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Studies on the Action of Penicillin. IV. Development of Penicillin Resistance by Gonococcus.*

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Among the several recent reports on the development of penicillin resistance by various microörganisms, only that by Bahn, Ackerman, and Carpenter[1] concerns gonococcus. They were able to enhance the penicillin tolerance of one of 5 strains of gonococcus sufficiently after 32 weeks to permit growth in blood broth containing 2 units of penicillin per ml. The other strains responded less readily, one scarcely at all. Lankford[2] found that among 203 freshly isolated strains of gonococcus none was resistant to more than 0.02 units of penicillin per ml.

As gonococci grow more readily on solid media than in broth, the following experiments were made by surface cultivation on agar containing known concentrations of penicillin.

Methods. Strains of gonococci were transferred daily to freshly prepared blood agar plates[3] containing increasing concentrations of penicillin.† Each transfer was made to 3 or 4 plates containing graded increments of penicillin and taken from the plate which had developed confluent growth during 24 hours incubation. This procedure was followed because it was found that enhancement of penicillin tolerance proceeded more rapidly when transfer was made from media containing the highest concentration of penicillin which permitted moderately abundant growth rather than from higher concentrations which produced only a few colonies.

The penicillin agar was prepared each day just before use because its growth inhibiting potency was found to diminish even in the icebox.

The results with one strain are plotted in Fig. 1. The penicillin tolerance; that is, the ability to multiply on penicillin agar, increased rapidly at first and reached its limit at 7.7 units per ml. Just before that occurred and at a time when the average daily increment had become small, a second series of transfers was begun by subcultivation onto penicillin-free agar. Thereafter, this second series was transferred back and forth from penicillin-containing to penicillin-free agar. As will be seen in the chart, this practice resulted in further and more rapid enhancement of penicillin tolerance which reached a level of 21 units per ml. The only detectable difference in this second series of transfers was more luxuriant growth on penicillin-free agar which provided a greater number of viable gonococci to be carried back onto penicillin agar. These results indicate that enhancement of penicillin resistance proceeds most rapidly when vigorous multiplication is maintained.

To test the validity of the foregoing explanation, a strain of gonococcus was repeatedly subjected to prolonged exposure to concentrations of penicillin which inhibited reproduction. To 2 cc volumes of blood broth containing varying concentrations of penicillin, approximately 5×10^6 gonococci per ml were

* A portion of the work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

1 Bahn, Jeanne M., Ackerman, Helen, and Carpenter, Charles M., Proc. Soc. Exp. Biol. and Med., 1945, **58**, 21.

2 Lankford, Charles E., *Am. J. Syph. Gon. and Ven. Dis.*, 1945, **29**, 56.

3 The medium is described by Miller, C. Phillip, Moeller, Velma, and Bohnhoff, Marjorie, Proc. Soc. Exp. Biol. and Med., 1945, **58**, 143.

† The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutics and Other Agents of the National Research Council.

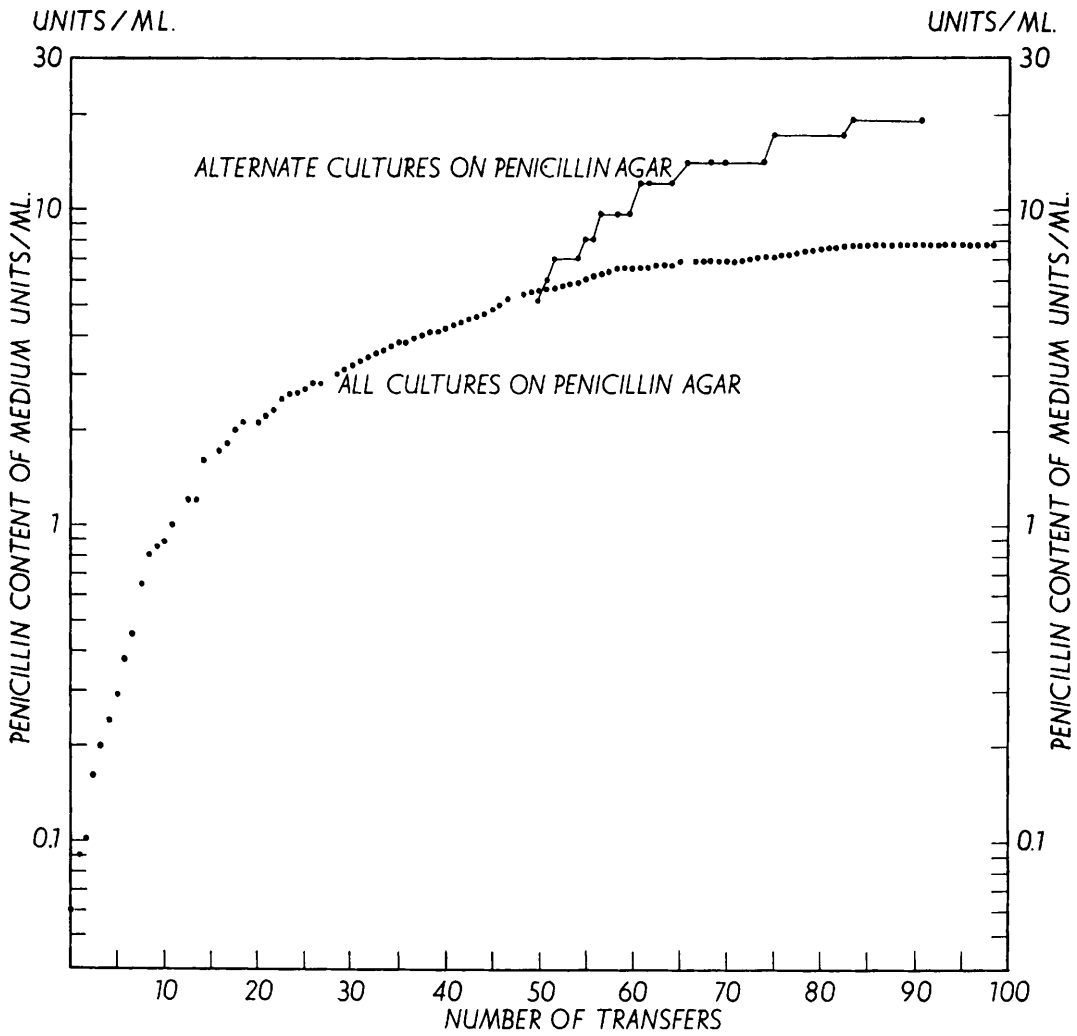

FIG. 1.

mixed in Wassermann tubes, incubated overnight, then centrifuged at high speed for one-half hour. The number of viable gonococci surviving was determined by culturing the centrifuged sediment onto freshly poured blood agar plates which were incubated in a candle jar and examined at 24 and 48 hours. After the first 24 hours of incubation, colonies developing from organisms surviving the highest concentration of penicillin were grown on penicillin-free agar and used for the next suspension in penicillin broth.

After 13 successive suspensions in penicillin-broth, the number of viable gonococci surviving exposure to each concentration of penicillin had not increased (beyond the limits of the error of the method) and indicate that the resistance of the strain had not been appreciably increased.

At the time of the first penicillin broth suspension and again after the 9th, the strain was tested for its ability to multiply on penicillin agar. The change was insignificant, being 0.04 and 0.05 units per ml, respectively.

The reliability of the method was checked by subjecting to this treatment organisms which had developed ability to multiply on agar containing different concentrations of penicillin. Multiplication always occurred in broth containing penicillin in the concentration just below that of the agar from which the inocula were taken.

Penicillin-fast strains of gonococcus showed distinct morphological changes, which were retained during subsequent cultivation on penicillin-free agar. They will be described in a subsequent report.

Penicillin resistance developed *in vitro* was not permanently retained during prolonged cultivation on penicillin-free agar. Its rate of loss has not yet been accurately determined.

Summary. Penicillin-fastness developed most rapidly in gonococci under conditions which permitted the greatest number of viable microörganisms to be transferred to a higher concentration of penicillin at each transfer. One strain of gonococcus acquired the ability to grow on media containing 21 units per ml. No appreciable increase in penicillin tolerance resulted from repeated exposure to bacteriostatic concentrations of penicillin.

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Studies on Action of Penicillin. V. Virulence of Penicillin Resistant Strains of Meningococcus.

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Several reports have appeared on the development of penicillin resistance *in vitro* and *in vivo* by staphylococci[1,2] and pneumococci,[3] but they do not agree on its effect on virulence.

Methods. The sensitivity of meningococci to the bacteriostatic action of penicillin* was determined by transferring them from routine media onto a series of blood agar plates[4] containing known concentrations of penicillin and incubating them in a candle jar. The highest concentration permitting the development of any colonies was considered the limit of that strain's resistance to penicillin. Increased resistance to penicillin was developed by the method described for gonococci.[5] Virulence was determined by inoculating mice intraperitoneally with mucin suspensions of meningococci.[6] Susceptibility to the therapeutic action of penicillin was estimated by infecting mice with 10,000-100,000 M.L.D. of meningococci and injecting them subcutaneously 3 and 6 hours thereafter with appropriate doses of penicillin.

Results. The natural resistance to penicillin was determined on 96 strains of meningococci and found to vary from 0.1 to 0.5 units per ml. Seven strains were selected at random for enhancement of penicillin resistance and were subcultured daily on media containing increasing concentrations of penicillin. The resistance of all 7 strains increased at about

1 McKee, Clara M., and Houck, Carol L., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 33.

2 Spink, Wesley W., Hall, Wendell H., and Ferris, Viola, *J. A. M. A.*, 1945, **128**, 555.

3 Schmidt, L. H., and Sesler, Clara L., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 353.

* The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

4 The medium is described by Miller, C. Phillip, Moeller, Velma, and Bohnhoff, Marjorie, PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 143.

5 Miller, C. Phillip, and Bohnhoff, Marjorie, PROC. SOC. EXP. BIOL. AND MED. (preceding communication).

6 Miller, C. Phillip, and Castles, Ruth, *J. Inf. Dis.*, 1936, **58**, 263.