

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.

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Inactivity of Nicotinuric Acid in Canine Blacktongue.

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It has been reported¹ 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 Graf³; 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.

remained healthy. Ten days after the last dose it began to decline rapidly and after 7 days more it died of severe blacktongue. These effects may be contrasted with those due to nicotinic acid itself. In our curative method a daily dose of 1 mg per kg for 5 days produces optimum response; and on basal diets of this type the daily dose needed for prevention of blacktongue is about 0.1 mg per kg.⁵

These results lead us to the conclusion that nicotinuric acid is not a blacktongue-preventive (and therefore not a pellagra-preventive) compound, but is to be grouped with the pyrazine acids,⁴ quinolinic acid,⁴ 3-amino-pyridine,⁶ 2-amino-nicotinic acid,⁷ and pyrimidine-4-carboxylic acid.⁷ These compounds are not regularly blacktongue-preventive but nevertheless are often able partially to replace nicotinic acid in the diet of the dog, sometimes for a considerable period.

The conclusion that nicotinuric acid is not to be ranked as a true blacktongue-preventive is borne out by the findings of Sarett,⁸ who has found that the dog maintained on a low intake of nicotinic acid is unable to hydrolyze nicotinuric acid, but excretes it completely without metabolic change.

This conclusion is of importance in the choice of an assay method for the quantitative estimation of nicotinic acid in biological materials. Snell and Wright⁹ have described a convenient and very sensitive microbiological assay, using the organism *Lactobacillus arabinosus* 17-5, and they point out that this organism utilizes nicotinuric acid as efficiently as nicotinic acid. It follows that their method is not suitable when the assay is being performed in order to evaluate the blacktongue- (or pellagra-) preventive activity of any material unless it is free from nicotinuric acid.

Moreover, some of the compounds enumerated above have been listed commercially as pellagra-preventive vitamins. Such a listing is potentially dangerous; nicotinuric acid and the other members of the group to which it belongs should not be classified with nicotinic acid and nicotinamide which can be depended upon to produce rapid clinical improvement in small doses.

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⁵ Birch, T. W., *J. Nutrition*, 1939, **17**, 281.

⁶ Subbarow, Y., Dann, W. J., and Meilman, E., *J. Am. Chem. Soc.*, 1938, **60**, 1510; Strong, F. M., Madden, R. J., and Elvehjem, C. A., *J. Am. Chem. Soc.*, 1938, **60**, 2564; Subbarow, Y., and Dann, W. J., *J. Am. Chem. Soc.*, 1938, **60**, 2565.

⁷ Handler, P., and Dann, W. J., unpublished observations.

⁸ Sarett, H. P., *J. Nutrition*, in press.

⁹ Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.