certified, by the experience of many workers, to fulfill these conditions are those known variously as

(a) No. 6571 of the National Collection of Type Cultures (England).
No. NRRL B-314 (Northern Regional Research Laboratory, Peoria, Illinois, U. S. A.).
No. 9144 of the American Type Culture Collection (Washington, D. C., U. S. A.).

and (b) No. 209-P, the Food and Drug Administration (Washington, D. C., U. S. A.).
No. NRRL B-313 (Northern Regional Research Laboratory, Peoria, Illinois, U. S. A.).
No. 6538 of the American Type Culture Collection, Washington, D. C., U. S. A.).

Both of these cultures may be obtained by application to the National Collection of Type Cultures, The Lister Institute, London, or to the American Type Culture Collection, Georgetown University, Washington, D. C.

(11) That the Conference recognizes that it may eventually become necessary and practicable to establish further standards made from other varieties of Penicillin; and recommends that, with a view to such further development, efforts should be made to make pure samples of other penicillins available for international exchange among research workers in this field.

These recommendations are signed by M. V. Veldee, R. P. Herwick and R. D. Coghill, *chairman*.

SPECIAL ARTICLES

PYRIDOXAMINE AND THE SYNTHESIS OF AMINO ACIDS BY LACTOBACILLI

Lactobacillus delbrückii LD5 requires 15 amino acids, namely, leucine, isoleucine, valine, cystine, tryptophane, tyrosine, phenylalanine, glutamic acid, threonine, aspartic acid, lysine, arginine, serine, methionine and alanine, for full growth in a basal medium of essentially the same composition as that described for *Lactobacillus casei*.[1] Little or no growth occurs if any of these amino acids, with the exception of the last two, is omitted; approximately half-maximum growth is obtained in the absence of either methionine or alanine. Replacement of the pyridoxine of the medium by an equal amount (2 γ per 10 cc medium) of pyridoxamine (or pyridoxal)[2,3] eliminates the requirement of *L. delbrückii* for lysine, threonine and alanine but not for any of the remaining 12 amino acids. Good growth occurs in the absence of any one of these amino acids or of all three (Table 1). Similar results were obtained with *L. casei* and *L. arabinosus* 17–5. However, the amino acid requirements of *Streptococcus lactis* R, which includes lysine, threonine and alanine,[4] are not affected by pyridoxamine except that the latter supports a small amount of growth in the absence of alanine.

TABLE 1

INFLUENCE OF PYRIDOXINE AND PYRIDOXAMINE ON AMINO ACID REQUIREMENTS OF LACTOBACILLUS DELBRÜCKII LD5

Amino acids omitted from basal medium	*L. delbrückii* LD5		
	No pyridoxine	Pyridoxine*	Pyridoxamine
Lysine	1.0†	2.0	8.9
Threonine	0.9	1.2	7.7
Alanine	0.8	4.5	8.5
Lysine, threonine, alanine	1.0	1.4	6.0
None	1.0	8.5	8.9

* Seitz filtered.
† The figures are the cc of 0.1 N acid formed in 10 cc of medium during incubation for 72 hours at 37°; growth is proportional to acid formation.

It was possible, therefore, to determine by means of *S. lactis* R whether lysine, threonine and alanine are synthesized by the lactobacilli when grown in media containing pyridoxamine. Cells of the three lactobacilli cultivated without lysine, threonine or alanine were hydrolyzed in sealed ampules with 10 per cent. HCl for 10 hours at 15 pounds pressure. Addition of portions of the neutralized cell hydrolysates to *S. lactis* media from which either lysine, threonine or alanine was omitted, permitted maximum growth of *S. lactis* indicating that all three amino acids were present in each lactobacillus.

Pyridoxamine fulfils functions other than those concerned with the formation of the three specified amino acids, since it is necessary for development of *L. delbrückii* and *L. casei* also in media containing those amino acids (Table 1). However, in such media, pyridoxine is equally effective. There are indications that pyridoxine is involved in protein[5] and tryptophane[6] metabolism in animals and that pyridoxine and pyridoxal function in the decarboxylation of tyrosine by *Streptococcus fecalis*.[7]

L. arabinosus differs from the other two lactobacilli in that it does not require added pyridoxine for development in an otherwise complete medium. Presumably it synthesizes pyridoxine or some similarly active compound, possibly pyridoxamine.[8] The necessity of adding the latter for growth of *L. arabinosus* in media lacking lysine, threonine and alanine may be

[1] Hutchings and Peterson, *Proc. Soc. Exp. Biol. Med.*, 52: 36, 1943.
[2] Snell, *Jour. Biol. Chem.*, 154: 313, 1944.
[3] Harris, Heyl and Folkers, *ibid.*, 154: 315, 1944. We are indebted to these investigators for supplies of the pyridoxine derivatives.
[4] Snell and Guirard, *Proc. Nat'l. Acad. Sci. (U. S.)*, 29: 66, 1943.
[5] McHenry and Gavin, *Jour. Biol. Chem.*, 138: 471, 1941.
[6] Lepkovsky, Roboz and Haagen-Smit, *ibid.*, 149: 195, 1943.
[7] Gunsalus and Bellamy, *Jour. Bact.*, 47: 413, 1944; *Jour. Biol. Chem.*, 155: 357, 1944.
[8] Bohonos, Hutchings and Peterson, *Jour. Bact.*, 44: 479, 1942.

due to synthesis of inadequate amounts of pyridoxamine. This is suggested by the fact that *L. casei* and *L. delbrückii* require ten times as much pyridoxamine for growth without the above three amino acids as with them.

An explanation is now available for the disagreement between investigators as to the essentiality of lysine and threonine for growth of *L. arabinosus*.[9] During heat sterilization of the medium sufficient pyridoxamine may be formed from the interaction of the pyridoxine and amino acids[2] to permit good growth of *L. arabinosus* in the absence of lysine or threonine. Autoclaving a medium deficient in lysine for 30 minutes instead of the customary 15 or 20 minutes permitted maximum growth, whereas the same medium autoclaved without pyridoxine and to which Seitz filtered pyridoxine was added, failed to support growth unless lysine was present.

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THE EFFECT OF UREA, URETHANE AND OTHER CARBAMATES ON BACTERIAL GROWTH[1,2]

UREA has been shown to have anti-bacterial properties by Peju and Rajat[3] and Foulger and Foshay[4] and to be effective in the treatment of infected wounds and diphtheria carriers by Symmers and Kirk.[5] Holder and MacKay[6,7] and Ilefeld[8] have reported favorable response of infected wounds to treatment with mixtures of urea and sulfonamides. Tsuchiya, Tenenberg, Clark and Strakosch[9,10,11] have recently shown that urea inhibits para-aminobenzoic acid and methionine, substances which antagonize the action of the sulfonamides. These findings could not be confirmed by Kirby[12] but have been confirmed by Lee, Epstein and Foley.[13]

Urethane has received very little attention with respect to its action on bacterial growth but has been found to depress the respiratory rate of yeasts and, in low concentrations, to stimulate, and in larger amounts to depress the luminescence and respiration of the luminescent bacteria.[17, 18, 19] Johnson[14] has claimed that urethane exerts an anti-sulfonamide effect on luminous bacteria but McIlwain[15] and Martin and Fisher[16] using streptococci in *in vitro* and *in vivo* studies could not confirm this work.

The investigations reported here indicate that urea and urethane are both bacteriostatic and bactericidal for many organisms, that they antagonize slightly the sulfonamide inhibitors and that they increase the solubility and bacteriostatic activity of the sulfonamides. Urethane appears to be greatly superior to urea in all respects.

Six per cent. urea and 3 per cent. urethane were found to be bacteriostatic for *E. coli* in veal infusion broth containing 50 per cent. horse serum. *Staphylococcus aureus* required higher concentrations of either drug in the same medium to produce a similar effect; 4 per cent. urethane and 10–12 per cent. urea inhibited growth of this organism. The bacteriostatic levels of both drugs were found to be lower in synthetic media. The growth of *Pneumococcus,* hemolytic *Streptococcus, Proteus vulgaris, E. typhi, Pseudomonas pyocyaneus, S. schotmulleri* and *S. paradysenteriae* (Flexner) in serum-veal infusion medium was found to be inhibited by 2 per cent. urethane and 6 per cent. urea, these being the final concentrations of the drugs in the medium. Several strains of some of these bacteria were examined and all were found to react in the same manner.

Studies of the effect of other urea derivatives such as propyl and butyl carbamate on the same group of organisms showed that bacteriostasis is produced by lower concentrations than those necessary with either urea or urethane. One to 2 per cent. propyl carbamate and 0.5 to 0.75 per cent. butyl carbamate were found to inhibit the growth of all the organisms listed above.

In a large number of bactericidal tests in which the bacteria enumerated above were exposed to various concentrations of either urea or urethane at a temperature of 37° C. and subcultures taken at periodic intervals, it was found that killing occurred after 5 to 15 minutes of contact with 10 per cent. urethane in

[9] Hegsted, *Jour. Biol. Chem.*, 152: 193, 1944.

[1] From the Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts.

[2] Aided by a grant from the Johnson Research Foundation, New Brunswick, New Jersey.

[3] G. Peju and J. Rajat, *Compt. rend. Soc. de Biol.*, 61: 477, 1906.

[4] J. H. Foulger and L. Foshay, *Jour. Lab. and Clin. Med.*, 20: 1113, 1934–35.

[5] W. St. C. Symmers and T. S. Kirk, *Lancet*, 2: 1684, 1915.

[6] H. G. Holder and E. A. MacKay, *The Military Surgeon*, 90: 509, 1942.

[7] H. G. Holder and E. A. MacKay, *Surgery*, 13: 677, 1943.

[8] F. W. Ilefeld, *Surg., Gynec. and Obst.*, 76: 427, 1943.

[9] H. M. Tsuchiya, D. J. Tenenberg, W. G. Clark and E. A. Strakosch, *Proc. Soc. Exp. Biol. and Med.*, 50: 262, 1942.

[10] D. J. Tenenberg, H. M. Tsuchiya, W. G. Clark and E. A. Strakosch, *Proc. Soc. Exp. Biol. and Med.*, 51: 247, 1942.

[11] H. M. Tsuchiya, D. J. Tenenberg, E. A. Strakosch and W. G. Clark, *Proc. Soc. Exp. Biol. and Med.*, 51: 245.

[12] W. M. M. Kirby, *Proc. Soc. Exp. Biol. and Med.*, 53: 109, 1943.

[13] S. W. Lee, J. A. Epstein and E. J. Foley, *Proc. Soc. Exp. Biol. and Med.*, 54: 107, 243, 245, 1943.

[14] F. H. Johnson, SCIENCE, 95: 104, 1942.

[15] H. McIlwain, SCIENCE, 95: 509, 1942.

[16] G. J. Martin and C. V. Fischer, SCIENCE, 95: 603, 1942.