13318

The Colorimetric Determination of Cystine in Tobacco Mosaic Virus Protein.

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In a study of the sulfur distribution in tobacco mosaic virus protein, Ross[1] found colorimetric cystine methods, the Sullivan[2] and the Folin-Marenzi[3] as modified by Lugg[4] unsatisfactory in that they gave low and variable results. With the Baernstein[5] method as modified by Kassell and Brand,[6] however, Ross obtained cysteine plus cystine values sufficiently high to account for most of the sulfur of the virus protein.

Since Ross' low and variable results with the colorimetric methods seem to be due to his type of hydrolysis and the use of norite as a decolorizing agent we obtained mosaic tobacco from Dr. D. Breese Jones of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. From this mosaic tobacco the virus protein was prepared by the isolation procedure of Stanley[7] with the trypsin treatment as recommended by Bawden and Pirie.[8] The precipitation, dialysis, concentration, and drying of the virus protein was as done by Ross.[1] The dried samples were kept *in vacuo* over phosphorus pentoxide at room temperature.

Of the dried sample, aliquots (usually 250 mg) were hydrolysed respectively with (A) 2.0 cc of 20% hydrochloric acid for 6 hours; (B) with a mixture of 1.0 cc con. hydrochloric acid and 1.0 cc 95% formic acid for 24 hours;[9] (C) with 6.0 cc of 6N sulfuric acid for 12 hours; (D) with 2.0 cc of 20% hydrochloric acid and 0.1 cc of 20% titanous chloride for 2 hours;[10] (E) with 6.0 cc of 57% hydriodic acid (sp. gr. 1.70) for 18 hours; (F) with 3.0 cc of 57% hydriodic acid and 3.0 cc of 95% formic acid for 24 hours. The

1 Ross, A. Frank, *J. Biol. Chem.*, 1940, **136**, 119.

2 Sullivan, M. X., *Pub. Health Rep., U.S.P.H.S.*, Supplement 78, 1929.

3 Folin, O., and Marenzi, A. D., *J. Biol. Chem.*, 1929, **83**, 103.

4 Lugg, J. W. H., *Biochem. J.*, 1932, **26**, 2160.

5 Baernstein, H. D., *J. Biol. Chem.*, 1936, **115**, 25, 33.

6 Kassell, B., and Brand, E., *J. Biol. Chem.*, 1938, **125**, 145.

7 Stanley, W. M., *J. Biol. Chem.*, 1936, **115**, 673.

8 Bawden, F. C., and Pirie, N. W., *Brit. J. Exp. Path.*, 1937, **18**, 275.

9 Miller, G. L., and duVigneaud, V., *J. Biol. Chem.*, 1937, **118**, 101.

10 Sullivan, M. X., and Hess, W. C., *J. Biol. Chem.*, 1937, **117**, 423.

hydrolysis with sulfuric acid and with hydriodic acid and the mixture of hydriodic acid and formic acid was done in a nitrogen atmosphere, that is, with nitrogen bubbling through the mixture during hydrolysis. There was considerable humin formation in (A) and (B), little in (C) and none in (D), (E), and (F).

(A) The humin in this hydrolysate was practically all in the insoluble form. The mixture was brought to pH 3.5 by 5 N sodium hydroxide added dropwise with stirring and the whole made to 20 cc with 0.1 N hydrochloric acid and filtered. The filtrate was slightly yellow and needed no decolorization.

(B) The hydrolysate was black. After evaporation to a syrup on the water bath it was made up to 20 cc with 0.1 N hydrochloric acid and filtered. The filtrate was a light tan, somewhat darker than (A), but the color was reducible so the filtrate needed no decolorization.

(C) The hydrolysate was neutralized to pH 3.5 and diluted to 25 cc with 0.1 N hydrochloric acid and filtered. The filtrate was golden yellow in color. The organic sulfur was mainly in the form of cystine but part was SH since it gave a good nitroprusside reaction without sodium cyanide. The solution on standing overnight gave no nitroprusside reaction without cyanide and so was determined as cystine.

(D) The hydrolysate was brought to pH 5, approximately, with 5 N sodium hydroxide and centrifuged from the precipitated titanous hydroxide. The precipitate was washed once with 5.0 cc of 0.1 N hydrochloric acid and again centrifuged. The combined solution was slightly yellow and contained only cysteine.

(E) and (F) The hydrolysates were concentrated to a syrup, neutralized with 5 N sodium hydroxide to pH 3.5 and diluted to 25 cc with 0.1 N hydrochloric acid. Both solutions were a light yellow.

The percentage cystine content of the virus protein with different methods of hydrolysis and the use of the Sullivan colorimetric method[2] and the Okuda iodometric method[11] are respectively (A) .47, .48; (B) .53, .48; (C) .67, .71; (D) .64, .64; (E) .73, .70; (F) .69, .69.

Ross' best value by the Baernstein hydriodic acid hydrolysis and iodometric titration was .70% cysteine or cystine when corrected for the recovery of cystine similarly treated.* In the hydriodic

11 Okuda, Y., *J. Biochem.*, Japan, 1925, **5**, 217.

* Such a correction, however, assumes that cystine in mixtures behaves like cystine alone.

acid hydrolysis the cysteine found by the Sullivan method without correction is .73% and by the Okuda method (after oxidizing and reducing with zinc and hydrochloric acid) .70%. The average value by the Sullivan method as determined from the different hydrolysates without humin formation ((C), (D), (E), and (F)) is .68%.

Summary. The Sullivan colorimetric method can be applied satisfactorily to hydrolysates of tobacco mosaic virus if humin formation is inhibited by hydrolyzing with hydrochloric acid-titanous chloride, or better with hydriodic acid.

13319 P

Inactivity of Nicotinuric Acid in Canine Blacktongue.

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It has been reported[1] on the basis of a single test that nicotinuric acid has a curative action in canine blacktongue, and reference has been made to the "antipellagra value" of nicotinuric acid in a recent review.[2]

We have tested the curative activity of a specimen of nicotinuric acid prepared from glycine ethyl ester and nicotinoyl-chloride by the procedure of Meyer and Graf[3]; melting point 240-41° (Meyer and Graf found 237-8°), neutral equivalent 184 (theoretical 182.7).

The arrangement of the test and basal diet were as previously described.[4] Four dogs were used. With the first 2 dogs curative tests were made by our standard procedure, with daily subcutaneous doses of 6 mg and 8 mg respectively per kg body weight for 5 days. The results were negative. In a third dog given 6 mg per kg a partial cure was obtained, and subsequently a preventive dose of 1 mg per kg daily was given, and the dog declined and died during this treatment. The fourth dog was given 3 mg per kg daily and was completely cured except that its normal weight was not fully restored. The dosing was continued for 60 days, during which time the dog

1 Woolley, D. W., Strong, F. M., Madden, R. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1938, **124**, 715.

2 Morgan, A. F., *Ann. Rev. Biochem.*, 1941, **10**, 353.

3 Meyer, H., and Graf, R., *Biochem. Z.*, 1930, **229**, 156.

4 Dann, W. J., Kohn, H. I., and Handler, P., *J. Nutrition*, 1940, **20**, 477.