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Pyridine Derivatives and Other Compounds as Growth-Promoting Substances for Dysentery Bacilli.*

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The activity of a number of pyridine derivatives as growth-promoting substances for dysentery bacilli has been reported by us.[1] More recently several additional compounds of special interest were secured and tested. These were: synthetic vitamin B_6, nicotinamide methiodide, pyrazine monocarboxylic acid, pyrazine 2,3-dicarboxylic acid, thiazole 5-carboxylic acid, thiazole 5-carboxylamide† and quinolinic acid.

Vitamin B_6 is a pyridine compound with the structure 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine.[2] It seemed of interest to determine whether this compound is capable of acting as a growth-promoting substance for dysentery bacilli when substituted for nicotinic acid or nicotinamide. Another pyridine compound of possible physiologic interest is nicotinamide methiodide which Karrer and Warburg[3] found could be reversibly oxidized and reduced, as can the coenzymes. The nicotinamide methiodide was prepared according to Karrer and coworkers[4] and identified by melting point. The preparation was recrystallized seven times from an alcohol-ether mixture.

Pyrazine monocarboxylic acid and pyrazine 2,3-dicarboxylic acid were included because of the recent report by Bills, McDonald and

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1 Dorfman, A., Koser, S. A., Reames, H. R., Swingle, K. F., and Saunders, F., *J. Inf. Dis.*, 1939, **65**, 163; Koser, S. A., Dorfman, A., and Saunders, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 311.

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2 Stiller, E. T., Keresztesy, J. C., and Stevens, J. R., *J. Am. Chem. Soc.*, 1939, **61**, 1237; Harris, S. A., Stiller, E. T., and Folkers, K., *ibid.*, **61**, 1242; Kuhn, R., and associates, *Ber.*, 1939, **72**, 305, 309, 310.

3 Karrer, P., and Warburg, O., *Biochem. Z.*, 1936, **285**, 297.

4 Karrer, P., Schwarzenbach, G., Benz, F., and Solmssen, U., *Helv. Chim. Acta*, 1936, **19**, 811.

Spies[5] that administration of these compounds produced a prompt improvement in the condition of pellagrins. The thiazole compounds are interesting because they are isoteric with nicotinic acid and nicotinamide. Schmelkes[6] has suggested that these isosteres may be interchangeable biologically.

All compounds were sterilized by filtration through sintered glass filters and added aseptically in a series of graded dilutions to a basal synthetic medium. This medium was the same as that employed in previous work[1] and consisted of 15 amino acids, glucose and inor-

TABLE I.

Compound	Molar concentrations	Growth-promoting effect for dysentery bacilli
Vitamin B_6	1×10^{-4} to 10^{-6}	No growth*
Nicotinamide methiodide	1×10^{-4} to 10^{-6}	" "
Pyrazine monocarboxylic acid	1×10^{-3} to 10^{-6}	" "
Pyrazine 2,3-dicarboxylic acid	1×10^{-3} to 10^{-6}	" "
Thiazole 5-carboxylic acid	1×10^{-3}	Slow growth, 4 to 18 days, with 9 of 10 cultures; one culture no growth.
	1×10^{-4}	Slow, light growth, 10 to 21 days, with 3 of 10 cultures; other 7 no growth.
	1×10^{-5}	No growth.
Thiazole 5-carboxylamide	1×10^{-3}	Slow growth, 5 to 21 days, with 5 of 10 cultures; other 5 cultures no growth.
	1×10^{-4}	Slow, light growth, 7 to 21 days, with 3 cultures; other 7 no growth.
	1×10^{-5}	No growth.
Quinolinic acid, recrystallized (filtered)	1×10^{-4}	Good growth, 2 days.
	1×10^{-5}	No growth.
Quinolinic acid, recrystallized (heated)	1×10^{-4}	Good growth, 24 hours.
	1×10^{-5}	Light or good growth, 24 hours.
	1×10^{-6}	Negative or light growth, 24 hr; good growth in 48 hr.
Controls:		
Nicotinic acid	1×10^{-6}	Good growth, 24 hr.
	1×10^{-7}	Light growth, 2 to 3 days; heavier growth 4 days.
	1×10^{-8}	No growth or occasional delayed growth, 10 to 14 days.
Nicotinamide	1×10^{-7}	Good growth, 24 hr.
	1×10^{-8}	Very light growth.

* All culture tubes in which growth did not appear were held at 37°C for 14 days and in many instances for 21 days before being recorded as negative.

5 Bills, C. E., McDonald, F. G., and Spies, T. D., *Southern Méd. J.*, 1939, **32**, 793.

6 Schmelkes, F. C., *Science*, 1939, **90**, 113.

ganic salts. Ten strains of dysentery bacilli belonging to the Flexner and Sonne subgroups were used. It was known from previous work (1) that these cultures either did not develop, or in a few instances, developed only to the extent of a light turbidity in the basal synthetic medium and (2) addition of small amounts of either nicotinamide or nicotinic acid to the synthetic medium exerted a pronounced growth-promoting effect so that cultures showed luxuriant growth within 24 hours.

The results are shown in Table I. Vitamin B_6, nicotinamide methiodide, and the two pyrazine compounds exerted no growth-promoting effect, even when used in relatively large amounts.

The two thiazole compounds showed a low order of activity; slow culture development appeared in the presence of relatively large amounts of these compounds while smaller amounts were negative. This finding confirms the report of Schmelkes[6] and is an interesting example of the replacement of a compound by an isostere. It will be noted, however, that on the basis of comparable effect the activity of thiazole 5-carboxylic acid is about one ten-thousandth or less of that of nicotinic acid. In this respect our results differ slightly from those of Schmelkes[6] who reported that thiazole 5-carboxylic acid was about one-tenth of one percent as active as nicotinic acid. In our experience thiazole 5-carboxylamide is slightly less active than its corresponding acid. Thus the difference in the order of activity between the thiazole amide and nicotinamide is even greater than that between the respective carboxylic acids.

Quinolinic acid was included because of the differing claims regarding its efficacy. It supported development of dysentery bacilli when used in relatively large amounts, 1×10^{-4} molar.[1] According to Lwoff and Querido[7] a similar concentration caused growth of *Proteus*. When *Staphylococcus aureus* was used no growth-promoting activity of quinolinic acid in concentrations up to 10^{-4} molar was observed either by Knight and McIlwain[8] or by Landy.[9] In canine blacktongue Woolley, *et al.*,[10] reported a single dose of 150 milligrams failed to alleviate the condition, but amounts of one gram given to pellagrins by Vilter and Spies[11] produced marked improvement.

A sample of commercial quinolinic acid was purified by repeated

7 Lwoff, A., and Querido, A., *C. R. Soc. Biol.*, 1939, **130**, 1569.

8 Knight, B. C. J. G., and McIlwain, H., *Biochem. J.*, 1938, **32**, 1241.

9 Landy, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 504.

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

11 Vilter, R. W., and Spies, T. D., *Lancet*, 1939, **2**, 423.

recrystallization. Tests of its growth-promoting activity for dysentery bacilli showed the activity decreased somewhat after the first recrystallizations but thereafter remained at a constant level. This low order of activity may perhaps be explained by decarboxylation at position 2 with formation of nicotinic acid. Heating a solution of the purified quinolinic acid in the autoclave for 30 minutes increased its activity approximately a hundred times.

These results lead us to believe that the curative effect obtained by Vilter and Spies when they fed quinolinic acid to pellagrins might be due to the presence of small amounts of nicotinic acid. As far as we know, no one has determined the minimum amount of nicotinic acid necessary to relieve the symptoms of pellagra; but in one report it was stated that 60 mg per day would effect a cure.[12] Spies, Bean and Ashe report that as little as 40 mg per day may be effective.[13] On this basis it would be possible to effect a cure with "quinolinic acid" if it contained as little as 4-6% nicotinic acid. From our experience commercial quinolinic acid may well contain this much nicotinic acid. At least a part of the activity of quinolinic acid is due to an impurity since the potency of quinolinic acid decreases on recrystallization.

Summary. Vitamin B_6, nicotinamide methiodide, pyrazine monocarboxylic acid and pyrazine 2,3-dicarboxylic acid showed no growth-promoting effect for dysentery bacilli when added to a basal synthetic medium. Thiazole 5-carboxylic acid and its amide exerted a low order of growth-promoting activity. The use of purified quinolinic acid in 1×10^{-4} molar concentration was uniformly followed by culture development. This may have been due to gradual conversion to nicotinic acid. Heating the quinolinic acid solution greatly increased its activity. The efficacy of quinolinic acid in curing pellagra may be due to contamination with small amounts of nicotinic acid.

12 Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. Am. Med. Assn.*, 1937, **109**, 2054.

13 Spies, T. D., Bean, W. B., and Ashe, W. F., *Ann. Internal Med.*, 1939, **12**, 1830.