

ANTIMICROBIAL AGENTS
AND CHEMOTHERAPY—1962

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY — 1962

63,383

4512

1962

Proceedings of the Second Interscience Conference on
Antimicrobial Agents and Chemotherapy,
Chicago, Illinois, October 31 — November 2, 1962

Editor

J. C. SYLVESTER

Editorial Board

Julius Berger
Nestor Bohonos
Maxwell Finland
David Gottlieb
Morton Hamburger
Mark H. Lepper
Joseph Lein

David Perlman
R. G. Petersdorf
E. L. Quinn
L. A. Rantz
M. J. Romansky
J. C. Sheehan
E. D. Weinberg

© 1963 American Society for Microbiology
115 Huron View Boulevard
Ann Arbor, Michigan

Library of Congress Catalog Card Number: 62-12476

Printed in the United States of America
by BRAUN-BRUMFIELD, INC.,
Ann Arbor, Michigan

Preface

The keen interest manifested in the First Interscience Conference on Antimicrobial Agents and Chemotherapy (1961) provided convincing evidence of the need and value of the meetings. Consequently, the conferences have been accepted as an important regular element of the affairs of the American Society for Microbiology. They can well continue to serve as a forum for discussion and announcement of information, problems, and ideas on control of pathogenic microorganisms, for the critical examination of available information and procedures, and for disclosure of promising new ventures and speculations that will serve to nourish progress.

Through will and design the conferences provide the means for all scientists interested in antimicrobial agents to exchange ideas on the subject. The Society heartily endorses and supports the combined participation of microbiologists, chemists, biochemists, clinicians, pharmacologists, pathologists, and others in the deliberations. It is anticipated that by breaking through the barriers of disciplines there will be improved communication, better understanding, and shortening of the period between disclosure and application.

The subject areas of the scientific reports of the Second Conference, included in this volume, are similar to those of the First Conference but with changes in accent. Among the subjects considered are: (i) composition, activity, and production of new antibiotics; (ii) synergistic effects of antimicrobial agents; (iii) control of microbial resistance to antibiotics; (iv) effects of physical state of drugs on their activity; (v) control of neoplastic growths; (vi) hospital septicemias; (vii) disinfection of hospital areas; (viii) chemotherapy of infectious diseases; and (ix) clinical evaluation of chemotherapeutic substances.

This volume reveals the present status and direction of the scientific maneuvers to control diseases by antimicrobial agents, and provides a prospect of the future. The annual volumes will serve as a record of progressive movement of the frontiers.

The effectiveness of the Second Conference and the importance of the material in this volume reflect the competence and devoted efforts of those who arranged the conference, in particular the Executive Committee under the chairmanship of J. C. Sylvester, and, of course, the participants.

Robert L. Starkey, *President*

TABLE OF CONTENTS

Preface	v
---------------	---

Infectious Diseases I

Maxwell Finland and Morton Hamburger, Conveners

Effect of Cycloheximide on the Immune Response. W. J. Cooney and S. G. Bradley.....	1
Changing Pattern of Septicemia in Infants and Children. Richard B. Johnston, Jr.	10
Rapid Detection of Lactose Fermentation by Single-Colony Isolates. Lois M. Bergquist, Ellen C. Thumann, and Ronald L. Searcy	16
Microbial Persistence Versus Reinfection in Recurrent Urinary Tract Infections. Calvin M. Kunin.....	21
In Vitro Effect of Buffered Solutions of Acetic Acid, Tri- clobisonium Chloride, Chlorhexidine Diacetate, and Chlor- hexidine Digluconate on Urinary Tract Pathogens. Richard H. Parker and Paul D. Hoeprich	26
Results of a Data-Processing Method Applied to the Clinical Bacteriology Laboratory. Esther L. Cheatle.....	35
Sequential Procedure for Screening Five-Drug Mixtures Against an Experimental <i>Salmonella choleraesuis</i> Infection in Mice. G. O. Gale and J. S. Kiser.....	43
"Pathogen-Free" Patient-Care Area. M. S. Rittenbury, D. M. Hume, and M. E. Hench.....	51
Evaluation of a Spray-Fog Technique with Quaternary Ammonium Disinfectant for Antimicrobial Control in Hospital Environments. Herman Friedman, Steven Syk, and David Laumann.....	66

Infectious Diseases II

Lowell Rantz and Donald Nichols, Conveners

Chemotherapy of Experimental Staphylococcal Disease in Mice. Gladys L. Hobby, Oscar Auerbach, and Lynn Ward ..	76
Subacute Intramuscular Staphylococcus Infection of the Mouse Leg for Drug Evaluation. George A. Hunt and Alvin J. Moses.....	87
Role of Phagocytosis in Immunity to Clumping Factor- Negative Staphylococci. Ralph Tompsett.....	100

Influence of Oxacillin on Staphylococcal Populations in Mouse Kidneys. Robert McCune	107
Mixed Infections with <i>Staphylococcus aureus</i> and <i>Candida</i> <i>albicans</i> . Mark H. Lepper, Bernhard Chomet, and Elvira Karklys	114
Therapeutic Observations in Experimental Ocular Infections. J. R. Regan, F. J. Sweeney, Jr., J. W. Sokolowski, Jr., J. P. Capelli, and E. L. Linegar	123
<i>Pseudomonas</i> Infections of the Cornea in Rabbits: an In Vivo Comparison of Polymyxin B and Colistin Sulfate. Philip C. Hessburg, Joseph P. Truant, and William P. Penn.	131
Effect of Antibiotic Nasal Ointments on Carrier States in Patients and on the Antibiotic Pattern of Organisms from Personnel Caring for These Patients. Mark H. Lepper, Harry F. Dowling, George G. Jackson, Harold W. Spies, and Jeanette Norsen	140
Influence of Three Penicillins and Cephalothin on Staphylo- cocci in Nasal Carriers. Henry Abramovitch and Emanuel Wolinsky	150
Development of Resistance to Fusidic Acid During Treat- ment of Nasal Carriers of Staphylococci. James Smith and Arthur White	155
Effect of the Usage of a Combination of Novobiocin and Erythromycin on the Susceptibility of Nasal Staphylococci. Mark H. Lepper and Agnes G. Lattimer	160
Clinical Trials with New Antifungal Agents. Communicable Disease Center Cooperative Mycoses Study	171

Chemistry of Antibiotics

Nestor Bohonos, Convener

Chemistry of the Duazomycins. II. Duazomycin B. Koppaka V. Rao	179
Chemistry of Zygomycin A: the Structure of Zygomycins A ₁ and A ₂ . Sueo Tatsuoka, Satoshi Horii, Takeshi Yamaguchi, Hiromu Hitomi, and Akira Miyake	188
Structural and Biosynthetic Studies on the Neomycins. Kenneth L. Rinehart, Jr., Martin Hichens, James L. Foght, and W. Scott Chilton	193
Chemical and Biological Properties of Capreomycin and Other Peptide Antibiotics. Earl B. Herr, Jr.	201
Chemistry and Biological Activities of the Tetracyclines. J. H. Boothe	213

Synthetic Antimicrobials

Eugene D. Weinberg, Convener

Antimicrobial Action of Some Amino Acids and Their Derivatives. I. Chemistry. Eli Seifter, Henry D. Isenberg, Edward Henson, and Marilyn Werble.	226
Antimicrobial Action of Some Amino Acids and Their Derivatives. II. Antimicrobial Activity. Henry D. Isenberg, Joshua Roth, Eli Seifter, and James I. Berkman	234
Mechanism of Antibacterial Action of N ¹ ,N ⁵ -Di-(3,4-dichlorobenzyl) biguanide. Vida Helms and Eugene D. Weinberg.	241
Hospital Floor Decontamination: Controlled Blind Studies in Evaluation of Germicides. Sydney M. Finegold, Edward E. Sweeney, Donald W. Gaylor, Doris Brady, and Lawrence G. Miller	250
Antitrichophyton Activity of Naphthlomates. Teruhisa Noguchi, Aritsune Kaji, Yoshinobu Igarashi, Akiyo Shigematsu, and Kanji Taniguchi	259
AS17665, a New Systemically Active Nitrofurantoin. J. C. Holper, R. H. Otto, E. T. Kimura, and R. R. Bower.	268
Chemotherapeutic Studies on 3-Amino-6-[(5-nitro-2-furyl)vinyl]-1,2,4-triazine and Related Compounds. Koji Miura	275
Inhibitory Effects of Colistin Sulfate and Seven Sulfonamides Against Gram-Negative Organisms. Joseph P. Truant and William P. Penn.	283
Effects of B.663, a Rimino Compound of the Phenazine Series, in Murine Leprosy. Y. T. Chang	294
Laboratory Studies and Clinical Pharmacology of Nalidixic Acid (Win 18,320). Mandel Buchbinder, Joe C. Webb, La Verne Anderson, and William R. McCabe	308

Clinical Evaluation I

George G. Jackson and Ralph Tompsett, Conveners

Effect of Penicillin Side Chain Structure on Staphylococcal Penicillinase Susceptibility. A. Gourevitch, T. A. Pursiano, and J. Lein.	318
Further Experiences with Ampicillin. Paul Bunn, John O'Brien, David Bentley, and Harvey Hayman	323
Laboratory Evaluation of Three New Penicillins Against <i>Staphylococcus aureus</i> . Howard E. Noyes, Jimmy R. Evans, and Alfred A. Serritella	334
Ampicillin: Antimicrobial Activity and Pharmacological Behavior with Reference to Certain Gram-Positive Cocci. E. L. Quinn, J. M. Colville, L. Ballard, D. Jones, and F. Debnam	339

In Vitro Evaluation of α -Aminobenzyl Penicillin (Ampicillin). Jack A. Barnett, Jay P. Sanford, Richard A. Ferguson, and Nancy E. Perry	350
Activity of Sodium Nafcillin [6-(2-Ethoxy-1-naphthamido-) Penicillanic Acid] Against Staphylococci In Vivo. James Smith and Arthur White.	354
Duration of Therapeutic Effectiveness of Nafcillin Compared with Potassium Penicillin G, Methicillin, and Oxacillin. Margaret W. Hopper, John A. Yurchenco, Anne Gillen, and George H. Warren	362
Comparative In Vitro Activity of Semisynthetic Penicillins Nafcillin and Oxacillin. Sanford B. Rosenman and George H. Warren	369
Concentration of Sodium Nafcillin in Pathological Synovial Fluid. Peter Viek	379

Clinical Evaluation II

Monroe Romansky and Gladys Hobby, Conveners

Blood Levels and Antistaphylococcal Titers Produced in Human Subjects by a Penicillinase-Resistant Penicillin, Nafcillin, Compared with Similar Penicillins. Alan C. Whitehouse, Jerome G. Morgan, Janet Schumacher, and Morton Hamburger	384
Therapeutic Effect of Oxacillin in Patients with Cystic Fibrosis. Nancy N. Huang, Kate Librenjak, and Robert H. High.	393
Absorption, Diffusion, and Excretion of a New Penicillin, Oxacillin. Aaron Prigot, Cleo J. Froix, and Emanuel Rubin	402
Parenteral Use of Oxacillin. Robert G. Brayton and Donald B. Louria	411
Increasing Incidence of Staphylococci Resistant to Kanamycin. James B. Grogan and Curtis P. Artz	420
Evaluation of a New Oral Preparation of Kanamycin, Kanamycin 3-Phenyl Salicylate, in Treatment of Infections of the Urinary Tract. Marvin Turck, Robert I. Lindemeyer, and Robert G. Petersdorf	425
Preliminary Report on Kanamycin 3-Phenyl Salicylate. Alexander M. Rutenburg and Harold L. Greenberg	435
Bacteriological Study of Colistin Therapy of Enteric Infections in Children. Gerald L. Saks and Erwin Neter	442
Assay of Colistin in Fecal Specimens. Samuel P. Gotoff and Mark H. Lepper	447
Clinical and Laboratory Evaluation of Colistin in Infants and Children. M. Flux, Harris D. Riley, Jr., E. C. Bracken, and M. I. Abbott	455

Colistin for Treating <i>Pseudomonas</i> Infections in Children. Lyal D. Asay and Richard Koch.....	466
--	-----

Clinical Evaluation III

Mark Lepper and E. L. Quinn, Conveners

Relationships Between the Concentrations of Various Penicillins in Plasma and Peripheral Lymph. W. F. Verwey and H. R. Williams, Jr.	476
Binding of Various Penicillins by Plasma and Peripheral Lymph Obtained from Dogs. W. F. Verwey and H. R. Williams, Jr.	484
Total Bilirubin and Serum Glutamic Oxalacetic Transaminase Content of Sera of Children Receiving Erythromycin Estolate and Phenoxymethyl Penicillin. Henry G. Cramblett and Hugh L. Moffet.	492
Nephrotoxicity of Amphotericin B: Observations on the Mechanism of Hypokalemia. Garabed Eknayan and Albert D. Roberts.	497
Methacycline in the Treatment of Gonorrhea in the Male. Milton Marmell, Joseph R. Sills, and Aaron Prigot	502
Oral Phenethicillin Treatment of Streptococcal Endocarditis: Autologous Serum Bactericidal Activity Related to Penicillinemia and Streptomycin. Roger P. Kennedy, John C. Perkins, and George G. Jackson	506
<i>Actinomyces bovis</i> Endocarditis: an Uncommon and Complex Problem. Edward W. Walters, Monroe J. Roman-sky, Arnold C. Johnson, and Steven J. Conway.	517
Intra- and Extraperitoneal Administration of Ristocetin and Polymyxin B. J. C. Sylvester, G. A. Olander, and V. Z. Hutchings	526
Comparative Pharmacodynamics, Urinary Excretion, and Half-Life Determinations of Nitrofurantoin Sodium. Hellmuth K. Reckendorf, Rudolf G. Castringius, and Helmut K. Spingler	531
Actinospectacin Serum Levels and Clinical Data. John M. Barry and Richard Koch.	538

New Antibiotics

Julius Berger, Convener

Protective Effect of Actinogan Against Experimental Infections in Mice. K. E. Price, G. A. Hunt, A. J. Moses, A. Gourevitch, and J. Lein	543
Lincomycin, a New Antibiotic. I. Discovery and Biological Properties. D. J. Mason, A. Dietz, and C. DeBoer.	554

Lincomycin, a New Antibiotic. II. Isolation and Characterization. R. R. Herr and M. E. Bergy	560
Lincomycin, a New Antibiotic. III. Microbiological Assay. L. J. Hanka, D. J. Mason, M. R. Burch, and R. W. Treick.	565
In Vitro and In Vivo Evaluation of Lincomycin, a New Antibiotic. Charles Lewis, Howard W. Clapp, and Joseph E. Grady	570
Chelocardin, a New Broad-Spectrum Antibiotic. I. Discovery and Biological Properties. T. J. Oliver, J. F. Prokop, R. R. Bower, and R. H. Otto.	583
Chelocardin, a New Broad-Spectrum Antibiotic. II. Isolation and Characterization. A. C. Sinclair, J. R. Schenck, G. G. Post, E. V. Cardinal, S. Burokas, and H. H. Fricke. . .	592
Capreomycin, a New Antimycobacterial Agent Produced by <i>Streptomyces capreolus</i> sp. n. W. M. Stark, C. E. Higgs, R. N. Wolfe, M. M. Hoehn, and J. M. McGuire	596
U-12898, a New Antibiotic. I. Discovery, Biological Properties, and Assay. D. J. Mason, A. Dietz, and L. J. Hanka.	607
U-12898, a New Antibiotic. II. Isolation and Characterization. M. E. Bergy, T. E. Eble, R. R. Herr, C. M. Large, and B. Bannister	614

Antibiotics - General

David Gottlieb, Convener

Influence of Freezing on the Activity Determination of Antibiotics and Sulfonamides in Human Serum and Tissue. H. P. Kuemmerle, P. Röttger, and H. Contzen	619
Spectrophotometric Assay for Penicillin in Aqueous and Protein Solutions. Michael W. Brandriss, Emmy L. Denny, Marget A. Huber, and Harry G. Steinman	626
Binding of Erythromycin, Novobiocin, Chloramphenicol, Chlortetracycline, and Nitrofurantoin by Serum Proteins. Horace H. Zinneman, Wendell H. Hall, Leland Hong, and Ulysses S. Seal	637
Influence of Penicillin on the Uptake of C ¹⁴ -Labeled Isoniazid by <i>Mycobacterium tuberculosis</i> . E. M. K. Vaichulis, E. E. Vicher, and M. V. Novak.	644
Prevention of the Development of Microbial Resistance to Drugs. W. T. Drabble and M. G. Sevag.	649
Penicillin Metabolites. G. N. Rolinson and F. R. Batchelor. . .	654
<i>Streptomyces lusitanus</i> and the Problem of Classification of the Various Tetracycline-Producing <i>Streptomyces</i> . Ivan Villax.	661

Factors Affecting the Biosynthesis of Griseofulvin.	
A. P. Bayan, U. F. Nager, and W. E. Brown.	669
Correlative Assays. L. J. Hanka and C. G. Smith.	677

Cephalosporins

John Sheehan and Joseph Lein. Conveners

A Specific Bio-Assay for Cephalosporin C in Fermentation Broths. C. A. Claridge and David L. Johnson	682
Structure-Activity Relationships Among 7-Acylamidocephalosporanic Acids. Robert R. Chauvette, Edwin H. Flynn, Bill G. Jackson, E. R. Lavagnino, Robert B. Morin, Richard A. Mueller, Richard P. Pioch, R. W. Roeske, C. W. Ryan, John L. Spencer, and Earle Van Heyningen	687
Blood and Tissue Distribution of Cephalothin. Chen-Chun Lee and Robert C. Anderson	695
Comparative Inhibition of 100 Strains of <i>Haemophilus influenzae</i> . Sarah H. W. Sell and Linda Arnold	702
Cephalothin, a New Semisynthetic Broad-Spectrum Antibiotic. Laboratory and Clinical Studies in 52 Patients (Preliminary Report). Edward W. Walters, Monroe J. Romansky, and Arnold C. Johnson	706
Studies of Cephalothin in Infants and Children. Harris D. Riley, Jr., E. C. Bracken, and M. Flux	716
Cephalosporin C and Cephalothin in Gram-Negative Infections. Kenneth N. Anderson and Robert G. Petersdorf. . . .	724

Cancer Chemotherapy

Chester Stock, Convener

Effects of Antibiotics, Antitumor Agents, and Antimetabolites on the Metabolism of Mammalian Cells in Tissue Culture. Peter Arnow, Sharon A. Brindle, Nancy A. Giuffre, and D. Perlman	731
Antitumor Properties of Phleomycin. W. T. Bradner and M. H. Pindell.	740
Effect of Hadacidin on a Transplantable Human Epidermoid Carcinoma. Philip C. Merker, Janet S. Sarino, Rica Anido, Matthew Bowle, and George W. Woolley	749
Isolation and Characterization of Roseolic Acid, an Antitumor Substance. Donald W. Renn, Imbi Truumees, and Koppaka V. Rao	760
BA-180265: a New Cytotoxic Antibiotic. Wen-Chih Liu, Walter P. Cullen, and Koppaka V. Rao	767

Sparsomycin, a New Antitumor Antibiotic. I. Discovery and Biological Properties. S. P. Owen, A. Dietz, and G. W. Camiener	772
Sparsomycin, a New Antitumor Antibiotic. II. Isolation and Characterization. A. D. Argoudelis and R. R. Herr.	780

Antibiotics - In Vitro Activity

G. B. Whitfield, Convener

Sensitivity of <i>Salmonella</i> , <i>Shigella</i> , and Enteropathogenic <i>Escherichia coli</i> Species to Cephalothin, Ampicillin, Chloramphenicol, and Tetracycline. Jorge Olarte, Emma Galindo, and Alicia Jaochin	787
Synergistic Action In Vitro of Tetracycline and Benzyl Penicillin Against Pathogenic Staphylococci. Leonardo Paredes and Joaquín de Pablo	794
Effect of Colistin on the Metabolism of <i>Pseudomonas aeruginosa</i> . Raam R. Mohan, Roland S. Pianotti, Ruth Leverett, and Benjamin S. Schwartz	801
Sulfonamide Potentiation of the Inhibitory Activity of Colistin on <i>Proteus vulgaris</i> . Frank J. Turner, Frances L. Lindo, Pasquale J. Storino, Joan M. Daly, Dorothea Allen, and Benjamin S. Schwartz	815
Protective Effect of Colistimethate Sodium Against Death Caused by Fecal Contaminants in Mice. James B. Grogan and Curtis P. Artz	827
Synergism of Erythromycin and Penicillin Against Resistant Staphylococci: Mechanism and Relation to Synthetic Penicillins. C. Evans Roberts, Jr., Lona S. Rosenfeld, and William M. M. Kirby	831
L-Phase Growth Induction as a General Characteristic of Antibiotic-Bacterial Interaction in the Presence of Serum. C. W. Godzeski, Gordon Brier, and D. E. Pavey	843
Survival In Vivo (In Ovo) of L-Phase Bacteria. Gordon Brier, Lee Ellis, and C. W. Godzeski	854
Factors Affecting the Activity of Antibiotic X-5079C Against <i>Histoplasma capsulatum</i> In Vitro. George W. Lones and Carl Peacock	861

International Integration of Antibiotic Sensitivity Tests

Arnold Branch, Moderator

Panel Discussion	867
Author Index	875
Subject Index	879

EFFECT OF CYCLOHEXIMIDE ON THE IMMUNE RESPONSE

W. J. COONEY AND S. G. BRADLEY

*Department of Microbiology,
University of Minnesota,
Minneapolis, Minnesota*

Abstract

The LD₅₀ values of saline solutions or suspensions of 6-mercaptopurine, cycloheximide, nystatin, griseofulvin, and colchicine, injected intraperitoneally into adult BALB mice, were found to be >300, 175, 250, >500, and 7 mg/kg, respectively. Each drug was tested for the power to suppress formation of antibody which neutralized actinophage. Only cycloheximide, given at 0.2 LD₅₀ daily for 1 week, markedly suppressed the early immune response; 6-mercaptopurine was slightly inhibitory. Suppression of antibody formation was greater when treatment with cycloheximide was begun before, rather than at the same time as, antigenic stimulation. A single dose of drug did not alter the immune response. Cycloheximide also inhibited the anamnestic response.

Specific suppression of the immune response may provide a useful tool for studying the mechanism of antibody formation, and may have practical application in tissue transplantation and autoimmune diseases. Impairment of the immune response may occur naturally, as in agammaglobulin anemia (Varco et al., 1955), or may be induced by X-radiation (Makinodan and Gengozian, 1959), cortisone treatment (Kass, Kendrick, and Finland, 1955), dietary deficiencies (Cannon, 1942; Axelrod and Pruzansky, 1955), and by immune tolerance (Smith, 1959). Several investigators have reported suppression of antibody production with drugs which are antagonists of nucleic acid metabolism (Sterzl, 1961; Nathan et al., 1961; Berenbaum, 1960). Suppression by the purine analogue 6-mercaptopurine has been studied extensively (Schwartz, Stack, and Damashek, 1958; Condie, Mennis, and Miller, 1961; Genghof and Battisto, 1961). Because nucleic acids are important for the initiation of antibody formation (Dutton, Dutton, and Vaughan, 1959; Burnet, 1956), any compound which inhibits their biosynthesis or function may also retard

development of the immune response. Cycloheximide, an antifungal antibiotic, is such a cytotoxic drug which inhibits nucleic acid synthesis in yeast and animal cells (Kerridge, 1958; Cooney and Bradley, 1962). This report is concerned with the effect of cycloheximide on the production of antibody which neutralizes actinophage. Other compounds tested for their effect on the immune response of the mouse were a known inhibitor, 6-mercaptopurine, two antifungal antibiotics, nystatin and griseofulvin, and the mitotic inhibitor, colchicine.

Materials and Methods

Adult BALB mice weighing about 20 g and guinea pigs weighing 400 to 750 g were used. To elicit antibody production, the animals were injected intraperitoneally with 10^{10} plaque-forming units of actinophage MSP8. In studying the early immune response, the mice were injected once; for hyperimmunization, five weekly injections were given. The phage had been purified by centrifugation, filtration, and chromatography (Kolstad and Bradley, 1962). After antigenic stimulation (5 days), blood samples were drawn from the retro-orbital plexus with a capillary tube moistened with heparin sodium; blood was allowed to clot, and serum was collected by centrifugation. All serum samples were heated to 56 C for 30 min and then diluted in 10% normal rabbit serum. Usually, early sera were diluted 1:6 and hyperimmune sera were diluted 1:6,000 to 1:60,000. For determining neutralizing activity, phage was added to the diluted serum to give 2×10^5 particles/ml. After 0, 0.5, 3, and 6 hr, samples of the reaction mixture were diluted 1:100 with cold peptone-yeast extract broth. Then, 0.1 ml of the final dilution was added to an inoculative suspension of the host, *Streptomyces venezuelae* S13, in molten peptone-yeast extract-agar medium and poured into petri plates containing a basal layer of the same medium (Bradley et al., 1961). After incubation at 30 C for 18 to 24 hr, plaque counts were made. Preimmune sera were taken from all animals and tested for neutralizing activity. Neutralizing activity has been expressed as a function of the percentage of surviving phage, using the diluent controls as the base line (Adams, 1959). Phage clearance from the blood was also used as an index of the immune response (Uhr, Finkelstein, and Bauman, 1962).

The compounds used in this study were dissolved or suspended in 0.15 M saline. Mice were given 0.2 LD₅₀ of a drug daily for 7 days; phage was administered on the third day and serum was collected on the eighth day. Variations of dosage and regimen were also employed. Groups of animals injected with phage alone and drug alone were included in each experiment. These controls were carried out to ensure that any alteration of neutralizing activity was an effect on the animals and not on the phage directly.

Results

Griseofulvin and 6-mercaptopurine were well tolerated by the mouse, whereas nystatin and cycloheximide were somewhat harmful and colchicine was very toxic (Table 1). Serum of preimmune animals and of animals receiving 0.2 LD₅₀ of a drug for 7 days did not inactivate actinophage; however, mice injected with phage developed substantial neutralizing activity within 5 days. The early immune response of mice stimulated with phage was markedly suppressed by treatment with cycloheximide (Fig. 1). Neutralizing activity in 6-mercaptopurine-treated antigen-stimulated animals was consistently lower than in untreated stimulated control animals. Raising the dose to 100 mg per kg per day and extending the treatment did not increase the inhibition. Similarly, more stringent regimens with colchicine, nystatin, and griseofulvin did not alter the immune response.

TABLE 1. *Toxicity of cycloheximide and other drugs*

Drug	Source	LD ₅₀ * mg/kg
Cycloheximide saline solution	California Biochemicals, Los Angeles, Calif.	175
	The Upjohn Co., Kalamazoo, Mich.	60†
6-Mercaptopurine saline suspension	California Biochemicals, Los Angeles, Calif. Nutritional Biochemicals Corp., Cleveland, Ohio	300
Colchicine saline solution	Nutritional Biochemicals Corp., Cleveland, Ohio	7
Griseofulvin saline suspension	Schering Corp., Bloomfield, N.J.	500
Nystatin saline suspension	E. R. Squibb & Sons, New Brunswick, N.J.	250

*BALB mice were injected intraperitoneally.

†Guinea pig.

Maximal suppression with 35 mg of cycloheximide per kg per day was observed when animals were treated daily at least 3 days prior to, and continuously after, injection of antigen. Omission of a single injection of drug permitted further development of the immune response. At least four injections of 0.2 LD₅₀ of cycloheximide were necessary for suppression to be clearly evident (Fig. 2). Retardation of antibody production was directly proportional to the daily amount of cycloheximide injected over a range of 10 through 60 mg per kg per day. Most animals succumbed to doses higher than 60 mg per kg per day, whereas all animals receiving 0.2 LD₅₀

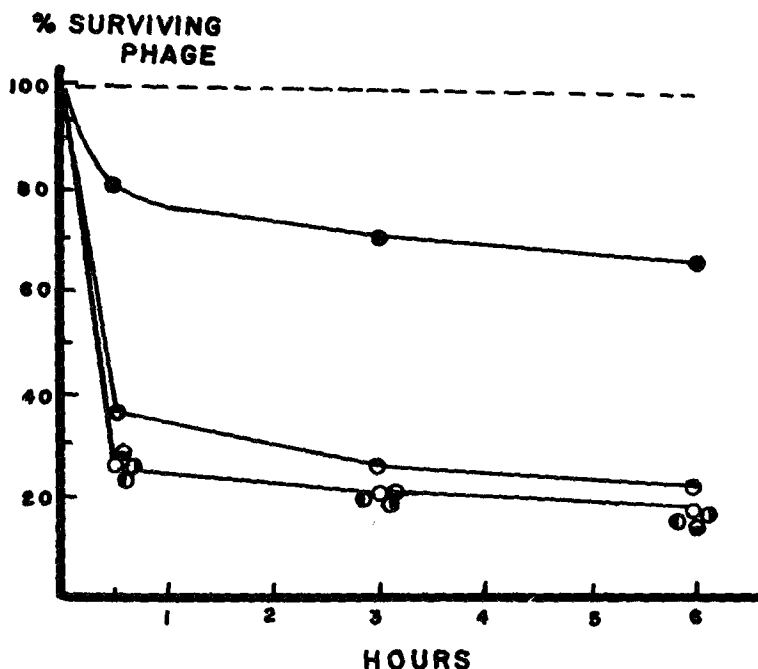


FIG. 1. Effect of cycloheximide (●), 6-mercaptopurine (○), nystatin (⊙), colchicine (⊖), and griseofulvin (⊙) on antibody production by mice receiving 9.2 LD₅₀ daily for 7 days and one injection of 10¹⁰ MSP8 on day 3. Preimmune sera and sera from unstimulated drug-treated mice (---) and sera from stimulated but untreated mice (○) served as controls.

per day survived. Mice receiving antigenic stimulation before or at the same time cycloheximide treatment was begun produced more neutralizing antibody than animals receiving several drug injections prior to presentation of the antigen (Table 2). Single injections of cycloheximide (up to 150 mg/kg) at various times before and after injection with antigen had no effect on antibody production. The inhibitory activity of cycloheximide on early antibody production in the guinea pig was comparable to that observed in the mouse.

The early immune response was effectively suppressed for 12 days in animals receiving daily injections of cycloheximide. Longer administration of drug usually resulted in death. When treatment was stopped after the seventh injection, neutralizing activity gradually increased during the subsequent 5 to 6 days until it equaled that of stimulated untreated mice. Restoration of immunological competence after having been suppressed by cycloheximide was also observed using phage clearance as an index of responsiveness. Phage was not isolated from the blood of control animals 2 days after injection but was isolated from the blood of mice receiving cycloheximide.

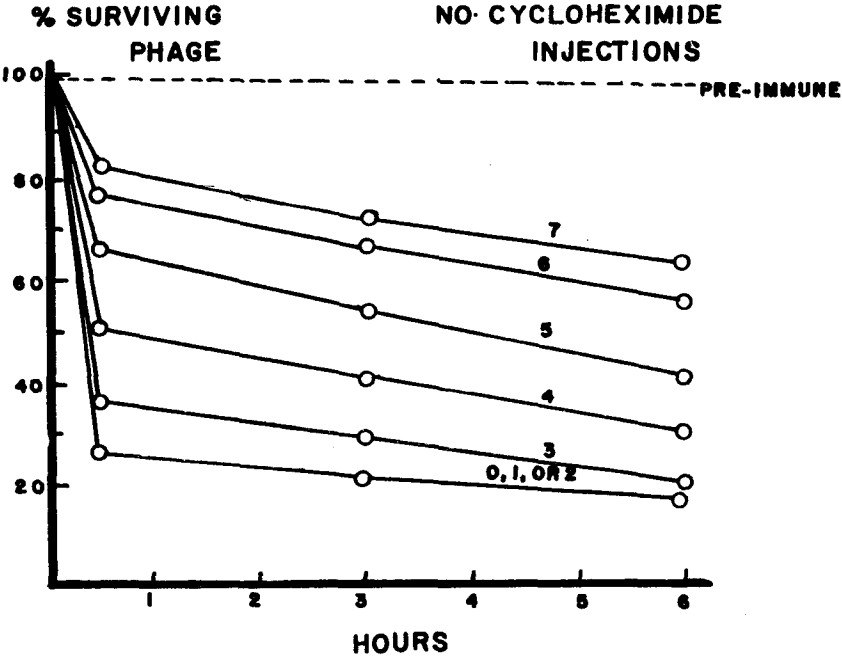


FIG. 2. Number of injections of 9.2 LD₅₀ cycloheximide required for suppression of synthesis of neutralizing antibody. A single injection of 10¹⁰ phage was given on day 3; drug injections were started on day 1 and continued for the time indicated. Mice were bled on day 8.

TABLE 2. Effect of treatment schedule on suppression of the early immune response by cycloheximide*

Day phage injected	Days treated with cycloheximide	Day bled	Neutralizing activity†
1	—	5	82
—	1 - 7	8	0
1	1 - 7	8	60
2	1 - 7	8	44
3	1 - 7	8	35
5	1 - 7	10	58
7	1 - 7	12	74
4	1 - 9	10	24

*BALB mice were given a single intraperitoneal injection of 10¹⁰ phage MSP8 and daily injections of 35 mg of cycloheximide/kg on the days indicated.

†Neutralizing activity of serum diluted 1:6 is expressed as percent phage destroyed in 6 hr.