ANTIMICROBIAL AGENTS AND CHEMOTHERAPY—1962

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

Editor

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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*

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EFFECT OF CYCLOHEXIMIDE ON THE IMMUNE RESPONSE

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Abstract

The LD_{50} 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 LD_{50} 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 LD_{50} 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 LD_{50} 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.	300
	Nutritional Biochemicals Corp., Cleveland, Ohio	
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 LD_{50} 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 LD_{50}

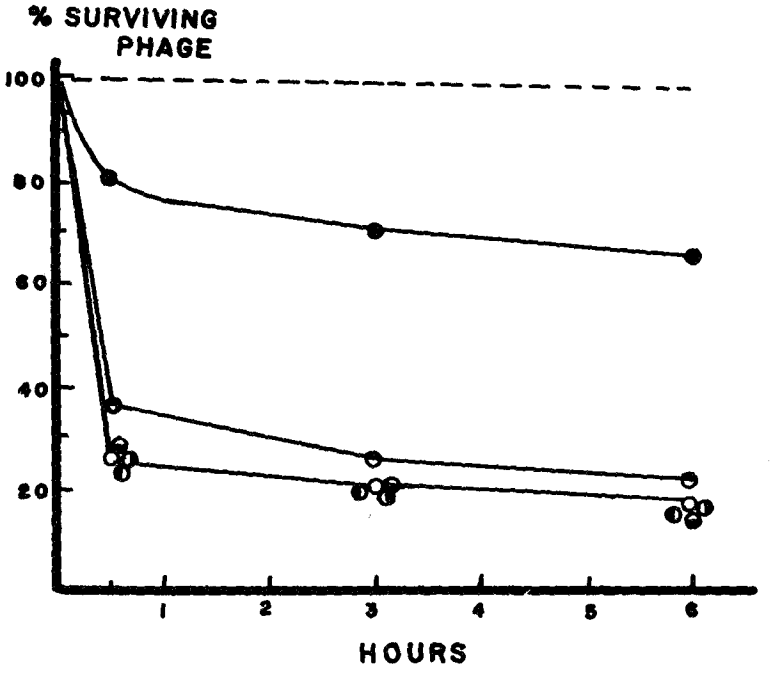

FIG. 1. *Effect of cycloheximide (●), 6-mercaptopurine (⊖), nystatin (⊜), colchicine (◑), and griseofulvin (◐) on antibody production by mice receiving 9.2 LD_{50} daily for 7 days and one injection of 10^{10} MSP8 on day 3. Preimmune sera and sera from unstimulated drug-treated mice (– – –) and sera 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.

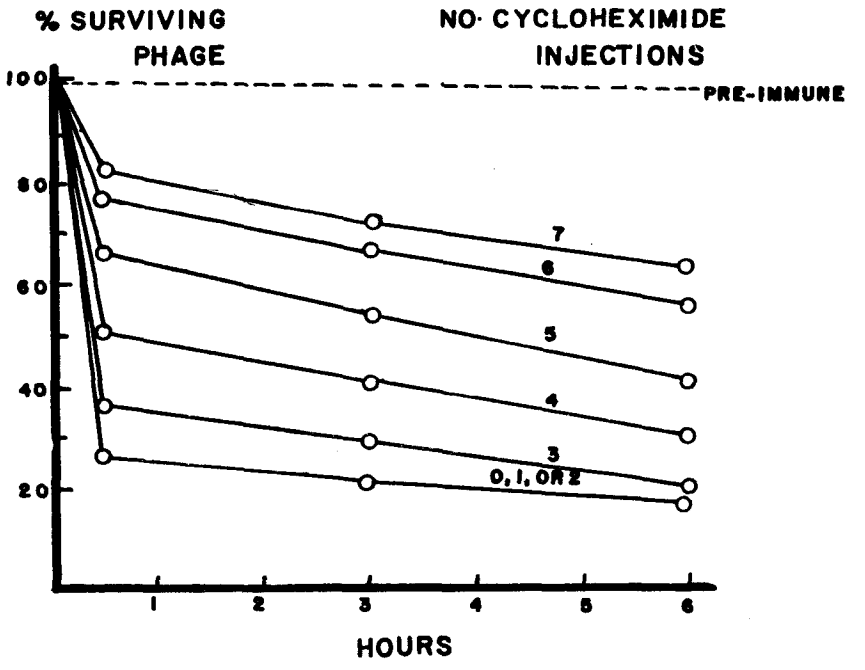

FIG. 2. *Number of injections of 9.2 LD_{50} cycloheximide required for suppression of synthesis of neutralizing antibody. A single injection of 10^{10} phage was given on day 3; drug injections were started on day 1 and continued for the time indicated. Mice were bled on day 8.*

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 10^{10} 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 per cent* phage destroyed in 6 hr.