MOLECULAR PHARMACOLOGY, 9, 304–310

The Effects of Antimalarial Drugs on Nucleic Acid Synthesis *in Vitro* in *Tetrahymena pyriformis*

K. A. CONKLIN, P. HEU, AND S. C. CHOU

Department of Pharmacology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822

(Received February 1, 1973)

SUMMARY

CONKLIN, K. A., HEU, P., AND CHOU, S. C.: The effects of antimalarial drugs on nucleic acid synthesis *in vitro* in *Tetrahymena pyriformis*. *Mol. Pharmacol.* **9**, 304–310 (1973).

Solubilized DNA and RNA polymerases were prepared from disrupted nuclei of *Tetrahymena pyriformis* and partially purified by gel filtration on Sephadex G-100. The enzymes were characterized and shown to carry out DNA replication and DNA-dependent RNA synthesis. The effects of three antimalarial drugs, quinine, primaquine, and chloroquine, on DNA and RNA syntheses by the intact nuclei and the solubilized polymerases were also investigated. For each drug dose-response curves were constructed, with each curve including the two drug concentrations which have previously been found to inhibit cell division by 80–90% and 100%, respectively, in *T. pyriformis*. At these two concentrations quinine and primaquine were shown to have relatively little inhibitory effect on the reactions of the test systems *in vitro* as compared to previously reported inhibition of precursor incorporation into nucleic acids of intact cells. These results indicate that the primary action of these two drugs in *T. pyriformis* may not be inhibition of nucleic acid synthesis, and that they may possess another mechanism for blocking precursor incorporation by *T. pyriformis in vivo*. Chloroquine, however, at the concentrations which block cell division, was shown to inhibit markedly the synthesis of both DNA and RNA by the solubilized polymerases and DNA synthesis by nuclei. These results indicate that inhibition of nucleic acid synthesis may, in part, account for the effects of this drug on *T. pyriformis*.

INTRODUCTION

Inhibition of DNA and RNA synthesis *in vitro* by chloroquine (1–3) and quinine (2, 3) supports the hypothesis that these agents act by inhibition of nucleic acid synthesis. This inhibition has been attributed to binding or intercalation of chloroquine (2–10) and quinine (2, 3, 11) with DNA, the interaction being studied by various physical parameters with solutions containing DNA and the antimalarial drugs. Primaquine has also been shown to bind to DNA (8, 10), and it is suggested that a portion of the antimalarial activity of this compound is related to inhibition of DNA function.

Our previous studies in *Tetrahymena pyriformis* have shown that these antimalarial drugs block the incorporation of nucleic acid precursors by intact cells (12). Similar results using intact cells have been observed with other test systems for chloroquine (2, 13–17) and quinine (2, 15–18), whereas primaquine had little effect on this

This is Contribution 1057 to the Army Research Program on Malaria. This investigation was supported by Contract DADA17-71-C-1116 with the United States Army Medical Research and Development Command.

process (15). However, in *T. pyriformis* (12) the three drugs were shown to inhibit uptake of nucleic acid precursors by the cells, and it was suggested that this inhibition of precursor uptake may account for the inhibition of incorporation. The present study was designed to determine whether the antimalarial drugs directly inhibit DNA and RNA synthesis *in vitro* in test systems from *T. pyriformis*.

MATERIALS AND METHODS

T. pyriformis, strain GL, was grown to a population of approximately 200,000/ml and harvested as described previously (19). Nuclei were prepared from 8 ml of packed cells by the procedure of Lee and Scherbaum (20), and assays for DNA and RNA syntheses in these organelles were carried out as described previously (21). The 0.25-ml reaction mixture for assay of RNA synthesis in nuclei contained 0.5 mM ATP, 0.05 mM each GTP and CTP, 0.5 μCi of [5-^{3}H]UTP (17.8 Ci/mmole; Schwarz BioResearch), 60 mM each KCl and NaCl, 25 mM Tris-HCl (pH 7.5), 1.5 mM $MgCl_2$, 5 mM mercaptoethanol, 0.25 M sucrose, and 1–3 $\times$ 10^6 nuclei. The 0.25-ml reaction mixture for assay of DNA synthesis in nuclei contained 0.5 mM ATP, 0.05 mM each dATP, dCTP, and dGTP, 2.5 μCi of [*methyl*-^{3}H]TTP (11.1 Ci/mmole; Schwarz BioResearch), 0.3 mM EDTA, 50 mM KCl, 25 mM Tris-HCl (pH 7.5), 1.5 mM $MgCl_2$, 5 mM mercaptoethanol, 0.25 M sucrose, and 1–3 $\times$ 10^6 nuclei. Reaction mixtures were incubated for 30 min at 28°.

Solubilized DNA and RNA polymerases were prepared from the isolated nuclei, all procedures being carried out at 2°. Nuclei from 8 ml of packed cell were suspended in 2 ml of a buffer containing 0.1 M Tris-HCl (pH 7.9), 3 mM $MgCl_2$, 10 mM mercaptoethanol, 0.6 mM EDTA, and 30% (v/v) glycerol (TMG buffer). The nuclei were lysed by addition of NaCl and KCl (concentrated solutions) to final concentrations of 0.15 M each. The lysate was centrifuged at 105,000 $\times$ *g* for 1 hr at 0°. The supernatant fraction was chromatographed on a Sephadex G-100 column (2 $\times$ 35 cm) which had been equilibrated with TMG buffer. The enzymes were eluted with TMG buffer, and peak fractions were pooled (fractions 15–18, Fig. 1). Assay procedures for the solubilized DNA and RNA polymerases were similar to those described for nuclei. The RNA polymerase reaction mixture (0.25 ml) contained 0.5 mM ATP, 0.05 mM each GTP and CTP, 0.5 μCi of [5-^{3}H]UTP (17.8 Ci/mmole), 50 mM Tris-HCl (pH 7.9), 1.5 mM $MgCl_2$, 1 mM $MnCl_2$, 5 mM mercaptoethanol, 0.3 mM EDTA, 15% (v/v) glycerol, 1–3 μg of protein (enzyme) as determined by the procedure of Lowry *et al.* (22), and 50 μg of native calf thymus DNA. The DNA polymerase reaction mixture (0.25 ml) contained 0.5 mM ATP, 0.05 mM each dATP, dCTP, and dGTP, 2.5 μCi of [*methyl*-^{3}H]TTP (11.1 Ci/mmole), 25 mM KCl, 50 mM Tris-HCl (pH 7.9), 1.5 mM $MgCl_2$, 5 mM mercaptoethanol, 0.3 mM EDTA, 15% (v/v) glycerol, 1–3 μg of protein (enzyme), and 50 μg of native calf thymus DNA. Both assay mixtures were incubated for 60 min at 28°.

RESULTS

Figure 1 shows the Sephadex G-100 elution pattern for the supernatant fraction of the disrupted nuclei which had been centrifuged for 1 hr at 105,000 $\times$ *g*. The first peak, which was within the void volume of the column, contained both the DNA and RNA polymerase activities. Both activities were proportionally similar within the peak. The four fractions containing the most activity were pooled for enzyme characterization and drug testing (fractions 15–18 in Fig. 1).

Tables 1 and 2, respectively, give the requirements for the DNA and RNA polymerase reactions by the solubilized enzymes, and Fig. 2 illustrates the time course for both reactions. Maximum DNA synthesis was dependent upon ATP, dATP, dGTP, dCTP, KCl, and native DNA, since omission of any component or replacement of the native DNA with an equal amount of heat-denatured DNA decreased precursor incorporation. These results are consistent with a DNA-dependent DNA polymerase reaction (DNA replication), and are similar to those described by Pearlman and Westergaard (23) except that they observed more activity with denatured DNA than with native DNA. The RNA synthesis reaction

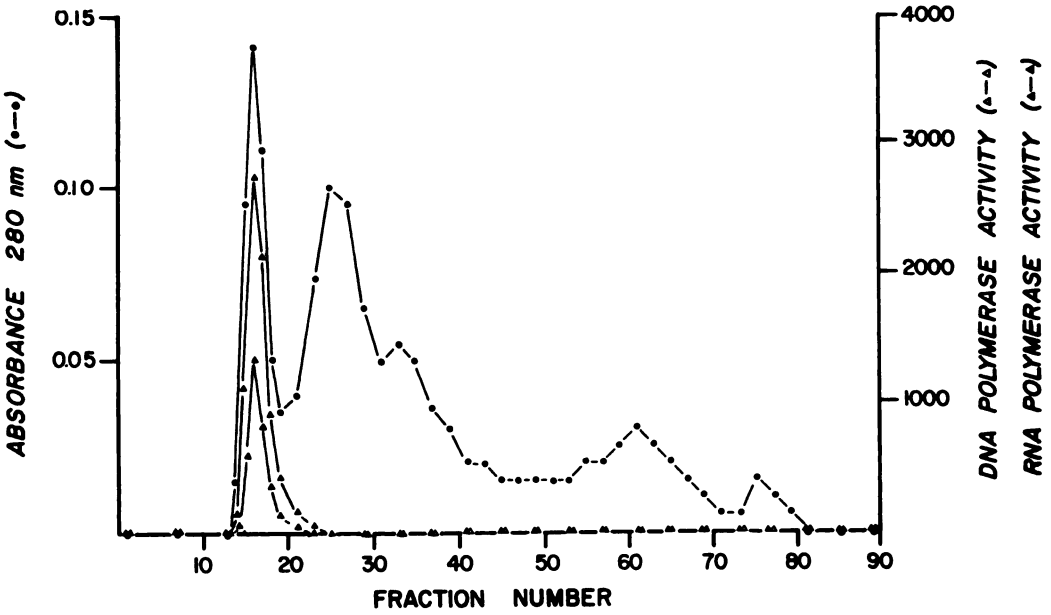

FIG. 1. *Sephadex G-100 elution pattern of DNA and RNA polymerases*

The 105,000 × *g* supernatant fraction from lysed nuclei was chromatographed on a column of Sephadex G-100, 2 × 35 cm, which was equilibrated with TMG buffer. The enzyme was eluted in fractions of 3.0 ml with TMG buffer, and 100-μl aliquots were assayed for DNA and RNA polymerase activity as described in MATERIALS AND METHODS. Activity is defined as counts per minute incorporated in the 60-min assay.

TABLE 1

Requirements for DNA polymerase reaction by enzyme solubilized from nuclei

One hundred microliters of the pooled fractions from the Sephadex G-100 column (fractions 15–18) were used in each reaction mixture. The complete reaction mixture is that described in MATERIALS AND METHODS for DNA synthesis by the solubilized enzyme. Each value is the mean of two determinations.

Reaction mixture	Radioactivity incorporated	Relative incorporation
	cpm/μg protein	%
Complete	644	100
Minus ATP	612	95
Minus dATP	386	60
Minus dCTP	245	38
Minus dGTP	335	52
Minus ATP, dATP, dCTP, dGTP	116	18
Minus KCl	193	30
Minus native DNA	13	2
Minus native DNA, plus heat-denatured DNA (50 μg)	270	42

by the solubilized enzyme required ATP, GTP, CTP, and Mn^{++} for maximum activity, and, in contrast to the work of Kurtz and Pearlman (24), more activity was observed with native than with denatured DNA. Actinomycin D was observed to inhibit RNA synthesis by 61% at 16 μM and 24% at 1.6 μM, and this effect is similar to that observed with *T. pyriformis in vivo* (25). These results are consistent with a DNA-dependent RNA polymerase reaction. The rate of both reactions was observed to decrease progressively with time over 180 min (Fig. 2). The DNA and RNA polymerase reactions of nuclei have previously been characterized (21).

Figures 3 and 4, respectively, illustrate the dose responses of the DNA and RNA polymerase reactions of nuclei and of the solubilized polymerases to the antimalarial drugs, and Tables 3 and 4 show in more detail the effects of the drugs at the concentrations which produce 80–90% inhibition (lower dose) and 100% inhibition of cell di-

TABLE 2

Requirements for RNA polymerase reaction by enzyme solubilized from nuclei

One hundred microliters of the pooled fractions from the Sephadex G-100 column (fractions 15–18) were used in each reaction mixture. The complete reaction mixture is that described in MATERIALS AND METHODS for RNA synthesis by the solubilized enzyme. Each value is the mean of two determinations.

Reaction mixture	Radioactivity incorporated	Relative incorporation
	cpm/μg protein	%
Complete	1200	100
Minus ATP	24	2
Minus CTP	480	40
Minus GTP	384	32
Minus ATP, CTP, GTP	3	0
Minus Mn^{++}	817	68
Minus native DNA	7	0
Minus native DNA, plus heat-denatured DNA (50 μg)	889	74
Plus actinomycin D, 16 μM	468	39
Plus actinomycin D, 1.6 μM	912	76

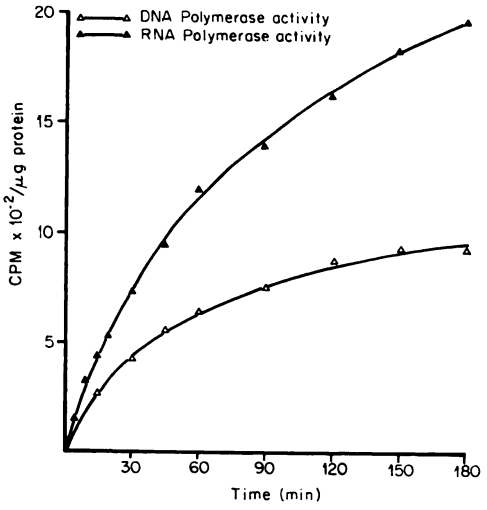

FIG. 2. *Time course of DNA and RNA polymerase reactions by solubilized polymerases*

Conditions were the same as described in MATERIALS AND METHODS for solubilized polymerases, with samples being assayed at the indicated times.

vision in synchronized *T. pyriformis*. With quinine, the ED_{50} for inhibition of DNA synthesis in nuclei was 3.0 mM and that for inhibition of RNA synthesis was greater than 10 mM. With the solubilized polymerases, the ED_{50} values for quinine were 2.2 mM and 2.7 mM for inhibition of DNA and RNA syntheses, respectively. However, at the concentrations which block cell division (Tables 3 and 4), quinine produced little inhibition of DNA or RNA synthesis in either system. The ED_{50} values for inhibition of DNA and RNA syntheses by primaquine were 1.2 mM and 2.3 mM in nuclei and 5.2 mM

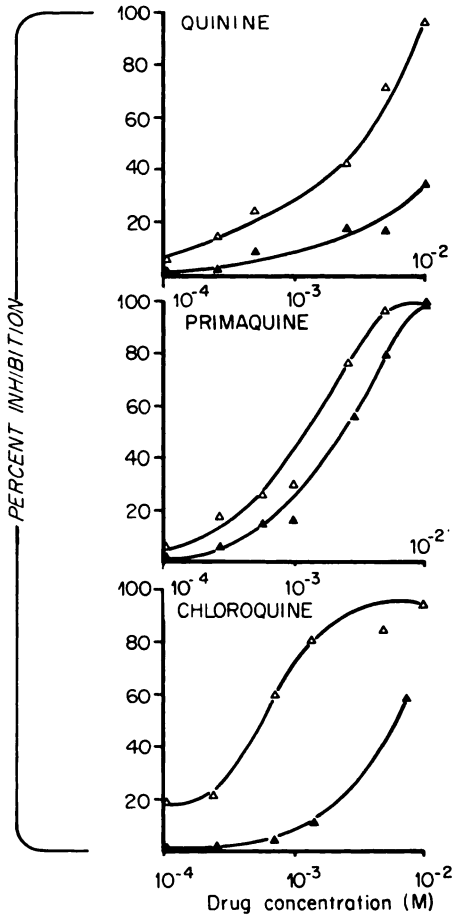

FIG. 3. *Dose-response curves for inhibition of DNA and RNA syntheses in isolated nuclei by antimalarial drugs*

Assay conditions were the same as described in MATERIALS AND METHODS, with the antimalarial drugs being added to the complete reaction mixtures for DNA and RNA syntheses in nuclei. △——△, DNA polymerase activity; ▲——▲, RNA polymerase activity.

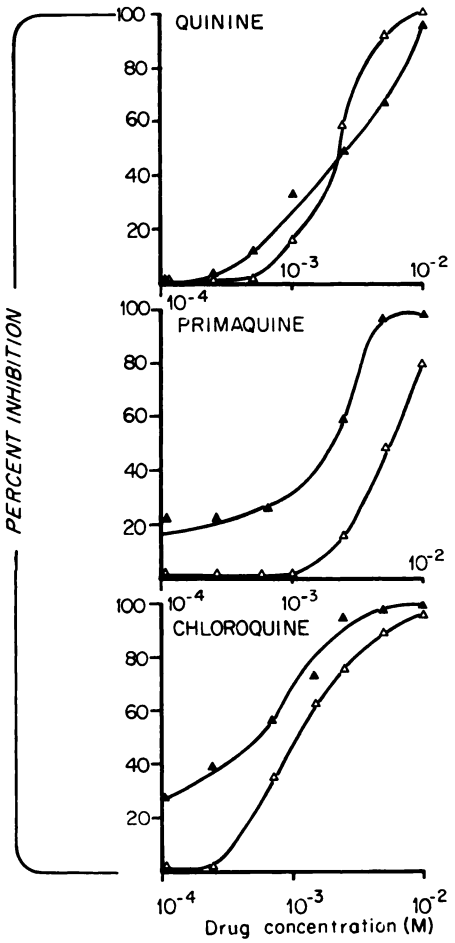

FIG. 4. *Dose-response curves for inhibition of DNA and RNA syntheses with solubilized polymerases by antimalarial drugs*

Assay conditions were the same as described in MATERIALS AND METHODS, with the antimalarial drugs being added to the complete reaction mixtures for DNA and RNA syntheses by the solubilized polymerases. △——△, DNA polymerase activity; ▲——▲, RNA polymerase activity.

and 2.2 mM for the reactions with the solubilized polymerases. Except for inhibition of RNA synthesis by the solubilized polymerase, primaquine was also found to have little inhibitory effect on any of the reactions at the concentrations which block cell division (Tables 3 and 4). With chloroquine, the ED_{50} values for inhibition of DNA and RNA syntheses were 0.58 mM and 5.8 mM in nuclei and 1.1 mM and 0.53 mM for the reactions with the solubilized polymerases. However, at the concentrations which inhibit cell division (Tables 3 and 4), chloroquine produced much greater inhibition than quinine or primaquine in all test systems except for RNA synthesis in nuclei. In that test system the degree of inhibition was similar for all three drugs.

DISCUSSION

At the concentrations which block cell division in *T. pyriformis* (Tables 3 and 4), the inhibitory effects of quinine and primaquine on DNA and RNA syntheses by nuclei and solubilized polymerases were considerably less than the inhibition of thymidine and uridine incorporation into nucleic acids by intact cells (12). At the lower concentration, quinine inhibited incorporation of DNA and RNA precursors *in vivo* by 46.5% and 63.7%, respectively (12), whereas the reactions were inhibited only 14% and 2% in nuclei and 1% and 2% with the solubilized polymerases. The failure of quinine to inhibit significantly these reactions *in vitro* is compatible with the results of others (3). Similar results were observed with primaquine in that the lower drug concentration, which inhibits precursor incorporation by 67.9% (DNA) and 87.5% (RNA) in intact cells (12), produces 18% (DNA) and 7% (RNA) inhibition in nuclei (Table 3) and zero (DNA) and 22% (RNA) inhibition of the solubilized polymerase reactions. The higher concentrations of both drugs produced only slightly greater inhibition. Although it is not known for certain whether the same enzyme is responsible for DNA synthesis *in vivo* and *in vitro*, or whether the reaction *in vitro* is measuring DNA replication or repair, these results indicate that quinine and primaquine may not inhibit nucleic acid synthesis. Therefore the drugs may possess another mechanism for blocking precursor incorporation by *T. pyriformis in vivo*. For primaquine, in support of this hypothesis are the observations that this drug binds only poorly to DNA (8) and that primaquine is a specific inhibitor not of nucleic acid but of protein biosynthesis in *Bacillus megaterium* (26).

Chloroquine, in contrast to the other two antimalarials studied, had a marked in-

TABLE 3

Effects of antimalarial drugs on DNA and RNA syntheses in isolated nuclei

Assay conditions were the same as described in MATERIALS AND METHODS, with the antimalarial drugs being added to the complete reaction mixture for DNA and RNA syntheses by nuclei. Each value is the mean of two determinations.

Drug	Concentration	DNA synthesis		RNA synthesis	
		Incorporation	Inhibition	Incorporation	Inhibition
	mM	*cpm/10^6 nuclei*	%	*cpm/10^6 nuclei*	%
None		748		12,690	
Quinine	0.25	645	14	12,450	2
	0.50	571	24	11,700	8
Primaquine	0.27	612	18	11,800	7
	0.54	556	26	10,910	14
Chloroquine	0.70	295	60	12,190	4
	1.40	145	81	11,390	11

TABLE 4

Effects of antimalarial drugs on DNA and RNA syntheses by solubilized DNA and RNA polymerases from nuclei

Assay conditions were the same as described in MATERIALS AND METHODS, with the antimalarial drugs being added to the complete reaction mixtures for DNA and RNA syntheses by the solubilized polymerases. Each value is the mean of two determinations.

Drug	Concentration	DNA synthesis		RNA synthesis	
		Incorporation	Inhibition	Incorporation	Inhibition
	mM	*cpm/μg protein*	%	*cpm/μg protein*	%
None		600		1192	
Quinine	0.25	596	1	1170	2
	0.50	642	0	966	9
Primaquine	0.27	624	0	931	22
	0.54	695	0	895	25
Chloroquine	0.70	396	34	525	56
	1.40	198	67	322	73

hibitory effect on three of the four test systems *in vitro* at the concentrations which block cell division (Tables 3 and 4). At the lower concentration the drug inhibited DNA synthesis by 60% in nuclei and 34% with the solubilized polymerase, and it inhibited RNA synthesis with the solubilized polymerase by 56%. These effects on DNA synthesis were greater than the inhibitory effect on thymidine (for DNA) incorporation by intact cells (27.3%), and the effect on RNA synthesis in the polymerase assay was almost the same as the inhibition of uridine (for RNA) incorporation (53.3%) in the assay *in vivo* (12). For chloroquine, the only result *in vitro* which markedly differed from the findings *in vivo* was inhibition of RNA synthesis by nuclei, since the assay *in vitro* showed only 4% inhibition. These results indicate that direct inhibition of nucleic acid

synthesis may account, in part, for the effects of chloroquine on *T. pyriformis*.

ACKNOWLEDGMENT

The authors would like to thank Sterling-Winthrop for their generous gift of primaquine diphosphate.

REFERENCES

1. S. N. Cohen and K. L. Yielding, *Proc. Nat. Acad. Sci. U. S. A.* **54**, 521–527 (1965).
2. F. E. Hahn, R. L. O'Brien, J. Ciak, J. L. Allison, and J. G. Olenick, *Mil. Med.* **131**, 1071–1089 (1966).
3. R. L. O'Brien, J. G. Olenick, and F. E. Hahn, *Proc. Nat. Acad. Sci. U. S. A.* **55**, 1511–1517 (1966).
4. J. L. Allison, R. L. O'Brien, and F. E. Hahn, *Science* **149**, 1111–1113 (1965).
5. L. W. Blodgett and K. L. Yielding, *Biochim. Biophys. Acta* **169**, 451–456 (1968).
6. S. N. Cohen and K. L. Yielding, *J. Biol. Chem.* **240**, 3123–3131 (1965).
7. N. B. Kurnick and I. E. Radcliffe, *J. Lab. Clin. Med.* **60**, 669–688 (1962).
8. C. R. Morris, L. V. Andrew, L. P. Whichard, and D. J. Holbrook, Jr., *Mol. Pharmacol.* **6**, 240–250 (1970).
9. F. S. Parker and J. L. Irvin, *J. Biol. Chem.* **199**, 897–909 (1952).
10. L. P. Whichard, C. R. Morris, J. M. Smith, and D. J. Holbrook, Jr., *Mol. Pharmacol.* **4**, 630–639 (1968).
11. R. D. Estensen, A. K. Krey, and F. E. Hahn, *Mol. Pharmacol.* **5**, 532–541 (1969).
12. K. A. Conklin and S. C. Chou, *J. Pharmacol. Exp. Ther.* **180**, 158–166 (1972).
13. J. Ciak and F. E. Hahn, *Science* **151**, 347–349 (1966).
14. H. Polet and C. F. Barr, *J. Pharmacol. Exp. Ther.* **164**, 380–386 (1968).
15. K. A. Schellenberg and G. R. Coatney, *Biochem. Pharmacol.* **6**, 143–152 (1961).
16. K. Van Dyke, C. Szustkiewicz, C. H. Lantz, and L. H. Saxe, *Biochem. Pharmacol.* **18**, 1417–1425 (1969).
17. C. H. Lantz and K. Van Dyke, *Biochem. Pharmacol.* **20**, 1157–1166 (1971).
18. D. H. Clarke, *J. Exp. Med.* **96**, 451–463 (1952).
19. K. A. Conklin, S. C. Chou, and P. Heu, *Biochem. Pharmacol.* **20**, 1877–1882 (1971).
20. Y. C. Lee and O. H. Scherbaum, *Nature* **208**, 1350–1351 (1965).
21. K. A. Conklin and S. C. Chou, *Proc. Helminthol. Soc. Wash.* In press.
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.* **193**, 265–275 (1951).
23. R. Pearlman and O. Westergaard, *C. R. Trav. Lab. Carlsberg* **37**, 77–86 (1969).
24. S. Kurtz and R. E. Pearlman, *Can. J. Biochem.* **50**, 154–157 (1972).
25. K. A. Conklin, S. C. Chou, S. Ramanathan, and P. Heu, *Pharmacology* **4**, 91–101 (1970).
26. J. G. Olenick and F. E. Hahn, *Antimicrob. Agents Chemother.* **1**, 259–262 (1972).