has been recently presented.[4,5] These various results would suggest that biotin has a role in the synthesis of a catalytic factor necessary for the formation of carbamyl phosphate.

In the present investigation, biotin deficiency in *Streptococcus lactis* 8043 has been found to result in greatly diminished ability to convert carbamyl phosphate and ornithine to citrulline, so that a role of biotin is indicated in the utilization of carbamyl phosphate for citrulline synthesis.

Cells of *S. lactis* were harvested after 24 hours of growth at 30° in a previously described medium[6] containing 200 mγ per ml. of calcium pantothenate and either 2 mγ or 0.01 mγ of biotin per ml. The cells were washed twice in fresh medium which was modified by eliminating arginine, purines, pyrimidines and biotin and adjusted to *p*H 8. To obtain cell-free preparations, the cells were suspended in the wash medium, 0.5 mg. of cells per ml., and exposed to sonic oscillations for 40 minutes. The disrupted cell suspensions were centrifuged at 5000 g. to obtain the cell-free supernatant as the enzyme preparation.

For assay, 0.1 ml. of this cell-free preparation (derived from 0.05 mg. of whole cells) was incubated for 2 hours at 30° with carbamyl phosphate (dilithium salt), 20 μM: magnesium chloride, 2.5 μM.; DL-ornithine, 20 μM.; and tris-(hydroxymethyl)-aminomethane buffer, 10 μM. at *p*H 8, in a total volume of 1 ml. The amount of citrulline produced was determined by a previously described method.[7] From the results (Table I), it is apparent that enzyme preparations from biotin deficient cells have considerably less ability to convert ornithine and carbamyl phosphate to citrulline than preparations from normal cells. In order to show that the decrease of activity of the biotin-deficient preparations was the result of biotin deficiency and not of reduced growth, cells were harvested after growth in medium in which the pantothenic acid concentration was lowered from 200 mγ to 2 mγ per ml. so that only half-maximal growth was obtained. Cell-free enzyme preparations from the pantothenic acid deficient cells showed no decrease in ability to form citrulline from carbamyl phosphate and ornithine.

TABLE I

EFFECT OF BIOTIN DEFICIENCY ON CITRULLINE SYNTHESIS

Cell-free enzyme preparations	Additional supplements	Per cent. conversion of ornithine to citrulline[c]
I[a]	None	7.7
I	N-Carbamyl-L-glutamic Acid (20 μM.)	9.2
I	N-Acetyl-L-glutamic Acid (20 μM.)	8.8
I	Biotin (1 γ)	7.7
I	Heated II (0.5 ml.)	7.2
II[b]	None	60.0

[a] I derived from cells cultured in a medium deficient in biotin (0.01 mγ/ml.). [b] II derived from cells cultured in a medium containing an excess of biotin (2 mγ/ml.). [c] Based on L-ornithine.

Supplements of biotin, biocytin, N-carbamylglutamic acid, N-acetylglutamic acid, glutamine, bicarbonate and adenosine triphosphate singly or in various combinations did not restore normal activity to the biotin-deficient preparations. Also, addition of a heated cell-free preparation from normal cells, hot water extract of pig liver or a heated residue of rat liver homogenate similarly failed to enhance the activity of the deficient preparations. Although biotin deficient cell-free preparations were not activated by these supplements, normal activity could be restored to biotin deficient cells in a biotin supplemented growth medium in a few hours.

Whether biotin functions directly as a compo nent or indirectly in the synthesis of the enzyme system and whether a common factor related to biotin is necessary for both the formation and trans fer of a carbamyl group remains to be determined.

(4) M. E. Jones, L. Spector and F. Lippmann, THIS JOURNAL, **77**, 819 (1955).

(5) J. M. Lowenstein and P. P. Cohen, *J. Biol. Chem.*, **220**, 57 (1956).

(6) J. M. Ravel, L. Woods, B. Felsing and W. Shive, *ibid.*, **206**, 391 (1954).

(7) R. M. Archibald, *ibid.*, **156**, 121 (1944).

(8) National Science Foundation Predoctoral Fellow, 1955–6.

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RECEIVED OCTOBER 30, 1956

PARAMAGNETISM OF THE SYSTEM S_8–SO_3–H_2O

Sir:

Yellow to blue solutions are formed when sulfur is dissolved in oleum,[1] and a blue solid, known as sulfur sesquioxide, arises when sulfur reacts with anhydrous liquid sulfur trioxide.[2] Recent evidence[3] indicates that the blue solid is a molecular compound with the formula $(SO_3.S)_x$. We have found that both the colored solutions and the blue solid are paramagnetic and contain at least two different paramagnetic species. The paramagnetism was detected by means of a paramagnetic resonance spectrometer operating at a wave length of 3.2 cm.[4]

Both the colored solutions and the blue solid in an excess of sulfur trioxide gave resonance spectra consisting of two well-resolved lines. For 0.01 *M* solutions of S_8 in 30% oleum, the total paramagnetic resonance intensity corresponds very roughly to ten unpaired electrons per S_8 molecule; but for 1 *M* S_8 in 30% oleum the intensity corresponds to only of the order of one unpaired electron per hundred S_8 molecules. The line widths (defined as the separation in gauss between inflection points of the absorption curve) and spectroscopic splitting factors (*g*-values) were the same for all the systems investigated. The low field line (I) had a width of 4 gauss and a *g*-value of 2.026 ± 0.003; and the high field line (II) had a width of 8 gauss and a *g*-value of 2.016 ± 0.003. The assignment of these two lines to two different species is justi-

(1) W. Ostwald and R. Auerbach, *Kolloid-Z.*, **38**, 336 (1926).

(2) L. Wohler and O. Wegwitz, *Z. anorg. allgem. Chem.*, **213**, 129 (1933).

(3) R. Appel, *Naturwissenschaften*, **40**, 509 (1953).

(4) J. M. Hirshon and G. K. Fraenkel, *Rev. Sci. Instr.*, **26**, 31 (1955).

fied by the fact that both the absolute and relative absorption intensities vary with time and with the concentration of sulfur and sulfur trioxide. For the 30% oleum solutions mentioned above, the intensity ratio of (II)/(I) increased with time and with decreasing concentration of sulfur. Similar two-line spectra were displayed by a mixture of sulfur, anhydrous aluminum chloride, and carbon tetrachloride.

The present data are insufficient to permit a conclusive interpretation of the behavior of sulfur in oleum but, if it is assumed that all the non-solvent sulfur is contained in paramagnetic molecules, then the data indicates that species (I) contains more sulfur than species (II). The similarity of the g-values for the paramagnetic resonance spectra of ultramarine (2.028),[5] liquid sulfur (2.024),[6] and the system S_8–SO_3–H_2O is discussed elsewhere.[6]

(5) D. M. Gardner and G. K. Fraenkel, THIS JOURNAL, **77**, 6399 (1955).

(6) D. M. Gardner and G. K. Fraenkel, *ibid.*, **78**, 3279 (1956).

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RECEIVED NOVEMBER 15, 1956

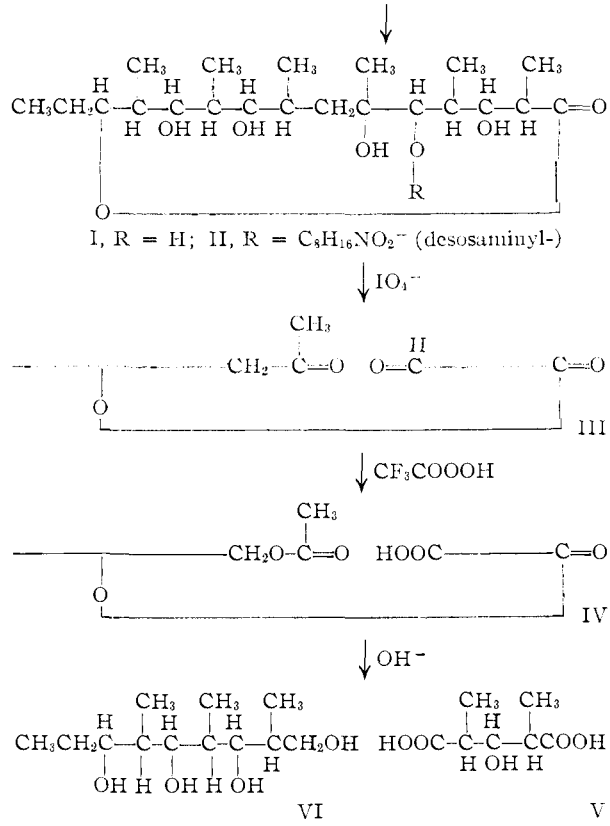
ERYTHROMYCIN. IX.[1] DEGRADATIVE STUDIES OF ERYTHROMYCIN B.

Sir:

A previous report[2] from this laboratory described the isolation and characterization of a second crystalline antibiotic, erythromycin B, from a culture of *Streptomyces erythreus*. The molecular formula $C_{37}N_{71}NO_{12}$ has been proposed,[3] and the presence of the sugars, desosamine[4] and cladinose,[5] has been demonstrated.[3] Erythromycin B and erythromycin[6] have very similar properties,[2,3,6] the principal difference being a greater acid stability of the former.

Degradative studies described below lead to structure I for dihydroerythronolide B,[7] the aglycone of dihydroerythromycin B.

Analytical data of purified erythromycin B, m.p. 198°, are consistent with the molecular formula $C_{37}H_{67}NO_{12}$ [Found: C, 62.09, 62.07; H, 9.46, 9.67; N, 1.99, 1.97; C-CH_3, 14.6; O-CH_3, 4.8; mol. wt., 730 (electrometric titration); pK_a' 8.8[8]], and the infrared (bands at 5.8 μ and at 5.9 μ) and ultraviolet (λ_{max} 289 mμ, ϵ = 36) spectra are consistent with the presence of a ketone and a lactone function. Erythromycin B N-oxide,[9] [Found: C, 60.79, 60.60; H, 9.44, 9.42; N, 1.98; pK_a' 5.4], prepared from the antibiotic and hydrogen peroxide, was stable in the presence of sodium metaperiodate.



Mild acid hydrolysis of erythromycin B N-oxide gave, in addition to cladinose ($C_8H_{16}O_4$), x-O-desosaminyl erythronolide B N-oxide,[7] m.p. 169–173° [Found: C, 60.63; H, 9.39; N, 2.59], also prepared from x-O-desosaminyl erythronolide B,[10] $C_{29}H_{53}NO_9$, m.p. 78–83° [Found: C, 62.26; H, 9.56. N, 2.33; mol. wt., 582; pK_a' 8.3], the product of mild acid hydrolysis of erythromycin B. This N-oxide still contains the ketone function (maximum at 285 mμ, ϵ = 37). It follows that the facile, acid-catalyzed spiro-ketal formation observed with erythromycin[11] does not occur with erythromycin B.

Reduction of erythromycin B with sodium borohydride followed by mild acid hydrolysis of the intermediate dihydroerythromycin B yielded 5(?)-O-desosaminyl dihydroerythronolide B (II), $C_{29}H_{55}NO_9$, m.p. 207–208° [Found: C, 62.21; H, 9.76; N, 2.62; C-CH_3, 18.0; mol. wt., 548; pK_a' 8.2; $\alpha^{25}D$ −1.7° (c, 1 in methanol)]. This lactone (II) formed the corresponding N-oxide,[9] m.p. 170–173° [Found: C, 59.45; H, 9.36; N, 2.46; pK_a' 5.2], which consumed no periodate.

Hydrolysis of II with 1 N hydrochloric acid in a two phase system with toluene gave, in addition to

(1) Previous paper in this series: Erythromycin. VIII, THIS JOURNAL, **78**, 6396 (1956).

(2) C. W. Pettinga, W. M. Stark and F. R. Van Abeele, *ibid.*, **76**, 570 (1954).

(3) R. K. Clark, Jr., and M. Taterka, *Antibiotics and Chemotherapy*, **5**, 206 (1955).

(4) R. K. Clark, Jr., *ibid.*, **3**, 663 (1953).

(5) P. F. Wiley and Ollidene Weaver, THIS JOURNAL, **78**, 808 (1956).

(6) J. M. McGuire, R. L. Bunch, R. C. Anderson, H. E. Boaz, E. H. Flynn, H. M. Powell and J. W. Smith, *Antibiotics and Chemotherapy*, **2**, 281 (1952). The Eli Lilly and Company trademark for the antibiotic erythromycin is "Ilotycin" (Erythromycin-Lilly).

(7) The names erythronolide B and dihydroerythronolide B are proposed for the aglycone portions of erythromycin B and dihydroerythromycin B, respectively.

(8) The pK_a' values reported herein were determined in 66% dimethylformamide solution.

(9) This N-oxide is isomeric with the corresponding tertiary amine in the erythromycin series.

(10) We have not been able to show the identity of this base with the corresponding base reported by Clark, *et al.* (reference footnote 3) for which the authors propose the molecular formula $C_{29}H_{55}NO_9$.

(11) Paper VI in this series: THIS JOURNAL, **78**, 388 (1956).