ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1978, p. 737-742
0066-4804/78/0014-0737$02.00

Vol. 14, No. 5
Printed in U.S.A.

Effect of Two Cancer Chemotherapeutic Agents on the Antibacterial Activity of Three Antimicrobial Agents

MARCIA R. MOODY,* MAUREEN J. MORRIS, VIOLA MAE YOUNG, LEMUEL A. MOYÉ III, STEPHEN C. SCHIMPFF, AND PETER H. WIERNIK

Baltimore Cancer Research Program, National Cancer Institute, and Research Microbiology and Infections Research Sections and Clinical Medicine Branch of the University of Maryland Hospital, Baltimore, Maryland 21201

Received for publication 16 August 1978

Cancer chemotherapeutic agents and antibacterial antibiotics are often given concomitantly. Daunorubicin, cytosine arabinoside, and three antibiotics (gentamicin, amikacin, and ticarcillin) were tested individually and in combinations to determine their antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli.* These cytotoxic agents are commonly employed in the therapy of acute nonlymphocytic leukemia for remission induction therapy, and these antimicrobial agents are used in infection therapy. The maximum concentrations of the two cytotoxic drugs were chosen to be twice the known peak plasma levels of commonly employed dosage schedules. Neither of the cancer chemotherapeutic agents, alone or in combination, demonstrated bactericidal activity at the levels tested. However, in the presence of these agents, the antimicrobial activity of gentamicin and amikacin, although not that of ticarcillin, was depressed for 11 of 15 *K. pneumoniae* strains and 8 of 15 *P. aeruginosa* strains, but for none of the strains of *E. coli.* This level of decreased activity occasionally resulted in a minimal inhibitory concentration of the tested aminoglycoside well above the standard serum levels. Daunorubicin was more likely to antagonize gentamicin than was cytosine arabinoside.

Patients with advanced cancer, especially those with acute leukemia who have been rendered granulocytopenic by their disease or therapy, are unusually susceptible to infection (2, 5, 9, 13, 21). Gram-negative bacilli are the etiological agents in most of these infections, i.e., 70% (13) to 80% (2); *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* are predominant among these causative agents (2, 21). Empiric antimicrobial regimens, often combinations of aminoglycosides, penicillin, and/or cephalosporin, directed against the predominant infectious agents are often used therapeutically in granulocytopenic patients (8, 11, 17, 20, 25). Certain of these combinations have been shown to be synergistic (12, 19, 22-24) or antagonistic (14) in vitro.

A possible influence on infectious microbial flora of patients with neoplasms is the cytotoxic agents used in their chemotherapeutic regimens. Methotrexate, cytosine arabinoside (AraC), 6-mercaptopurine, and cyclophosphamide have all been shown to have antimicrobial activity against *K. pneumoniae* and *E. coli,* but not against *P. aeruginosa* (7); methotrexate has also been shown to have activity against group A streptococci (16). Whereas such preferential inhibition of patient flora by cytotoxic agents might help to explain why an unaffected microorganism such as *P. aeruginosa* is associated with a high infection rate, the fact that inhibitory concentrations of these drugs in vitro were higher than levels generally achievable in the blood after infusion (3, 4, 10) should preclude this possibility. Therefore, the current study was undertaken, first to determine if two cytotoxic agents commonly used for the treatment of leukemia exhibit antimicrobial activity at readily achievable serum levels when tested against those bacteria that most frequently cause infection in granulocytopenic cancer patients, and second to determine whether these agents exert any effect on the antimicrobial activity of some of the antibiotics most often used to treat infections in these patients.

MATERIALS AND METHODS

Bacteria. Microorganisms in this study were recovered from clinical specimens collected from patients at the Baltimore Cancer Research Center. Fifteen strains each of *P. aeruginosa* and *K. pneumoniae* and 14 strains of *E. coli* were used as test organisms. To ascertain the effects of the cytotoxic agents and the antibiotics on bacterial strains of varying suscep-

tibilities, test strains were selected which ranged from susceptible to resistant as determined by disk susceptibility testing.

Cancer chemotherapeutic agents and antibiotics. Based on their current utilization at the Baltimore Cancer Research Center, the cytotoxic agents daunorubicin (DNR) and AraC, in the injectable form used for patient therapy, were selected to be tested individually and in combination with each other for their antimicrobial activity against the test bacteria. In addition, DNR and AraC were each combined with three antibiotics (amikacin, gentamicin, and ticarcillin) commonly used in therapeutic regimens to determine their effect on the antimicrobial activity of these antibiotics.

Susceptibility testing methods. Minimal inhibitory concentrations (MICs) of the antibiotics and/or chemotherapeutic agents were determined by the microtiter technique of MacLowry et al. (15). Combined activity of an antibiotic and a chemotherapeutic agent was evaluated by a checkerboard titration technique. Serial twofold dilutions of the antimicrobial agents in volumes of 50 μl per well were made in Mueller-Hinton broth. The concentration of gentamicin and amikacin ranged from 96 to 0.48 μg/ml, and that of ticarcillin ranged from 6,400 to 3.13 μg/ml. Serial twofold dilutions of DNR and AraC were prepared in Mueller-Hinton broth in test tubes (17 by 100 mm); concentrations of the DNR were 4 to 0.125 μg/ml, and those of AraC were 3.2 to 0.1 μg/ml. The various dilutions of these cytotoxic agents were then added in constant volumes of 25 μl of the previously serially diluted antibiotics.

Bacteria that had been grown overnight in Mueller-Hinton broth at 37°C were adjusted to a McFarland no. 3 standard, and 25 μl of the resultant suspension was added to each well. Those control wells that received only antimicrobial agents or cancer chemotherapeutic agents and bacterial suspension were adjusted to a final volume of 100 μl by the addition of Mueller-Hinton broth. The final concentrations of the antimicrobial agents were: gentamicin and amikacin, 24 to 0.011 μg/ml; ticarcillin, 1,600 to 0.75 μg/ml. Final concentrations of DNR were 0.8 to 0.025 μg/ml and of AraC, 1 to 0.032 μg/ml. The highest concentrations of the cytotoxic agents whenever possible were based upon achievable blood levels (1, 10).

The microtiter plates then were rotated on a flat surface to ensure that adequate mixing of the contents had occurred, sealed with a self-adhesive sheet, and incubated at 37°C for 24 h. The lowest concentration of combined agents which prevented visible turbidity was recorded as the MIC.

To compare the antibacterial effects of the antibiotic-chemotherapeutic agent combination on organisms of varying susceptibilities to the antibiotic alone, the fractional inhibitory concentration (FIC) of drug against each organism was determined as defined by Elion et al. (6). The FIC is the ratio of the MIC of a drug in combination to the MIC of the drug alone. When the FIC is equal to or less than 0.25, synergy is suggested, and when the FIC is equal to or greater than 4, antagonism is suggested.

Statistical analysis. Linear regression analyses were done by a computerized statistical program. The regression coefficients for the cytotoxic agents and the antibiotics were compared by the test statistic: $t = (b_1 - b_2)/^2(S_{b_1} - {}_{b_2})$. A value equal to or less than 0.05 was considered a significant difference.

RESULTS

Antimicrobial activity of cancer chemotherapeutic agents. DNR and AraC, either alone or in combination, exerted no antimicrobial activity against *K. pneumoniae, E. coli,* or *P. aeruginosa* at the highest levels tested.

Antimicrobial activity of antibiotics in the presence of AraC and DNR. The MICs of gentamicin against the strains of *E. coli* ranged from 0.09 to 6 μg/ml; those against *P. aeruginosa,* from 0.011 to >24 μg/ml; and those against *K. pneumoniae,* from 0.023 to >24 μg/ml. MICs of amikacin against the three species ranged from 6 to >24 μg/ml, 0.75 to >24 μg/ml, and 1.5 to >24 μg/ml, respectively. Ticarcillin MICs ranged from 3.12 to >1,600 μg/ml for *E. coli,* from 12.5 to 1,600 μg/ml for *P. aeruginosa,* and from 200 to >1,600 μg/ml for *K. pneumoniae.*

The effect of the addition of AraC and DNR on the MICs of gentamicin against the three bacterial species is shown in Fig. 1. Both AraC and DNR exerted an antagonistic effect on the susceptibility of the *K. pneumoniae* strains to gentamicin that was directly related to the concentration of the cytotoxic agent. Antagonism against *K. pneumoniae* was demonstrated even at the lowest concentration of DNR and AraC tested. Consequently, the rates at which the concentrations of the cytotoxic agents affected the mean FICs for *K. pneumoniae* were significantly greater than for the effects on *P. aeruginosa* ($P < 0.001$) and *E. coli* ($P < 0.001$).

The effect of the chemotherapeutic agents on amikacin MICs for *P. aeruginosa* and *K. pneumoniae* could not be considered antagonistic as defined by our criteria (Fig. 2). Nor was the antimicrobial activity of ticarcillin affected by the two agents (Fig. 3).

The mean FIC against each strain of the three species, *P. aeruginosa, K. pneumoniae,* and *E. coli,* was determined, and the mean FIC against each species was then found by dividing the sum of the mean FICs against each strain by the total number of each species tested. These FICs and their standard deviations are shown in Table 1. The addition of the two cancer chemotherapeutic agents to ticarcillin did not alter the susceptibility of any species to this antibiotic, nor did these additions affect the MICs of either aminoglycoside tested against *E. coli.* However, as previously mentioned, when the immunosuppressive agents were added to gentamicin and

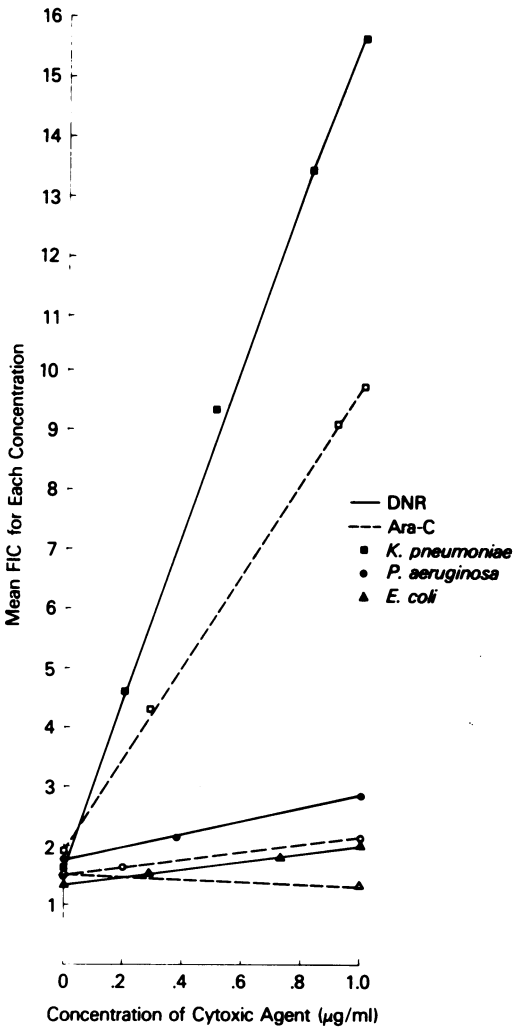

FIG. 1. *Effect of AraC and DNR on the susceptibilities of three microbial species to gentamicin.*

tested against *K. pneumoniae,* antagonistic activity was demonstrated. As the rather large standard error for *P. aeruginosa* and gentamicin with the cytotoxic agents would indicate, there were occasional individual strains of *P. aeruginosa* that required an MIC that was increased by fourfold or higher in the presence of the combined drugs over the MIC in the presence of the antibiotic alone.

In Table 2 are shown those individual strains (11 *K. pneumoniae* and 8 *P. aeruginosa*) for which one or more concentrations of the cytotoxic agent, when combined with an aminoglycoside, caused fourfold or greater increases in the MICs over those obtained with antibiotics alone. The antagonism was often demonstrated only at higher concentrations of the cytotoxic agents. Usually the combination of either cytotoxic agent with amikacin resulted in only fourfold increases in the MICs. However, when there were three or more cytotoxic agent concentrations in which the MIC was eight or more fold greater than the MIC of the antibiotic alone, the mean FIC for the strain was equal to or greater than 4, which indicated an overall antagonistic effect. Such antagonism was shown for eight *K. pneumoniae* strains and one *P. aeruginosa* strain by DNR and gentamicin, whereas AraC

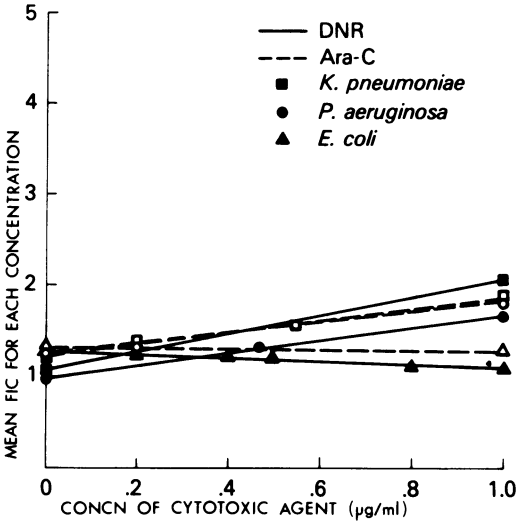

FIG. 2. *Effect of AraC and DNR on the susceptibilities of three microbial species to amikacin.*

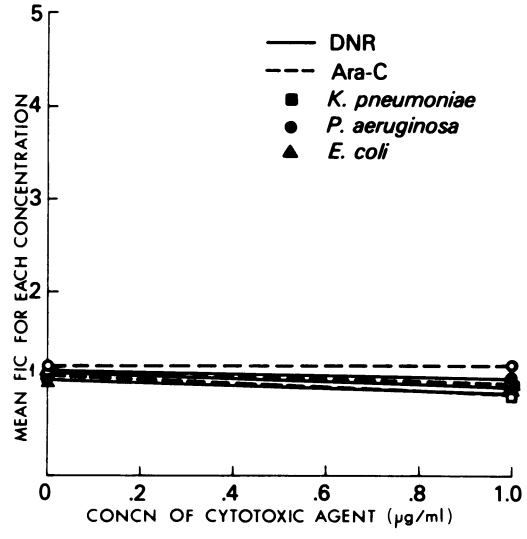

FIG. 3. *Effect of AraC and DNR on the susceptibilities of three microbial species to ticarcillin.*

TABLE 1. *Mean FICs of three antibiotics tested in combination with AraC or DNR against three bacterial species*

Species	Mean FIC[a]		
	Amikacin	Gentamicin	Ticarcillin
P. aeruginosa			
DNR	1.23 ± 0.08	2.08 ± 0.67[b]	1.05 ± 0.07
AraC	1.44 ± 0.17	1.68 ± 0.36[b]	1.22 ± 0.10
K. pneumoniae			
DNR	1.41 ± 0.10	5.79 ± 1.69	1.0 ± 0.01
AraC	1.43 ± 0.19	4.35 ± 1.26	0.98 ± 0.01
E. coli			
DNR	1.21 ± 0.01	1.58 ± 0.21	1.04 ± 0.07
Arac	1.31 ± 0.11	1.42 ± 0.14	1.09 ± 0.06

[a] Average FIC for a strain/total number of bacterial strains tested, ± standard error.
[b] Rare individual strains had two or more dilutions in which the MIC was four or more fold greater in the presence of cytotoxic agents than the MIC of the antibiotic alone.

TABLE 2. *Strains of K. pneumoniae and P. aeruginosa which showed changes in MIC when aminoglycosides were combined with cytotoxic aents*[a]

Organism	Gentamicin			Amikacin		
	MIC (μg/ml)	DNR	AraC	MIC (μg/ml)	DNR	AraC
K. pneumoniae						
5982	0.02	A	A	1.5	I	I
2840	0.09	A	A	1.5	I	I
7106	0.09	A	I	6.0	—	—
6976	0.09	A	A	3.0	—	—
4375	0.01	A	A	1.5	I	I
3886	0.09	A	I	3.0	(I)	—
9641	0.18	A	I	6.0	(I)	—
8689	0.18	A	I	12.0	—	—
2692	0.37	I	I	6.0	—	—
5143	0.05	I	I	6.0	—	—
6482	0.18	I	I	>24.0	—	—
P. aeruginosa						
5477	0.37	A	I	12.0	—	—
5424	0.37	—	A	>24.0	—	—
5487	0.37	I	I	>24.0	—	—
6323	0.18	I	I	0.75	I	I
6322	0.37	I	—	3.0	—	(I)
6324	0.01	—	I	0.75	—	I
6307	>24.0	—	—	6.0	—	(I)
5516	3.0	(I)	—	>24.0	—	—

[a] A, Three or more dilutions in which the MIC was eight or more fold greater than the MIC of the antibiotic alone. I, No more than two dilutions in which MIC was four- to eight-fold greater than the MIC of the antibiotic alone. —, MIC same as MIC of antibiotic only. Parentheses indicate that MICs were above clinically achievable serum levels, and hence the strain was resistant.

and gentamicin were antagonistic for four of the eight *K. pneumoniae* strains and for another *P. aeruginosa* strain. No such antagonism was noted with the amikacin-cytotoxic agent combination. In most instances, the increase in MIC did not alter the susceptibility of the strain to the point that it was resistant to the antibiotic. However, two strains of *K. pneumoniae* and three strains of *P. aeruginosa* had MIC ranges above clinically achievable serum levels, and hence were termed resistant, as a result of the DNR- or AraC-antibiotic combination.

On the whole, when gentamicin and DNR were antagonistic for a strain, the AraC combi-

nation was also antagonistic for that strain; antagonism with one agent, but not the other, was noted with only four strains of *P. aeruginosa.*

DISCUSSION

Two of the most frequently used cancer chemotherapeutic agents for the treatment of acute nonlymphocytic leukemia, when combined at levels clinically achievable in serum with gentamicin or amikacin, exerted antagonistic activity against some strains of *K. pneumoniae* and *P. aeruginosa,* but not against *E. coli*; the same concentrations of the agents did not affect the antimicrobial activity of ticarcillin against these species. The findings would seem to indicate that the administration of these chemotherapeutic agents to patients with advanced cancer, who are receiving either of the aminoglycosides could interfere with effective therapy against *K. pneumoniae* or *P. aeruginosa* infection. The magnitude of antagonism was usually insufficient, however, to raise the MICs of the antibiotics above the clinically achievable serum levels, although in five instances, antagonism was sufficient to yield a resistant MIC. Whether the effect of these agents is upon the bacteria or upon the antibiotic is not known.

DNR is an agent that is capable of intercalating into DNA, and AraC is a pyrimidine analog that can become incorporated into the DNA of the cell. It is possible that changes in bacterial DNAs render bacteria more resistant to antibiotics. The strains that were affected by one drug-antibiotic combination were usually affected by the other combination, which would seem to indicate a basic change in the character of the strain. This DNA alteration might cause an antibiotic-inactivating enzyme or enzymes to be produced, either directly or indirectly, by a depression of the operon that controls enzyme production, since the susceptibility to ticarcillin was not altered. Although this is an appealing theory, there are arguments against it. The only enzyme capable of inactivating both amikacin and gentamicin is aminoglycoside acetyltransferase, whereas enzymes other than acetyltransferase are capable of inactivating gentamicin (18). If production of this enzyme was responsible for the antagonism observed, amikacin susceptibilities should have been affected more often, but gentamicin was the antibiotic most affected. The majority (but not all) of the *K. pneumoniae* strains and about half of the *P. aeruginosa* strains had significantly altered MICs; none of the *E. coli* strains showed any such change. Therefore, the agents would have to be capable of selective DNA intercalation to explain the greater number of *K. pneumoniae* strains with a change in MICs. Since the MICs of the antibiotics were rarely increased to the resistant range, it is unlikely that an inactivating enzyme was responsible, or resistance to the antibiotic would have been more prevalent.

A more likely explanation is that the cancer chemotherapeutic drugs affect the antibiotics directly. The antagonism appeared to be dose related, since most instances of antagonism were noted in the higher concentration of the chemotherapeutic agent. Consequently, as more agent was present, the more likely it was that the antibiotic would be affected. That neither gentamicin nor amikacin was totally inactivated suggests minor but sufficient changes in the antibiotic structure, which could result in alteration of the active antibiotic form and thus in diminished activity. Gentamicin and amikacin have similar chemical structures, but the data suggest that the structure of gentamicin is more prone to alteration by the chemotherapeutic agents, especially by DNR. Further investigations in progress to evaluate the effects of DNR and AraC on other aminoglycosides indicate that they are affected as well.

Although these in vitro studies could be at variance with results obtained in patients, the possible clinical significance of interaction between cancer chemotherapeutic agents and antibiotics is intriguing. Since patients undergoing cancer chemotherapy frequently have impaired host defenses, are subject to infection, and therefore receive antibiotics, the effect of such chemotherapy on the antimicrobial activity of the antibiotics could be important in either the pathogenesis of an opportunistic organism or the treatment of established infection. Thus, although these antitumor agents have no antimicrobial activity against specific bacteria that are frequently recovered from patients with acute leukemia, DNR and AraC are capable of occasionally inhibiting the antimicrobial activity of two aminoglycosides, gentamicin and amikacin. The results of this study would suggest that, should a patient be receiving concurrent DNR or AraC plus gentamicin or amikacin while infected with *K. pneumoniae,* the lack of prompt therapeutic response should lead to reevaluation of antimicrobial therapy.

ACKNOWLEDGMENTS

All drugs were kindly supplied by Clarence Fortner, Clinical Research Pharmacy Service, Baltimore Cancer Research Center, National Cancer Institute.

LITERATURE CITED

1. **Alberts, D. S., N. R. Bachur, and J. L. Holtzman.** 1971. Pharmacokinetics of daunomycin in man. Clin. Pharm. Ther. 12:96-104.

2. **Bodey, G. P.** 1975. Infections in cancer patients. Cancer Treatment Rev. **2**:89–128.
3. **Chabner, B. A., and R. C. Young.** 1973.Threshold methotrexate concentration for in vivo inhibition of DNA synthesis in normal and tumorous target tissues. J. Clin. Invest. **52**:1804–1811.
4. **Coffey, J. J., C. A. White, A. B. Lesk, W. I. Rogers, and A. A. Serpick.** 1972. Effect of allopurinol on the pharmacokinetics of 6-mercaptopurine (NSC 755) in cancer patients. Cancer Res. **32**:1283–1289.
5. **Dilworth, J. A., and G. L. Mandell.** 1975. Infections in patients with cancer. Semin. Oncol. **2**:349–359.
6. **Elion, G. B., S. Singer, and G. H. Hitchings.** 1954. Antagonists of nucleic acid derivatives. VIII. Synergism in combination of biochemically related anti-metabolites. J. Biol. Chem. **208**:471–488.
7. **Goldschmidt, M. C., and G. P. Bodey.** 1972. Effect of chemotherapeutic agents upon microorganisms isolated from cancer patients. Antimicrob. Agents Chemother. **1**:348–353.
8. **Hahn, D. M., S. C. Schimpff, V. M. Young, C. L. Fortner, H. C. Standiford, and P. H. Wiernik.** 1977. Amikacin and cephalothin: empiric regimen for granulocytopenic cancer patients. Antimicrob. Agents Chemother. **12**:618–624.
9. **Hersh, E. M., G. P. Bodey, B. A. Nies, and E. J. Freireich.** 1965. Causes of death in acute leukemia: a ten-year study of 414 patients from 1954–1963. J. Am. Med. Assoc. **193**:105–109.
10. **Ho, D. H. W., and E. Frei, III.** 1971. Clinical pharmacology of 1-β-D-arabinopuranyl cytosine. Clin. Pharm. Ther. **12**:944–945.
11. **Klastersky, J., A. Henri, C. Hensgens, and D. Daneau.** 1974. Gram negative infections in cancer: study of empiric therapy combining carbenicillin-cephalothin with and without gentamicin. J. Am. Med. Assoc. **227**:45–48.
12. **Kluge, R. M., H. C. Standiford, B. Tatem, V. M. Young, S. C. Schimpff, W. H. Greene, F. M. Calia, and R. B. Hornick.** 1974. The carbenicillin-gentamicin combination against *Pseudomonas aeruginosa*. Correlation of effect with gentamicin sensitivity. Ann. Intern. Med. **81**:584–586.
13. **Levine, A. S., S. C. Schimpff, R. G. Graw, and R. C. Young.** 1974. Hematologic malignancies and other marrow failure states: progress in the management of complicating infections. Semin. Hematol. **11**:141–202.
14. **Luboshitzky, R., T. Sacks, and J. Michel.** 1973. Bactericidal effect of combinations of antibiotics on *Klebsiella-Enterobacter-Serratia*. Chemotherapy **19**:354–364.
15. **MacLowry, J. D., M. J. Jaqua, and S. T. Selepak.** 1970. Detailed methodology and implementation of a semiautomated serial dilution microtechnique for antimicrobial susceptibility testing. Appl. Microbiol. **20**:46–53.
16. **Metcalfe, D., and W. T. Hughes.** 1972. Effects of methotrexate on Group A beta hemolytic streptococci and streptococcal infection. Cancer **30**:588–593.
17. **Middleman, E. A., A. Watanabe, H. Kaizer, and G. P. Bodey.** 1972. Antibiotic combinations for infections in neutropenic patients. Cancer **30**:573–579.
18. **Neu, H. C.** 1976. Molecular modifications of antimicrobial agents to overcome drug resistance, p. 87–111. *In* F. E. Hahn (ed.), Acquired resistance of microorganisms to chemotherapeutic drugs, vol. 20. S. Karger, New York.
19. **Phair, J. P., C. Watanakunakorn, and T. Bannister.** 1969. In vitro susceptibility of *Pseudomonas aeruginosa* to carbenicillin and gentamicin. Appl. Microbiol. **18**:303–306.
20. **Schimpff, S. C., W. Satterlee, V. M. Young, and A. Serpick.** 1971. Empiric therapy with carbenicillin and gentamicin for febrile patients with cancer and granulocytopenia. N. Engl. J. Med. **284**:1061–1065.
21. **Schimpff, S. C., V. M. Young, W. H. Greene, G. D. Vermeulen, M. R. Moody, and P. H. Wiernik.** 1972. Origin of infection in acute nonlymphocytic leukemia. Significance of hospital acquisition of potential pathogens. Ann. Intern. Med. **77**:707–714.
22. **Smith, C. B., P. E. Dans, and J. N. Wilfert.** 1969. Use of gentamicin in combinations with other antibiotics. J. Infect. Dis. **119**:370–377.
23. **Sonne, M., and E. Jawetz.** 1969. Combined action of carbenicillin and gentamicin on *Pseudomonas aeruginosa* in vitro. Appl. Microbiol. **17**:893–896.
24. **Standiford, H. C., A. C. Kind, and W. M. M. Kirby.** 1969. Laboratory and clinical studies of carbenicillin against gram-negative bacilli, p. 286–291. Antimicrob. Agents Chemother. 1968.
25. **Valdivieso, M., G. P. Bodey, M. A. Burgess, and V. Rodriguez.** 1976. Therapy of infections in neutropenic patients. Results with gentamicin in combination with cephalothin or chloramphenicol. Am. J. Med. Sci. **270**:453–463.