ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1993, p. 106–110
0066-4804/93/010106-05$02.00/0

Vol. 37, No. 1

In Vitro Activities of Nucleoside Analog Antiviral Agents against Salmonellae

STEVEN J. SPERBER,[1]†* EVAN L. FEIBUSCH,[1] ALDO DAMIANI,[1]‡ AND MELVIN P. WEINSTEIN[1,2]

Departments of Medicine[1] and Pathology,[2] UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903-0019

Received 20 July 1992/Accepted 5 November 1992

Zidovudine (ZDV) has antibacterial activity against many members of the family *Enterobacteriaceae*, including *Salmonella* species, and may be responsible for a decrease in the frequency of salmonellosis in persons infected with human immunodeficiency virus (HIV). Other nucleoside analogs, such as didanosine (2′,3′-dideoxyinosine [ddI]) and zalcitabine (2′,3′-dideoxycytidine [ddC]), which have undefined anti-*Salmonella* activity, increasingly are being used in the treatment of HIV infection. To evaluate the anti-*Salmonella* activity of the antiviral agents ZDV, ddI, ddC, and acyclovir (ACV), we determined MICs for 39 nontyphoidal *Salmonella* blood isolates. ZDV (MIC for 50% of strains tested [MIC_{50}], 0.5 μg/ml; MIC range, 0.125 to 4 μg/ml) and ddI (MIC_{50}, 8 μg/ml; MIC range, 2 to 125 μg/ml) had concentration-dependent activity. Anti-*Salmonella* activity was not observed for ddC or ACV. Nine *Escherichia coli* blood isolates were inhibited by ZDV (MIC_{50}, 0.125 μg/ml; MIC range, 0.031 to 1 μg/ml) to a greater degree than they were by ddI (MIC_{50}, 62.5 μg/ml; MIC range, 31 to >62.5 μg/ml). Inoculum size affected susceptibility to ZDV and ddI for *Salmonella* and *E. coli* isolates. Resistance to ZDV or to ddI could be induced in vitro in *Salmonella* isolates, but cross-resistance was not observed. These results indicate that at concentrations achieved during the treatment of HIV infection, ZDV has activity against nontyphoidal salmonellae, although resistance can develop. ddI, ddC, and ACV at currently used dosages would not be expected to be effective in the prevention or treatment of *Salmonella* infections.

Nucleoside analogs, which have been under investigation for decades as antimicrobial agents (33), are the major class of antiviral compounds in use today. Zidovudine (ZDV; 3′-azido-2′,3′-dideoxythymidine) also has potent in vitro antibacterial activity against many members of the family *Enterobacteriaceae*, including *Salmonella* species (11, 17, 23, 31). Intracellular growth of *Salmonella typhimurium* within macrophages is inhibited by ZDV (13). Orally administered ZDV has been shown to be as effective in mice as ampicillin in the treatment of *Escherichia coli* ascending pyelonephritis and at least as effective as trimethoprim in systemic *E. coli* infections (17), and subcutaneous ZDV prevents lethal *Salmonella dublin* infection in calves (17). ZDV resistance, however, has been identified among *Salmonella* and *E. coli* strains exposed to ZDV in vitro (11, 21) and in vivo (17, 24, 31).

Recurrent bacteremia with nontyphoid strains of *Salmonella* is one of the criteria for the diagnosis of AIDS (3). Despite adequate antibacterial therapy, frequent relapses have been reported (32). It has been suggested that the recent decrease in frequency of human immunodeficiency virus (HIV)-associated *Salmonella* bacteremia may be attributable to the widespread use of ZDV (6, 13, 31). Increasingly, other nucleoside analogs, such as 2′,3′-dideoxyinosine (ddI; didanosine) and 2′,3′-dideoxycytidine (ddC; zalcitabine), which have undefined anti-*Salmonella* activity, are being used in the treatment of HIV infection.

We compared the in vitro activities of the three antiretroviral agents ZDV, ddI, and ddC and the nucleoside analog acycloguanosine (acyclovir [ACV]) against bacteremia-producing nontyphoid *Salmonella* species. After the observation that ZDV and ddI were active against *Salmonella* species, we determined the effect of the *Salmonella* and *E. coli* inoculum size on antibacterial activity. We also sought to extend previous observations regarding bacterial resistance to ZDV (17, 21, 22, 24, 31) by assessing cross-resistance after inducing resistance in vitro individually to ZDV and to ddI.

(This work was presented in part at the 92nd General Meeting of the American Society for Microbiology, New Orleans, La., 26 to 30 May 1992.)

MATERIALS AND METHODS

Antimicrobial agents. The antimicrobial agents used in this study were ZDV (no. A2169; Sigma Chemical Co., St. Louis, Mo.), ddC (no. D5782; Sigma), ddI (no. D0162; Sigma), and ACV (no. A4669; Sigma). Each was prepared in sterile distilled water as a stock solution at a concentration of 1,280 μg/ml, except for ddI, which was prepared at a concentration of 1,000 μg/ml.

Bacterial strains. The bacteria used in this study were 39 nontyphoid *Salmonella* blood isolates recovered in the Microbiology Laboratory from 33 patients at Robert Wood Johnson University Hospital from 1981 to August 1991 and 9 *E. coli* blood isolates recovered from 9 patients between April and August 1991. Eight of the *Salmonella* isolates were from five patients with documented HIV infections. None of the patients were receiving antiretroviral therapy at the time of salmonellosis. All isolates were stored at −70°C until retrieved for testing.

Determination of MICs. MIC determinations were carried

* Corresponding author.
† Present address: Infectious Diseases Section, Hackensack Medical Center, Johnson Hall—Room 121, Hackensack, NJ 07601.
‡ Present address: Department of Medicine, Thomas Jefferson University Hospital, Philadelphia, PA 19107.

out in vitro by the broth microdilution method (25); fresh antimicrobial agents were added to the wells daily. The standard inoculum size was 5×10^5 CFU/ml. The drugs and concentrations with which each *Salmonella* isolate was studied were as follows: ZDV, 0.004 to 4 μg/ml; ddC, 0.024 to 25 μg/ml; ddI, 0.12 to 125 μg/ml; and ACV, 0.6 to 640 μg/ml. The concentrations of each drug were prepared in serial twofold dilutions.

Effect of inoculum size. The effect of inoculum size on the activity of ZDV and ddI was characterized by using nine *Salmonella* blood isolates and nine *E. coli* blood isolates. Cultures were prepared in supplemented Mueller-Hinton broth (Oxoid Limited, Basingstoke, Hampshire, England) and allowed to grow to the turbidity of a 0.5 McFarland standard (5×10^7 CFU/ml). The inoculum was then diluted in 10-fold steps to reach a final concentration of 5×10^3 CFU/ml. Each of these sequential 10-fold dilutions was then tested for susceptibility to ZDV and ddI as per the broth microdilution method described above, except that the maximum ddI concentration was 62.5 μg/ml.

In vitro resistance study. An in vitro study to induce resistance individually to ZDV and to ddI was carried out in cation-supplemented Mueller-Hinton broth by using several of the previously studied *Salmonella* isolates at an inoculum size of 5×10^5 CFU/ml. The test strain initially was exposed to subinhibitory concentrations (four- to five-fold below the MIC) of either ZDV or ddI and allowed to grow for 16 to 24 h at 37°C. The resulting growth was then serially subcultured into fresh cation-supplemented Mueller-Hinton broth that contained double the previous concentration of the antimicrobial agent, such that seven isolates were exposed to up to 16 μg of ZDV per ml and two isolates were exposed to up to 125 μg of ddI per ml. The ZDV and ddI MICs for the resulting postexposure growth, as well as for the original isolate, were then determined by using concentrations of ZDV of up to 16 μg/ml and of ddI of up to 250 μg/ml.

RESULTS

Activity of ZDV, ddC, ddI, and ACV against *Salmonella* isolates. At the concentrations tested, ZDV and ddI had concentration-dependent inhibitory activity against all 39 *Salmonella* isolates (Fig. 1). The MIC for 50% of strains tested (MIC_{50}) of ZDV was 0.5 μg/ml, and the MIC_{50} of ddI was 8 μg/ml (Table 1). There was no correlation between degree of susceptibility to ZDV and degree of susceptibility to ddI. For example, the isolate least susceptible to ddI (MIC, 125 μg/ml) had a ZDV MIC of 1 μg/ml. The isolate least susceptible to ZDV (MIC, 4 μg/ml) had a ddI MIC of 16 μg/ml. Inhibitory activity against any of the 39 *Salmonella* isolates by up to 25 μg of ddC per ml or 640 μg of ACV per ml was not noted. Against several of the isolates, concentrations of ddC as high as 2,548 μg/ml or concentrations of ACV as high as 2,560 μg/ml were used, and in all cases no inhibitory effect was observed.

Effect of inoculum size of salmonellae and *E. coli* on antibacterial activity of ZDV and ddI. For the nine *Salmonella* isolates studied, the inhibitory effects of ZDV and ddI were inversely related to the size of the bacterial inoculum (Table 2). Against 5×10^7 CFU/ml, ZDV at 4 μg/ml had no measurable inhibitory effect, whereas against 5×10^6 CFU/ml the same ZDV concentration inhibited six of nine *Salmonella* isolates. At an inoculum size of 5×10^5 CFU/ml or smaller, all *Salmonella* isolates were inhibited by 4 μg of ZDV per ml. Similarly, ddI at 62.5 μg/ml inhibited all of the *Salmonella* isolates when the inoculum size was smaller than or equal to 5×10^4 CFU/ml, but none of the isolates were inhibited when the inoculum size was 5×10^6 CFU/ml or larger (Fig. 2).

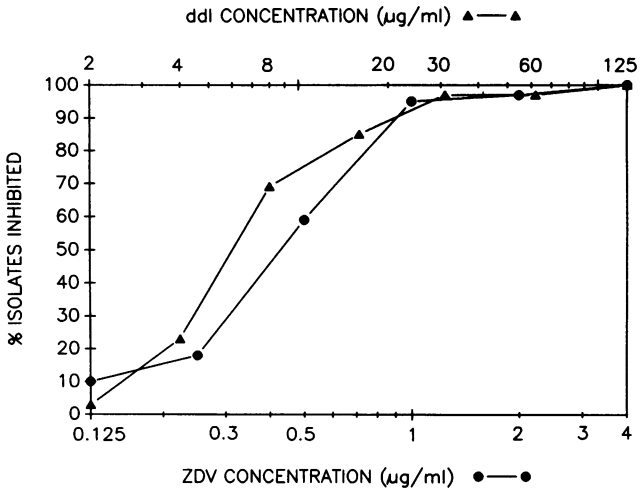

FIG. 1. Inhibition of 39 nontyphoid *Salmonella* isolates by ZDV or ddI.

ZDV was also active (MIC_{50}, 0.125 μg/ml) against the nine *E. coli* isolates studied (Table 1). ddI was less active against the *E. coli* isolates (MIC_{50}, 62.5 μg/ml) than it was against the *Salmonella* isolates (Table 1). Increasing the size of the *E. coli* inoculum resulted in decreased inhibitory activity of ZDV and ddI (Fig. 2); the magnitude of these differences, however, appeared to be less for ddI than for ZDV because of its decreased activity against the *E. coli* isolates at the concentrations studied (Table 2).

In vitro resistance. By passaging *Salmonella* isolates, initially susceptible to ZDV and ddI, in serially higher concentrations of each drug, several resistant strains were isolated (Table 3). The ZDV-resistant isolates remained susceptible to ddI. Similarly, the ddI-resistant isolates remained susceptible to ZDV.

DISCUSSION

The antimicrobial spectrum of activity of the nucleoside analog agents ZDV, ddI, ddC, and ACV with regard to their antiviral activity has been well described (2, 5, 10). We evaluated these agents for activity against nontyphoid *Salmonella* blood isolates and observed that only ZDV was active against most *Salmonella* isolates (MIC range, 0.125 to 4 μg/ml) at concentrations achievable in plasma (0.4 to 1.1

TABLE 1. Activity of nucleoside analog antiviral agents against 39 *Salmonella* and 9 *E. coli* isolates

Organism (*n*)[a]	Drug	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
Salmonellae (39)	ZDV	0.5	1	<0.125–4
	ddI	8	31	2–125
	ddC	>25	>25	>25
	ACV	>640	>640	>640
E. coli (9)	ZDV	0.125		0.031–1
	ddI	62.5		31–>62.5

[a] *n*, number of isolates tested.

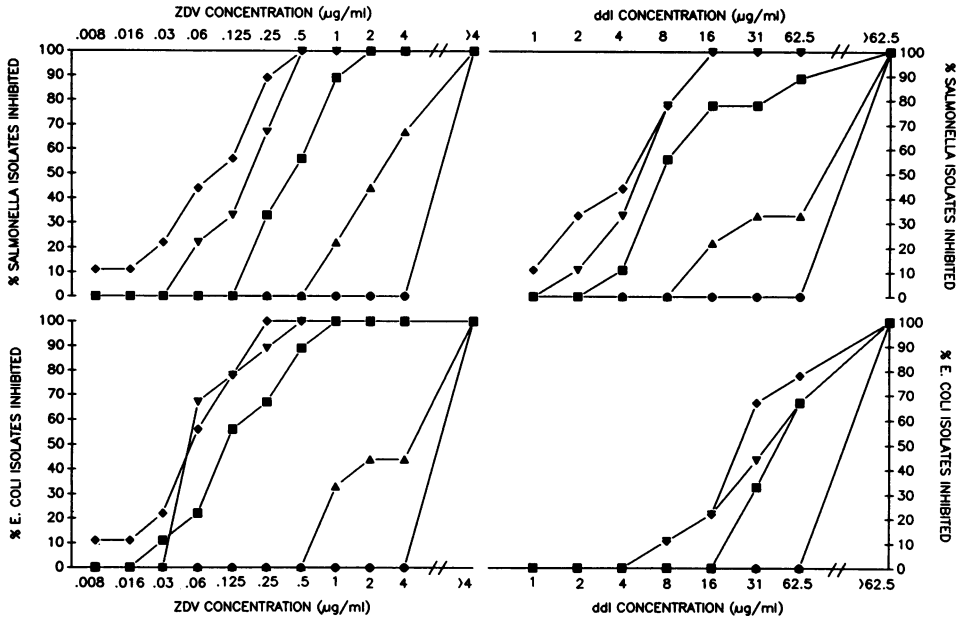

FIG. 2. Effect of *Salmonella* and *E. coli* inoculum size on inhibitory activities of ZDV and ddI. Inoculum concentrations (CFU per milliliter) for nine *Salmonella* and nine *E. coli* isolates were 5×10^3 (◆), 5×10^4 (▼), 5×10^5 (■), 5×10^6 (▲), and 5×10^7 (●).

μg/ml) with the dosages currently recommended for the treatment of HIV infection (9, 14, 34). These results are consistent with ZDV MICs for ZDV-naive *Salmonella* strains of 0.03 to 2 μg/ml as reported by others (11, 17, 31). ddI, which also inhibited all *Salmonella* isolates studied, required considerably higher drug concentrations (MIC range, 2 to 125 μg/ml). For the most part, these concentrations are greater than peak ddI levels (1.5 to 2.3 μg/ml) achievable with currently used oral dosages (7, 12). Activity against salmonellae relative to activity against HIV was similar for ZDV and ddI. In contrast, ddC, the most potent of these agents against HIV in vitro (2), had no measurable activity against any of the *Salmonella* isolates at 25 μg/ml, a concentration many times greater than that necessary to inhibit HIV in vitro (2) and much higher than peak levels achieved with oral dosages of the drug (20). ACV at concentrations of 640 μg/ml did not inhibit any of the *Salmonella* isolates. This concentration is approximately 1,000-fold greater than peak levels achieved after oral administration of dosages effective in the treatment of herpes simplex virus infections (8).

ZDV had activity against the *E. coli* blood isolates that was similar (MIC range, 0.031 to 1 μg/ml) to that against the *Salmonella* blood isolates. These results are consistent with previously reported ZDV MICs of 0.0025 to 1 μg/ml for ZDV-naive *E. coli* species (11, 17). ddI activity against the *E. coli* isolates (MIC range, 31 to >62.5 μg/ml) was poorer than that against the *Salmonella* isolates, especially at lower concentrations and with larger bacterial inocula. Beskid et al. reported that 2'3'-dideoxyadenosine, a prodrug with the same active product as ddI, has activity against several *E. coli* strains at 63 to 500 μg/ml (1). An effect of *Salmonella* and *E. coli* inoculum size on the MICs of ZDV and ddI was observed, as has previously been noted with a variety of antibacterial agents against a number of bacterial species (35).

Nucleoside analogs must be converted to triphosphate moieties for antiviral activity, necessitating anabolic phosphorylation catalyzed by specific enzymes (10, 36). The differential antibacterial activities of the nucleoside analogs may be due, at least in part, to differences in activity of the enzyme necessary for the initial phosphorylation to an active drug. In mammalian cells, ZDV is initially phosphorylated to the monophosphate by thymidine kinase. *E. coli* and *Salmonella* species possess thymidine kinase (30). Mono-, di-, and triphosphate derivatives of ZDV have been found in *E. coli* exposed to ZDV (11). Of these, ZDV-triphosphate has the

TABLE 2. Effect of *Salmonella* and *E. coli* inoculum size on ZDV and ddI antibacterial activity[a]

Organism	Drug	MIC_{50} (μg/ml) at inoculum concn (CFU/ml) of:				
		5×10^3	5×10^4	5×10^5	5×10^6	5×10^7
Salmonellae	ZDV	0.125	0.125	0.5	4	>4
	ddI	8	8	8	>62.5	>62.5
E. coli	ZDV	0.0625	0.0625	0.125	>4	>4
	ddI	31	62.5	62.5	>62.5	>62.5

[a] Nine nontyphoid *Salmonella* and nine *E. coli* isolates were studied.

TABLE 3. Susceptibilities of *Salmonella* isolates grown in ZDV- or ddI-supplemented broth

Growth condition (*n*)[a]	Mean MIC (μg/ml)	
	ZDV	ddI
Broth only[b] (7)	0.5	4
ZDV[c] (7)	>16	4
ddI[d] (2)	1	>250

[a] *n*, number of isolates.
[b] Grown in cation-supplemented Mueller-Hinton broth only.
[c] Grown in increasing concentrations of ZDV of up to 16 μg/ml.
[d] Grown in increasing concentrations of ddI of up to 125 μg/ml.

greatest antibacterial activity (11). ddI is converted in cells to ddI-monophosphate by 5′-nucleotidase or deoxyguanosine kinase (15, 36). 5′-Nucleotidase activity has also been associated with *E. coli* (26) and to a more variable degree with strains of *Salmonella* (19, 27). ddC undergoes initial phosphorylation by deoxycytidine kinase (4, 36). Deoxycytidine and deoxyguanosine kinase activities have not been detected for *E. coli* or salmonellae (16, 28, 29). The monophosphorylation of ACV to ACV-monophosphate requires virally specified thymidine kinase. Cellular thymidine kinase has been demonstrated to be inactive on ACV (10), although cellular 5′-nucleotidase is also capable of phosphorylating ACV to a small degree if large concentrations of the drug are present (10, 18).

None of the *Salmonella* strains used in our study were isolated from patients receiving antiretroviral therapy. *Salmonella* and *E. coli* isolates obtained from ZDV-naive patients have previously been shown to be susceptible to ZDV in vitro (24, 31). We and others (11, 21, 22) have been able to induce resistance in *Salmonella* species to ZDV in vitro. ZDV-resistant *E. coli* organisms have been detected in ZDV-treated mice with *E. coli* pyelonephritis (17). Recently, ZDV-resistant *E. coli* and *Salmonella* isolates have been recovered from patients treated with ZDV (24, 31). Acquired ZDV resistance of salmonellae and *E. coli* has been shown to result from loss of thymidine kinase activity (11, 21, 22, 24). We were also able to induce resistance in *Salmonella* isolates in vitro to ddI. That the development of resistance in these bacteria results from nucleoside-specific changes is supported by the observations that the ZDV-resistant *Salmonella* isolates were not cross-resistant to ddI and that the ddI-resistant *Salmonella* isolates were not resistant to ZDV.

These results suggest that ZDV, which has activity against salmonellae within macrophages (13), may play a role in the suppression of salmonellosis in patients infected with HIV, although microbial resistance can develop. Despite the fact that cross-resistance was not demonstrated, ddC and ddI at currently used dosages would not be expected to have clinical usefulness in the treatment or prevention of *Salmonella* infections. If the decreased incidence of salmonellosis in patients with HIV infection is attributable to the antibacterial activity of ZDV, a rise in *Salmonella* infections might be expected as alternative antiviral therapies are employed.

ACKNOWLEDGMENTS

We thank Karen Jones and Judy Rothberg for technical assistance.

REFERENCES

1. **Beskid, G., B. Eskin, R. Cleeland, J. Siebelist, A. J. Cappetta, A. D. Hill, and R. H. Geiger.** 1981. Antibacterial activity of 2′,3′-dideoxyadenosine in vivo and in vitro. Antimicrob. Agents Chemother. **19:**424–428.
2. **Broder, S.** 1990. Antiretroviral therapy in AIDS. Ann. Intern. Med. **113:**604–618.
3. **Centers for Disease Control.** 1987. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. Morbid. Mortal. Weekly Rep. **36**(Suppl. 1S):1S–15S.
4. **Cooney, D. A., M. Dalal, H. Mitsuya, J. B. McMahon, M. Nadkarni, J. Balzarini, S. Broder, and D. G. Johns.** 1986. Initial studies on the cellular pharmacology of 2′,3′-dideoxycytidine, an inhibitor of HTLV-III infectivity. Biochem. Pharmacol. **35:**2065–2068.
5. **Dahlberg, J. E., H. Mitsuya, S. B. Blam, S. Broder, and S. A. Aaronson.** 1987. Broad spectrum antiretroviral activity of 2′,3′-dideoxynucleosides. Proc. Natl. Acad. Sci. USA **86:**2469–2473.
6. **Dellamonica, P., E. Bernard, C. Pradier, J. Durant, H. Etesse-Carsenti, P. Elbaze, V. Mondain, and M. Carles.** 1991. AZT—does it prevent occurrence of salmonelloses in AIDS patients? J. Intern. Med. **229:**103.
7. **Dolin, R., J. S. Lambert, G. D. Morse, R. C. Reichman, C. S. Plank, J. Reid, C. Knupp, C. McLaren, and C. Pettinelli.** 1990. 2′,3′-Dideoxyinosine in patients with AIDS or AIDS-related complex. Rev. Infect. Dis. **12:**S540–S551.
8. **Douglas, J. M., C. Critchlow, J. Benedetti, G. J. Mertz, J. D. Connor, M. A. Hintz, A. Fahnlander, M. Remington, C. Winter, and L. Corey.** 1984. A double-blind study of oral acyclovir for suppression of recurrences of genital herpes simplex virus infection. N. Engl. J. Med. **310:**1551–1556.
9. **Drew, R. H., S. Weller, H. A. Gallis, K. A. R. Walmer, J. A. Bartlett, and M. R. Blum.** 1989. Bioequivalence assessment of zidovudine (Retrovir) syrup, solution, and capsule formulations in patients infected with human immunodeficiency virus. Antimicrob. Agents Chemother. **33:**1801–1803.
10. **Elion, G. B.** 1986. History, mechanism of action, spectrum and selectivity of nucleoside analogs, p. 118–137. *In* J. Mills and L. Corey (ed.), Antiviral chemotherapy: new directions for clinical application and research. Elsevier, New York.
11. **Elwell, L. P., R. Ferone, G. A. Freeman, J. A. Fyfe, J. A. Hill, P. H. Ray, C. A. Richards, S. C. Singer, V. B. Knick, J. L. Rideout, and T. P. Zimmerman.** 1987. Antibacterial activity and mechanism of action of 3′-azido-3′-deoxythymidine (BW A509U). Antimicrob. Agents Chemother. **31:**274–280.
12. **Hartman, N. R., R. Yarchoan, J. M. Pluda, R. V. Thomas, K. S. Marczyk, S. Broder, and D. G. Johns.** 1990. Pharmacokinetics of 2,3′-dideoxyadenosine and 2′,3′-dideoxyinosine in patients with severe human immunodeficiency virus infection. Clin. Pharmacol. Ther. **47:**647–654.
13. **Herrmann, J. L., and P. H. Lagrange.** 1992. Intracellular activity of zidovudine (3′-azido-3′-deoxythymidine, AZT) against *Salmonella typhimurium* in the macrophage cell line J774-2. Antimicrob. Agents Chemother. **36:**1081–1085.
14. **Hochster, H., D. Dieterich, S. Bozzette, R. C. Reichman, J. D. Connor, L. Liebes, R. L. Sonke, S. A. Spector, F. Valentine, C. Pettinelli, and D. D. Richman.** 1990. Toxicity of combined ganciclovir and zidovudine for cytomegalovirus disease associated with AIDS. Ann. Intern. Med. **113:**111–117.
15. **Johnson, M. A., and A. Fridland.** 1989. Phosphorylation of 2′,3′-dideoxyinosine by cytosolic 5′-nucleotidase of human lymphoid cells. Mol. Pharmacol. **36:**291–295.
16. **Karlstrom, O., II.** 1970. Inability of *Escherichia coli* B to incorporate added deoxycytidine, deoxyadenosine, and deoxyguanosine into DNA. Eur. J. Biochem. **17:**68–71.
17. **Keith, B. R., G. White, and H. R. Wilson.** 1989. In vivo efficacy of zidovudine (3′-azido-3′-deoxythymidine) in experimental gram-negative-bacterial infections. Antimicrob. Agents Chemother. **33:**479–483.
18. **Keller, P. M., S. A. McKee, and J. A. Fyfe.** 1985. Cytoplasmic 5′-nucleotidase catalyzes acyclovir phosphorylation. J. Biol. Chem. **15:**8664–8667.
19. **Kier, L. D., R. Weppelman, and B. N. Ames.** 1977. Resolution and purification of three periplasmic phosphatases of *Salmonella typhimurium*. J. Bacteriol. **130:**399–410.
20. **Klecker, R. W., Jr., J. M. Collins, R. C. Yarchoan, R. Thomas, N. McAtee, S. Broder, and C. E. Myers.** 1988. Pharmacokinetics of 2′,3′-dideoxycytidine in patients with AIDS and related disorders. J. Clin. Pharmacol. **28:**837–842.
21. **Lewin, C. S., R. Allen, and S. G. B. Amyes.** 1990. Zidovudine-resistance in *Salmonella typhimurium* and *Escherichia coli*. J. Antimicrob. Chemother. **25:**706–708.
22. **Lewin, C. S., R. A. Allen, and S. G. B. Amyes.** 1990. Mechanisms of zidovudine resistance in bacteria. J. Med. Microbiol. **33:**235–238.
23. **Lewin, C. S., and S. G. B. Amyes.** 1989. Conditions required for the antibacterial activity of zidovudine. Eur. J. Clin. Microbiol. Infect. Dis. **8:**737–741.
24. **Lewin, C. S., B. Watt, R. Paton, and S. G. B. Amyes.** 1990. Isolation of zidovudine resistant *Escherichia coli* from AIDS patients. FEMS Microbiol. Lett. **70:**141–144.
25. **National Committee for Clinical Laboratory Standards.** 1990.

Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically—second edition; approved standard. NCCLS document M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.

26. **Neu, H. C.** 1967. The 5′-nucleotidase of *Escherichia coli*. J. Biol. Chem. **242:**3896–3904.
27. **Neu, H. C., and J. Chou.** 1967. Release of surface enzymes in *Enterobacteriaceae* by osmotic shock. J. Bacteriol. **94:**1934–1945.
28. **Neuhard, J.** 1968. Pyrimidine nucleotide metabolism and pathways of thymidine triphosphate biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **96:**1519–1527.
29. **Neuhard, J., and P. Nygaard.** 1987. Purines and pyrimidines, p. 445–473. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
30. **Saito, H., H. Tomioka, and S. Ohkido.** 1985. Further studies on thymidine kinase: distribution pattern of the enzyme in bacteria. J. Gen. Microbiol. **131:**3091–3098.
31. **Salmon, D., P. Detruchis, C. Leport, E. Bouvet, D. Karam, M.-C. Meyohas, J. P. Coulaud, and J.-L. Vilde.** 1991. Efficacy of zidovudine in preventing relapses of *Salmonella* bacteremia in AIDS. J. Infect. Dis. **163:**415–416.
32. **Sperber, S. J., and C. J. Schleupner.** 1987. Salmonellosis during infection with human immunodeficiency virus. Rev. Infect. Dis. **9:**925–934.
33. **Suhadolnik, R. J.** 1970. Nucleoside antibiotics, p. 96–122, 271–297. John Wiley and Sons, New York.
34. **Surbone, A., R. Yarchoan, N. McAtee, M. R. Blum, M. Maha, J.-P. Allain, R. V. Thomas, H. Mitsuya, S. N. Lehrman, M. Leuther, J. M. Pluda, F. K. Jacobsen, H. A. Kessler, C. E. Myers, and S. Broder.** 1988. Treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex with a regimen of 3′-azido-2′,3′-dideoxythymidine (azidothymidine or zidovudine) and acyclovir. Ann. Intern. Med. **108:**534–540.
35. **Tilton, R. C., L. Liberman, and E. H. Gerlach.** 1973. Microdilution antibiotic susceptibility test: examination of certain variables. Appl. Microbiol. **26:**658–665.
36. **Yarchoan, R., H. Mitsuya, C. E. Myers, and S. Broder.** 1989. Clinical pharmacology of 3′-azido-2′,3′-dideoxythymidine (zidovudine) and related dideoxynucleosides. N. Engl. J. Med. **321:**726–737.