

14453

A Color Reaction of Ascorbic Acid with Nicotinamide and Nicotinic Acid.

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Since reactions between different vitamins are always of interest, it appears worthwhile to report the following observations. In the course of performing a number of spot tests, it was observed that a yellow color was produced when a mixture of ascorbic acid and nicotinamide was moistened with water and stirred into a thick paste. The formation of the canary yellow color was almost immediate. Nicotinic acid gave a similar color reaction with ascorbic acid although the reaction proceeded more slowly. None of the other vitamins that were tested gave this reaction; these included pyridoxine hydrochloride, thiamine,

calcium pantothenate, and para-amino benzoic acid.

Failure to note this reaction previously in any of the numerous mixtures of vitamins used as dietary supplements probably was due to the common practice of including riboflavin in such preparations. The deep yellow color of the riboflavin would mask that of the reaction.

Whether the reaction alters the biologic activity of the vitamins or represents one of the functions of ascorbic acid in the body is not known.

14454

A Color Reaction of Ascorbic Acid with Derivatives of Pyridine, Piperidine, Quinoline, and Iso-Quinoline.

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Recently it was shown by T. H. Milhorat¹ that ascorbic acid will react with nicotinamide and nicotinic acid with the production of a yellow color. A number of other vitamins tested gave no color reaction with the ascorbic acid. The present investigations show that many pyridine derivatives, other than nicotinamide and nicotinic acid, show this color formation with ascorbic acid, and for the numerous compounds tested so far the reaction has been specific for certain definite chemical structures.

While the color formation usually can be observed when ascorbic acid is mixed with

these substances in solution, the simplest and most satisfactory method is to mix a small amount of each on a white porcelain spot plate and stir into a paste after the addition of a drop or two of water. In most instances, the color formation is almost immediate; in the case of a few substances a short period of from 10 to 20 seconds might be required for the full development of the color.

Observations. A large number of substances representing various types of chemical structure were tested. The chemical compounds which gave a yellow color reaction with ascorbic acid are shown in Fig. 1. The list of substances giving no color reaction is too long to reproduce here but those most important for the purposes of studying the specifi-

¹ Milhorat, T. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1944, **55**, 52.