Development of Streptomycin-resistant Variants of Meningococcus[1]

C. PHILLIP MILLER and MARJORIE BOHNHOFF

Department of Medicine, University of Chicago

In an attempt to explain the rapidity with which meningococci develop a very high degree of streptomycin resistance during two or three subcultivations on media containing increasing concentrations of the drug (*6*), the following observations were made.

A series of plates was prepared containing streptomycin[2] in graded concentrations from 10 to several thousand μg./ml. of media, and the surface of each was inoculated with a heavy seeding of meningococci. The inocula were carefully spread by means of small glass beads which were rolled back and forth and discarded after the inocula were evenly distributed. The plates were examined at the end of 24, 48, and 72 hours incubation. The results of a typical experiment are presented diagrammatically in Fig. 1.

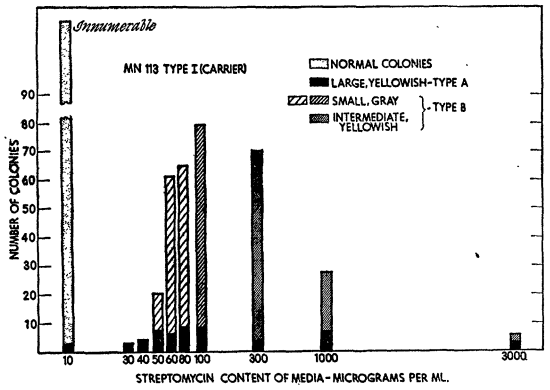

FIG. 1. Diagrammatic representation of colonies developing from equivalent inocula of meningococci on graded concentrations of streptomycin. Density in cross-hatching indicates differences in size and color of Type B colonies.

Normal meningococcus colonies developed on the lowest concentrations of streptomycin (10–20 μg./ml.) but were lacking on concentrations above 40 μg./ml. On concentrations higher than that, two types of colonies unlike the normal were observed. These have been designated variants A and B. Their properties are summarized in Table 1.

Both variants have developed from 16 of 18 strains of meningococcus and on all of 25 preparations of streptomycin, including several of a high degree of purity.

The type A variant appears in approximately equal numbers on all concentrations of the drug, but the numbers of colonies per plate vary considerably from strain to strain. It seems highly probable, therefore, that this type of colony arises from streptomycin-resistant mutants which are appearing regularly in the original bacterial population.[3] This variant differs from the original culture in size and color of colony and in being streptomycin resistant *in vitro* and *in vivo*. It multiplies on any medium which supports growth of normal meningococci. It is as virulent for mice as the original culture from which it arose, and produces infection against which maximal doses of streptomycin afford no protection.

TABLE 1

SUMMARY OF CHARACTERISTICS OF MENINGOCOCCUS VARIANTS A AND B

	Type A	Type B
Colonial appearance		
Size	Large on all concentrations of streptomycin	Small on low concentrations; medium on higher concentrations
Color	Yellowish	Pearl gray on low concentrations; slightly yellowish on higher concentrations
Viability on all concentrations of streptomycin	Grows readily	Grows readily on concentrations above 5 μg.ml.
Viability on streptomycin-free media	Grows readily	No growth*
Virulence for mice	Fully virulent	Nonvirulent except in streptomycin-treated mice
Effect of streptomycin treatment on experimental infection in mice	No protection from maximal doses	Promotes development of fatal meningococcal sepsis

* Among many subcultures onto streptomycin-free media only 4 single colonies grew out slowly.

Variants presumably of the same sort have been described by Alexander and Leidy (*1*) from *Hemophilus influenzae* and by Chandler and Schoenbach (*5*) from other microorganisms.

Colonies of the type B variant have one characteristic in common: They all require streptomycin for reproduction *in vitro* and *in vivo*. This variant is not virulent for mice; *i.e.* intraperitoneal inoculations with mucin suspensions (*7*) failed to produce meningococcal infection. However, mice thus inoculated and treated with adequate doses of streptomycin (500–5,000 μg.) developed fatal meningococcal sepsis. Meningococci

[1] This investigation was supported jointly by the U. S. Navy, Office of Naval Research, and the University of Chicago. It also received assistance from the Antibiotic Study Section, Research Grants Division, U. S. Public Health Service, and from the A. B. Kuppenheimer Foundation of the University of Chicago.

A preliminary report of the study was presented at the Conference on Antibiotic Research held at Washington, D. C., on February 1, 1947 under the auspices of the Antibiotics Study Section, National Institute of Health.

[2] Preparations of streptomycin were supplied by the Antibiotics Study Section, National Institute of Health, U. S. Public Health Service; the Division of Penicillin Control and Immunology, Food and Drug Administration; Abbott Laboraties; Eli Lilly & Company; Merck & Company; Chas. Pfizer & Company; and E. R. Squibb & Son.

[3] The authors are indebted to Sewall Wright and Thomas Park, Department of Zoology, University of Chicago, for their helpful suggestions and criticisms of the genetic aspects of this problem.

could be recovered regularly from their hearts' blood in cultures made on streptomycin-containing media but not in duplicate cultures made on streptomycin-free media.

The results of these mouse inoculations indicate that (1) the type B variants are nonvirulent for (untreated) mice; (2) they require streptomycin for their reproduction *in vivo* as well as *in vitro*; and (3) their dependence on streptomycin for growth persists after multiplication within the body of an infected animal host.

The origin of the type B variant is difficult to explain except as current mutation. It never appeared on media containing less than 40 μg./ml., but once it had developed, it could be subcultured on concentrations as low as 5 μg./ml. It could not be grown on less even after repeated transfer on media containing that concentration. The rare occurrence of a single colony on streptomycin-free media represents an exception to the rule and seems most likely to be the result of mutation back to normal.

The numbers of colonies developing from equivalent inocula are always greatest on concentrations between 100 and 400 μg./ml., whether the seedings are made from a parent (stock) strain, as illustrated in Fig. 1, or from a subculture of type B variant taken from a high or a low concentration of streptomycin. This fact seems to indicate that all the B variants are alike genetically and that the higher reproductive rate at those concentrations reflects a physiological response to the drug rather than a differential induction of the variants at different concentrations.

The variation in color and size of their colonies in relation to the concentration of the drug is additional evidence that streptomycin directly affects the physiology of the bacterial cells. On 60–100 μg./ml. they are small and pearl gray. On higher concentrations, they develop greater size and acquire a distinctly yellowish tinge, resembling the large variants described as type A. Small, gray colonies taken from low concentrations and transferred onto higher concentrations grow as medium-sized, pigmented colonies. Conversely, pigmented colonies taken from high and subcultured onto low concentrations develop as small gray colonies.

Benham (*4*) has noted the stimulating action of streptomycin on the O_2 uptake of typhoid bacilli. Welch, Price, and Randall (*9*) report that small doses of streptomycin increased the mortality rate of mice infected with typhoid bacilli.

Studies on the growth requirements of mutants isolated from cultures of *Bacillus coli* after treatment with bacteriophage (*2*) or X-ray (*8*) have demonstrated a variety of deficiencies in their metabolic processes. Similar observations have been made on mutants induced in *Neurospora* by X-ray (*3*).

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Irritating Effects of 9,9-Dibromofluorene

John R. Sampey, Anne B. King, Thomas A. Roe, Jr., and S. J. Childress[1]

Furman University, Greenville, South Carolina

Alfred Cavendish (*1*) has reported the irritating effects of 9-bromofluorene. In the further study of the photochemical bromination of fluorene (*2*), one of us (A. B. K.) worked several weeks with 9-bromofluorene without any irritation, but on the first exposure to 9,9-dibromofluorene, a severe skin eruption developed.

The irritation set in shortly after crystallization of a sample of 9,9-dibromofluorene from hot glacial acetic acid. Red blotches appeared first on the back of the left hand and the inner left wrist, and after a few hours red streaks developed on the face and ear. The rash spread gradually, and after three weeks it covered both forearms and face completely, and one eye was swollen shut.

Administration of benadryl relieved the severe itching at once, and within a few days the face peeled, and the blotches on the hands and arms began to dry up. Six weeks from the time of exposure, small red blotches remain only at the points of initial contact.

A second member of the group suffered some inflammation and itching of the hands following one exposure to 9,9-dibromofluorene, but the condition cleared up within a week, without medical attention.

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dl-α-Amino-ε-Hydroxy Caproic Acid in the Rat

R. Gingras, Edouard Pagé, and Roger Gaudry

Department of Biochemistry, Medical School, Laval University, Quebec, Canada

In the course of the synthesis of lysine from dihydropyran, one of the authors (R. Gaudry) prepared dl-α-amino-ε-hydroxy caproic acid by hydrolysis of 5-δ-hydroxy butyl hydantoin. Since this amino acid differs from lysine only because of its hydroxyl group instead of the ε-amino group, it was thought of interest to investigate its biological properties.

Young albino rats averaging 66 grams in weight were first placed on a diet of the following percentage composition: zein, 10; dl-tryptophane, 0.2; cellu-flour, 2; soybean oil, 4; salt mixture, 4; sucrose, 79.65; choline chloride, 0.15. Each 100 grams of ration contained: thiamine-HCl, 0.4; riboflavin, 0.4; pyridoxine-HCl, 0.5; calcium pantothenate, 3.0; nicotinic acid, 3.0; inositol, 10.0; and 2-methyl-1,4-naphthoquinone, 0.1 mg.

After 24 days on this diet, weight changes were very small (Table 1), and the following additions were made to the ration at the expense of the sucrose: Group I (Zt), 0.6 per cent zein; Group II (Ztl), 0.6 per cent l-lysine; and Group III (Ztl-OH), 1.5 per cent dl-α-amino-ε-hydroxy caproic acid. Eleven days

[1] Acknowledgment is made of a grant from the Carnegie Foundation for the Advancement of Teaching.