dryness and the residue taken up in 10 cc of acetone and 10 cc of a 0.1 N solution of NaOH. When complete solution is effected with moderate heating, the volume is brought to 50 cc by the addition of 30 cc of 0.1 N NaOH. The solution is placed in the cold and pregnandiol completely precipitates when the solution is thoroughly chilled. The pregnandiol is collected by filtration with suction and the precipitate is washed with warm water. The precipitate is dissolved in a minimum amount of hot acetone and 2 volumes of 0.1 N NaOH added. This is put in the cold to effect precipitation. A third precipitation using a minimum amount of ethyl alcohol and two volumes of water usually give a pure product.

The essential points in the procedure are: (1) The liberation of the free form of pregnandiol from its conjugation form by enzyme action during incubation; (2) the insolubility of pregnandiol in aqueous solutions such as urine; (3) the purification of pregnandiol by precipitation from alkaline acetone.

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Effect of Nicotinic Acid, Its Isomers and Related Compounds upon Nutrition of *Staphylococcus aureus.*

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(Introduced by N. Paul Hudson.)

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Nicotinic acid has aroused widespread interest with the discovery of its rôle as a co-enzyme in tissue metabolism,[1, 2] later by the finding of its activity as a growth factor for *Staphylococcus aureus*,[3, 4] and most recently by its dramatic effect in the cure of canine black-tongue[5] and human pellagra.[6, 7, 8] In a series of unpublished ex-

1 Warburg, O., and Christian, W., *Biochem. Z.*, 1936, **287**, 291.

2 Schlenk, F., and Euler, H. v., *Arkiv. Kemi, Mineral Geol.*, 1936, 12B, No. 20.

3 Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 731.

4 Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 966.

5 Elvehjem, C. A., Madden, R. J., Strong, F. M., and Wooley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

6 Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, J. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 405.

7 Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. A. M. A.*, 1937, **109**, 2054.

8 Spies, T. D., Cooper, C., and Blankenhorn, M. A., *J. A. M. A.*, 1938, **109**, 622.

periments we have been able to confirm the work of Knight[3,4] in demonstrating the necessity of nicotinic acid and amide for the growth of *S. aureus*. Knight,[4] by testing a number of compounds closely related to thiamine, found that they could not replace it as a growth requirement of this microörganism, indicating a high degree of specificity. The importance of similar knowledge concerning compounds related to nicotinic acid is self-evident.

Using methods given in the chemical literature, we have prepared a series of compounds closely related to nicotinic acid. This series included the 2 isomers (picolinic and isonicotinic acids), sodium and ammonium salts, and ethyl ester of nicotinic acid, as well as the mono- and di-substituted nicotinic amides. The physical and chemical properties of the prepared compounds were in agreement with those cited in the literature and in general indicated a high degree of purity.

For the purpose of testing the utilization of this series of compounds by *S. aureus*, the amino acid glucose medium of Fildes, *et al.*,[9] was employed. The compounds were tested in the presence of an excess of thiamine (0.05 γ per 10 cc of medium) using an 18-hour culture of *S. aureus*.

The tabulation of the results is in Table I. All tests were repeated 2 or more times with consistent results.

The most important finding was the inability of the isomers of nicotinic acid to support the growth of *S. aureus*. The effect of substitution on the activity of the amide was marked, inasmuch as the unsubstituted amide exhibited a high degree of activity, the mono-substituted amide had a moderate effect (somewhat less than that of nicotinic acid), and the di-substituted compound manifested no activity. The contrast in the action of nicotinic acid (pyridine 3:carboxylic acid) and quinolinic acid (pyridine 2-3:dicarboxylic acid) is also of interest.

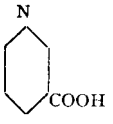
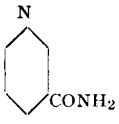
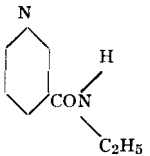
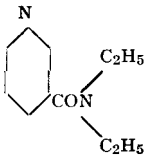
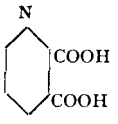
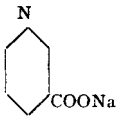
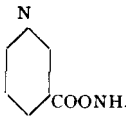
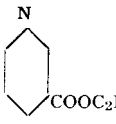
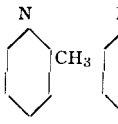
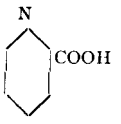
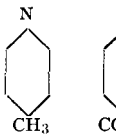
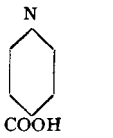
The significance of these findings with respect to tissue metabolism and animal nutrition remains to be determined.

Summary. Neither of the 2 isomers of nicotinic acid was able to replace nicotinic acid itself in the nutrition of *S. aureus*, when tested in concentrations up to 20 γ per 10 cc of medium. Reduction in the activity of nicotinic amide was brought about by introduction of an ethyl group. Complete loss of activity was evident by introduction of 2 ethyl groups into nicotinic amide. Pyridine 2-3:dicarboxylic acid was inactive as a factor in the growth of *S. aureus*.

[9] Fildes, P., Richardson, G. M., Knight, B. C. J. G., and Gladstone, G. P., *Brit. J. Exp. Path.*, 1936, **17**, 481.

TABLE I.

Effect of Nicotinic Acid and Related Compounds on Growth of *S. aureus* in synthetic medium.

Quantity of Material Tested Gamma per 10 cc of medium	 Pyridine 3-Carboxylic Acid	 Pyridine 3-Carboxylic Amide	 Pyridine 3-Carboxylic Ethyl Amide	 Pyridine 3-Carboxylic Diethyl Amide	 Pyridine 2-3-Dicarboxylic Acid	 Sodium Pyridine 3-Carboxylate	 Ammonium Pyridine 3-Carboxylate	 Ethyl Pyridine 3-Carboxylate	 2-Methyl Pyridine	 Pyridine 2-Carboxylic Acid	 4-Methyl Pyridine	 Pyridine 4-Carboxylic Acid
20	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
15	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
10	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
5	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
2.5	++++	++++	+++	—	—	++++	++++	++	—	—	—	—
1.0	+++	++++	++	—	—	+++	+++	±	—	—	—	—
.5	++	++++	+	—	—	++	++	—	—	—	—	—
.1	±	++	—	—	—	±	—	—	—	—	—	—
.05	—	+	—	—	—	—	—	—	—	—	—	—
.01	—	±	—	—	—	—	—	—	—	—	—	—

++++ = Maximum growth, other + signs proportional to amount of growth estimated by opacity. ± = Trace. — = No Growth.
Incubated for 48 hours at 37.5°C.
†0.05 gamma thiamine in each tube.