Chapter 15. Antiviral Agents

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Introduction - A sign of the advances in antiviral chemotherapy is the large number and variety of review articles published on the subject. Clinical applications for available antiviral agents are covered in reports by Becker,[1] Diana and Pancic,[2] Kaufman,[3] Pradalies and Leynadier,[4] Waldman,[5] James,[6] and Hermans.[7] The review by Maugh[8] discusses the history and present status of antiviral agents in the U.S.A. More generalized reviews on the trends in antiviral research are covered in reviews by Becker,[9] Galabov,[10] Meyer and colleagues,[11] and Hoffmann.[12] The papers presented at two symposia, "Antivirals with Clinical Potential" and the "Third Conference on Antiviral Substances," have been published[13,14] and provide a broad overview of both interferon and its inducers and compounds with antiviral activity.

Antiviral agents are currently available for clinical use against herpes- and influenza-caused infections. This has resulted in a series of papers related to clinical treatment of the disease. Reports by Rawls[15] and Dostal, *et al.*[16] cover the treatment of herpesvirus infections, with others covering the treatment of herpes skin infections[17] and herpes-caused encephalitis.[18] Testing and model systems for herpesvirus studies have also appeared with descriptions of test methods for herpes keratitis[19] and for intranasal testing.[20] A study of the polypeptides and antigens of herpesvirus has been used as a guide for studies of chemotherapy and epidemiology of herpes infections.[21] The chemoprophylaxis and prevention of respiratory diseases are discussed in articles by Jackson and Stanley[22] and Batten.[23] Douglas,[24] Knight,[25] and Dolin[26] report on influenza as a disease and cover methods for its control. More specifically, Couch[27] provides an assessment of amantadine's use for influenza and Hoffman and Dixon[28] review the problem of influenza in hospitals and methods for control.

The control of herpesvirus disease remains a major target of virus chemotherapy with many studies aimed at this and other DNA viruses. A comparison of the activities of the better known antiherpes compounds in tissue culture and *in vivo* was reported by Collins and Bauer,[29] in the hairless mouse model by Harris and Boyd,[30] and in hamsters by Renis.[31] The utility of nucleoside analogs for treatment of non-neoplastic diseases was discussed by Calabresi, *et al.*[32] while Fox discussed the pharmacology and chemistry of inhibitors of herpes replication.[33]

Adenine Arabinoside (Ara-A, Vidarabine) - A breakthrough in the treatment of herpes simplex encephalitis with the use of Ara-A (1) was reported by Whitley and colleagues in a collaborative NIAID study.[34] In 28 clinical biopsy-proved cases, i.v. administration reduced mortality from 70 to 28%, and if started early enough, prevented subsequent debilitating neurological sequela. Treatment started after severe neurological damage had occurred could prevent death, but could not reverse the damage. Another clinical

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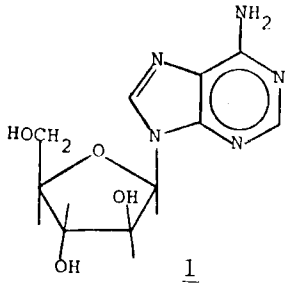
study on six patients with severe herpesvirus infections showed a clinical response and cure of the infection by a 15 mg/kg/day i.v. treatment.[35] In double-blind and open studies of the treatment of IDU refractory herpetic keratitis, Ara-A treatment provided clinical improvement and appeared useful for the treatment of this disease.[36] Other clinical studies have reported a degree of efficacy on herpes zoster in immunosuppressed[37] patients. In two cases of severe chickenpox and one of *Purpura fulminans*, rapid cure was reported from i.v. Ara-A treatment.[38] No effect on the human cellular immune mechanism was seen from treatment with this compound, an important consideration in an infection where host defense is of such importance.[39] Although these studies are encouraging, the insolubility of Ara-A, its requirement for i.v. administration, and its rapid *in vivo* deamination to the less active ara-hypoxanthine (Ara-Hx) pose severe problems for widespread use and have resulted in continuing research to provide a better agent.



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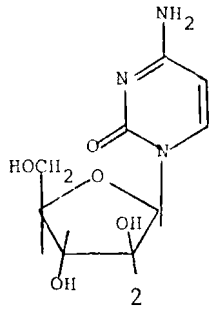
One promising compound is adenine arabinoside monophosphate (Ara-AMP) which is more soluble and has been shown to build high intra-ocular levels in rabbits dosed i.v. with small amounts of the compound.[40] Other studies have shown that Ara-A and Ara-AMP ointment were able to prevent fatal herpesvirus encephalitis when used as an ointment and neither caused skin irritation.[41] Treatment of established herpetic keratitis in rabbits indicated that Ara-AMP was superior to Ara-A and low doses of Ara-Hx or Ara-HxMP.[42] Due to the higher solubility of Ara-AMP, greater doses could be administered. A comparison study in mice infected with three different herpesvirus hominis infections showed Ara-AMP to be superior to Ara-A which was superior to phosphonoacetic acid.[43] Almost the reverse was observed in the treatment of genital herpesvirus hominis infections in mice where PAA ointment was effective whereas neither Ara-A nor Ara-AMP ointment provided protection.[44] Several studies have been carried out comparing the relative activities of Ara-A and Ara-Hx,[45,46,47] with Ara-A found to be ten times or more active than Ara-Hx. In the presence of coformycin, an adenine deaminase inhibitor, Ara-A in tissue culture was 35-70 times more potent than Ara-Hx.[48] In order to establish the role of antibody in control of herpesvirus infections, Ara-A was tested in mouse herpes encephalitis infections with and without rabbit immune globulin. The combination produced greater efficacy than either treatment alone, suggesting that severe infections may require a combination therapy for control.[49]

Cytosine Arabinoside (Cytarabine, Ara-C) - In spite of its higher toxicity and immunosuppressive action,[50] there is continued interest in its clinical application. In an open study it was reported that intravenous doses of cytarabine (2) reduced the shedding of type 1 and 2 herpesvirus to 4 days in 15 of 17 patients whereas without treatment, shedding would be expected to continue for several weeks.[51] An uncontrolled study of cytarabine in 61 patients with herpes zoster suggested a beneficial effect, but a randomized double-blind study in 30 patients provided no evidence of a benefit. In fact, the blister healing time was greater for the treated group.[52]

Another open study on herpes zoster in patients with malignancies reported 9 of 10 to be benefited by single daily i.v. doses of cytarabine.[53] Studies on the use of cytarabine against herpes encephalitis in children and infants indicated a beneficial effect if given early enough;[54,55] however, it would appear that vidarabine is a better agent. Neither cytarabine nor IDU were effective in treatment of cytomegalovirus infections of newborn mice indicating that they would be of little value in treatment of this disease in human neonates.[56] In an effort to bypass some of the toxicity of these agents yet retain antiviral efficacy, albumin conjugates of IDU and Ara-C were made and tested in ectromelia-infected mice.[57] Less toxicity and more activity was observed than with the free compounds.



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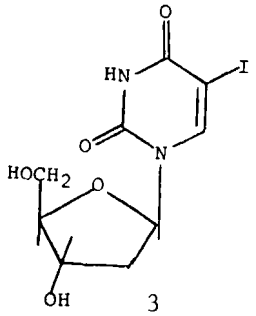
Idoxuridine (IDU - 5-Iodo-2'-deoxyuridine) - Idoxuridine (3) was used successfully in the treatment of recurrent genital herpes when applied topically in a solution of dimethyl sulfoxide.[58] Twenty percent idoxuridine gave the best response, although 5% solutions were better than placebo. Recurrence rates were not affected by these treatments. A similar effect was seen with this combination for the treatment of zoster lesions.[59] A study of 6-azauridine and IDU showed about a 50% response on relapsing genital herpes for either alone, but an 82% response for a combination of the two.[60] The present status of idoxuridine for herpes infections is discussed by Stuart-Harris[61] and Grupper.[62]



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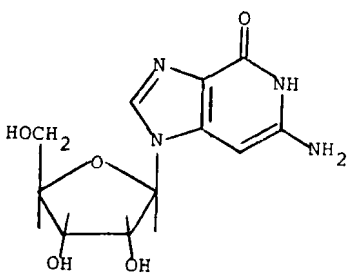
Other Purine and Pyrimidine Analogs - In continuing effort to obtain increased activity and safety, a large number of purine and pyrimidine analogs have been tested in laboratory systems. Among these are 5,6-dihydro-5-azathymidine with prophylactic and therapeutic activity against herpes infections in mice,[63] 5-iodo-2'-deoxycytidine (IDC) with therapeutic activity equal or better than IDU against herpetic keratitis in rabbits and man,[64] and 5-trifluoromethyl-2'-deoxyuridine, which was well tolerated and showed a dose-dependent protection of mice infected intracerebrally with type 1 herpesvirus, but was inactive against encephalitis in mice due to EMC, an RNA virus.[65] A combination of 2-deoxy-D-glucose and 3-deazauridine provided a synergistic effect against Japanese B encephalitis in tissue culture with no increased cytotoxicity, again suggesting combinations of agents for virus chemotherapy.[66] Continued studies with 3-deazaguanine (4) and its nucleoside and nucleotide suggested broad-spectrum activity with nine RNA viruses and seven DNA viruses sensitive in tissue culture. *In vivo* activity was demonstrated against influenza A and B and parainfluenza virus infections[67] as well as against Friend leukemia



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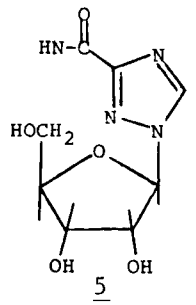
splenomegaly in mice. More basic studies have attempted to determine the mechanism of action for a number of active analogs. Arabinosylthymine (Ara-T), which has selective activity against herpesvirus, was shown to be phosphorylated and activated only by a virus-induced pyrimidine deoxyribonucleoside kinase and was not a substrate for normal cell kinases.[68] A similar effect was demonstrated in the case of 5-halogenated deoxycytidine analogs which were 10-100 times less toxic to cells than the corresponding 5-halogenated uridine analogs. The former could only be phosphorylated by a virus-induced kinase whereas normal cells contained an activating kinase for the latter compounds.[69,70] A new thymidine analog, 5'-amino-2',5-dideoxy-5-iodouridine (AIU), has been shown to be a potent inhibitor of herpesvirus, but has essentially no cellular toxicity.[71] It appears to selectively block an intracellular event during virus replication, an event occurring 4-6 hours after infection, and has no effect on infected or uninfected cell RNA synthesis, although blocking DNA synthesis in infected, but not uninfected, cells.

Ribavirin (Virazole - 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide) - Oral doses (600 mg/day) (5) showed no demonstrable effect in volunteers challenged with influenza A virus[72] and a marginal effect on volunteers challenged with influenza B.[73] Dosage at 1000 mg/day resulted in a reduction in signs and symptoms in volunteers infected with the Victoria strain of influenza A, but had no effect on antibody response or virus shedding.[74]



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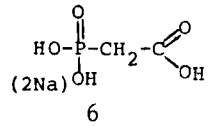
In ferrets, 100 mg/kg/day, but not lower doses, caused a marked reduction in symptoms from influenza A challenge infection. However, this was also accompanied by an immunosuppressive effect.[75] Activity by aerosol or i.p. administration to mice infected with influenza A and parainfluenza has been reported.[76,77]

Mode-of-action studies show that virus RNA synthesis is inhibited by ribavirin and this can be reversed by guanosine or xanthosine.[78] Additional studies with cell-free influenza virus ribonucleic acid polymerase showed that the inhibition is not by ribavirin but by its 5'-triphosphate, indicating the necessity for cellular phosphorylation for activity.[79] A general effect of the compound on cellular processes as a mode of action is suggested by the fact that no resistance to ribavirin could be obtained in herpesvirus after 10 passages, whereas idoxuridine gave a high degree of resistance under similar passage.[80]

Phosphonoacetic Acid (PAA) - Disodium phosphonoacetic acid (6) in a cream base proved to be too irritating at effective levels for use in treating herpesvirus lesions in monkeys.[81] It, but none of its analogs, was active against Marek's disease virus replication in tissue culture. However, it was ineffective against the disease in chickens.[82] PAA produced a significant effect when applied topically on superficial herpetic keratitis of rabbits against both IDU-sensitive and



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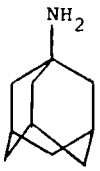
-resistant strains.[83] Mode-of-action studies indicate it is active on Marek's disease herpesvirus and on the virus-induced DNA polymerase where it acts by binding with the pyrophosphate site of the enzyme.[84] Herpesvirus mutants resistant to PAA can be produced and the DNA polymerase isolated from these mutants, although of the same molecular weight, are also resistant.[85,86] A review of the data on PAA to 1977 discussed some possible problems with PAA, the development of resistance, and the chance that the agent would be sequestered like pyrophosphate causing long-term complications.[87]

Amantadine HCl (1-Adamantanamine HCl) - Until 1976, FDA clearance for amantadine HCl (7) was for the prophylaxis of influenza A2 (Asian) infection only. In 1976 this was broadened to include use for the prevention and symptomatic management of infections caused by influenza A viruses. A report of the VIII Influenza Workshop[88] covered much of the work to 1975. More recent reports assess the use of amantadine HCl for influenza.[89,90] Double-blind controlled studies showed that amantadine HCl was effective in reducing the incidence of 1973 and 1974 influenza A outbreaks, but did not provide activity against influenza B occurring at the same time.[91] In another study, illness due to an influenza A outbreak was significantly reduced in students treated prophylactically. However, during a post-treatment period with influenza still present, an accelerated rate of illness occurred in the previously protected group, indicating treatment should be continued until the outbreak is over.[92] A study of students with natural influenza A infection indicated that the therapeutic use of amantadine HCl resulted in an accelerated physiologic improvement with a significant increase in helium-oxygen flow rate.[93] Influenza A/New Jersey/8/76 (HswlNl) was found to be inhibited by amantadine HCl in tissue culture, *in ovo*, and in mice,[94] and amantadine HCl was also inhibitory to the formation of virus-induced polypeptides in tissue culture.[95] High doses of amantadine HCl given orally to ferrets produced no activity against influenza A infection and caused toxic effects. In contrast, low doses, 6 mg/kg, provided as an aerosol, were consistently effective and without side effects.[96] Aerosols of amantadine, rimantadine (8) and ribavirin have also been effective for treatment of influenza A infections in mice.[97] Studies on the formation of amantadine- and rimantadine-resistant variants of influenza suggest this is due to a selection of resistant particles from a nonhomogeneous population and not due to a mutagenic effect.[98] More recent studies on the mode of action of these compounds suggest a possible effect on virus-associated RNA-dependent RNA polymerase which occurs at an early phase of infection.[99,100]



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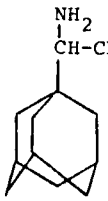
Rimantadine HCl (α-Methyl-1-adamantanemethylamine HCl) - In the USSR, rimantadine HCl (8) (available as Remantadini) is used as an antiviral chemotherapeutic agent due to good therapeutic activity and good tolerance. Although many studies have been carried out in the USSR, most of the literature on these studies is not available in the U.S. Available reports of clinical studies have shown that prolonged administration of the drug is safe, well tolerated,[101] and also effective and well tolerated against

influenza A infections, including the influenza A/Victoria/3/76 type.[102,103,104] Aerosol administration of the compound to mice infected with influenza A virus significantly increased survival rates.[105,106] A combination of rimantadine and ribavirin provided a synergistic effect[107] in both tissue culture and mouse infections with influenza A. Rimantadine was highly active against the 1976 influenza A/New Jersey isolate in a chick embryo test[108] and other studies in tissue culture[109,110] suggest that rimantadine has a direct effect on influenza virus RNA-dependent RNA polymerase and that this is the mode of action of the compound.



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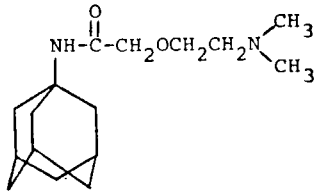
Tromantadine HCl (Viru-Merz) - Continued studies abroad with tromantadine HCl (9) indicate clinical efficacy against primary and recurring herpesvirus infections in man.[111,112] Used as an ointment it was reported to provide an excellent response in acute and chronic eye trachoma infections with little evidence of irritation[113] Low toxicity and minor irritation was reported from topical and systemic applications. Tissue culture studies indicate it is active at the stage of virus penetration or uncoating rather than at an inhibition of nucleic acid synthesis.[114]



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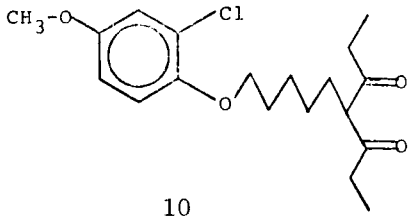
Isoprinosine (Paracetamidobenzoic Acid Salt of Inosine Dimethylaminoisopropanol) - This agent continues to be controversial. In a study to determine its effect on the immune system, it was found to have a direct effect on the proliferation of lymphocytes, but with an action different from other immunopotentiating agents such as levamisole.[115] Results from a clinical study in Brazil indicated activity against herpes simplex and herpes zoster infections.[116] However, in 1977 the FDA terminated the IND on isoprinosine due to a lack of evidence of efficacy.[117]

Vitamin C (Ascorbic Acid) - Additional trials have failed to provide substantive evidence of activity of vitamin C against the common cold. A co-twin study of 44 school-aged twins showed no significant overall effect of ascorbic acid, although treated girls in the youngest groups had shorter and less severe illnesses.[118] The study was double-blind, but several of the mothers reported tasting the capsules and thus knew who was receiving ascorbic acid. In order to check on earlier positive studies, a double-blind trial of vitamin C was performed in 868 Navajo school children. No effect was observed, and it was concluded that vitamin C was not an effective prophylactic or therapeutic agent.[119] Overall, an analysis of the clinical studies indicate a lack of significant effect of vitamin C on the common cold.[120]

Arildone {Win 38,020 [4-(6-((2-chloro-4-methoxy))phenoxyhexyl)-3,5-heptonedione]} - Arildone (10) was the most active of a series of β-diketones against a number of both RNA and DNA virus strains[121] with activity shown both in tissue culture and in animal studies. It is

reported to be effective topically against herpesvirus types 1 and 2 in the rabbit eye and guinea pig skin.[122]



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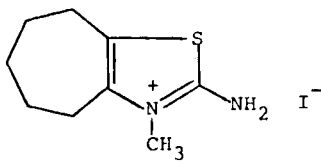
Bonapthon (6-Bromonaphthaquinone) - A study showed bonapthon to reduce pneumonia and improve symptoms in mice infected with influenza virus when given orally and to have a beneficial effect on herpetic keratitis in rabbits when applied as an ointment.[123] In contrast, other laboratory studies showed no activity and some toxicity,[124] the lack of activity being confirmed in a clinical trial with influenza.[103]

Other Compounds Tested Clinically - A number of compounds, including an interferon inducer, have been tested by intranasal administration to volunteers infected with rhinoviruses. A new antiviral agent, 1-phenyl-3-(4-phenyl-2-thiazolyl)guanidine was ineffective in moderating clinical illness due to rhinovirus type 44 challenge.[125] Three unrelated compounds with antirhinovirus activity in tissue culture - SKF-40491 {4-(8-amino-7-chloro-5-methyl-5H-as-triazino[5,6-b]indol-3-yl)amino-2-methyl-2-butanol}; GL R9-338 (3-α-naphthyl-5-diethylcarbamoyl-1,2,4-oxadiazole); and RP 19326 (2,6-diphenyl-3-methyl-2,3-dihydroimidazo[2,1-b]thiazole) were given intranasally to volunteers infected with rhinovirus types 3 or 9. All caused a reduction in mean viral titer from nasal washing and RP 19326 also provided a slight reduction in symptoms.[126] The lack of a striking response was thought due to inability to maintain adequate concentrations of compound. A similar study was carried out with an interferon inducer, CP 20961 (N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)propanediamine), which was found to induce interferon, and if timing and number of doses were correct, also reduce clinical symptoms of a rhinovirus challenge.[127] In such challenge studies the timing can be carefully controlled which is not the case in natural infections.

Compounds with *In Vivo* Antiviral Activity - D-Glucosamine, an inhibitor of the glycosylation of the viral coat, decreased tumor growth due to Rous sarcoma virus in chicks and quails and increased survival of mice infected with influenza when administered by i.p. injection, but provided essentially no protection to mice when administered intranasally.[128] A new compound, AMCT (11), was active on influenza A virus strains in tissue culture and in experimental mouse infections, but was inactive on influenza B. Timing experiments indicated it acts at a very early stage of virus growth (penetration or uncoating). These results are similar to those observed with amantadine HCl.[129]



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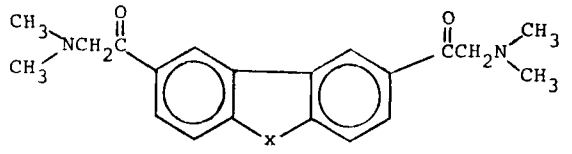
Interferon Inducers - The two most active analogs (12) of a series of bis-basic substituted polycyclic aromatic compounds have provided significant amounts of interferon in mice and afforded protection against infection with both RNA and DNA viruses.[130,131] BL 3849A (13) has a similar effect and is also active in mice against RNA and DNA viruses by oral

administration.[132] CMA (10-carboxymethyl-9-acridanone Na salt) protected



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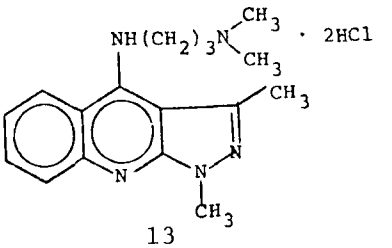

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mice infected with a variety of viruses and produced an interferon-like substance in mouse cell culture and mice.[133]

Conclusion - Antiviral chemotherapy has come of age. Broadened and more effective applications of available and new agents will require a continuing effort by researchers on methods for best treating virus diseases and on bringing this to the attention of physicians and public health authorities.

References

1. Y. Becker in "Antiviral Drugs - Monographs in Virology," Vol. 11, S. Karger, N.Y., 1976.
2. G. D. Diana and F. Pancic, Angew. Chem. Int. Ed. Engl., 15 (2), 410 (1976).
3. H. E. Kaufman, Int. J. of Dermatol., 16, 464 (1977).
4. A. Pradalier and F. Leynadier, Gaz. Med. Fr., 82 (32), 3795 (1975).
5. R. H. Waldman and J. D. Lee, Compr. Ther. 3 (7), 10 (1977).
6. D. G. James, Practitioner, 217 (1300), 603 (1976).
7. P. E. Hermans, Mayo Clin. Proc., 52 (11), 683 (1977).
8. T. H. Maugh, II, Science (Wash.), 192 (4235), 128 (1976).
9. Y. Becker, Isr. J. Med. Sci., 11 (11), 1135 (1975).
10. A. S. Galabov, Forsch., 26 (2), 169 (1976).
11. F.-P. Meyer, H. Walther and L. Domner, Biol. Rundsch. 13 (6), 358 (1975).
12. C. E. Hoffmann, Ann. Rep. Med. Chem. 11, 128 (1976).
13. T. C. Merigan, J. Inf. Dis., 133, A1 (1976).
14. E. C. Herrmann, Jr., Ed., Ann. N.Y. Acad. of Sci. 284, 1 (1977).
15. W. E. Rawls, JAMA, 235 (13), 1365 (1976).
16. V. Dostal, D. Fanta and R. J. Reiss-Gutfreund, Wein Klin Wochenschr. 89 (22), 741 (1977).
17. E. B. Smith, JAMA, 235 (16), 1731 (1976).
18. M. Longson, J. Antimicrob. Chemo. 3 (Suppl. A), 115 (1977).
19. M. G. Falcon and B. R. Jones, *ibid*, 3 (Suppl. A), 83 (1977).
20. E. DeClercq and M. Luczak, J. Inf. Dis., 133, A226 (1976).
21. D. H. Watson and R. W. Honess, J. Antimicrob. Chemo. 3, (Suppl. A), 33 (1977).
22. G. G. Jackson and E. D. Stanley, JAMA, 235 (25), 2739 (1976).
23. J. Batten, Postgrad. Med. J. 52, 571 (1976).
24. R. G. Douglas, Jr., Hosp. Pract. 11 (12), 43 (1976).
25. V. Knight, Dis.-M. XX (11), Year Book Medical Publishers, Inc., Chicago, Ill. (1976).
26. R. Dolin, Amer. Family Physician 14, 72 (1976).
27. R. B. Couch, J. Inf. Dis. 7 (9), 4 (1977).
28. P. C. Hoffman and R. E. Dixon, Ann. Intern. Med. 87 (6), 725 (1977).
29. P. Collins and D. J. Bauer, J. Antimicrob. Chemo. 3 (Suppl. A), 73 (1977).
30. S. R. B. Harris and M. R. Boyd, *ibid*, 3 (Suppl. A), 91 (1977).
31. H. E. Renis, Antimicrob. Ag. Chemother. 11 (4), 701 (1977).
32. P. Calabresi, C. H. Doolittle, III, G. H. Heppner, and C. J. McDonald, Ann. N.Y. Acad. Sci., 255, 190 (1975).
33. R. W. Fox, J. Antimicrob. Chemo. 3 (Suppl. A), 23 (1977).
34. R. J. Whitley, S.-J. Soong, R. Dolin, G. J. Galasso, L. T. Ch'ien, C. A. Alford and Coll. Study Group, New Eng. J. Med. 297, 289 (1977).

35. M. D. Aronson, C. F. Phillips, D. W. Gump, R. J. Albertini and A. Phillips, JAMA, 235 (13), 1339 (1976).
36. D. Pavan-Langston and R. A. Buchanan, Trans. Am. Acad. Ophthalmol. Otolaryngol., 81 (5), 813 (1976).
37. R. J. Whitley, S.-J. Soong, R. Dolin, G. J. Galasso, L. T. Ch'ien, C. A. Alford and the Coll. Study Group, New Eng. J. Med. 297 (6), 1193 (1976).
38. B. Juel-Jensen, J. Antimicrob. Chemo. 2, 261 (1976).
39. R. W. Steele, I. A. Capa, M. M. Vincent, S. A. Hensen and R. E. Keeney, Antimicrob. Ag. Chemother. 7 (2), 203 (1975).
40. D. Pavan-Langston, R. D. North, Jr., P. A. Geary, and A. Kinkel, Arch. Ophthalmol. 94 (9), 1585 (1976).
41. R. J. Klein and A. E. Friedman-Kien, Antimicrob. Ag. Chemother. 12 (5), 577 (1977).
42. H. E. Kaufman and E. D. Varnall, *ibid*, 10 (6), 885 (1976).
43. E. R. Kern, J. T. Richards, J. C. Overall, Jr., and L. A. Glasgow, *ibid*, 13 (1), 53 (1978).
44. E. R. Kern, J. T. Richards, J. C. Overall, Jr., and L. A. Glasgow, J. Inf. Dis. 135 (4), 567 (1977).
45. B. B. Williams, E. J. Bailey and A. M. Lerner, J. Lab. Clin. Med. 89 (4), 687 (1977).
46. Y. J. Bryson and J. D. Connor, Antimicrob. Ag. Chemother. 9 (3), 540 (1976).
47. C. Shipman, Jr., S. H. Smith, R. H. Carlson and J. C. Drach, *ibid*, 9 (1), 120 (1976).
48. P. M. Schwartz, C. Shipman, Jr., and J. C. Drach, *ibid*, 10 (1), 64 (1976).
49. C. T. Cho, K. K. Feng and N. Brahmacupta, J. Inf. Dis. 133 (2), 157 (1976).
50. P. Peller, O. Goetz and M. Rath, Infection 4 (3), 130 (1976).
51. B. Juel-Jensen, J. Antimicrob. Chemo. 3 (Suppl. A), 125 (1977).
52. C. E. Orfanos, A. Weese, U. Runne, J. Kratka and G. Goerz, Dtsch. Med. Wochenschr. 102 (9), 312 (1977).
53. S. Kernbaum, Br. Med. J. 1 (6003), 224 (1976).
54. B. Wolman and M. Longson, Acta Paediatr. Scan. 66 (2), 243 (1977).
55. B. Lagerkvist and H. Ekelund, Scand. J. Infect. Dis. 7 (2), 81 (1975).
56. D. K. Kelsey, E. R. Kern, J. C. Overall, Jr., and L. A. Glasgow, Antimicrob. Ag. Chemother. 9 (3), 458 (1976).
57. P. G. Balboni, A. Minia, M. P. Grossi and G. Barbanti-Brodano, Nature 264, 181 (1976).
58. J. D. Parker, J. Antimicrob. Chem. 3 (Suppl. A), 131 (1977).
59. T. Nasemann, Hautarzt 27 (9), 463 (1976).
60. R. M. Bikbulatov and V. N. Grebenyuk, Vopr. Virusol. (2), 228 (1977).
61. C. Stuart-Harris, J. Antimicrob. Chemo. 2 (2), 112 (1976).
62. C. Grupper, Z. Hautkr. 52 (8), 483 (1977).
63. G. E. Underwood and S. D. Weed, Antimicrob. Ag. Chemother. 11 (4), 765 (1977).
64. G. Takenaka, Nichidai Igaku Zasshi 35 (4), 427 (1976).
65. D. W. Clough and J. R. Parkhurst, Antimicrob. Ag. Chemother. 11 (2), 307 (1977).
66. D. R. Woodman and J. C. Williams, *ibid*, 11 (3), 475 (1977).
67. L. B. Allen, J. H. Huffman, P. D. Cook, R. B. Meyer, Jr., R. K. Robins and R. W. Sidwell, *ibid*, 12 (1), 114 (1977).
68. J. F. Aswell, G. P. Allen, A. T. Jamieson, D. E. Campbell and G. A. Gentry, *ibid*, 12 (2), 243 (1977).
69. I. Schildkraut, G. M. Cooper and S. Greer, Mol. Pharmacol. 11, 153 (1975).
70. M. J. Dobersen, M. Jerkofsky, and S. Greer, J. Virol. 20 (2), 478 (1976).
71. Y. C. Cheng, B. Goz, J. P. Neenin, D. C. Ward and W. H. Prusoff, *ibid*, 15 1284 (1975).
72. A. Cohen, Y. Togo, R. Khakoo, R. Waldman and M. Sigel, J. Inf. Dis. 133, A114 (1976).
73. Y. Togo and E. A. McCracken, *ibid*, 133, A109 (1976).
74. C. R. Magnussen, R. G. Douglas, Jr., R. F. Betts, F. K. Roth and M. P. Meagher, Antimicr. Ag. Chemother. 12 (4), 493 (1977).
75. R. J. Fenton and C. W. Potter, J. Antimicrob. Chem. 3, 263 (1977).
76. R. F. Berendt, J. S. Walker, J. W. Dominik and E. L. Stephen, Antimicrob. Ag. & Chemother. 11 (6), 1069 (1977).
77. E. W. Larson, E. L. Stephen and J. S. Walker, *ibid*, 10 (4), 770 (1976).
78. C. Scholtissek, Arch. Virol. 50 (4), 349 (1976).
79. B. Eriksson, E. Helgstrand, N. G. Johansson, A. Larsson, A. Misiorny, J. O. Noren, L. Philipson, K. Stenberg, G. Stening, S. Stridh and B. Oberg, Antimicrob. Ag. Chemo. 11 (6), 946 (1977)
80. L. B. Allen and C. M. Fingal, *ibid*, 12 (1), 120 (1977).
81. A. E. Palmer, W. T. London and J. L. Sever, *ibid*, 12 (3), 510 (1977).
82. L. F. Lee, K. Nazerian, S. S. Leinbach, J. M. Reno and J. A. Boezi, J. Natl. Cancer Inst. 56 (4), 823 (1976).
83. R. F. Meyer, E. D. Varnell and H. E. Kaufman, Antimicrob. Ag. Chemo. 9 (2), 308 (1976).
84. S. S. Leinbach, J. M. Reno, L. F. Lee, A. F. Isbell and J. A. Boezi, Biochemistry (Wash.), 15 (2), 426 (1976).

85. Y. Becker, Y. Asher, Y. Cohen, E. Weinberg-Zahlering and I. Shlomai, Antimicrob. Ag. Chemo. 11 (5), 919 (1977).
86. B. Fridlander, N. Chejanovsky and Y. Becker, *ibid*, 13 (1), 124 (1978).
87. J. May, S. M. Brown, A. T. Jamieson, F. J. Rixon, H. Moss, D. A. Dargan and J. H. Subak-Sharpe, *ibid*, 3, 63 (1977).
88. R. B. Couch and G. G. Jackson, J. Inf. Dis. 134 (5), 516 (1976).
89. R. B. Couch, *ibid*, Sept. (1977).
90. A. Chanin, JAMA, 236 (18), 2050 (1976).
91. A. W. Galbraith, J. Antimicrob. Chemo. 1 (Suppl.), 81 (1975).
92. R. L. Muldoon, E. D. Stanley and G. G. Jackson, Amer. Rev. Resp. Dis. 113, 487 (1976).
93. J. W. Little, W. J. Hall, R. G. Douglas, Jr., R. W. Hyde and D. M. Speers, Ann. Inter. Med. 85, 177 (1976).
94. R. R. Grunert and C. E. Hoffmann, J. Inf. Dis. 136 (2), 297 (1977).
95. J. S. Oxford and G. C. Schild, FEMS Microb. Letters 1, 223 (1977).
96. R. J. Fenton, C. Bessell, C. R. Spilling and C. W. Potter, J. Antimicrob. Chemother. 3, 463 (1977).
97. J. S. Walker, E. L. Stephen and R. O. Spertzel, J. Inf. Dis. 133, A140 (1976).
98. V. I. Ilyenko, Vopr. Virusol. (2), 199 (1975).
99. V. A. Kalninya and M. K. Indulen, Acta. Virol. 20, 343 (1976).
100. J. H. Skehel, A. J. Hay and J. A. Armstrong, J. gen. Virol. 38, 97 (1977).
101. V. Mazhinskaya and R. Antipova Khimi, Khimio. Grippai, Mater. Vses. Simp. 1st, 92 (1972).
102. V. F. Krylov, A. A. Alekseeva, T. Ia. Liarskaia, T. G. Poliakova and L. M. Kupriashina, Vopr. Virusol. (2), 186 (1976).
103. N. P. Obrosova-Serova, L. M. Kupryashina, G. V. Shtunderendo, G. S. Churkin, E. A. Severin, and G. A. Galegov, *ibid*, (3), 295 (1977).
104. A. Denys, S. Szram, W. T. Kaczewski, H. Niedzielska, J. Bochenska, M. Kulawczyk, L. Szymanski and E. Zujewski, Acta. Microbiol. Polonica Ser. A V(22), 219 (1973).
105. J. B. Arensman, J. W. Dominik and D. E. Hilmas, Antimicrob. Ag. Chemo 12 (1), 40 (1977).
106. J. S. Walker, E. L. Stephen and R. O. Spertzel, J. Inf. Dis. 133, A140 (1976).
107. G. Galegov, N. Pushkarskaya, N. Obrosova-Serova and V. Zhdanov, Experientia 33 (7), 905 (1977).
108. N. L. Pushkarskaya, V. K. Blinova, R. Ya. Podchernyaeva, N. P. Obrosova-Serova, G. A. Galegov, D. K. L'vov and V. M. Zhdanov, Vopr. Virusol (3), 369 (1977).
109. G. Galegov, A. Yatsna, G. Linitskaya and N. Pushkarskaya, Biochemistry 41, 2 (1976).
110. V. A. Kalninya and M. K. Indulen, Acta virol. 20, 343 (1976).
111. P. M. Ahumada, Ther. Ggw. 116 (1), 100 (1977).
112. D. Fanta, Wien Med. Wschr. 126, 315 (1976).
113. M. M. Hassan, Rev. Int. Trach. 53 (3-4), 105 (1976).
114. Wien. Klin. Wochenschr. 85 (37), 614 (1973). (Authors not stated.)
115. J. W. Hadden, E. M. Hadden and R. G. Coffey, Infect. & Immun. 13 (2), 1382 (1976).
116. R. D. Azulay and E. Azulay, Rev. Bras. Med. 32 (2), 105 (1975).
117. Scrip, p. 16, Sept. 1977.
118. J. Z. Miller, W. E. Nance, J. A. Norton, R. L. Wolen, R. S. Griffith and R. J. Rose, JAMA 237 (3), 248 (1977).
119. J. L. Conlehan, S. Eberhard, L. Kapner, F. Taylor, K. Rogers, and P. Garry, New Eng. J. Med. 295, 973 (1976).
120. Editorial, Brit. Med. J. 1 (6010), 606 (1976).
121. G. D. Diana, J. Med. Chem. 20 (6), 757 (1977).
122. F. Pancic, B. Steinberg, G. Diana, W. Gorman, and P. Came, 17th Intersci. Conf. Antimicrob. Ag. Chemo., New York (Oct. 12-14), 1977 Abst. #289.
123. I. S. Nikolaeva, S. N. Kutchak, N. S. Bogdanova and G. N. Pershin, Farmakol. Toksikol. (Moscow) 3, 336 (1976).
124. N. Gustman, I. Zastavnii, L. Manakhova and I. Balandin, Vopr. Virusol (6), 738 (1975).
125. Y. Togo, F. E. Durr and D. A. Laurenzana, Med. Microbiol. Immunol. 163 (1), 37 (1977).
126. S. E. Reed, J. W. Craig and D. A. J. Tyrrell, J. of Inf. Dis. 133, A128 (1976).
127. E. D. Stanley, G. G. Jackson, V. A. Dirda and M. Rubenis, *ibid*, 133, A121 (1976).
128. F. Floc'h and G. H. Werner, Arch. Virol. 52, 169 (1976).
129. K. Shinkai and T. Nishimura, Ann. Rep. Sankyo Res. Lab. 28, 93 (1976).
130. W. L. Albrecht, J. Med. Chem. 20 (3), 364 (1977).
131. A. A. Carr, *ibid*, 19 (9), 1142 (1976).
132. E. R. Kern, J. R. Hamilton, J. C. Overall, Jr., and L. A. Glasgow, Antimicrob. Ag. Chemo. 10 (4), 691 (1976).
133. M. J. Kramer, R. Cleeland and E. Grunberg, *ibid*, 9 (2), 233 (1976).