Growth Inhibition of a Biotinless Mutant of *Escherichia coli* by Purines and Pyrimidines.* (20795)

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It is well known that the biotin requirement of certain microorganisms can partially be replaced by aspartic acid(1), oleic acid(2), or a combination of these two substances (3,4). For *Lactobacillus arabinosus*, oxalacetate effectively replaces aspartate but other members of the Krebs cycle do not(5). For the "biotinless" mutant, *Escherichia coli* 58, which is an aerobic organism, a variety of four carbon dicarboxylic acids are effective in partially replacing the requirement for biotin(6). These observations led to the conclusion that biotin is involved in the synthesis of 4 carbon dicarboxylic acids from pyruvate and carbon dioxide(5,7,8). It has been postulated that biotin also plays a role in purine synthesis since biotin-deficient rats fix less $C^{14}O_2$ into their tissue guanine than do normal rats(9). When experiments were conducted to determine the biotin-sparing action of purines and pyrimidines for *E. coli* 58 it was found that these substances were inhibitory. In the presence of biotin, however, the purines and pyrimidines did not inhibit growth of the mutant and they were likewise innocuous toward the parent wild type *E. coli*, K 12.

Experimental. Escherichia coli 58,† (Gray and Tatum(10)) was carried on nutrient agar slants to which biotin was added (0.3 mγ per 10 ml slant). The slants were incubated at 37°C for 20-24 hours. The basal experimental medium(11) contained K_2HPO_4 7 g, KH_2PO_4 2 g, $(NH_4)_2SO_4$ 1 g, Na citrate 0.5 g, and $MgSO_4$ 0.1 g per liter. Growth experiments were conducted in Evelyn tubes. One ml of sterile 1% glucose was added to each tube containing 9 ml of the autoclaved basal medium. All additions were made at the expense of the basal medium. The tubes were inoculated with two drops of a cell suspension (adjusted to give a galvanometer reading of 91-93 with the Evelyn colorimeter, filter 620), made from 20-24-hour slants. Incubation was at 37°C without shaking. The *amount of growth* was measured in the Evelyn colorimeter using a 620 filter and setting the uninoculated tube at 100. Readings were taken after 20-24 hours incubation except in the growth curve experiments where readings were made at several intervals from 10-144 hours. The results are expressed as per cent of maximum growth calculated from density values. The positive control containing the basal medium and biotin was taken as 100%. The results reported represent averages of duplicate tubes. All experiments were repeatedly confirmed. The average per cent transmission value of the negative control (basal medium) was 95, of the positive control (basal medium + biotin) was 70, and that of the basal medium + aspartic acid was 82. The purine and pyrimidine compounds were obtained from the following companies: adenylic acid a and b, adenosine, adenine, thymine, guanine, and xanthine from Schwarz Laboratories; adenosine-5-phosphate, Bischoff; cytosine, Dougherty Chemicals; uracil, Eastman Kodak Co.; hypoxanthine, Nutritional Biochemicals Co. The free bases and nucleosides were autoclaved with the medium; nucleotides were sterilized by filtration and added to the tubes just before inoculation.

Results. The biotin requirement of *E. coli* 58 can be partially replaced by aspartic acid, the 4-carbon dicarboxylic acids, α-ketoglutarate or citrate(6). When attempts were made to improve growth of this organism in the absence of biotin by further supplementing the medium with other metabolites, it was found that purines and pyrimidines were inhibitory in biotin-free media but not in the presence of biotin.

As shown in Fig. 1, very low concentrations

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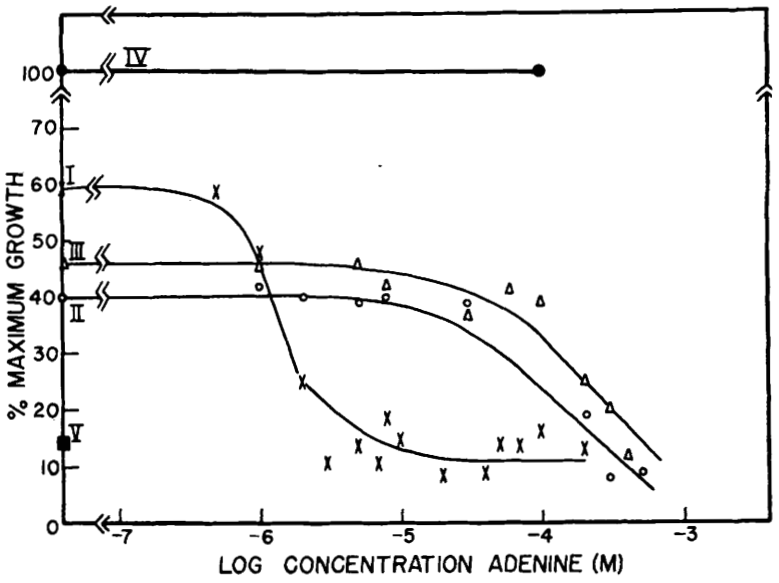

FIG. 1. Effect of adenine on the growth of *E. coli* 58.

The tubes contained:

I. Basal medium + 1 mg/ml DL-aspartic acid.
II. + " " succinic acid.
III. + " " α-ketoglutaric acid.
IV. + 0.2 mγ/ml biotin.
V. Basal medium.

of adenine (10^{-5} M), added to media containing aspartate, depressed growth to the level achieved on the basal medium. Some variation in the amount of inhibition at higher levels of adenine was observed. When growth was supported by supplementing the basal medium with succinate or α-ketoglutarate, the biotinless mutant was less sensitive to adenine although higher concentrations of the latter were inhibitory. Adenine did not inhibit growth when biotin was supplied (IV, Fig. 1). In other experiments, adenosine, adenosine-3′-phosphate (yeast adenylic acid isomer b), adenosine-5′-phosphate and adenosinetriphosphate inhibited growth of the biotinless mutant to about the same extent as an equimolar quantity of adenine. Nearly complete inhibition was obtained with 6×10^{-4} M concentrations of the nucleotides. With the exception of adenosinetriphosphate, these compounds were more strongly inhibitory in aspartate-containing media than in media containing succinate, malate or α-ketoglutarate. Adenosinetriphosphate inhibited growth even when succinate or α-ketoglutarate were added to replace, partially, the biotin requirement. Biotin overcame the inhibitory effect of adenosine and its derivatives for *E. coli* 58. These compounds did not depress the growth of *E. coli* K 12. Adenosine-2′-phosphate (yeast adenylic acid isomer a) did not inhibit growth of the biotinless mutant, but did not reverse the inhibition by adenine even when added at concentrations 10 times higher than adenine.

The effect of hypoxanthine is shown in Fig. 2. Inhibition was pronounced in media in which aspartate partially replaced the biotin requirement. When this partial requirement was met by succinate (II, Fig. 2) or α-ketoglutarate (III, Fig. 2), inhibition by hypoxanthine was negligible.

Fig. 3 presents the effect of uracil, cytosine, thymine, and xanthine on the growth of *E. coli* 58. A concentration of 10^{-6} to 10^{-5} M of either uracil or cytosine brought growth down to the level attained on the unsupplemented basal medium. Higher amounts of thymine (10^{-4} M) had to be added to cause significant inhibition and the concentration of

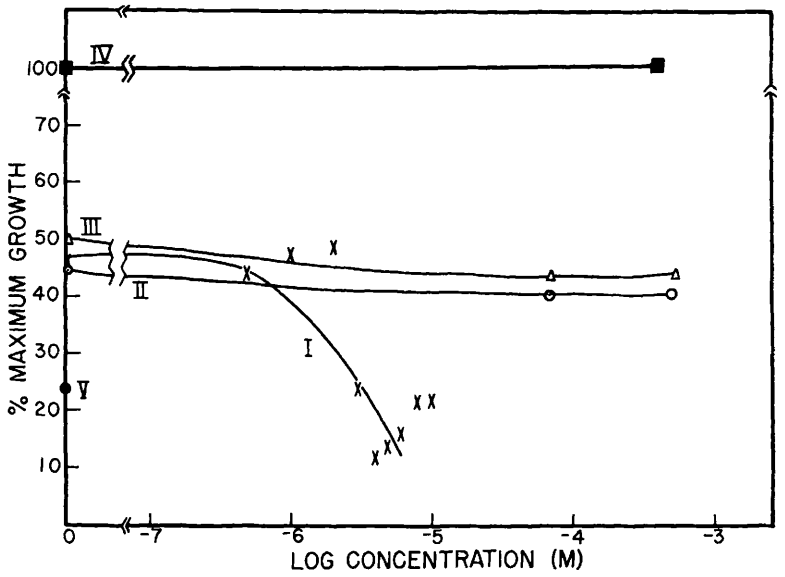

FIG. 2. Effect of hypoxanthine on the growth of *E. coli* 58.

The tubes contained:

I. Basal medium + 1 mg/ml DL-aspartic acid + hypoxanthine.
II. + 1 " succinic acid + hypoxanthine.
III. + 1 " α-ketoglutaric acid + hypoxanthine.
IV. + 0.2 mγ/ml biotin.
V. Basal medium.

xanthine had to be raised to 10^{-3} M before a similar effect was obtained. The data presented in Fig. 3 are from experiments in which the biotin requirements of the organism were partially replaced by aspartic acid. In two experiments in which the biotin requirements were partially replaced by succinic or α-ketoglutaric acid, uracil, cytosine, thymine, and xanthine at concentrations of 10^{-6} to 10^{-4} M depressed growth only slightly.

Additions of guanine failed to inhibit growth of *E. coli* 58 in the various media; however, owing to the great insolubility of guanine at a neutral pH, this substance could not be compared on an equimolar basis with the other inhibitors. Combinations of guanine and various pyrimidines did not reverse the inhibiting effect of adenine. None of the purine or pyrimidine bases inhibited growth of the wild type *E. coli*, K 12, when grown on either the basal medium or with supplements of aspartate or succinate.

Rates of growth of *E. coli* 58 up to 144 hours incubation are shown in Fig. 4. Both the rate of growth and the total growth finally achieved in media containing aspartate were depressed by adenine. The addition of biotin reversed both effects.

It has been consistently observed that in the basal medium and in the basal + aspartic acid, a secondary rapid growth phase takes place after the stationary phase has been reached. The suggestion that the mutant is able to synthesize some biotin after prolonged incubation was confirmed by assay((6) *Lactobacillus arabinosus*) of the cells grown in biotin-free media. Subcultures from these tubes, however, retain their biotin requirement.

Discussion. There are several plausible explanations of the inhibitory effect of purines and pyrimidines on the biotinless mutant of *E. coli*, but no logical explanation can be offered for the greater inhibition by these compounds in the presence of aspartate as compared with succinate or α-ketoglutarate. It is possible that the purines and pyrimidines inhibit by competitive interference with normal intermediary metabolites, for example, in the elaboration of the nucleotide units(12,13)

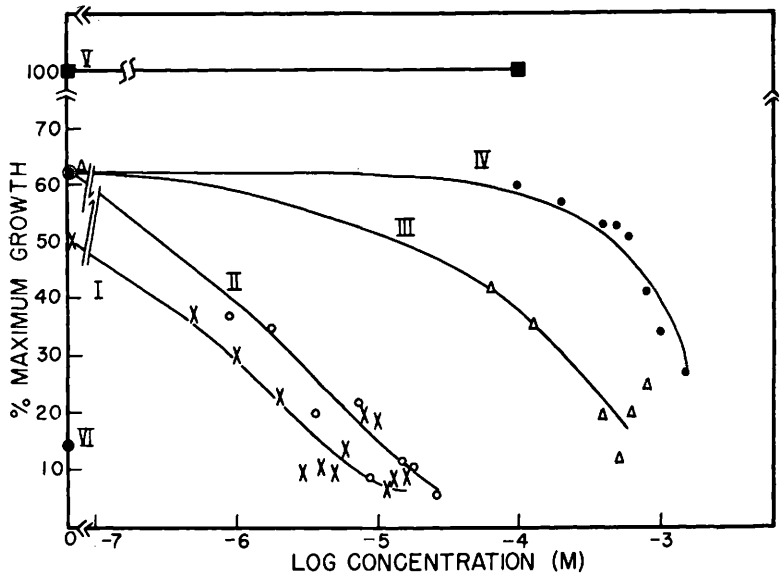

FIG. 3. Effect of uracil, cytosine, thymine and xanthine on growth of *E. coli* 58.
The tubes contained:

I. Basal medium + 1 mg/ml DL-aspartic acid + uracil.
II. + cytosine.
III. + thymine.
IV. + xanthine.
V. + 0.2 mγ/ml biotin
(plus any of the above purines or pyrimidines).
VI. Basal medium.

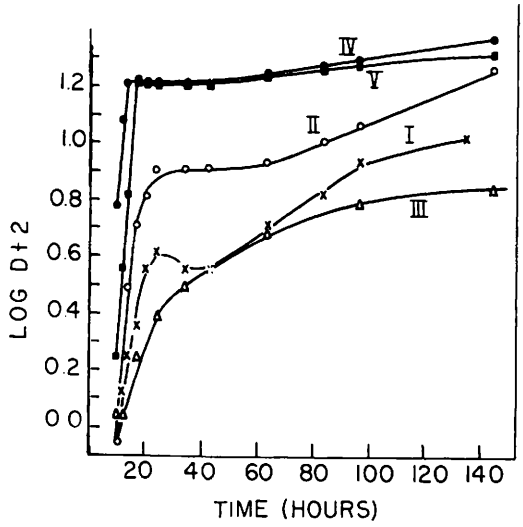

FIG. 4.
Inhibition of growth of *E. coli* 58 by adenine.
The tubes contained:

I. Basal medium.
II. Basal medium + 1 mg/ml DL-aspartic acid.
III. Basal medium + 1 mg/ml DL-aspartic acid + 10^{-4} M adenine.
IV. Basal medium + 1 mg/ml DL-aspartic acid + 10^{-4} adenine + 0.2 mγ/ml biotin.
V. Basal medium + 0.2 mγ/ml biotin.

which participate in nucleic acid synthesis. Pierce and Loring(14) have reported that adenosine inhibits growth of a pyrimidine-deficient mutant of *Neurospora* and that uridine and cytidine reversed the inhibitory effect. In the present work no such reversal could be obtained.

An inhibitory effect of adenine, guanine, and uracil on the growth of *Streptococcus faecalis* in the absence of serine was reported by Holland and Meinke(15). The inhibition could also be reversed by adequate levels of folic acid and in this respect is somewhat analogous to the one reported here.

Summary. The growth of the biotinless mutant, *Escherichia coli* 58, in a medium containing aspartic acid to replace partially the biotin requirement, was inhibited by adenine, adenosine, adenosine-3′- or 5′-phosphate, ATP, uracil, cytosine, hypoxanthine, and, to a lesser extent, by thymine and xanthine. Adenosine-2′-phosphate was not inhibitory. None of these compounds was inhibitory when biotin was added to the growth medium of strain 58,

nor were they inhibitory to the parent strain *E. coli* K 12 which does not require biotin. When succinate or α-ketoglutarate, instead of aspartate, were added to the basal medium, adenine and its derivatives were less inhibitory and the other purines and pyrimidines did not significantly inhibit the growth of the biotinless mutant.

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Effects of Growth Hormone upon Erythropoiesis in the Hypophysectomized Rat.* (20796)

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Chronic treatment with the growth principle fails to prevent(1-5) or overcome(6) the anemia characteristic of the hypophysectomized rat. Despite this lack of influence upon the peripheral blood values, two of the above groups of investigators(1,6) have found that somatotrophin (STH) induces considerable repair of the hypoplastic marrow of the hypophysectomized rat. The present research was undertaken to determine, in more detail, the influence of STH upon erythropoiesis and to uncover the cause for the discrepancy observed between the behavior of the peripheral blood and bone marrow elements.

Materials and methods. Twelve male rats of a modified Long-Evans strain, hypophysectomized 2 months previously and weighing 150-200 g at the beginning of the experiment, were employed. They were fed a complete laboratory ration and kept at temperatures of 70-80°F. Six of the rats received daily subcutaneous injections, for 7 days, of one mg of the Raben-Westermeyer(7) STH preparation‡ dissolved in 0.2 cc water acidified with 0.1 N HCl to pH 3.0. The 6 control hypophysectomized animals were injected with 0.2 cc acid water for the same period of time. Hematologic analyses were conducted shortly before and at the termination of the 7-day period. Reticulocyte counts were made from wet mounts stained with brilliant cresyl blue. One thousand cells were counted for each determination. Quadruplicate hematocrit val-

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