polymerization[6] in ether solution in a sealed quartz tube placed in front of a 100-watt quartz mercury arc, but the product darkened in the tube unless precautions were taken to shield the polymer from the ultraviolet radiation.

Oxidation-reduction polymerization[7] using the recipe: 75 cc. of deoxygenated water, 2 g. of Cetab, 10 cc. of 1.0 *N* sulfuric acid, 13 g. of vinyl bromide, 1 cc. of 0.01 *N* ferrous sulfate in *N*/10 sulfuric acid and 1 cc. of 0.01 *N* hydrogen peroxide gave no yield, possibly as a consequence of the difficulty of deoxygenating a monomer which boils at 15.8°. Some conversion was obtained with potassium persulfate as catalyst, using the procedure described by Kolthoff and Dale.[8]

The polyvinyl bromide obtained by solution polymerization was insoluble in Bu_3N (cohesive energy density 44), petroleum ether (52), carbon tetrachloride (73), nitromethane (143), ethyl alcohol (260), and methyl alcohol (346); swelled in toluene (75), benzene (81) and acetone (91); and dissolved in methyl ethyl ketone (80), dioxane (91), $C_6H_5NO_2$ (102) and C_5H_5N (105). From these data, we estimate that the cohesive energy density[9] of polyvinyl bromide is about 90, approximately the same as that of polyvinyl chloride.[10]

Addition of polyvinyl bromide to pyridine in a conductance cell at 25° produced electrolyte, as shown by a rapid increase in conductance. Reaction with triethylamine and with trimethyl amine in methanol also lead to electrolyte formation. No polyelectrolyte was produced, however; in every case, an insoluble red-to-dark-brown precipitate was obtained, and tertiary amine hydrobromide was found in the supernatant liquid. This result shows that tertiary amines dehydrohalogenate polyvinyl bromide[11] in preference to adding to it to form a polymeric quaternary salt. Two facts favor the elimination of hydrogen bromide: first, steric hindrance militates against the insertion of an R_3N group at every other carbon of the polymer chain; and, second, any single elimination of hydrobromic acid produces labile allylic bromines (—CHBr·CH=CH—) in the chain, which are more reactive than the original bromine atoms of the polymer. The tendency thus is to produce conjugated segments in the polymer chain. If the elimination of hydrogen bromide is purely statistical,[12] occasional methylene groups will be isolated, which will break the conjugation. From the deep color and the insolubility of the product, however, we conclude that the conjugated segments must be quite long, in analogy to the higher members[13] of the $R(CH{=}CH)_nR'$ series.

(6) C. S. Marvel and E. H. Riddle, THIS JOURNAL, **62**, 2666 (1940).

(7) J. H. Baxendale, M. G. Evans and J. K. Killiam, *J. Polymer Sci.*, **1**, 466 (1946); A. G. Evans and E. Tyrrall, *ibid.*, **2**, 387 (1947).

(8) I. M. Kolthoff and W. J. Dale, THIS JOURNAL, **67**, 1672 (1945).

(9) J. H. Hildebrand, "Solubility of Non-Electrolytes," A. C. S. Monograph 17, Reinhold Publishing Corp., New York, N. Y., 1936.

(10) P. M. Doty and B. H. Zimm, Bulletin of the High Polymer Clinic, Polytechnic Institute of Brookyn, N. Y., 1946.

(11) C. R. Noller and R. Dinsmore, THIS JOURNAL, **54**, 1025 (1932).

(12) R. F. Boyer, *J. Phys. Colloid Chem.*, **51**, 80 (1947).

(13) R. Kuhn and A. Winterstein, *Helv. Chim. Acta*, **11**, 87 (1928); G. Compton and W. Bergmann, *J. Org. Chem.*, **12**, 363 (1947).

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Inhibition of Growth of *Escherichia coli* by 4-Aminopteroylglutamic Acid and its Reversal

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Recent investigations[1-5] have shown that thymidine and certain other desoxyribosides can replace vitamin B_{12} as a growth factor for various lactic acid bacteria. In the present study, the relation of these desoxyribosides to the reversal of the inhibitory effect of 4-aminopteroylglutamic acid[6] (4-amino PGA), a potent antagonist of PGA, was measured. It was found that the growth of *Escherichia coli* was inhibited by high levels of 4-amino PGA in spite of the fact that the organism does not need PGA for growth. The inhibition was found to be reversed by liver extract or thymidine but not by PGA, *p*-aminobenzoic acid, vitamin B_{12}, thymine, guanine, hypoxanthine, adenosine, adenylic acid, cytidylic acid or the desoxyribosides of guanidine and hypoxanthine (Table I).

It was also found that *Lactobacillus leichmannii* 313, which needs both PGA (or *p*-aminobenzoic acid) and vitamin B_{12} for growth, was inhibited by 4-amino PGA. This inhibition was reversible by PGA at low levels of 4-amino PGA, up to 5 γ per ml., but at high levels of 4-amino PGA, 25 γ per ml., PGA was ineffective while thymidine produced a reversal. The desoxyribosides of guanine and hypoxanthine were ineffective as reversing agents, although these two compounds produced approximately maximum growth in the presence of PGA if 4-amino PGA and B_{12} were omitted. In the absence of 4-amino PGA, thymidine produced an incomplete growth response, about 50% of maximum, if PGA and *p*-aminobenzoic acid were omitted, with or without the addition of vitamin B_{12}. The response to guanine desoxyriboside or hypoxanthine desoxyriboside under these conditions was 25 to 33% of maximum.

Experimental

The liver extract was a commercial antipernicious-anemia preparation, 10 units per ml. The medium used

(1) Shive, Ravel and Eakin, THIS JOURNAL, **70**, 2614 (1948).

(2) Snell, Kitay and McNutt, *J. Biol. Chem.*, **175**, 473 (1948).

(3) Hoffmann, Stokstad, Franklin and Jukes, *ibid.*, **176**, 1467 (1948).

(4) Kitay, McNutt and Snell, *ibid.*, **177**, 993 (1949).

(5) Hoff-Jorgensen, *ibid.*, **178**, 525 (1949).

(6) Seeger, Smith and Hultquist, THIS JOURNAL, **69**, 2567 (1947).

TABLE I

EFFECT OF VARIOUS SUPPLEMENTS ON GROWTH OF *E. coli* ON COMPLETE MEDIUM PLUS 4-AMINO PGA

Addition per ml. basal medium[a]	Optical density after 16 hours at 37° Expt. 1	Expt. 2	Addition per ml. basal medium[a]	Optical density after 16 hours at 37° Expt. 1	Expt. 2
None	0.09	0.05	100 γ thymidine	..	0.32
3.0 c. mm. liver extract	.16	.20	10 γ guanine desoxyriboside	0.10	.06
10 c. mm. liver extract	.27	.28	100 γ guanine desoxyriboside	..	.09
100 c. mm. liver extract	..	.38	10 γ hypoxanthine desoxyriboside	.09	.06
3 γ thymine	.06	..	100 γ hypoxanthine desoxyriboside	..	.08
50 γ thymine	..	.08	0.01 γ vitamin B_{12}	.08	.07
0.1 γ thymidine	.14	.12	0.1 γ vitamin B_{12}	..	.10
1.0 γ thymidine	.24	.18	0.4 mg. pteroylglutamic acid	..	.08
10 γ thymidine	..	.26	0.5 mg. *p*-aminobenzoic acid	..	.06

[a] Landy–Dicken[b] medium with dextrose in place of sucrose *plus* 0.15 mg. (Expt. 1) or 0.25 mg. (Expt. 2) 4-amino PGA per ml. of culture medium. Final volume 2 ml. per tube. Inoculum: 24 hour culture on broth,[c] washed once and diluted 1:10. Reading with 4-amino PGA omitted, about 0.4. [b] Landy and Dicken, *J. Lab. Clin. Med.*, **27**, 1086 (1942). [c] Teply and Elvehjem, *J. Biol. Chem.*, **157**, 303 (1945).

with *Lactobacillus leichmannii* was similar to that of Snell and co-workers[2] but with thioglycolic acid, 0.2 mg. per ml.[7] When 25 γ of 4-amino PGA per ml. and 0.2 γ of PGA were added, the following are typical of optical densities obtained at 18 hours: no supplement, 0.02; 200 γ PGA, 0.04; 0.01 γ B_{12}, 0.05; 2 γ thymidine, 0.58; 2 γ guanine desoxyriboside, 0.08; 2 γ hypoxanthine desoxyriboside, 0.04; 100 γ thymine, 0.10; 100 γ thymine *plus* 2 mγ vitamin B_{12}, 0.29. The addition of 2 mγ of vitamin B_{12} did not augment the growth obtained with the desoxyribosides. When 4-amino PGA, PGA and *p*-aminobenzoic acid were omitted from the basal medium the following optical densities were obtained: no supplement, 0.03; 2 mγ B_{12}, 0.27; 2 γ guanine desoxyriboside, 0.37; 2 γ hypoxanthine desoxyriboside, 0.37; 2 γ thymidine, 0.52; 2 mγ B_{12} *plus* 0.2 γ PGA, 1.5; 2 γ guanine desoxyriboside *plus* 0.2 γ PGA, 1.1; 2 γ hypoxanthine desoxyriboside *plus* 0.2 γ PGA, 1.1; 2 γ thymidine *plus* 0.2 γ PGA, 1.2.

Discussion.—The inhibitory effects of low levels of 4-amino PGA for certain organisms are reversible by PGA but high levels of the antagonist produce toxic effects which are not so reverse.[8,9,10] The present investigation shows that these toxic effects may in certain instances be reversed by thymidine, which may indicate that PGA has a role in the formation of thymidine. The inhibitory effect of 4-amino PGA on the growth of *E. coli* conceivably may be due to the entrance of this substance into the cell to displace endogenously-formed PGA from this role. The inhibitory effect of "x-methyl-PGA" upon *Leuconostoc mesenteroides* 8293 was found by Shive and co-workers[11] to be reversed by either PGA or thymidine. After the present experiments were completed, Sauberlich[12] reported inhibition of the growth of *Leuconostoc citrovorum* 8081 by 4-amino PGA and reversal by thymidine. We are indebted to Dr. J. O. Lampen for thymidine, to Dr. H. M. Kalckar for guanine desoxyriboside and to Dr. E. E. Snell for hypoxanthine desoxyriboside.

(7) Stokstad, Dornbush, Franklin, Hoffmann, Hutchings and Jukes, *Fed. Proc.*, **8**, 257 (1949).

(8) Franklin, Stokstad and Jukes, *Proc. Soc. Exp. Biol. Med.*, **67**, 398 (1948).

(9) Oleson, Hutchings and SubbaRow, *J. Biol. Chem.*, **175**, 359 (1948).

(10) Philips and Thiersch, *J. Pharmacol.*, **95**, 303 (1949).

(11) Shive, Eakin, Harding, Ravel and Sutherland, THIS JOURNAL, **70**, 2299 (1948).

(12) Sauberlich, *Fed. Proc.*, **8**, 247 (1949).

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The Reduction of 2-Acetylpyridine to 2-Ethylpyridine

BY ARTHUR FURST

Recently Gregg and Craig[1] reported that the melting point of the picrate of 2-ethylpyridine made by two different methods—hydrogenation of 2-vinylpyridine and the action of methyl iodide on lithium picoline—did not agree with that obtained by Bergstrom and McAllister[2] who made it by the addition of ethylmagnesium bromide to pyridine. In confirmation of the work of the former authors 2-acetylpyridine was reduced to 2-ethylpyridine by three different methods, namely, Clemmensen, Wolff–Kishner, and Huang-Minlon. The results of the boiling points, melting points, mixed melting points, and fusion analysis[3] show that these reduction products agree in all respects with each other and with the compound obtained by Gregg and Craig by hydrogenation of 2-vinylpyridine.

Experimental

The 2-acetylpyridine, from Dougherty Chemical Co., was redistilled: picrate m. p. 131°; lit. 131°.[4]

Clemmensen Reduction.—A solution of 2-acetylpyridine (0.12 mole) in 70 ml. of concentrated hydrochloric acid was vigorously refluxed in contact with amalgamated zinc (0.77 mole).[5] After ten hours 50 ml. more acid was added, and refluxing was continued an additional five hours. The solution was made basic with solid hydroxide, filtered through a stainless steel funnel, extracted with benzene, dried and fractionated. The colorless distillate that came over at 144° was caught in a 50% alcoholic solution

(1) E. C. Gregg and D. Craig, THIS JOURNAL, **70**, 3138 (1948).

(2) F. W. Bergstrom and S. H. McAllister, *ibid.*, **52**, 2845 (1930).

(3) N. Goetz-Luthy, *J. Chem. Education*, **26**, 159 (1949).

(4) H. Maier-Bode and J. Altpeter, "Das Pyridin und seine Derivative," Edwards Brothers, Inc., Ann Arbor, Mich., 1934, p. 197.

(5) E. L. Martin in "Organic Reactions," Vol. I, R. Adams, Ed. John Wiley and Sons, Inc., New York, N. Y., 1942, p. 163.