9832 P

# Nicotinic Acid as an Essential Growth-Substance for Dysentery Bacilli.*

STEWART A. KOSER, A. DORFMAN AND FELIX SAUNDERS.

*From the Department of Bacteriology and Parasitology and the Department of Biochemistry, University of Chicago.*

Recent evidence has shown that nicotinic acid is a compound of considerable biologic importance. Current interest in this compound can be attributed primarily to the work of Warburg,[1] Euler[2] and their associates, which demonstrated that the amide of nicotinic acid is a constituent of the coenzyme from horse blood and the cozymase of yeast.

To those interested in the nutritive requirements of microörganisms this work has been of considerable significance as it supplied a clue to the chemical identity of one of the essential substances, or "growth-factors," needed for development of certain of the more exacting bacteria. In studies of *Staphylococcus aureus* Knight[3] demonstrated that a combination of nicotinic acid and vitamin $B_1$ (thiamin chloride) was effective in replacing a concentrate prepared from yeast. Neither substance would suffice in the absence of the other, but when both were supplied the staphylococcus developed in a culture-medium containing only known compounds. For growth of the diphtheria bacillus Mueller[4] found that nicotinic acid could replace one of several fractions obtained from liver. A combination of nicotinic acid, *beta*-alanine and, for some strains of the organism, pimelic acid was effective in promoting growth in the absence of tissue extract preparations.[5] Nicotinic acid without *beta*-alanine was relatively ineffective.[5]

The work reported here deals with the growth-promoting effect of nicotinic acid upon dysentery bacilli. It is of interest for two reasons: first, through the use of nicotinic acid it is possible to cultivate dysentery bacilli in a solution of known chemical com-

* The present investigation was aided by a grant to the University of Chicago from the Rockefeller Foundation.

1 Warburg, O., Christian, W., and Griese, A., *Biochem. Z.*, 1935, **279**, 143; **282**, 157.

2 Euler, H. v., Albers, H., and Schlenk, F., *Z. physiol. Chem.*, 1935, **234**, I; **237**, I; Euler, H. v., and Schlenk, F., *Z. physiol. Chem.*, 1937, **246**, 64.

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

4 Mueller, J. H., *J. Bact.*, 1937, **34**, 429.

5 Mueller, J. H., and Cohen, S., *J. Bact.*, 1937, **34**, 381.

pounds and second, nicotinic acid alone is strikingly effective without the addition of any other "accessory" factor.

The essential rôle of nicotinic acid was demonstrated by the use of a synthetic culture-medium consisting of 15 amino-acids,† dextrose, and several inorganic salts.[6] In such a medium many dysentery strains failed to grow. Upon the addition of nicotinic acid,‡ however, development of the organisms took place as indicated with the several representative cultures shown in Table I. With 0.1 microgram per cc. of medium all cultures developed promptly and in several of the tests the luxuriance of growth after 24 hours at 37°C. closely approached that observed in meat infusion broth. Amounts of 0.04 microgram were sufficient to support visible growth of all cultures within 24 hours, while 0.01 microgram or in some instances 0.004 or even 0.002 microgram sufficed for slower multiplication.

Preliminary tests with several derivatives of nicotinic acid were also made in a similar manner. Nicotinic acid amide showed activity in slightly higher dilution than that of the acid, methyl nicotinate

TABLE I.

Effect of Nicotinic Acid upon Development of Dysentery Bacilli in a Synthetic Medium.

| Amount of nicotinic acid added, micrograms per cc. of medium | Flexner Development after days | | | Hiss Y Development after days | | | Strong Development after days | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 |
| none | — | — | — | — | — | — | — | — | — |
| 0.4 | +++ | +++ | +++ | ++ | ++ | ++ | +++ | +++ | +++ |
| 0.2 | +++ | +++ | +++ | ++ | ++ | ++ | +++ | +++ | +++ |
| 0.1 | +++ | +++ | +++ | ++ | ++ | ++ | +++ | +++ | +++ |
| 0.04 | + | +++ | +++ | + | ++ | ++ | +++ | +++ | +++ |
| 0.02 | — | +++ | +++ | — | + | ++ | ++ | +++ | +++ |
| 0.01 | — | ? | +++ | — | — | ++ | — | + | +++ |
| 0.004 | — | — | +* | — | — | — | — | — | —* |
| 0.002 | — | — | — | — | — | — | — | — | —* |

All tubes were inoculated lightly from suspensions of the respective organisms in a buffered inorganic salt solution, thus carrying over smaller numbers of cells than is the case when inoculations are made in the usual way directly from an agar slant.

— = no visible growth; + to +++ = light to very pronounced turbidity.

Observations were made at intervals after 4 days. In certain instances development of the cultures appeared later, or became more pronounced, upon continued incubation. These are shown by an *.

† The amino-acids were used as purchased without any additional purification.

[6] Koser, S. A., Finkle, R. D., Dorfman, A., Gordon, Mary V., and Saunders, F., *J. Inf. Dis.*, 1938, in press.

‡ Commercial samples of nicotinic acid were found to be impure and were subjected to a process of purification before use.

compared favorably with the acid while the ethyl ester was less active, requiring about 0.4 microgram per cc. of medium to exert growth-promoting effect.

Some additional observations have shown that different strains of dysentery bacilli exhibit different nutritional requirements. In a small collection of cultures an occasional strain was encountered that was able to develop in the synthetic medium without nicotinic acid. If this or a related compound is needed by these organisms they presumably must be able to synthesize it. Differences in amino-acid requirements became apparent on changing the composition of the synthetic medium. When a simpler medium containing asparagine, tryptophane, cystine, dextrose and inorganic salts was substituted for the more elaborate medium containing 15 amino-acids, some but not all of the dysentery strains refused to develop even in the presence of nicotinic acid. Evidently certain of the omitted amino-acids were needed by these cultures.

Several cultures of the Sonne type were also used. These developed to a certain extent in the synthetic medium without any added factor and produced a light but visible clouding which rarely became more pronounced with continued incubation. Upon the addition of nicotinic acid to the synthetic medium all of the Sonne dysentery cultures produced a much more luxuriant growth with pronounced turbidity within 24 hours.

In previous work[6,7] the writers found that fractions prepared from spleen, liver, yeast, and other sources contained growth-promoting substances for dysentery bacilli. The same effect has more recently been observed with certain brands of gelatin,[8] and it has also been demonstrated that the activity for dysentery bacilli can be extracted with benzene from some gelatins. Amounts of benzene extract supplying 0.1 to 0.2 micrograms of total solids per cc. of medium supported visible growth of several strains of dysentery bacilli in a synthetic medium in which the organisms were unable to develop. Nicotinic acid alone is capable of replacing gelatin and the tissue-extract preparations. Whether the activity of our tissue fractions and of certain brands of gelatin is due to nicotinic acid itself is uncertain since the presence of nicotinic acid in these preparations has not been definitely determined.

[7] Koser, S. A., and Saunders, F., *J. Inf. Dis.*, 1935, **56**, 305; 1936, **58**, 121.

[8] Koser, S. A., Chinn, B. D., and Saunders, F., in press.