suggest receptor bonding interactions involving van der Waals and hydrophobic bonding, and possibly charge-transfer complexing. These factors are being further investigated.

The kinetics of ChA inhibition are being studied with the most potent inhibitor, I. The most specific inhibitor of ChA, IV, is being employed for the investigation of the inhibition of ChA *in vivo* on various physiological functions. All the compounds which inhibit ChA show reversible non-competitive inhibition.

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REFERENCES

1. D. NACHMANSOHN in *Cholinesterases and Anticholinesterase Agents* (Ed. G. B. KOELLE), p. 41. Springer, Berlin (1963).
2. F. W. BERG, T. R. NORTON and R. A. SEIBERT, *J. org. Chem.* **10**, 452 (1945).
3. A. P. GRAY, W. L. ARCHER, E. E. SPINNER and C. J. CAVALLITO, *J. Am. chem. Soc.* **79**, 3805 (1957).
4. E. A. WAGSTAFF, *J. chem. Soc.* 276 (1934).
5. H. E. HECK, *Justus Liebigs Annln Chem.* **613**, 171 (1958).
6. J. C. SMITH, C. J. CAVALLITO and F. F. FOLDES, *Fedn Proc.* **25**, 320 (1966).
7. R. E. MCCAMAN and J. M. HUNT, *J. Neurochem.* **12**, 253 (1965).
8. W. N. ALDRIDGE and A. N. DAVISON, *Biochem. J.* **55**, 763 (1953).
9. C. J. CAVALLITO, M. D. NAPOLI and T. B. O'DELL, *Archs int. Pharmacodyn. Thér.* **149**, 188 (1964).

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The stoichiometry of erythromycin binding to ribosomal particles of *Staphylococcus aureus*

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ERYTHROMYCIN, a macrolide antibiotic, inhibits bacterial protein synthesis, both *in vivo*[1] and *in vitro*.[2,3] Recently, erythromycin was reported to be bound to ribosomes isolated from *Escherichia coli*[4] and *Bacillus subtilis*.[5] Some evidence suggests that the inhibition of protein synthesis is related to the binding of erythromycin to the ribosomes,[5,6] but the number of erythromycin molecules bound per ribosome was not established. Because these data on binding of erythromycin to ribosomes would appear more significant if a definite number of erythromycin molecules were bound to each ribosome, the stoichiometry of the binding was studied.

S. aureus 209P was grown in Brain Heart Infusion (Difco) and harvested at the early expoential phase of growth. Ribosomes were isolated and washed twice with standard buffer by the method for

E. coli,[7] as modified for the isolation of *S. aureus* ribosomes.[8] The standard buffer contained: Tris, 10 mM, pH 7·5; NH_4Cl, 50 mM; $Mg(OAc)_2$, 16 mM; and dithiothreitol, 0·1 mM. The dissociated ribosomes were obtained by dialyzing ribosomes overnight in standard buffer containing only 0·1 mM Mg^{2+}.

Binding of ^{3}H-erythromycin ($1{\cdot}58 \times 10^6$ cpm/μmole) to ribosomes was determined by sucrose gradient centrifugation. Linear sucrose gradients (5–20%) were layered with 0·2 to 0·3 ml of sample (total, 5 ml) and spun at 4° for 120 min (intact ribosomes) or 210 min (dissociated ribosomes) at 30,000 rpm in the SW-39 rotor of the model L ultracentrifuge. Fractions of 0·14 ml were collected and diluted with 1·0 ml water. Optical density was read at 260 mμ. After addition of 1·0 ml of 3 M potassium phosphate, pH 9·5, the ^{3}H-erythromycin was extracted with three 2·0-ml portions of ether. The combined ether layers, which give complete recovery of erythromycin, were dried over anhydrous sodium sulfate, poured into counting vials, evaporated, and counted by the scintillation method in a Packard Tri-Carb model 3002 with approximately 28 per cent efficiency for tritium.

The sedimentation coefficients of *S. aureus* ribosomal particles determined by ultracentrifugation were found to be essentially idnetical to those of *E. coli*.[8] Therefore, the molecular weights of *S. aureus* ribosomes were assumed to be the same as *E. coli* ribosomes ($2{\cdot}6 \times 10^6$, $1{\cdot}8 \times 10^6$, and $0{\cdot}8 \times 10^6$ for 70S, 50S, and 30S ribosomes respectively).[9] By orcinol and biuret methods,[10, 11] one O.D.$_{260}$ unit of the *S. aureus* ribosomes was equivalent to 53 μg of RNA and 28 μg of protein. From these values, one O.D.$_{260}$ unit represents 15·5 $\mu\mu$mole 100S ribosomes, 31 $\mu\mu$mole 70S ribosomes, 45 $\mu\mu$mole 50S ribosomes, or 101 $\mu\mu$mole 30S ribosomes.

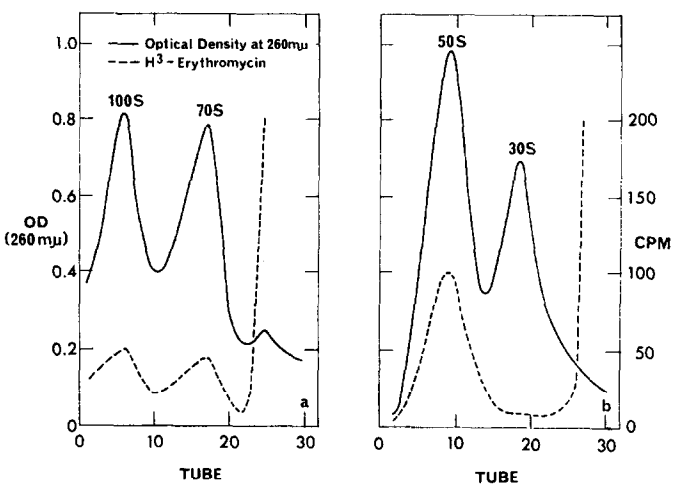

FIG. 1. Binding of ^{3}H-erythromycin to the ribosomal particles. The reaction mixtures, in a volume of 0·3 ml, containing 20 O.D.$_{260}$ units of *S. aureus* ribosomes (a), or dissociated ribosomes (b) and 0·068 μmole of ^{3}H-erythromycin ($1{\cdot}5 \times 10^6$ cpm/μmole) were incubated at 35° for 10 min. Samples of 0·2 ml were layered over sucrose gradients made in 16 mM Mg^{2+} (a) or 0·1 mM Mg^{2+} (b) buffer.

Sucrose gradient centrifugation showed that ^{3}H-erythromycin was bound to 100S, 70S, and 50S ribosomes, but not to the 30S ribosomes (Fig. 1a, b).

Table 1 summarizes the stoichiometric relation between erythromycin and the ribosomal particles. Results from three experiments showed that each 70S or 50S ribosome bound from 1·1 to 1·4 molecules of erythromycin. The 100S ribosomes, which contain two 70S ribosomes, bound 2·7 molecules of erythromycin. The binding of erythromycin to 30S ribosomes was negligible.

These experiments show that erythromycin binds specifically to the 50S subunit of *S. aureus* ribosomes. One molecule of erythromycin was bound to each 50S subunit. The 70S ribosome containing one 50S subunit and the 100S ribosome containing two 50S subunits bound approximately one and two molecules of erythromycin respectively. The exact site of erythromycin binding is not known, but the site of joining the 30S to the 50S particle can be ruled out because erythromycin was able to bind

TABLE 1. STOICHIOMETRY OF ^{3}H-ERYTHROMYCIN BINDING TO RIBOSOMAL PARTICLES*

Ribosome	($\mu\mu$mole)	^{3}H-erythromycin ($\mu\mu$mole)	Ratio erythromycin/ribosome
100S	16·8	45·0[a]	2·7
70S	26·3	36·1[a]	1·4
	31·7	41·2[b]	1·3
	29·1	33·6[c]	1·2
50S	50·4	63·4[a]	1·3
	54·9	62·1[b]	1·1
	66·2	70·6[c]	1·1
30S	94·6	3 4[a]	0·04

* The twice-washed ribosomes of *S. aureus* (16–20 O.D.$_{260}$ units) were incubated with (a) 0·14 μmole, (b) 0·07 μmole, or (c) 0·014 μmole of ^{3}H-erythromycin ($1{\cdot}58 \times 10^6$ cpm/μmole) at 35° for 10 min. The binding of ^{3}H-erythromycin to ribosomal particles was analyzed by sucrose gradient centrifugation. The O.D. and radioactivity of peak fractions were used to calculate the molar ratio between ribosomes and erythromycin.

to the 70S ribosome. The binding of erythromycin to ribosomes does not depend on the concentration of Mg^{2+}, since at high Mg^{++} (16 mM) or low Mg^{2+} (0·1 mM) concentrations erythromycin was bound to ribosomes to the same extent.

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REFERENCES

1. T. D. BROCK and M. L. BROCK, *Biochim. biophys. Acta* **33**, 274 (1959).
2. S. B. TAUBMAN, A. G. SO, F. E. YOUNG, G. W. DAVIE and J. W. CORCORAN, *Antimicrob. Agents Chemother.* 395 (1963).
3. A. D. WOLFE and F. E. HAHN, *Science* **143**, 1445 (1964).
4. K. TANAKA, H. TERAOKA, T. NAGIRA and M. TAMAKI, *Biochim. biophys. Acta* **123**, 435 (1966).
5. S. B. TAUBMAN, N. R. JONES, F. E. YOUNG and J. W. CORCORAN, *Biochim. biophys. Acta* **123**, 438 (1966).
6. K. TANAKA and H. TERAOKA, *Biochim. biophys. Acta* **114**, 204 (1966).
7. M. W. NIRENBERG and J. H. MATTHAEI, *Proc. natn. Acad. Sci. U.S.A.* **47**, 1588 (1961).
8. J. C.-H. MAO, *J. Bact.* **94**, 80 (1967).
9. A. TISSIÈRES, J. D. WATSON, D. SCHLESSINGER and B. R. HOLLINGWORTH, *J. molec. Biol.* **1**, 221 (1959).
10. W. C. SCHNEIDER, *Meth. Enzym.* **3**, 680 (1957).
11. E. LAYNE, *Meth. Enzym.* **3**, 450 (1957).