Constant Treatment Technic for Inducing Antibiotic Resistance in Streptococci.* (19844)

HORACE M. GEZON† AND GEORGE R. COLLINS. (Introduced by C. Phillip Miller.)

From the Department of Pediatrics, University of Chicago.

The biochemical and biological properties of beta hemolytic streptococci resistant to various antibiotics have been under study in this laboratory for some time(1-7). Essential to this work has been the production of resistant organisms with a minimum of time and effort and with assurance of the integrity of the strains. Heretofore, the technic utilized was the serial transfer of organisms on blood agar plates containing progressively increasing concentrations of antibiotic. When several strains were subcultured simultaneously on the same plates, the obvious hazard existed of mixing strains by inadvertent substitution, by confluence of growth or by spattering while inoculating. Resistance was also difficult to maintain if plating sequences were interrupted for periods exceeding 3 days.

In the present report a new technic is described in which the bacterial strain was confined in a semi-permeable porous vessel suspended in sterile liquid medium containing antibiotic. This permitted the continuous action of antibiotic on the growing culture and the free exchange of nutritive and catabolic substances. The method was devised to ensure the integrity of the stain throughout the period of the experiment and to determine whether or not antibiotic resistance could be induced more rapidly and with greater ease than with the laborious plate-to-plate technic. This method is designated the constant-treatment technic.

Materials and methods. An inverted hollow porcelain filter candle‡ was suspended in a 250-ml centrifuge bottle containing 100 ml of Todd-Hewitt broth. An inoculum of 0.1 ml of a 24-hour broth culture of streptococcus was placed in the center of the candle and streptomycin, aureomycin, bacitracin or penicillin was added to the external medium immediately or 24 hours later. The system was incubated at 37°C. Samples were removed at intervals from within the filter by means of a platinum loop and the sensitivity of the organism determined by means of the whole-plate technic. One strain each of group A, B, and C beta hemolytic streptococci, previously studied by the plate-to-plate technic (1-7) and designated as S3, T7 and U1, respectively, were employed. Streptomycin, aureomycin, bacitracin, and penicillin of single lots and freshly prepared in physiological saline were used both in the experimental systems and in the sensitivity test plates. The systems were usually set up in groups of 3 for each strain and each antibiotic with the initial concentration of antibiotic constant for all; one was kept as a control and 2 received subsequent increments of antibiotic, the exact amount being based upon the sensitivity of the sample of organisms most recently withdrawn. Additions of antibiotic to the external medium were made at intervals of one to 10 days, but no nutritive broth was added to the systems. The range of initial concentrations and the variations in the subsequent additions are given in Table I.

Results. A. *Comparison of the plate-to-plate technic with the constant-treatment technic.* The results obtained by the constant-treatment and the plate-to-plate methods in represenative experiments are given in Table II. With the former, in most instances, resistance was induced to streptomycin, aureomycin, and bacitracin in streptococci to the same or greater magnitude than obtained with the latter. In every instance considerably less time was required for this transformation. In the case of penicillin, however, no significant change in resistance was induced regardless of the concentration of antibiotic employed, the subsequent additions of

* This investigation was supported by research grants from the National Institutes of Health, U. S. Public Health Service and Abbott Laboratories.

† Present address: Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa.

‡ Selas No. 02 porosity with maximum pore size of 0.85 μ.

TABLE I. Antibiotic Concentration in External Medium.

	Streptomycin, μg/ml	Aureomycin, μg/ml	Bacitracin, u/ml	Penicillin, u/ml
Initial conc. of antibiotic				
Range	5–100	.08–5	.005–5	.005–.1
Optimal range*	5–25	.1–.5	.005–1	—
Subsequent additions				
Range	10–10000	.08–80	.005–80	.005–1
Optimal range*	10–500	1–15	1–30	—

* Optimal range: the range of conc. of antibiotics at which an increase in resistance resulted.

TABLE II. Constant-Treatment vs. Plate-to-Plate Technic for Production of Resistant Streptococci.

Antibiotic	Whole-plate technic MFC*	Whole-plate technic Days†	Constant-treatment technic MFC	Constant-treatment technic Days
	Group A (S3 strain)			
Streptomycin	140	60	480	43
Aureomycin	6	60	10	11
Bacitracin	40	60	3.3	11
	Group B (T7 strain)			
Streptomycin	400	60	400	20
Aureomycin	12	60	10	6
Bacitracin	15	60	200	13
	Group C (U1 strain)			
Streptomycin	3000	57	300	24
Aureomycin	10	60	10	11
Bacitracin	30	60	60	32

* Max fold change in resistance.

† No. of transfers reported in previous publications(1-7) converted to avg time to accomplish this change.

antibiotic or the length of time the organism was exposed to the drug.

B. *Effect of concentration of streptomycin and duration of exposure on induced resistance.* On occasions, group A, B, and C organisms became highly resistant to streptomycin after exposure to concentrations of streptomycin that were sub-inhibitory for the parent strains. A resistance far greater than the cumulative amount of streptomycin to which the organisms had been exposed was reached consistently for members of each group. The early response of identical inocula to similar constant-treatment conditions was not uniform. In one system, for example, employing the group C organism, an extremely low concentration of streptomycin induced a high degree of resistance (60-fold within 24 hours) while no resistance was evident in an identical member of the pair. The final sensitivities, however, after 45 days of treatment and frequent additions of streptomycin, were the same, *i.e.*, greater than 12,000 μg/ml, or a maximum fold change of 240. In another experiment on the same group C organism, this degree of resistance was reached in 24 days with only the initial sub-inhibitory quantity of streptomycin and no subsequent additions. At the completion of this experiment the concentration of streptomycin in the sterile external medium had dropped to less than 1/16th of the inhibitory level for that organism.

Discussion and conclusions. Employing the constant-treatment technic, strains of group A, B, and C beta hemolytic streptococci became resistant individually to streptomycin, aureomycin, and bacitracin. The magnitude of the acquired resistance compared favorably with that obtained by the plate-to-plate method. The advantages of complete bacterial isolation during study, as well as more rapidly induced resistance, make the constant-treatment technic the method of choice. In contrast to these results, penicillin resistance was not acquired using this method. This may mean that penicillin has a different mode of action or that the conditions of the experiment were not suitable. Repeated efforts with varying conditions suggest that the latter explanation is less likely to be the correct one.

The lack of uniformity in the early response of beta hemolytic streptococci to similar constant-treatment conditions in the streptomycin studies can be explained in terms of genetic selection by the presumption that even in samples of inocula drawn at random from the same culture the distribution of resistant mutants varies(8). The fact that the final resistances, after a long period of treatment, are the same supports this presumption. However, the acquisition of high resistance after

brief exposure to sub-inhibitory concentrations and the progressive acquisition of the maximum resistance in an environment where the concentration of streptomycin dwindles from a sub-inhibitory level to still lower values are not as readily explained by genetic selection.

No concise range for the management of the constant-treatment technic systems can be given. However, in a given series, involving the same organism and the same antibiotic, the maximum fold change will be virtually equal, although reached, perhaps, by one system in a shorter period of time and/or with exposure to smaller concentrations of antibiotic.

Summary. 1. A new method is described for the production of resistant bacteria by continuous exposure to an antibiotic in a liquid environment. 2. The constant-treatment technic rapidly produces streptomycin-, aureomycin-, or bacitracin-resistant variants of an isolated streptococcus, but fails to produce any significant decrease in streptococcal sensitivity to penicillin. 3. The responses of streptococci to varying degrees of exposure to streptomycin are discussed.

The authors wish to express their indebtedness to Dr. C. Phillip Miller for many helpful suggestions on this study.

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Received September 19, 1952. P.S.E.B.M., 1952, v81.

Effects of Antibiotics in Mice on Weight and Thorium Dioxide Uptake of Spleen and Liver.* (19845)

DON W. ESPLIN† AND STANLEY MARCUS.

From the Department of Bacteriology, College of Medicine, University of Utah, Salt Lake City.

It has been demonstrated in a number of studies(1-3) that the antibacterial host defense mechanisms play a supporting role in the antibiotic treatment of bacterial infections. In addition to independent participation of the host defense mechanisms, some studies suggest(4,5) that certain antibiotics may act directly upon the cellular defenses of the body to increase their effectiveness. It is also possible that antibiotics may, through direct action decrease the effectiveness of certain host defense mechanisms. In this regard Munoz and Geister(6) have shown that aureomycin inhibits the phagocytic activity of leukocytes of human blood. Lepin, Barski and Maurin(7) have observed that both aureomycin and chloramphenicol, in concentrations attainable in the body, retard the rate of growth of fibroblasts.

In the present investigation several antibiotics were tested for their effect, whether of enhancement or inhibition, on the phagocytic activity of the reticulo-endothelial cells of the liver and spleen of the mouse. The ability of these cells to take up ThO_2 from the circulating blood was used as an indication of their phagocytic ability.

Materials and methods. Laboratory albino mice of mixed sexes were used in all experiments. Prior to each experiment the mice,

* The work reported in this paper was carried out under research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service and in part from the University of Utah Medical Research Fund.

† Present address, Laboratory of Microbiology, University of Texas, Medical Branch, Galveston.