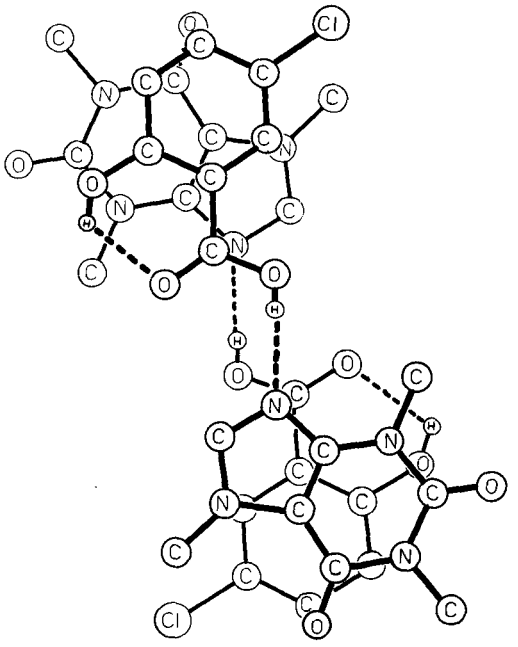

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in relation to the caffeine molecule. This arrangement is consistent with the ideas presented above for polarization bonding. A more detailed account of this material will be published at a later date.

In conclusion, it is felt that in order to propose molecular models for xanthine complexes of pharmaceutical interest, one should take into account polarization interactions. Studies on other pyrimidine and purine complexes are being carried out in this laboratory to shed more light on this problem.

(1) Higuchi, T., and Lach, J. L., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 349(1954).
(2) Higuchi, T., and Zuck, D. A., *ibid.*, **41**, 10(1952).
(3) *Ibid.*, **42**, 132(1953).
(4) Guttman, D. E., and Athalye, M. Y., *ibid.*, **49**, 687 (1960).
(5) Harbury, H., and Foley, K. A., *Proc. Natl. Acad. Sci. U. S.*, **44**, 114(1956).
(6) Eckert, T., *Arch. Pharm.*, **295**, 233(1962).
(7) Pullman, A., and Pullman, B., *Proc. Natl. Acad. Sci. U. S.*, **44**, 1197(1958).
(8) Donbrow, M., and Jan, Z. A., *J. Pharm. Pharmacol.*, **17**, Suppl. 129s(1965).
(9) Schnaare, R. S., and Martin, A. N., *J. Pharm. Sci.*, **54**, 1707(1965).
(10) Wallwork, S. C., *J. Chem. Soc.*, **1961**, 494.
(11) Mulliken, R. S., *J. Phys. Chem.*, **56**, 801(1952).
(12) Briegleb, G., "Zwischenmolekular Krafte und Molekulstrukture," Enke, Stuttgart, Germany, 1937, through *Reference 10.*
(13) Wallwork, S. C., and Hardin, T. T., *Acta Cryst.*, **6**, 791 (1953).
(14) Hanson, A. W., *ibid.*, **16**, 1147(1963).
(15) Sakurai, T., *ibid.*, **19**, 320(1965).
(16) Damiani, A., De Santis, P., Giglio, E., Kiquori, A. M., Puliti, R., and Ripamonti, A., *ibid.*, **19**, 340(1965).

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Keyphrases

Complexes–xanthine
Xanthine complexes–bonding mechanism
Polarization bonding–complex formation effect
Caffeine, 5-chlorosalicylic acid complex–crystal structure

Effect of Certain Tetracycline Analogs on Phenylalanine-^{14}C Incorporation by *Escherichia coli* B Cell-free Extracts

Sir:

This communication reports the effects of several tetracycline analogs on messenger RNA (mRNA) and polyuridylic acid (poly U)-directed phenylalanine-^{14}C incorporation by *E. coli* B cell-free extracts. This study was undertaken to determine (*a*) if mRNA and poly U-directed amino acid incorporation are differentially sensitive to inhibition by the tetracyclines, and (*b*) to examine structure-activity relationships in this series of drugs.

The tetracyclines have been observed (1) to inhibit protein synthesis *in vivo* (*Staphylococcus aureus*). Franklin (2) has studied the incorporation of leucine-^{14}C into polypeptides by rat liver or *E. coli* cell-free extracts and has observed that the tetracyclines inhibit the transfer of amino acids from the aminoacyl-tRNA complex to the growing polypeptide. Suarez and Nathans (3) showed that tetracycline inhibits protein synthesis in *E. coli* cell-free extracts and impedes the binding of aminoacyl-tRNA to mRNA-ribosome complex. Hierowski (4) and Maxwell (5) have made similar observations.

The relative inhibitory activity of the tetracycline analogs was examined at a drug concentration of 1.79×10^{-4} *M*. *E. coli* cell-free extracts and reaction mixtures were prepared according to Nirenberg (6). Incubations were terminated after 70 min. by the addition of 5%

TABLE I—EFFECT OF TETRACYCLINE ANALOGS ON mRNA-DIRECTED PHENYLALANINE-^{14}C INCORPORATION BY *E. Coli* B CELL-FREE EXTRACTS[a]

Inhibitor	Phenylalanine-^{14}C Incorporation (dpm./mg. protein ± S.E.)	Percent Inhibition
Experiment 1		
Control	476 ± 47	..
Isotetracycline	367 ± 39[b]	23
Tetracycline	316 ± 20[b]	34
4-Epitetracycline ammonium salt	478 ● 7[c]	..
Pyrrolidinomethyl-tetracycline	402 ± 6[c]	16
4-Dedimethylamino-tetracycline	424 ± 47[c]	11
Experiment 2		
Control	968 ± 45	..
Tetracycline	603 ± 64[b]	38
7-Chlorotetracycline	697 ± 52[b]	30

[a] Each incubation flask contained in 0.70-ml. total volume: *E. Coli* B cell-free extract (1.40–2.0 mg. protein), phosphoenolpyruvate (5.4 × 10^3 μM), 2-mercaptoethanol (4.2 μM), magnesium acetate (5.2 × 10^3 μM), phosphoenolpyruvate kinase (1.2 EU), aminoacid-^{12}C mixture (143 μM in each of 19 aminoacids, phenylalanine excepted), D, L-3′-phenylalanine-^{14}C (143 μM, 0.57 μc.), adenosine-5′-triphosphate (761 μM), guanosine-5′-triphosphate (23 μM), potassium chloride (3.6 × 10^4 μM), and Tris (7.1 × 10^4 μM). pH = 7.8. Four or five samples per group. [b] $p < 0.05$. [c] Not significantly different from control, $p > 0.05$.

trichloroacetic acid. Protein content was determined by the Lowry method and radioactivity determination made using a liquid scintillation spectrometer. Data were corrected for quenching by use of the internal standard technique.

In the mRNA-directed system (Table I), with tetracycline assigned an activity of 100, the relative activities of the analogs were: (*a*) 7-chlorotetracycline (80), (*b*) isotetracycline (67), (*c*) pyrrolidinomethyltetracycline (47), (*d*) 4-dedimethylaminotetracycline (32), and (*e*) 4-epitetracycline ammonium salt (0). These findings suggest that the nature of the substituents on C_2, C_3, and C_4 are relatively more important than those on C_{11} and C_{12} of the drug molecule.

In the case of poly U-directed phenylalanine-^{14}C incorporation (Table II) the relative activities of each analog *versus* tetracycline (100) were: (*a*) pyrrolidinomethyltetracycline (102), (*b*) 7-chlorotetracycline (98), (*c*) 4-epitetracycline ammonium salt (93), (*d*) 4-dedimethylaminotetracycline (43), and (*e*) isotetracycline (0). The relatively low inhibition by 4-dedimethylaminotetracycline and isotetracycline suggested that the C_4-dimethylamino group and/or C_{11}-keto and C_{12}-hydroxyl groups are important in the presence of artificial messenger.

TABLE II—EFFECT OF TETRACYCLINE ANALOGS ON POLY U-DIRECTED PHENYLALANINE-^{14}C INCORPORATION BY *E. Coli* B CELL-FREE EXTRACTS[a]

Inhibitor	Phenylalanine-^{14}C Incorporation (dpm. mg. protein ± S.E.)	Percent Inhibition
Control	2174 ± 108	...
Isotetracycline	2178 ± 144[c]	...
Tetracycline	1009 ± 53[b]	54
4-Epitetracycline ammonium salt	1082 ± 67[b]	50
Pyrrolidinomethyl-tetracycline	980 ± 36[b]	55
4-Dedimethylamino-tetracycline	1660 ± 131[b]	23
7-Chlorotetracycline	1018 ± 104[b]	53

[a] Incubation mixtures prepared as in Table I, except that 1.04 × 10^4 μM magnesium acetate and polyuridylic acid (20 mcg./ml.) were used. Five samples per group. [b] $p < 0.01$. [c] Not significantly different from control, $p > 0.05$.

These data suggest that there may be different modes of action of the tetracycline antibiotics in the inhibition of mRNA and poly U-directed phenylalanine-^{14}C incorporation by *E. Coli*. Further studies on the nature of the differential inhibition are in progress.

(1) Gale, E. F., and Folkes, J. P., *Biochem. J.*, **53**, 493 (1953).
(2) Franklin, T. J., *Biochem. J.*, **90**, 624(1964).
(3) Suarez, G., and Nathans, D., *Biochem. Biophys. Res. Commun.*, **18**, 743(1965).
(4) Hierowski, M., *Proc. Natl. Acad. Sci. U. S.*, **53**, 594 (1965).
(5) Maxwell, J. H., *Biochim. Biophys. Acta*, **138**, 337(1967).
(6) Nirenberg, M. W., "Methods in Enzymology," vol. VI. Colowick, S. P., and Kaplan, N. O., eds., Academic Press Inc., New York, N. Y., 1963, pp. 17–23.

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