when brain hormone was added to concentrations of ecdysone which would not induce pupation alone. (Pupation was not induced by brain hormone alone.) Brain hormone therefore may have a direct action on the tissues when acting synergistically with ecdysone in addition to its tropic action on the prothoracic gland.

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Inhibition of Growth of Streptococci by Adenine.* (26590)

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Bernheimer *et al.*(1) described a synthetic medium for cultivation of *Streptococcus pyogenes,* specifically strain C203S. For several years we have used this medium with success for cultivation of strains C203 and C203S. Recently, however, we experienced difficulty in growing 2 other strains of *S. pyogenes* (S23 and C203U) in the same medium and had occasion to delete adenine and uracil from the medium. The 2 strains which had earlier failed to grow now showed growth comparable to that of strain C203S. On further testing it appeared that the inhibition of strains S23 and C203U was due to adenine. The inhibition could be reversed in the normal growth period by addition of hypoxanthine, xanthine or guanine or their corresponding nucleotides. When adenine was present with uracil in the medium growth eventually occurred but the lag phase was prolonged to several hours. A somewhat similar phenomenon was observed by Pierce and Loring(2) using a pyrimidine deficient mutant of *Neurospora crassa.* The growth of this organism could be inhibited by adenine and yeast adenylic acid. In this case however the reversal of inhibition was brought about by the pyrimidines cytidine or uridine. They further found that guanine caused a similar though less marked inhibition of growth of the mold.

Strain C203U, which is inhibited by adenine, is a mutant of strain C203S which is not. The only reported difference between the 2 strains is that C203U fails to produce streptolysin-S (Bernheimer)(3).

Methods. The organisms used were strains C203S, C203U, S94, and S23 of *S. pyogenes.* These organisms were kindly supplied by Dr. Cornelia A. Eddy, Dept. of Microbiology, Tulane Univ. School of Medicine. The bacterial stock cultures were carried on Pia egg slants at 4°C, transferred monthly. All inocula were prepared in heart infusion broth incubated at 37°C. Incubation time varied

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TABLE I. Effect of Adenine Concentration on Growth of *S. pyogenes* (C203U).

Adenine conc., μg/ml	O.D. of culture (20 hr)
0 (control)	.54
.1	.62
.5	.51
1	.46
5	.09
10	.10
50	.11

from 8 to 10 hours. The inoculum after incubation was centrifuged, washed twice with physiological saline and diluted with saline to an optical density of approximately 0.4, on a B and L "Spectronic 20" photometer. One drop of this suspension was used as inoculum for 10 ml of the test medium. The synthetic medium was prepared according to the procedure of Bernheimer *et al.*(1) using Bacto-Casamino acids as a source of amino acids. Adenine was deleted from the medium. The original medium was supplemented with inositol, 14 mg per liter and PABA, 10 mg per liter.

Additions to the medium in these experiments were sterilized separately. Ten milliliters of medium were used in each assay tube and each assay was carried out in triplicate. The results presented represent the average growth, in terms of optical density, of the triplicate analysis. In any case where the tubes showed deviation greater than 10% from the average the determination was repeated.

Results. To determine more quantitatively the amount of adenine causing the inhibition, various concentrations of adenine were added to the basal medium with the results noted in Table I.

TABLE II. Reversal of Adenine Inhibition by Yeast Nucleic Acid.

Yeast nucleic acid, μg/ml	O.D. of culture (20 hr)
No adenine, no Y.N.A.	.56
5	.10
10	.11
50	.22
100	.31

All tubes but controls contained 10 μg adenine/ml.

Based upon the earlier experiments of Pierce and Loring(2) we assumed that perhaps an analog inhibition type of phenomenon was occurring. A hydrolysate of yeast nucleic acid was therefore tested. The results are given in Table II. This shows a partial effect of the whole nucleic acid.

By paper electrophoresis of the above hydrolsate (0.01 M PO_4-buffer pH 7.0, for 3 hr at 400 Volts in a Spinco Model R electrophoresis cell) and elution of the ultraviolet quenching areas of the strip it was found that the active materials migrated similarly to known samples of hypoxanthine and xanthine. These materials were therefore tested along with guanine and uric acid (Table III). The inability of uric acid to reverse the inhibition is perhaps understandable since it is an end product of purine metabolism and is not converted metabolically to the purine bases.

TABLE III. Reversal of Adenine Inhibition by Other Purines.

Addition compound		O.D. of culture (20 hr)
No addition		.62
Adenine only		.09
Xanthine	(25 μg/ml)	.70
Hypoxanthine	(" ")	.68
Guanine	(" ")	.58
Uric acid	(" ")	.10

All tubes but controls contained 10 μg adenine/ml.

TABLE IV. Reversal of Adenine Inhibition by Xanthine.

Xanthine, μg/ml	O.D. of culture (20 hr)
No adenine, no xanthine	.77
0	.08
1	.09
2	.27
3.1	.66
6.2	.69
12.5	.77
25	.78
50	.77

All tubes but controls contained 10 μg adenine/ml.
Inhibition ratio approx. 3:1.

The inhibition to antagonist ratio is shown for adenine and xanthine in Table IV. Experiments with the other 2 purines gave essentially the same results. These values are

TABLE V. Effect of Adenine on Growth of 4 Strains of *S. pyogenes*.

Strains	O.D. of culture (20 hr)	Strains	O.D. of culture (20 hr)
C203U (A)	.66	S94 (A)	.82
" (B)	.07	" (B)	.88
" (C)	.54	" (C)	.68
C203S (A)	.97	S23 (A)	.61
" (B)	.92	" (B)	.08
" (C)	.96	" (C)	.45

(A)—Basal without adenine. (B)—Basal + adenine (5 μg/ml). (C)—Basal + adenine (5 μg/ml) + xanthine (2.5 μg/ml).

rather unusual in that the inhibition can be overcome by approximately one-third the molar concentration of antagonist.

To determine whether the growth inhibition by adenine was a more general phenomenon, not limited solely to strain S23, we tested 3 other strains, C203U, C203S, and S94, with the results shown in Table V. It is apparent that 2 of the 4 strains are inhibited by adenine and the inhibition, in both cases, is overcome by xanthine.

Using the same assay procedures we have further demonstrated that adenylic acid in approximately the same concentration as adenine causes inhibition of growth. The inhibition is also reversed by the nucleotides of the 3 purines previously shown to be antagonists to adenine. Whether the cells are able to cleave the nucleotides to the free bases, and whether the free bases are the specific functioning moieties as inhibitor and antagonist, we do not know.

Discussion. There have been few reports in the literature of inhibition of microbial growth by adenine. In none of these has the inhibition been reversed by other purines, rather the general effect described has been that reported by Pierce and Loring(2) that all the purines have a similar inhibitory effect. Recently Levin and Magasanik(4) have demonstrated the repression, by adenine, of inosinicase synthesis in a non-purine requiring strains of *Salmonella typhimurium* and *Aerobacter aerogenes*. On the basis of this observation, the following hypothesis is offered as a possible explanation of our results. Adenine may suppress the formation or inhibit the activity of an enzyme in the common pathway of purine synthesis in a manner comparable to the reported effect of adenine on inosinicase. Addition of other purines which are metabolized and inter-converted beyond the block may overcome this effect by permitting a continuation of nucleic acid synthesis, thus removing the adenine and consequently the inhibitor. We have no experimental evidence for this but since our strain of *S. pyogenes* does not require purines for growth it presumably has the necessary enzymes for the several known interconversions of the various purines and their corresponding nucleotides. Obviously more work is needed before a more definite statement can be made regarding the specific site of inhibition of growth of these cells by adenine.

Summary. Adenine, at a concentration of 5 μg/ml or above in a synthetic medium, has been shown to inhibit growth of 2 strains of *S. pyogenes*, S23 and C203U. The inhibition can be overcome by addition of xanthine, guanine or hypoxanthine at approximately one-third the molar concentration of the inhibitor.

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