reverse is the case. The cultures reach optimum growth density in 4 to 5 days according to the size of the inoculum. If transferred to the ice box at this point they remain active for 10 days to 2 weeks. Transplants have been made as late as the 15th day of culture. One strain has been carried through 19 generations during a period of 150 days. The 18th subculture of this strain was still fully virulent for chickens and caused fatal infections.

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Action of Sulfamido Compounds upon *M. lysodeikticus* and Lytic and Bactericidal Activities of Lysozyme.

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In contrast to many antiseptics used in surgical practice, sulfanilamide and its derivatives do not interfere with the lytic action of bacteriophage. In view of the fact that lysozyme has certain similarities to bacteriophage, experiments were carried out to determine what effects these chemotherapeutic substances have upon the lytic and bactericidal activity of lysozyme. Furthermore, the effects of sulfanilamide upon the growth of *M. lysodeikticus* were studied. Finally, experiments were done to determine whether or not lysozyme and sulfamido compounds act synergistically, as do sulfamido compounds and other anti-microbial agents, *e. g.*, immune serums, bacteriophage and certain disinfectants.

As a source of lysozyme egg white powder* was used. A strain of *M. lysodeikticus* was obtained through the courtesy of Dr. Marshall L. Snyder, Ann Arbor, Michigan. Brain heart infusion broth (Difco) was used as culture medium. Physiological saline solution and citric acid-disodium phosphate buffer, as recommended for blood cultures by Nelson,[1] were also employed.

The strain of *M. lysodeikticus* was grown on plain agar slants at 37°C. Suspensions were prepared either in physiological saline solution, distilled water or buffer solution.

* The egg white powder was kindly supplied by Stein, Hall & Company, Inc., New York, New York.

1 Nelson, C. I., *J. Infect. Dis.*, 1940, **66**, 113.

Lysis of the organism was appraised grossly. Viability of the organisms was determined by seeding plain agar plates with a standard loopful of the material; the plates were incubated at 37°C for 72 hours.

The effect of sulfanilamide upon the growth of *M. lysodeikticus in vitro* was first investigated. Brain heart infusion broth containing sulfanilamide in concentrations ranging from 1 mg % to 1000 mg % as well as broth as a control (volume 5 cc) were seeded with 0.2 cc of a heavy suspension of *M. lysodeikticus* in one experiment, and with 0.2 cc of a light suspension in another. The tubes were incubated at 37°C for 48 hours. Growth was noted at various intervals. It was found that in a concentration of 1000 mg %, sulfanilamide completely prevented the growth of *M. lysodeikticus*, provided small numbers of organisms were used for seeding purposes. In concentrations of 10 mg % to 100 mg % this drug only slightly delayed the growth of the organism.

The effect of sulfanilamide, sulfapyridine and sulfathiazol on the lytic activity of lysozyme (egg white) toward *M. lysodeikticus* was then investigated. The drugs were dissolved and the organisms suspended in physiological saline solution, distilled water, or citric acid-disodium phosphate buffer (pH 7.2) solution. The latter was used in order to exclude interference of different pH values. The results of all experiments were essentially identical. A typical experiment follows:

Sulfanilamide in concentrations of 0.1 mg % to 1000 mg % and sulfapyridine in concentrations of 0.01 mg % to 100 mg % were mixed with egg white powder (100 mg %). Controls without sulfanilamide and sulfapyridine and controls without egg white were included. The mixtures (volume 4.0 cc) were kept for 5 minutes at 55°C in part I of the experiment and for 24 hours at 55°C in part II. Then a suspension of *M. lysodeikticus* (volume 0.2 cc) was added, and the test tubes were incubated at 55°C. Clearing was noted at various intervals. The experiment revealed that neither sulfanilamide, sulfapyridine nor sulfathiazol interfered with the lytic activity of egg white toward *M. lysodeikticus*, even if these drugs were allowed to act upon lysozyme for 24 hours at 55°C. Experiments carried out at 37°C revealed essentially the same findings.

These results, therefore, indicate that lysozyme belongs to that group of enzymes or enzyme-systems that are not inhibited by drugs of the type of sulfanilamide. Although the rôle of lysozyme as a defense factor against microbial infections is still a moot

question, the demonstration that sulfamido compounds even in high concentration do not interfere with lysozyme activity *in vitro* gives indirect evidence that the administration of these chemotherapeutic substances probably does not affect its lytic or bactericidal activities *in vivo*.

In another series of experiments the susceptibility to the action of lysozyme of *M. lysodeikticus* that was previously exposed to the action of sulfamido compounds was determined. The experiments revealed that egg white powder caused lysis of the organisms irrespective of whether they were grown in the presence or absence of sulfanilamide.

The question arises as to whether or not sulfamido compounds and lysozyme act synergistically and together exert greater bactericidal effects than does either agent alone. A typical experiment follows: Egg white powder in concentrations of 100 mg %, 50 mg %, and 20 mg % in (a) buffer solution and in (b) buffer solutions containing sulfapyridine in a concentration of 100 mg %, was incubated at 55°C for 2 hours. At this state of the experiment cultures were made in order to test for sterility. Then a suspension of *M. lysodeikticus* in buffer solution was added. The tubes were incubated at 37°C for 2 weeks. Lysis and survival of the organisms were determined at various intervals. The experiment revealed that neither egg white in a concentration of 100 mg % or less nor sulfapyridine caused loss of viability of the organisms even after an incubation period of 15 days. In contrast, egg white in concentrations of 50 mg % and above used together with sulfapyridine killed the organisms after 8 days' incubation. Essentially the same results were obtained with sulfanilamide in a concentration of 1000 mg %. It may be concluded, therefore, that the combined use of sulfanilamide or sulfapyridine and lysozyme results in somewhat greater bactericidal effects than that of either compound alone.

Several experiments were designed in order to compare the action of sulfamido compounds upon lysozyme with other commonly used disinfectants. In concentrations that did not cause visible precipitation of egg white (lysozyme), phenol (1:100); bichloride of mercury (1:100,000); merthiolate (1:10,000); and zephiran (1:100) did not interfere with the lytic action of lysozyme. In contrast, tincture of iodine in concentrations that did not cause visible precipitation of egg white, definitely inhibited the lytic activity of lysozyme.

Summary. (1) Sulfanilamide in high concentration (1000 mg %) exerts bacteriostatic activity toward small numbers of *M.*

lysodeikticus. (2) Sulfanilamide in concentrations up to 1000 mg % and sulfapyridine and sulfathiazol in concentrations up to 100 mg % do not interfere with the lytic activity of lysozyme (egg white). (3) The combined use of lysozyme (egg white) and sulfamido compounds (sulfanilamide and sulfapyridine) exerts somewhat greater bactericidal effects upon *M. lysodeikticus* than either agent alone.

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II. Effects of Sulfanilamide and Sulfapyridine upon Experimental Streptococcal Infections.

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Having obtained a suitable method for determining the virulence of *Streptococcus hemolyticus* in small laboratory animals, we proceeded to study the therapeutic effect of sulfanilamide and sulfapyridine upon infection. The infection consisted of injecting 0.03 cc of an 18-hour serum broth culture intracerebrally. In the experiment on the effect of sulfanilamide upon hemolytic streptococcal infection, strain SA_{17} was used. Two different dilutions of organisms were given and for each dilution the animals were divided into 2 groups. One group received the treatment and the other group served as a control. Each group consisted of 6 white mice and 3 hamsters. In the study on the effect of sulfapyridine upon hemolytic streptococcal infection, strain 232 was used instead. The animals were similarly divided and similarly infected except that only white mice were used and the number of animals employed for each group was 8. In the case of sulfanilamide, 3 mg in 1 cc normal saline was given intraperitoneally 2, 6, 10, 14, 24, 32, 50, 72, 96, 120, and 144 hours after the infection. The control group received parallel injections of 1 cc of normal saline each time. In the case of sulfapyridine, the drug was given by the oral route. The drug was suspended in a mixture containing one part of mucilage of tragacanth and one part of distilled water. The dosage was ½ cc containing 30 mg of the drug. The first treatment was given at the time of infection and subsequently 7, 24, 48, and 72 hours after the infection. The controls received similar amounts of diluted mucilage of tragacanth at the same time. All the animals were kept under observation for 2