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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.

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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. Milhorat[1] 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 specifici-

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

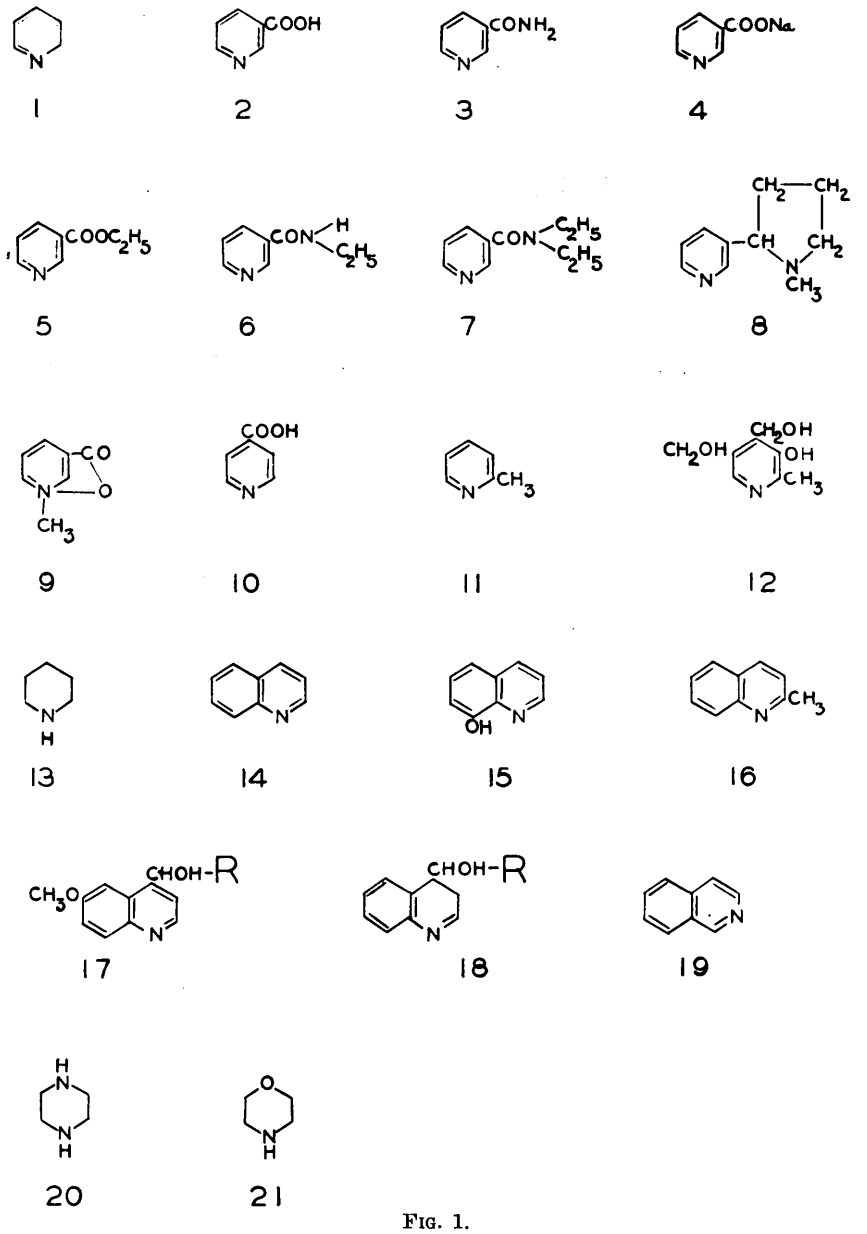

FIG. 1.
Substances Giving a Yellow Color Reaction with Ascorbic Acid.
1. Pyridine; 2. nicotinic acid; 3. nicotinic acid amide; 4. sodium nicotinate; 5. ethyl nicotinate; 6. nicotinyl monoethylamide; 7. nicotinyl diethylamide; 8. nicotine; 9. trigonelline; 10. isonicotinic acid; 11. alpha-picoline; 12. pyridoxine (vitamin B_6); 13. piperidine; 14. quinoline; 15. 8-hydroxyquinoline; 16. quinaldine; 17 quinine and quinidine and their various salts (hydrochloride, dihydrochloride, sulphate, hydrosulphate, ethyl carbonate); 18. cinchonine and cinchonidine; 19. isoquinoline; 20. piperazine; 21. morpholine.

ty of the reaction are illustrated in Fig. 2. Other compounds giving no reaction included tryptophane, histidine, cystine, cysteine, methionine, leucine, isoleucine, valine, norvaline, proline, aminoacetic acid, aspartic acid, threonine, lysine, alanine, phenylalanine, ornithine, arginine, various guanidine derivatives, ephedrine and several chemically related compounds, adenine, picrotoxin, strychnine, brucine, veratrine, physostigmine, sparteine, digi-

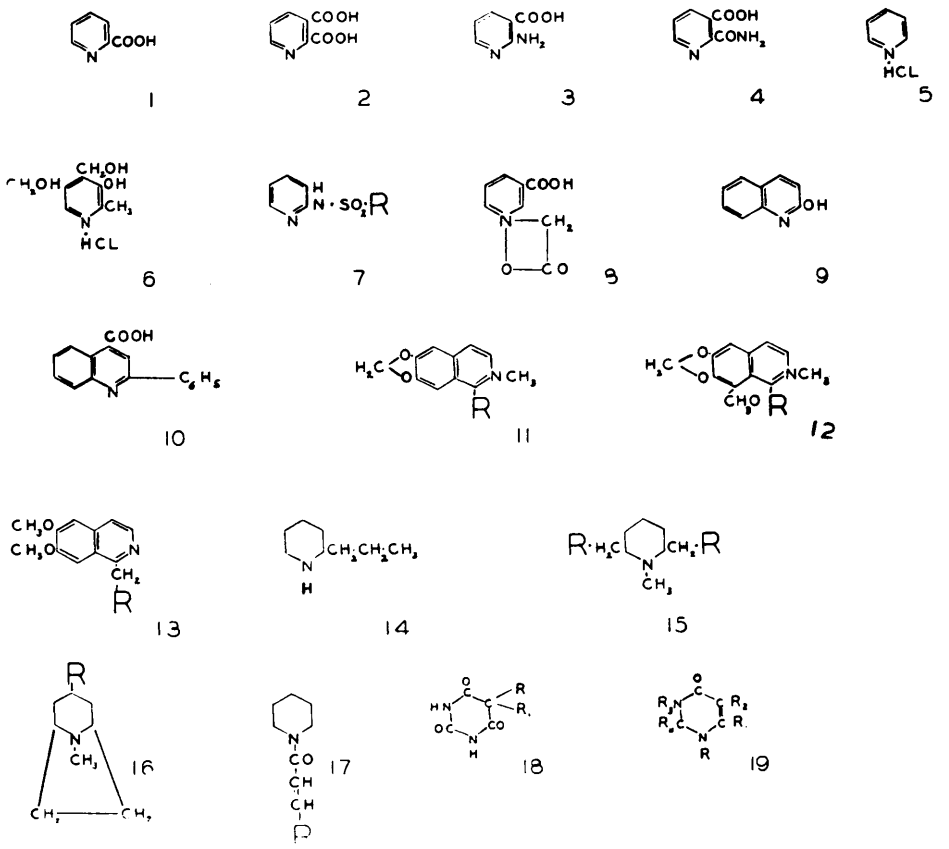

FIG. 2.
Some of the Substances Giving No Color Reaction with Ascorbic Acid.
1. Picolinic acid; 2. quinolinic acid; 3. 2-amino nicotinic acid; 4. quinolinamic acid; 5. pyridine hydrochloride; 6. pyridoxine hydrochloride; 7. sulfapyridine; 8. pyridine-betaine-3-carboxylic acid; 9. 2-hydroxy-quinoline; 10. cinchophen; 11. hydrastine; 12. narcotine; 13. papaverine; 14. coniine; 15. lobeline; 16. atropine and hyoscyamine; 17. piperine; 18. phenobarbital and other derivatives of barbituric acid; 19, caffeine, theobromine, guanine, uric acid.

talin, phenol and various derivatives, benzoic acid, hydroquinone, menthol, camphor, isoborneol, anthracene, thiamine, pantothenic acid, vitamin K, and several sterols.

In general, the observations indicate that the color reaction occurs when the alpha-carbon of the pyridine ring is not occupied by a radical other than the methyl group. These conditions hold also with the piperidines, quinolines, and iso-quinolines that were investigated. The radicals interfering with the reaction are shown in Fig. 2.

The two betaines, trigonelline and pyridine-betaine-3-carboxylic acid are of interest in that an intense yellow color was produced when they were treated with strong alkali alone. This reaction was not observed with any of the other derivatives of nicotinic acid. No color formation was observed when ascorbic acid was mixed with piperine which has a substitution on the nitrogen atom; however, treatment of piperine with acid alone produced a brilliant yellow color. Ascorbic acid gave a yellow color with trigonelline, but not with pyridine-betaine-3-carboxylic acid. Failure of this latter substance to give the color reaction with ascorbic acid might be due to steric hindrance or the presence of a strongly acidic radical attached to the nitrogen atom. It has also been shown that the hydrochlorides of pyridine and pyridoxine do not react with ascorbic acid, whereas the free bases do.

Picolinic acid, quinolinic acid, and cinchophen, substances in which an acid radical is

attached to the alpha-carbon, did not react with color formation with ascorbic acid unless the acid radical was neutralized. Of the many substances investigated, morpholine and piperazine were the only compounds which gave the color reaction and were not derivatives of pyridine. However, both compounds may be considered as piperidine in which the CH_2 group in the gamma position has been replaced by either O or NH. In both instances the development of color was slow in contrast with the almost immediate formation of color in the case of nicotinamide and other derivatives of pyridine.

Other sugars and related substances substituted for ascorbic acid gave no color reaction with nicotinamide; those tested included glucose, levulose, xylose, mannose, lactose, galactose, sorbitol, arabinose, inositol, glycogen, and gluconic acid.

The reducing property of the ascorbic acid, as determined by titration with dichlor-phenol-indo-phenol, was found to be unchanged by the color reaction with nicotinamide. Furthermore, the effect of an ingested dose of 25 mg ascorbic acid on the urinary output of the vitamin in a fasting dog was essentially the same whether or not the ascorbic acid had reacted with nicotinamide before its administration to the animal. Whether the biologic activity of nicotinamide is affected by the reaction is not known.

Summary. Ascorbic acid reacts with formation of yellow color with nicotinamide, nicotinic acid, and a number of other derivatives of pyridine. In general, a radical other than the methyl group attached to the alpha-carbon atom interferes with the reaction. Similar conditions were observed in the case of the piperidines, quinolines, and iso-quinolines that were investigated. The effect on the reaction of radicals attached to the nitrogen atom and the influence of neutralization of a carboxyl group in the alpha position are discussed.

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Vitamin E and Length of Life of Rats Fed a Diet with Fatally Low Protein Content.*

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The experiments reported here were originally planned as part of an investigation designed to determine whether a condition simulating exudative diathesis could be induced in rats fed with a vitamin E-deficient diet. The exudative diathesis occurs in chicks when the diet is deficient in vitamin E and contains highly unsaturated fatty acids, such as those provided by cod liver oil, and, within certain limits, can be accelerated by a high carbohydrate-low protein ratio in the diet.[1] Since it is also known from clinical medicine that a tendency to edema is enhanced when the diet is high in carbohydrate and low in protein, it was thought that a drastic change of the vitamin E-deficient diet in this same direction might precipitate similar symptoms in rats. Although no condition resembling exudative diathesis occurred in these animals it was observed that adequacy of vitamin E enabled the rats to survive longer on the protein-insufficient diet than was the case when the diet was also lacking in vitamin E. Although further study of this problem is not immediately contemplated it seems worthwhile reporting the experimental data since the results may offer some additional suggestions concerning the physiological functions of vitamin E.

Procedure. Groups of 6 newly weaned rats equally distributed according to weight in the

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[1] Dam, H., *J. Nutr.*, in press.