Effect of Antibiotics on Development of Resistance to Hemolytic Streptococcus Infection.* (19428)

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It is current medical practice to administer early, intensive chemotherapy in all respiratory infections ascribed to hemolytic streptococci in part at least to prevent the possible development of the hypersensitive state. The question arises whether such treatment might also affect the development of resistance to bacterial infection and thus alter the natural history of streptococcal disease. A laboratory model has been employed to investigate this point.

A recent report from this laboratory(1) described a subacute infection in mice induced by the inoculation of beta hemolytic streptococci into the thigh muscle(2). This infection was characterized by the development of a progressive, hard swelling at the site of infection for 4 or 5 days, then dissemination of bacteria throughout the body, toxemia, and death between the 5th and 8th day after inoculation. The mortality in untreated groups was about 95% with death occurring in all animals which had developed a local lesion. Treatment with antibiotics reduced the death rate in proportion to the effective dose of the drug. Surviving mice usually developed some induration of the thigh early in the treatment period. In some animals this local lesion regressed during therapy, in others it persisted and several weeks later streptococci could be cultured from the muscle lesion. When such survivors were challenged with an otherwise lethal intraperitoneal infection with homologous organisms they survived in significant numbers. The present report describes an experiment which was undertaken to evaluate this phenomenon.

Experimental. White Swiss mice from a heterologous stock weighing from 20 to 25 g were used. The infection was produced by the beta hemolytic, group A, type 3 streptococcus strain C203 used in previous work (1,2). The bacteria were grown in 5% sheep blood broth; virulence was maintained by weekly passage through mice. The LD50 for intraperitoneal infection was 0.2 ml of a 10^{-6} dilution of an 18-hour culture containing about 60-100 organisms or chains. Commercial crystalline potassium penicillin G was dissolved in sterile 0.85% sodium chloride solution to make a stock solution of 100,000 units/ml which was stored at 4°C during the treatment period. Aureomycin hydrochloride (Lot 7-6116), supplied by Dr. S. S. Hardy, Lederle Laboratories, was dissolved in 0.85% sodium chloride solution and sterilized by Seitz filtration to make a stock solution of 2.5 mg/ml which was quick-frozen and stored at —20°C. Suitable saline dilutions for injection were prepared daily from the stock solutions. Mice were infected by injecting 0.1 ml of a 10^{-4} dilution in broth of an 18-hour culture of the streptococcus into the left thigh. Treatment with antibiotics was started 24 hours after infection and continued at 24-hour intervals for 5 doses. The dose of antibiotic was administered intraperitoneally in a volume of 0.2 ml. Four groups of 100 mice each received respectively a total of 150 and 1500 μg of penicillin and 250 and 2500 μg of aureomycin. A control group of 20 mice was left untreated. All animals were observed for 16 days after infection or 11 days after the last treatment, and deaths were recorded. In the survivors the persistence at the site of infection of an indurated lesion was noted and those demonstrating it were marked with a dye. All the survivors were then challenged with an intraperitoneal inoculation of 0.2 ml of a 10^{-4} dilution in broth of an 18-hour culture of the homologous streptococcus strain. This dose contained about 100 LD50's and was known from previous experience to give a mortality of 95-100%. Following this chal-

* Supported in part by a grant from the Committee on Research of the University of California School of Medicine.

TABLE I. Persistence of Streptococci Related to Resistance to Subsequent Homologous Challenge Infection.*

Treatment over 5 days	Initial subacute intramusc. infection				Survivors challenged with acute intraper. infection			
	Infected No.	Survivors No.	Survivors with lesions No.	%	Infected No.	Survivors No.	%	Survivors with lesions No.
Penicillin, total 150 μg	100	67	32	47.8	67	30	44.8	21
Aureomycin, total 250 μg	100	80	13	16.2	80	16	20	11
Penicillin, total 1500 μg	100	78	7	9	78	14	17.9	6
Aureomycin, total 2500 μg	100	96	6	6.3	96	4	4.2	3
Control for initial infection	20	0	—	—	—	—	—	—
Control for challenge infection	—	—	—	—	25	0	.0	—

* Mice were infected intramuscularly with beta hemolytic streptococcus strain C203 and treated with the specified antibiotic 5 days; 16 days after the first infection survivors were challenged with an intraperitoneal inoculation of the homologous strain.

lenge infection the mice were observed for 7 days and deaths were recorded. Survivors were rechallenged twice in the same manner at intervals of 7 days. Groups of 20 normal mice were included as controls with each challenge infection. Statistical significance of the results was calculated by the χ^2 method.

Results. The relationship of antibiotic treatment resulting in survival of the initial subacute infection to the subsequent resistance to homologous reinfection is summarized in Table I. The drug schedules are arranged in order of the increasing curative effect on the initial infection: penicillin 150 μg < aureomycin 250 μg = penicillin 1500 μg < aureomycin 2500 μg. The differences in survival rates are significant ($p < 0.01$) between the least effective dose, the intermediate doses, and the most effective dose. Of the 67% of animals surviving with 150 μg of penicillin about half showed persisting lesions, whereas of the 96% alive after treatment with 2500 μg of aureomycin only 6% had such evidence of persisting local infection. All untreated animals died.

The challenge infection was administered intraperitoneally 16 days after the initial infection. All normal control mice died. In the animals infected with the streptococcus for the second time survival of the challenge appeared to be inversely related to the efficacy of a given drug schedule in eradicating the organisms from the animal. Thus in the aureomycin treated group in which 96% had survived and only 6% developed local lesions with the initial infection, all but 4% proved to be completely susceptible to the homologous reinfection. It is significant that 3 of the 4 survivors were among the 6 animals which had local lesions ($p = 0.01$). Conversely resistance to the challenge was considerable (44.8%) in the group where treatment with a small dose of penicillin had saved only 67% of the animals permitting the development of gross local lesions in half of them. In the intermediate groups also the survival from the challenge appeared to be roughly proportional to the development of local lesions during the initial infection.

The survival rates of animals subjected repeatedly to homologous challenge are shown in Table II. The first reinfection apparently weeded out the susceptible animals so that the resistance offered to the subsequent reinfections by the surviving groups appears more solid.

One group of 20 normal mice was given the challenge intraperitoneal infection followed in 3 hours by treatment with 120 μg of penicillin intraperitoneally in 0.2 ml of saline solution.

TABLE II. Increasing Resistance to Homologous Challenge as a Result of Repeated Infection.

Mice	First challenge*			Second challenge			Third challenge		
	Infected No.	Survivors No.	%	Infected No.	Survivors No.	%	Infected No.	Survivors No.	%
Survivors of previous infection	321	64	19.9	64	44	68.8	44	37	84.1
Control	25	0	.0	—	—	—	—	—	—
,,	—	—	—	20	0	.0	—	—	—
,,	—	—	—	—	—	—	20	0	.0

* See Table I.

Seven days later the challenge infection was repeated on the 15 mice which survived the first infection. All of these mice died of the reinfection within the same time interval in which the groups of 20 normal control mice died when infected in the same manner. This confirms previous experience that mice given an acute intraperitoneal infection and treated with antibiotics within a few hours of this infection did not acquire resistance to reinfection.

Discussion. The experiment demonstrates that the less effective the treatment of the initial subacute streptococcal infection is, provided it permits significant numbers of survivors, the greater is the resistance of such survivors to subsequent homologous challenge. Correlated with this increased ability to withstand challenge is the presence of a chronic lesion at the site of injection from which streptococci can be regularly cultured. The significance of this persisting lesion is further demonstrated by the fact that among 321 animals challenged 58 or 18% had local lesions, whereas in the 64 survivors of the challenge such lesions occurred in 41 or 64% ($p < 0.01$). Thus it appears that antibiotic treatment which rapidly and completely eliminated the infecting microorganism interfered significantly with the development of homologous immunity.

It is of interest in this connection to note that the rapid and complete elimination of hemolytic streptococci from the respiratory tract of man significantly interferes with the production of antibodies(3-5). If the intensive administration of chemotherapeutic agents in streptococcal infections of man should likewise effectively prevent the development of antibacterial immunity as well as the hypersensitivity, the natural history and epidemiology of streptococcal disease might be significantly altered in the future(6).

Summary. A subacute infection of mice with hemolytic streptococci was treated with antibiotics in such a way as to interfere to a varying degree with the persistence of the bacteria in the tissue. Striking differences were noted in the subsequent susceptibility of survivors to a homologous challenge. The acquired resistance of the animals was inversely proportional to the efficacy of the treatment regimen in rapidly eliminating the infecting microorganisms in the initial infection.

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Received February 25, 1952. P.S.E.B.M., 1952, v79.