In this paper we present data on the spreading properties of PDHA prepared from *in vitro* hydrolysis of streptococcal hyaluronic acid by testicular hyaluronidase.

Materials and methods. Bacterial hyaluronic acid was isolated by Dr. George H. Warren from a virulent strain of group A *Streptococcus pyogenes* obtained from the Hospital of the Rockefeller Institute. It contained 3.67% N and was free from S, P, and halogen. Purified bovine vitreous humor hyaluronate and human umbilical cord hyaluronate were prepared by Dr. Harvey E. Alburn. Streptococcal hyaluronate before treatment with the enzyme was the most viscous and vitreous humor hyaluronate the least viscous preparation. PDHA was prepared by incubating streptococcal hyaluronic acid with testicular hyaluronidase* for 15 minutes at 38°C. The incubation mixture was autoclaved for forty minutes at 104°C and the resulting precipitate removed by centrifugation and discarded. The supernatant was found to be free from hyaluronidase by the turbidimetric assay and therefore contained less than 0.1 TRU/ml. Incubation of the substrate with the enzyme for one hour resulted in supernatants that had no significant spreading activity. Spreading tests were made on albino rabbits of both sexes weighing between 2.3 kg and 2.9 kg. The intradermal injections were made through a 26 gauge, one-half inch needle into the lumbar and scapular regions clipped free from hair. The injections consisted of 0.1 ml of 1% trypan blue in physiological salt solution (PSS) mixed with 0.1 ml of test solution. Measurements of the large diameter of spread (D) and the small diameter of spread (d) were made with vernier caliper immediately after injection and 15, 30, and 60 minutes later. The area of spread was computed from the formula for the area of an ellipse, $A_{sq\ mm} = \frac{D \times d \times \pi}{4}$. Each concentration of a substance was tested on a minimum of 6 rabbits. Each rabbit received a control injection of PSS. The injection sites for the control and materials to be tested were randomized. The comparative efficiency of hyaluronidase and PDHA to facilitate hypodermoclysis of PSS was carried out in rabbits. Six rabbits received

* Wydase®—Wyeth Laboratories, Inc.—7000TRU/mg N.