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Inhibition of Deoxyribonucleic Acid Synthesis in Ehrlich Ascites Cells by Chloramphenicol

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SUMMARY

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The effect of D(−)-*threo*-chloramphenicol and a number of its analogues on protein, RNA, and DNA biosynthesis in isolated Ehrlich-Lettré mouse ascites cells was examined. The methylsulfonyl analogue (thiamphenicol) and the sulfamoyl analogue (Tevenel) inhibited DNA synthesis slightly in both the presence and absence of glucose. The methylthio analogue inhibited all three processes; glucose reversed the inhibition at lower concentrations of the analogue, indicating that inhibition was secondary to the inhibition of NADH oxidation. Chloramphenicol also inhibited all three processes; the inhibition of protein and RNA synthesis was reversed by glucose, but the inhibition of DNA synthesis was slightly greater in the presence of glucose. The inhibition of DNA synthesis by chloramphenicol was similar under aerobic and anaerobic conditions and was not the result of an effect on the [^{3}H]thymidine nucleotide pool. Inhibition required whole cells; it was slight with lysed cells, isolated nuclei, or purified DNA polymerases. The *N*-trifluoroacetyl analogue and the L(+)-*threo* isomer displayed patterns of inhibition of protein and DNA synthesis similar to that of chloramphenicol. The results are consistent with the possibility that chloramphenicol inhibits DNA synthesis in whole cells in the presence of glucose because the antibiotic undergoes metabolism, possibly reduction, in cells, and that a *p*-nitro group is important for this effect.

INTRODUCTION

Chloramphenicol has two well-established modes of action in mammalian cells. At low concentrations (below 100 μM) it inhibits mitochondrial protein synthesis (1–4), and at high concentrations (about 1 mM) it inhibits the oxidation of NADH (4–6). The actions of a number of chloramphenicol analogues have also been studied. The *p*-methylsulfonyl analogue (thiamphenicol) is as strong an inhibitor of mitochondrial protein synthesis as chloramphenicol (2, 7), but it is a weaker inhibitor of NADH oxidation (6). Chloramphenicol and this analogue cause a common, dose-related, reversible erythroid depression (8, 9) which probably results from the inhibition of mitochondrial protein synthesis (2, 10, 11), but only chloramphenicol

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causes the rare, usually irreversible aplastic anemia of unknown origin (8, 9, 12). Interest in other modes of action therefore continues.

Recently Yunis *et al.* (7) have reported that chloramphenicol is a much stronger inhibitor of DNA synthesis in human lymphoblastoid cells than its methylsulfonyl analogue. The concentrations of chloramphenicol and its methylsulfonyl analogue necessary to inhibit DNA synthesis (7, 13, 14) are about the same as those which inhibit NADH oxidation (5, 6). We previously showed that the inhibition of total protein synthesis in Ehrlich-Lettré ascites cells by chloramphenicol and its analogues results from inhibition of NADH oxidation along with a drop in cellular ATP levels (15); hence it is possible that the inhibition of DNA synthesis is also a secondary effect. We have therefore examined the relationship between the inhibition of DNA synthesis and that of NADH oxidation by chloramphenicol, its methylsulfonyl analogue, and some other analogues. In agreement with Yunis *et al.* (7), we have found that chloramphenicol inhibits DNA synthesis in mammalian cells and in addition have shown that the effect requires whole cells and is under some but perhaps not all conditions distinct from the inhibition of NADH oxidation.

METHODS

Materials. All chemicals were reagent grade where possible, and all solutions except nucleotides were sterilized. The following compounds were gifts: D(−)- and L(+)-*threo*-chloramphenicol, from Dr. M. E. Machamer of Parke, Davis; the *p*-methylthio and *p*-methylsulfonyl analogues, from Drs. C. W. Birkett and R. A. Cutler of Sterling-Winthrop; the *p*-sulfamoyl analogue (Tevenel), from Dr. C. E. Hoffman, du Pont; and the *N*-trifluoroacetyl analogue, from Dr. C. Hansch, Pomona College. [5-^{3}H]Uridine (29 Ci/mmole) was obtained from Amersham/Searle, and [*methyl*-^{3}H]thymidine (20 Ci/mmole), L-[*U*-^{14}C]leucine (325 mCi/mmole), and [5-^{3}H]dCTP (22.9 Ci/mmole), from New England Nuclear. Deoxyribonucleoside triphosphates were purchased from Sigma Chemical Company.

Cells. Ehrlich-Lettré hyperdiploid ascites cells (16) were obtained from Dr. T. D. Hauschka, Roswell Park Memorial Institute. They were maintained by intraperitoneal injection of 0.25 ml of ascites fluid containing 1.7×10^8 cells/ml in 8–10-week-old female Swiss-Webster mice and used 6 or 7 days after injection. At 5 days or earlier there were insufficient cells for experiments, and at 8 days or more the rate of DNA synthesis was lower than at 6 or 7 days.

Incubations. Cells were removed from mice, centrifuged at $125 \times g_{max}$ for 10 min, and washed once in sterile phosphate-buffered NaCl containing calcium and magnesium without antibiotics (17). Cells were incubated in triplicate in test tubes, in a volume of 0.2 ml at a concentration of 5×10^6/ml in Earle's medium (17) buffered with 27.5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.4, without phenol red (17). Glucose at 0.01 M and antibiotics were added as indicated. Incubations with radioactive precursors were carried out at 37° for 20 min in a shaking water bath at 110 oscillations/min. Anaerobic conditions were maintained where indicated by a constant stream of oxygen-free nitrogen. Linear incorporation for at least 1 hr was achieved by diluting the specific radioactivity of the usually highly radioactive precursor.

Macromolecular synthesis in whole cells. The precursors used were 0.41 μM [^{3}H]thymidine (7 Ci/mmole), 0.34 μM [^{3}H]uridine (7.7 Ci/mmole), and 15 μM [^{14}C]leucine (30 mCi/mmole) for DNA, RNA, and protein synthesis, respectively. For DNA or RNA synthesis, incorporation was stopped by adding 50 μl of ice-cold, saturated tetrasodium pyrophosphate, followed by 3 ml of ice-cold 5% trichloracetic acid containing 160 mg of thymidine or uridine per liter. The precipitate was collected on 2.4-cm glass fiber filters (grade 934AH, Reeve Angel), washed three times with 3 ml of ice-cold 5% trichloracetic acid and 1 ml of ice-cold water, and dried. Protein synthesis was stopped by adding 50 μl of ice-cold, saturated tetrasodium pyrophosphate, followed by 3 ml of 5% trichloracetic acid saturated with DL-leucine at room temperature. After heating at 90° for

15 min, followed by cooling, the precipitate was collected and washed as for DNA or RNA synthesis. Radioactivity was determined using 3 ml of a toluene-based scintillation fluid and a Nuclear-Chicago mark 1 scintillation spectrophotometer. The efficiency of counting was 8% for ^3H and 50% for ^{14}C.

DNA synthesis in cell lysates, isolated nuclei, and purified DNA polymerase. Cells were lysed, and DNA synthesis by cell lysates was determined by the method of Tseng and Goulian (18), except that dCTP was used as the precursor at 1 μCi/ml. Incubations of 0.1 ml with 4×10^7 lysed cells/ml were carried out at 37° for 20 min.

Nuclei were prepared by the method of Widnell and Tata (19), except for cell lysis. Cells were swollen in 0.01 M NaCl–1.5 mM $MgCl_2$–10 mM Tris-HCl, pH 7.4, at 0° for 10 min and disrupted with an Ultra-Turrax homogenizer (20). After disruption, sucrose was added to 0.25 M, and nuclei and cell debris were recovered at 750x*g* for 12 min. Nuclei were resuspended in 10 ml of 2.4 M sucrose–1 mM $MgCl_2$ (pH 7.4) and layered over 8 ml of 2.4 M sucrose–1 mM $MgCl_2$ (pH 7.4) in three tubes of a Beckman SW 41 rotor. After centrifugation at 38,000 $\times$ g_{av} for 1 hr, the nuclei were recovered from the interface and suspended in 1 ml of 0.25 M sucrose–1 mM $MgCl_2$, pH 7.4. They appeared free of whole cells and cell debris. Incubations were performed at 37° for 15 min in 0.075 ml of medium containing 140 μg of nuclear protein, 25 mM sucrose, 100 mM Tris-HCl (pH 8.1), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 1 mM dATP, 1 mM dGTP, 1 mM TTP, and 1 mCi of [5-^3H]dCTP (specific activity, 22.9 Ci/mmole). Incorporation was linear for at least 30 min and was determined as described for DNA synthesis in whole cells. Protein was determined by the method of Lowry *et al.* (21).

The effects of chloramphenicol and its methylthio analogue were also tested with isolated DNA polymerases α and β from rat thymus, using activated DNA template (22, 23). These experiments were kindly performed by Dr. Peter Penner, Department of Biochemistry, Memorial University of Newfoundland.

Previously incubated cells. DNA synthesis in cells was directly examined after prior labeling of the thymidine nucleotide pools as described by Scholtissek (24, 25). The thymidine nucleotide pools were labeled by incubating ascites cells at 4° for 30 min at 5 times the usual concentration in the standard medium containing 0.01 M glucose and 5 times the usual concentration of [^3H]thymidine. Cells were washed once in phosphate-buffered NaCl and incubated at 37° for 20 min in the standard medium with 0.01 M glucose and appropriate antibiotics. Incorporation of [^3H]- thymidine nucleotides into DNA was determined as described above.

The accumulation of [^3H]thymidine into the cellular nucleotide pools was determined with a 2-ml reaction mixture containing cells and [^3H]thymidine at the concentration and under the conditions of the preliminary incubation. The cells were then washed twice with phosphate-buffered NaCl and extracted with 0.1 ml of 0.5 N $HClO_4$ for 1 hr at 0°. The precipitate was removed, and the supernatant solution was neutralized with 0.25 volume of 2 N KOH to give a pH of about 7. After 2 hr at 0° to remove $KClO_4$, 25 μl of the supernatant solution were chromatographed on Whatman No. 1 paper with isobutyric acid–concentrated NH_4OH-H_2O (66:1:33, by volume) as solvent. Spots of TTP, TDP, TMP, and thymidine were first spotted as markers. The paper was cut, and radioactivity was determined using a toluene-based scintillation fluid.

RESULTS

Effects of chloramphenicol and its analogues on protein, RNA, and DNA biosynthesis. We have previously found that when Ehrlich-Lettré ascites cells are incubated in a buffered salts medium, chloramphenicol inhibits protein synthesis and glucose reverses the effect (15). We showed that the inhibition of protein synthesis results from inhibition of NADH oxidation and a drop in the level of cellular ATP; glucose, through glycolysis and the shuttle of reducing equivalents into mitochondria, restores cellular ATP levels, permitting protein synthesis (6, 15). By extension,

comparison of the effects of chloramphenicol and its isomers and analogues on any energy-requiring processes in whole cells in the presence and absence of glucose can be used to determine whether any inhibition is secondary to inhibition of energy metabolism.

The effects of chloramphenicol and several of its analogues, each at 2 mM, on DNA synthesis in ascites cells in the presence and absence of glucose and, from previous studies (15), on respiration in the absence of glucose, are shown in Table 1. Only chloramphenicol and the methylthio analogue were strongly inhibitory. The extent of inhibition of DNA synthesis and respiration in the absence of glucose was approximately parallel for all drugs except chloramphenicol, which inhibited DNA synthesis more than expected from its effect on respiration. Glucose reduced the inhibition of DNA synthesis by the methylthio analogue (Table 1). This is consistent with the inhibition being secondary to inhibition of energy metabolism. In contrast, glucose did not reduce the inhibition of DNA synthesis by chloramphenicol. Both the greater than expected inhibition of DNA synthesis and the lack of an effect of glucose suggested that chloramphenicol acts by a mechanism in addition to that of inhibiting respiration. This was examined in more detail by determining the effect of chloramphenicol and its methylthio analogue on the biosynthesis of protein, RNA, and DNA (Figs. 1 and 2). Comparison of Fig. 1A and B with Fig. 2A and B shows that the methylthio analogue was a stronger inhibitor of the biosynthesis of

TABLE 1

Effects of chloramphenicol and its analogues on DNA synthesis in the presence and absence of glucose, and on respiration in ascites cells

The inhibitors were present at 2 mM, and glucose, at 0.01 M. The control value for DNA synthesis was 1.9×10^3 cpm/10^6 cells. The results for respiration, measured in the absence of glucose, were from previous studies (15).

Compound	[^{3}H]Thymidine incorporation		Respiration, no glucose
	+ Glucose	− Glucose	
	% control		
Chloramphenicol	28.1	29.4	48.4
Methylthio analogue	63.7	20.9	18.8
Methylsulfonyl analogue	87.6	87.5	80.9
Sulfamoyl analogue	91.4	90.3	88.0

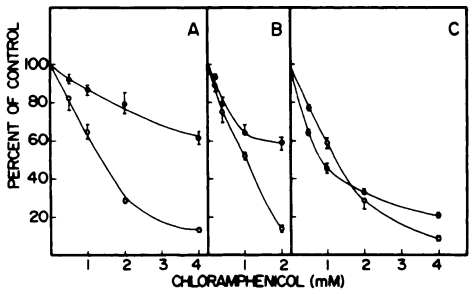

FIG. 1. *Effect of D-chloramphenicol on protein synthesis (A), RNA synthesis (B), and DNA synthesis (C) in Ehrlich-Lettré ascites cells in the presence and absence of glucose*

Cells were incubated and macromolecular synthesis was determined as described in METHODS in the presence (●) and absence (○) of 0.01 M glucose. The control values were: for protein synthesis, 4×10^3 cpm/10^6 cells (with and without glucose); for RNA synthesis, 5.5×10^2 cpm/10^6 cells (with and without glucose); and for DNA synthesis, 7×10^2 cpm/10^6 cells (with glucose) and 1.6×10^3 cpm/10^6 cells (without glucose). The points and bars show the means and standard errors for two or three experiments, each in triplicate. This is the case in all subsequent figures except where indicated.

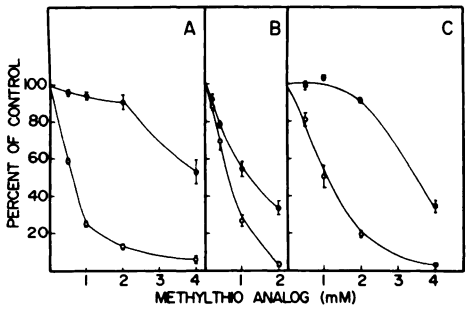

FIG. 2. *Effect of p-methylthio analogue on protein synthesis (A), RNA synthesis (B), and DNA synthesis (C) in Ehrlich-Lettré ascites cells in the presence and absence of glucose*

Cells were incubated and macromolecular synthesis was determined as described in METHODS in the presence (●) and absence (○) of 0.01 M glucose. The control values were similar to those of Fig. 1.

protein and DNA than chloramphenicol. Glucose reversed the inhibition to different extents in all cases. Although most of the inhibition of protein synthesis was reversed below an antibiotic concentration of 2 mM, the reversal was not as great for chloramphenicol. In contrast to its effects on protein and RNA synthesis, chloramphenicol at lower concentrations inhibited DNA synthesis at least as strongly as its methylthio analogue. Below 2 mM drug, glucose slightly increased the extent of inhibition by chloramphenicol, whereas it removed the inhibition by the methylthio analogue. Chloramphenicol inhibited DNA synthesis under both aerobic and anaerobic conditions in the presence of glucose (Fig. 3) but was less inhibitory to protein synthesis under both conditions.

Several particular features of the results should be noted. The methylthio analogue inhibited protein and RNA synthesis to about the same extent but inhibited DNA synthesis less. The incorporation of [^{3}H]thymidine into DNA was less in the presence of glucose than in its absence, as stated in the legend to Fig. 1. It was also found that the level of [^{3}H]TTP was lower in cells incubated in the presence of glucose, and this probably explains the apparently lower incorporation. In our earlier studies (15) a more complete reversal by glucose of the inhibition of protein synthesis by chloramphenicol was observed with 10–12-day-old ascites cells than with the 6- or 7-day-old cells used here. Repetition of the experiments with 11-day-old cells gave only 20% inhibition with 4 mM chloramphenicol in the presence of glucose, compared with 10% previously (15) and 40% in Fig. 1A.

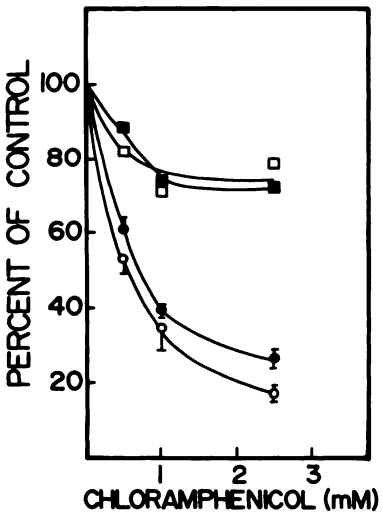

FIG. 3. *Effect of* D*-chloramphenicol on protein and DNA synthesis under aerobic and anaerobic conditions*

Cells were incubated and macromolecular synthesis was determined as described in METHODS in the presence of glucose. □ and ■, protein synthesis under anaerobic and aerobic conditions, respectively; ○ and ●, DNA synthesis under anaerobic and aerobic conditions, respectively. Control values were 1.2×10^4 and 1.3×10^4 cpm/10^6 cells for protein synthesis under aerobic and anaerobic conditions, respectively, and 1.4×10^3 and 1.6×10^3 cpm/10^6 cells for DNA synthesis under aerobic and anaerobic conditions, respectively.

Importance of the p-nitro group. From the results of the comparison of chloramphenicol and its three analogues, it is possible that a *p*-nitro group might be important for the inhibition of DNA synthesis. This was examined further with an analogue of chloramphenicol in which the *N*-dichloroacetyl group is replaced by a *N*-trifluoroacetyl group, and with the L isomer. Both are weaker inhibitors of NADH oxidation than chloramphenicol (6, 26). As shown in Fig. 4A and C, both are also weaker inhibitors of protein synthesis and glucose reverses the inhibition, as seen with chloramphenicol (Fig. 1A). Both the analogue and the isomer were weaker inhibitors of DNA synthesis (Fig. 4B and D, respectively). As with chloramphenicol, glucose increased the inhibition at lower concentrations of the drugs but decreased it at higher concentrations. The over-all pattern of these effect is therefore similar for all compounds with a *p*-nitro group.

Effects of chloramphenicol and its methylthio analogue on DNA synthesis in whole cells and cell-free systems. Chloramphenicol could inhibit DNA synthesis per se or one of the steps from thymidine to DNA. To examine the effect of chloramphenicol on DNA synthesis per se, the TTP pools of cells were first labeled in the absence of DNA synthesis at 4° and in the presence of glucose (normal ATP levels), and then the cells were incubated in the presence of antibiotics at 37°. Cells incu-

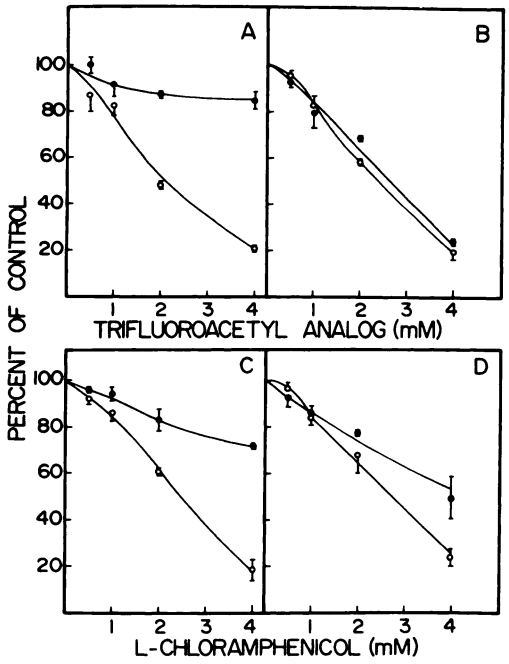

FIG. 4. *Effect of N-trifluoroacetyl analogue and L isomer of chloramphenicol on protein and DNA synthesis in the presence and absence of glucose*

Cells were incubated and macromolecular synthesis was determined as described in METHODS in the presence (●) and absence (○) of glucose. A and B. Effect of the *N*-trifluoroacetyl analogue on protein and DNA synthesis, respectively. C and D. Effect of the L isomer on the same respective processes. Control values were similar to Fig. 1.

bated at 4° for 30 min in the presence of glucose accumulated [^{3}H]thymidine as TTP (63.1%), TDP (12.8%), TMP (12.9%), and thymidine (11.2%). After washing, the cells were placed at 37° for 20 min in the presence of glucose and either chloramphenicol or its methylthio analogue. As shown in Fig. 5, chloramphenicol inhibited DNA synthesis, and the inhibition was greater than that caused by the methylthio analogue, especially at the lower concentrations of the drugs. It was also found that chloramphenicol did not significantly affect the labeling of the thymidine nucleotide pools or the activity of cytosolic thymidine kinase, measured as described by others (27, 28). It is concluded that chloramphenicol probably does not inhibit thymidine uptake or phosphorylation.

The possible site of action of chloramphenicol was examined further in cell-free DNA-synthesizing systems. The results of studies with cell lysates and isolated nuclei are shown in Fig. 6A and B, respec-

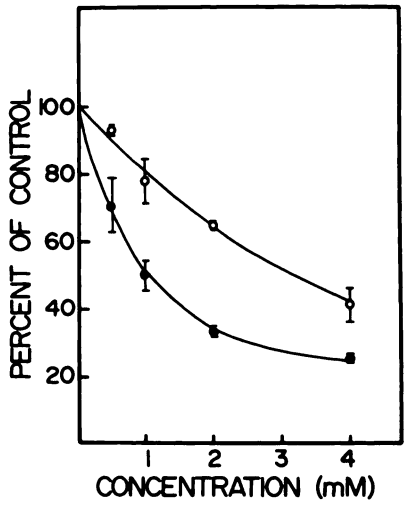

FIG. 5. *Effect of chloramphenicol and its p-methylthio analogue on DNA synthesis on cells with previously labeled TTP pool*

Cells were incubated with [^{3}H]thymidine and glucose at 4° for 30 min, washed, and then incubated again for 20 min at 37° in the presence of chloramphenicol (●) or its *p*-methylthio analogue (○). The control value was 1 × 10^3 cpm/10^6 cells. The results are the averages of two experiments.

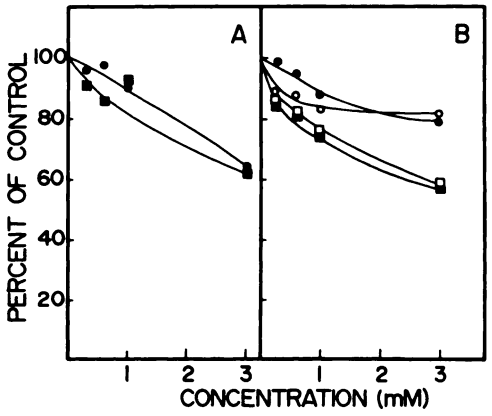

FIG. 6. *Effect of chloramphenicol and its p-methylthio analogue on DNA synthesis in cell lysates (A) and isolated nuclei (B)*

The experimental protocol is described in METHODS. The control values were 4 × 10^2 cpm/10^6 lysed cells (A) and 1.2 × 10^4 cpm/mg of nuclear protein (B). ○ and ●, chloramphenicol; □ and ■, *p*-methylthio analogue. In both cases the solid symbols indicate the presence of glucose.

tively. In neither case was there much inhibition. The methylthio analogue was more inhibitory than chloramphenicol in isolated nuclei. Glucose did not affect inhibition in isolated nuclei (Fig. 6B); its effect was not tested in cell lysates (Fig. 6A). A pattern of inhibition similar to that with isolated nuclei was seen with isolated DNA polymerase α of rat thymus, whereas neither compound inhibited DNA polymerase β. The effect of glucose was not tested with the isolated enzymes.

DISCUSSION

It has been well established that chloramphenicol inhibits mitochondrial protein synthesis (1–4) and NADH oxidation (4–6) in mammalian cells. It was further reported that chloramphenicol inhibits DNA synthesis (7, 13). Our results confirm this and further show that inhibition occurs only in whole cells. Although chloramphenicol does not act by inhibiting the formation of TTP, as shown by the preliminary incubation experiment, it is possible, although less likely, that it inhibits the formation of one of the precursors for DNA synthesis rather than DNA synthesis per se. It is not known why whole cells are required for inhibition, but a possibility is considered below.

The studies with chloramphenicol and its analogue indicate the complex nature of the inhibition of DNA synthesis. In the absence of glucose the inhibition of DNA synthesis is secondary to the inhibition of NADH oxidation by the methylthio analogue, and probably by chloramphenicol. In the presence of glucose, which restores cellular ATP levels (15), inhibition of DNA synthesis is reversed for the methylthio analogue but chloramphenicol is still inhibitory. The simplest hypothesis is that chloramphenicol can inhibit DNA synthesis in two ways: indirectly, by inhibiting NADH oxidation; and in another, as yet uncharacterized, manner. In the absence of glucose the former mechanism predominates. In the presence of glucose the inhibition due to the former mechanism is partly or completely reversed, as for protein and RNA synthesis and as seen with the methylthio analogue, and a new type of inhibition predominates. Reversal of inhibition of protein synthesis by glucose is not as great with chloramphenicol at lower concentrations as with the methylthio analogue, indicating that the inhibitory effect of chloramphenicol might be general although it is most striking for DNA synthesis.

The similar patterns of inhibition by D- and L-chloramphenicol and the trifluoroacetyl analogue and the lack of inhibition by the other analogues is consistent with the possibility that the *p*-nitro group is important for inhibition, as suggested earlier (13). Manyan *et al.* (13), examining a more extensive series of analogues, found that some analogues without a *p*-nitro group inhibited DNA synthesis in human lymphoblastoid cells incubated in the presence of glucose. As it is not known whether all these analogues act by the same mechanism, studies only with analogues cannot definitively demonstrate whether the *p*-nitro group is important for the inhibition of DNA synthesis by chloramphenicol itself. This must be resolved by determining the molecular mechanisms of the effect of chloramphenicol. In this regard, it is possible that the inhibition of DNA synthesis by chloramphenicol in the presence of glucose reflects metabolism of the drug in cells. Compounds containing a *p*-nitro group are known to undergo reduction in mammalian cells, including ascites cells, causing single-strand breaks in DNA (29). Chloramphenicol is reduced by microsomal nitroreductase (30, 31) and undergoes other, as yet unclarified, metabolic changes (32–34). Analogues without a *p*-nitro group, except perhaps ones with another electronegative group, would not be expected to undergo similar reduction. The trifluoroacetyl analogue and the L isomer are weaker inhibitors possibly because they are poorer substrates for metabolism or because cells are less permeable to them. If cellular metabolism is necessary, this would explain why chloramphenicol inhibits DNA synthesis more strongly than its methylthio analogue only in whole cells. However, the possibility that the cell lysate system lacks the step sensitive to chloramphenicol cannot be ex-

cluded. In whole cells the role of glucose could be to supply reducing equivalents (32). If reduction is important, inhibition in nitrogen would be expected to be greater than in air (29, 31, 33). This was not observed. Further studies are necessary to test the validity of this hypothesis.

REFERENCES

1. Kroon, A. M. (1965) *Biochim. Biophys. Acta*, 108, 275-284.
2. Freeman, K. B. (1970) *Can. J. Biochem.*, 48, 479-485.
3. Schatz, G. & Mason, T. L. (1974) *Annu. Rev. Biochem.*, 43, 51-88.
4. Beattie, D. S. (1971) *Sub-cell. Bichem.* 1, 1-23.
5. Freeman, K. B. & Haldar, D. (1968) *Can. J. Biochem.*, 46, 1003-1008.
6. Freeman, K. B. (1970) *Can. J. Biochem.*, 48, 469-478.
7. Yunis, A. A., Manyan, D. R. & Arimura, G. K. (1973) *J. Lab. Clin. Med.*, 81, 713-718.
8. Yunis, A. A. & Bloomberg, G. R. (1964) *Prog. Hematol.*, 4, 138-159.
9. Yunis, A. A. (1973) *Semin. Hematol.*, 10, 225-234.
10. Yunis, A. A., Smith, U. S. & Restrepo, A. (1970) *Arch. Intern. Med.*, 126, 272-275.
11. DeVries, H. & Kroon, A. M. (1970) *Biochim. Biophys. Acta*, 204, 531-541.
12. Keiser, G. & Buchegger, U. (1973) *Helv. Med. Acta*, 37, 265-278.
13. Manyan, D. R., Arimura, G. K. & Yunis, A. A. (1975) *Mol. Pharmacol.*, 11, 520-527.
14. Schwarz, M. A. & Firkin, B. S. (1976) *Med. J. Aust.*, 1, 687-690.
15. Haldar, D. & Freeman, K. B. (1968) *Can. J. Biochem.*, 46, 1009-1017.
16. Hauschka, T. S., Grinnell, S. T., Révész, L. & Klein, G. (1957) *J. Natl. Cancer Inst.*, 19, 13-31.
17. Martin, E. M., Malec, J., Coote, J. L. & Work, T. S. (1961) *Biochem. J.*, 80, 585-597.
18. Tseng, B. Y. & Goulian, M. (1975) *J. Mol. Biol.*, 99, 317-337.
19. Widnell, C. C. & Tata, J. R. (1964) *Biochem. J.*, 92, 313-317.
20. Freeman, K. B. (1965) *Biochem. J.*, 94, 494-501.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, 193, 265-275.
22. Moranelli, F. & Penner, P. E. (1975) *Can. J. Biochem.*, 53, 574-582.
23. Weissbach, A., Baltimore, D., Bollum, F., Gallo, R. & Korn, D. (1975) *Eur. J. Biochem.*, 59, 1-2.
24. Scholtissek. C. (1967) *Biochim. Biophys. Acta*, 145, 228-237.
25. Scholtissek, C. (1967) *Biochim. Biophys. Acta*, 145, 238-246.
26. Freeman, K. B. (1977) *Antimicrob. Agents Chemother.*, in press.
27. Kit, S., Leung, W.-C. & Trkula, D. (1973) *Arch. Biochem. Biophys.*, 158, 503-513.
28. Kit, S., Leung, W.-C. & Kaplan, L. A. (1973) *Eur. J. Biochem.*, 39, 43-48.
29. Olive, P. L. & McCalla, D. R. (1975) *Cancer Res.*, 35, 781-784.
30. Fouts, J. R. & Brodie, B. B. (1957) *J. Pharmacol. Exp. Ther.*, 119, 197-207.
31. Mason, R. P. & Holtzman, J. L. (1974) *Pharmacologist*, 16, 277.
32. Bonanomi, L., Della Bella, D. & Gazzaniga, A. (1975) *Pharmacol. Res. Commun.*, 7, 437-441.
33. Krishna, G. & Bonanomi, L. (1971) in *Drug Interactions* (Morselli, P. L., Garattini, S. & Cohen, S. N., eds.), pp. 173-180, Raven Press, New York.
34. Pohl, L. R., Reddy, B. G., Bonanomi, L. & Krishna, G. (1975) *Pharmacologist*, 17, 231.