experiments, CTC represents a maximum of 93%, 70% and 80% of the total terminal tetracyclines in the cases of BC-41, S-580, and S-1055, respectively. No appreciable quantities of chlorinated organic materials other than CTC were detected in fermented mashes to which excess chloride[36] originally had been added.

Mutants S-580 and S-1055 were isolated from cultures of BC-41 and possess, respectively, 65–70% and 80–85% of the total tetracyclines potential of BC-41 under the uniform fermentation conditions used for this study. All are descendents through a series of mutation treatments of the original *S. aureofaciens* A-377 isolate of Duggar. *S. aureofaciens* A-377 and most of its progeny studied are members of Class I. Both Class I and Class II contain mutants differing substantially in their morphology and physiology.

Biosynthetic TC has been isolated and found identical in its properties with TC produced by the catalytic hydrogenation of CTC.[4] BTC produced by a BC-41 fermentation has been separated by partition chromatography (butanol-chloroform moving over stationary Celite-aqueous HCl) from the TC concurrently produced and has been isolated as the crystalline hydrochloride. Small, variable quantities of CTC were present in the BTC preparations, arising from the efficient utilization by BC-41 of traces of chloride for CTC even in the presence of large excesses of bromide. A variety of counter-current extraction, partition chromatographic, and paper strip chromatographic systems useful in separating CTC, TC, oxytetracycline[5] and their quatrimycin isomers[2] failed to separate mixtures of varying ratios of CTC and BTC. The properties of BTC·HCl, corrected for

TABLE I

ULTRAVIOLET SPECTRA IN 0.1 *N* H_2SO_4

BTC·HCl		CTC·HCl	
λ max.	ε max. × 10^{-3}	λ max.	ε max. × 10^{-3}
368 mμ	9.15	368 mμ	10.7
260	18.9	265	18.3
228	17.2	228	17.6

TABLE II

HALF LIVES IN ACID AND ALKALI

Conditions	BTC	CTC	TC
*p*H 10.0 carbonate buffer, 22°	10.9 min.	18.6	>600
0.2 *N* H_2SO_4 (aq.), 100°	18.8	8.2	<2

the CTC·HCl present, are: $[\alpha]D$ (0.5% in 0.03 *N* aq. HCl): BTC·HCl, −205°; CTC·HCl, −235°. Solubility in water (25°): BTC·HCl, 1.36%; CTC·HCl, 1.40%. Solubility in dry *n*-butanol (25°): CTC·HCl, 0.013%; BTC·HCl, 0.038%. M.p.: BTC·HCl, browns at 218°, dec. at 235°; CTC·HCl, dec. at 210°: Response on a weight basis of BTC·HCl in terms of CTC·HCl to analytical procedures: turbidimetric (*S. aureus*), 95%; turbidimetric (*E. coli*), 90%; fluorometric,[6] 26.0%; Hiscox,[7] 45.0%; spectrophotometric (368 mμ, 0.1 *N* H_2SO_4), 77.0%. Catalytic hydrogenation (palladium on carbon, 1.1 atmosphere of hydrogen) of BTC has yielded TC.

A BC-41 fermentation containing no chloride except chloride[36] in concentration less than 93% that stoichiometrically equivalent to the total tetracyclines potential of the system has given an essentially quantitative conversion of chloride[36] to 7-Cl^{36}-TC; partition chromatographic separation of this material from the TC also produced and crystallization has given an over-all yield of 53% from chloride[36] to pure, crystalline 7-Cl^{36}-TC·HCl possessing essentially the same molar radioactivity as the HCl^{36} available from the Atomic Energy Commission.

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PREPARATION OF BORON MONOXIDE AT HIGH TEMPERATURES

Sir:

In a series of attempts to produce elemental boron of very high purity for use in a study of certain borides,[1] considerable difficulty was experienced in removing the last traces of impurity. This was found to be true particularly in the case of boron produced by the direct reduction of boron trioxide. It has been suggested that this residual impurity may be oxygen combined with the boron as a suboxide.[2,3,4] Since the literature concerning the nature of the suboxide is limited, a study was made of its preparation and properties.

Boron monoxide has been prepared from $H_4B_2O_4$ by dehydration[5,6,7] and at high temperatures by Zintl[8] who prepared it by heating a mixture of boron and ZrO_2 at 1800° in vacuum. But he and others[9] have stated that it cannot be produced by heating a mixture of boron and B_2O_3 due to the higher vapor pressure of B_2O_3 compared to that of the monoxide. We have found that boron monoxide of high purity can be obtained by this method at temperatures as low as 1050°.

Our procedure was to heat the B–B_2O_3 mixtures in a tantalum crucible, covered with a perforated tantalum lid, and suspended in an all-glass vacuum system. The heating chamber was an air cooled Pyrex cylinder provided with a reëntrant water cooled cold finger which served as a condenser. Heat was generated in the tantalum crucible by means of a high frequency induction unit.

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The B_2O_3 and B were mixed in various ratios, ball milled and pressed into pellets. The pellets were thoroughly baked out in the system by preheating at 900° until a pressure of less than 10^{-3} mm. was obtained. When the temperature was increased to above 1050° and the pressure had dropped to 10^{-4} mm. or less, an amber glassy deposit began to form on the wall of the glass heating chamber and on the metal cold finger above the heated crucible.

The most favorable ratio of boron to oxygen in the mixture was found to lie between 3:1 and 4:1. In this range a yield of 65% (based on available oxygen) was obtained in two hours at 1350° with an average yield of 0.5 g. per run. The purity of the product, expressed as the ratio B:O, fell within the limits of 1:1.00 and 1:1.014. The material which condensed on the cold finger was found to be pyrophoric in a few instances in more than 100 production runs and ignited spontaneously in the course of its removal.

Boron monoxide obtained by this method is a polymorphic glass which gave an X-ray diffraction pattern showing only amorphous rings. Attempts to develop a crystalline pattern by heating the polymer at temperatures below 400° were unsuccessful. At temperatures above 450° its color changed as it disproportionated to boric oxide and a black amorphous insoluble non-reducing solid which was probably boron. The rate of disproportionation increased with temperature. The density of the polymer, determined by the flotation method, is 1.765 ± 0.001 g./cm.^{3}. No solvent has been found for the polymer. It reacts vigorously with water and alcohols with the evolution of hydrogen which contains traces of boranes. The rate of reaction with alcohols decreases with increasing molecular weight of the alcohol. The resulting solutions in water and alcohol have reducing character which decreases with aging. The rate of decrease in reducing ability is a function of *p*H and is affected by the presence of dissolved oxygen in the solutions.

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INTERCONVERSION OF PURINES IN THE BIOSYNTHESIS OF NUCLEIC ACID ADENINE AND GUANINE AND OF HISTIDINE[1]

Sir:

Cell free extracts of *Aerobacter aerogenes* and of *Escherichia coli* were found to convert IMP[2] to GMP by the following reactions:

$$\text{IMP} + \text{DPN}^+ \longrightarrow \text{XMP} + \text{DPNH} + \text{H}^+ \quad (1)^3$$

$$\text{XMP} + \text{ATP} + \text{NH}_4 \longrightarrow \text{GMP} + \text{AMP} + \text{PP} \quad (2)$$

The enzymes catalyzing these reactions have been purified 30 and 50 fold, respectively. The crude extracts are also capable of converting IMP to an adenine derivative. Extracts of *A. aerogenes* strain P-14, a guanine requiring mutant which excretes xanthosine,[4] are unable to aminate XMP (reaction 2). Consequently, it appears that reactions 1 and 2 are essential steps in the biosynthesis of nucleic acid guanine. They are also essential in the conversion of exogenous adenine to nucleic acid guanine, since mutant P-14 incorporates adenine into xanthosine, but not into nucleic acid guanine.[5]

The following observations indicate that the conversion of exogenous guanine to nucleic acid adenine does not proceed through a reversal of these steps: (a) Reactions (1) and (2) are not reversible; (b) xanthine, which is converted to both nucleic acid adenine and guanine in mutant PD-1 blocked at an early stage of purine biosynthesis[6] (Table I, Exp. 2), cannot be converted to either of the nucleic acid purines in P-14, but is excreted exclusively as xanthosine (Table I, Exp. 8); (c) exogenous guanine is a precursor of nucleic acid adenine in P-14[5] (Table I, Exp. 7).

TABLE I

INCORPORATION OF VARIOUS PRECURSORS INTO NUCLEIC ACID PURINES AND HISTIDINE

Exp.	Strain	Supplement added	—Relative specific activity— Guanine	Adenine	Histidine
1[a]	PD-1[b]	Guanine-8-C^{14}	100	100	
2	PD-1	Xanthine-8-C^{14}[c]	103	103	0
3	PD-1	Guanine-2-C^{14}[c]	100	40	38
4	PD-1	$C^{14}O_2$[d]	0.3	0.4	2.5
5	PD-1	$HC^{14}OOH$[d]	0.1	0.1	1.7
6	PD-1	Glycine-2-C^{14}[d]	0.7	7.4	6.4
7[a]	P-14[e]	Guanine-8-C^{14}	97	38	
8	P-14	Xanthine-8-C^{14}[c]	0	0	
9	P-14	Guanine-2-C^{14}[c]	96	24	19
10	HP-1[f]	Xanthine-8-C^{14}[c]	107	94	0
11	HP-1	Guanine-2-C^{14}[c]	110	106	99[g]
12	HP-1	Histidine-2-C^{14}[d]	0	2	65[g]

[a] See reference 5. [b] *A. aerogenes* mutant requiring guanine, adenine, hypoxanthine, xanthine or 4-amino-5-imidazole carboxamide. [c] Kindly supplied by Drs. G. B. Brown and M. E. Balis, Sloan-Kettering Institute, New York. [d] Unlabeled guanine added. [e] *A. aerogenes* mutant requiring guanine or 2,6-diaminopurine. [f] *E. coli* mutant requiring guanine or xanthine; spared by histidine. [g] Label located in position 2 of the imidazole ring.

The pathway of the conversion of guanine to adenine was explored by experiments with C^{14} labeled compounds (Table I). In strain PD-1 carbon 8 of exogenous guanine or xanthine is transferred to nucleic acid adenine without dilution (Expt. 1, 2), while carbon 2 of guanine contributes only 40% (Exp. 3). The methylene carbon of glycine, but not formate or CO_2, is incorporated into nucleic acid adenine when guanine is the purine source (Exp. 4, 6). Similar results are obtained in strain P-14, except that here some of the nucleic acid adenine is formed by synthesis *de novo*[5] (Exp. 7–9).

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(2) Abbreviations: IMP, inosine-5'-phosphate; DPN$^+$ and DPNH, oxidized and reduced diphosphopyridine nucleotide; XMP, xanthosine-5'-phosphate; ATP, adenosine triphosphate; GMP, guanosine-5'-phosphate; AMP, adenosine-5'-phosphate; PP, pyrophosphate.

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