16704

# Antibiotic Studies on Beta Hemolytic Streptococci. IV. Penicillin Resistance Induced in Mice and Embryonated Eggs.

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

*From the Department of Pediatrics, The University of Chicago.*

Induced *in vivo* penicillin resistance has been demonstrated in only a few species of bacteria. Miller and Bohnhoff[1] were able to induce penicillin resistance in a strain of meningococcus by serial passage in mice when the animals received sub-protective doses of penicillin. After 12 passages, the $PD_{50}$ was increased 100 fold, and the *in vitro* resistance was increased 50 fold. After 60 passages, 170 times as much penicillin was needed to protect 50% of the mice as was required for the first passage. Schmidt and Sesler[2] using two strains of pneumococcus demonstrated a 4 fold rise in penicillin resistance after 7 passages in mice that had been treated with penicillin. In previous reports from this laboratory,[3-5] the development of *in vitro* resistance to penicillin was demonstrated for 28 strains of groups A, B, and C beta hemolytic streptococci. The group A organisms developed resistance very slowly, the group B relatively quickly, and the group C intermediately. The maximum change induced in the group A organisms after 60 transfers was 17 fold, in group B, 190 fold, and in group C, 16 fold. Milzer[6] and his coworkers have recently shown that patients who had received oral penicillin had resistant strains of beta hemolytic streptococci present in their nasopharynx. Typing and grouping were not made, nor were sensitivities determined on the strains before therapy was started. It would be impossible, therefore to say that the organisms that developed resistance were the strains present at the outset.

*Technic.* A total of 6 strains of beta hemolytic streptococci, 2 each of groups A, B, and C were used for the present study. The same strains, designated $S_2$, $S_3$, $T_3$, $T_5$, $U_1$, and $U_2$, were used in our previous reports.[3-5] All were obtained from patients who had received no penicillin therapy.

For mouse passage, 0.03 ml of an 18-hour culture of the organism in veal infusion broth containing 5% defibrinated sheep's blood was injected intracerebrally into each of 6 mice. The animals, in pairs, received 3 injections of penicillin G at 2½-hour intervals. Initially, the total doses arbitrarily assigned were 150, 300, and 450 units given subcutaneously in 0.2 ml amounts. Twenty-four hours after infection, a surviving animal for each of the penicillin dosages was sacrificed, and broth cultures made of the brain and cerebrospinal fluid. The positive culture from the animal receiving the highest quantity of penicillin was used for the next serial passage. The dosage schedule was adjusted for each transfer, so that two animals received the same amount, two a larger amount, and two a smaller amount than that of the mouse from which the culture had been obtained. These changes were approximately 25% for the group A, and up to 100% for the group B and C organisms.

Virulence studies were made on the parent strain, the parent strain after 15 passages in normal mice, and the same strain after 15 passages in animals that had received subprotective doses of penicillin. The technic used for this was the same as that described in a previous communication.[3] The parent and resistant organisms were tested at inter-

1 Miller, C. P., and Bohnhoff, M., *J. Inf. Dis.*, in press.

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

3 Gezon, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 208.

4 Gezon, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 212.

5 Gezon, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 215.

6 Milzer, A., Kohn, K. H., and MacLeon, H., *J. Am. Med. Assn.*, 1948, **136**, 536.

TABLE I.
Sterilizing Dosage of Penicillin in Mice Infected Intracerebrally with Streptococci.

| Group | Strain | Daily dosage in units Mean of 5 passages 1-5 | 6-10 | 11-15 |
|---|---|---|---|---|
| A | $S_2$ | 360 | 540 | 780 |
| A | $S_3$ | 480 | 660 | 630 |
| B | $T_3$ | >1860 | >21,000 | >30,000 |
| B | $T_5$ | >1860 | >21,000 | >30,000 |
| C | $U_1$ | >1860 | >21,000 | >30,000 |
| C | $U_2$ | >1860 | >21,000 | >30,000 |

vals, usually the fifth, tenth, and fifteenth passages, for *in vitro* penicillin sensitivity by the whole plate method.

Fertile hens' eggs, after incubation at 37.5°C for 10-13 days, were infected in the chorio-allantoic space with 0.1 ml of an 18-hour broth culture of streptococcus. This was followed immediately by penicillin G through the same burr hole and at approximately the same depth. Initially, the doses arbitrarily assigned were 0.1, 0.2, and 0.3 units of penicillin per egg in 0.1 ml amounts. Usually 2 eggs were used for each dosage, and 2 as untreated controls. After twenty-four hours incubation all eggs were harvested through the air sac and chorio-allantoic fluid streaked onto blood agar plates. Growth from the egg that had received the largest amount of penicillin was inoculated into broth for the next serial passage. The penicillin dosage was adjusted for each passage, so that 2 eggs received the same amount, 2 approximately 25% more, and 2 approximately 25% less than that of the egg from which the culture had been obtained. *In vitro* sensitivity tests were performed at the fifth, tenth, fifteenth, twentieth, and twenty-fifth passage by the whole plate technic.

*Results.* The results in mice are given in Table I. It may be seen that for the 2 group A organisms no significant increase in the penicillin requirement for bacterial sterilization was found between the first and fifteenth passage in mice.

With group B and group C streptococci, a positive culture was obtained in every instance from the brain of animals receiving the maximum dose of penicillin. A dosage of 30,000 units of penicillin was reached by the ninth passage. It was maintained at this level for the next 7 passages, as this was close to the toxic dose for mice. Positive cultures were also obtained from mice that had been infected with the parent strains and treated with 30,000 units of penicillin. For this reason, the means of the first to fifth and sixth to tenth passages in Table I should not be interpreted as demonstrating an increase in resistance to penicillin. While this may be true, our data do not prove it.

When the group A, B, and C organisms were tested by *in vitro* technics, there was no difference in sensitivity between the parent strains and those obtained after 15 passages in treated mice.

The mouse virulence of all 6 parent strains, the same strains after 15 passages in normal mice, and after 15 passages in penicillin treated mice, were essentially alike.

The results in eggs are given in Table II. For the group A organisms there was no appreciable difference in the amount of penicillin necessary to sterilize the chorio-allantoic fluid in the first and the twenty-fifth passage in both strains studied. In contrast, both group B and C organisms showed a progressively increased resistance to penicillin on serial passages in treated eggs. Approximately 30 to 40 times as much penicillin was required to sterilize the chorio-allantoic fluid for the group B strains on the twenty-fifth passage as was needed for the first. For the group C organisms this difference was 12 and 3 fold respectively. This apparent change in penicillin resistance was not reflected in the *in vitro* sensitivities as all 6 strains maintained their initial sensitivities throughout the 25 serial passages.

*Conclusion.* Two strains of group A streptococci failed to acquire resistance to penicillin after repeated exposures to sublethal amounts either in embryonated hens' eggs or in mice. With 2 strains each of group B and C organisms, however, in eggs there was a definite increase in penicillin resistance. In mice this same increase may have occurred,

TABLE II.
Sterilizing Dosage of Penicillin in Eggs Infected in the Chorio-Allantoic Space with Streptococci.

| Group | Strain | Daily dosage in units Mean of 5 passages | | | | |
|---|---|---|---|---|---|---|
| | | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 |
| A | $S_2$ | 0.19 | 0.13 | 0.17 | 0.24 | 0.28 |
| A | $S_3$ | 0.34 | 0.32 | 0.32 | 0.34 | 0.30 |
| B | $T_3$ | 1.70 | 2.80 | 1.76 | 3.60 | 5.60 |
| B | $T_5$ | 0.42 | 0.66 | 1.52 | 3.80 | 6.60 |
| C | $U_1$ | 0.25 | 0.18 | 0.54 | 0.52 | 0.66 |
| C | $U_2$ | 0.30 | 0.22 | 0.36 | 0.60 | 2.18 |

but we failed to demonstrate it through limitations of the method. The increasing penicillin resistance *in vivo* of the group B and C streptococci was not associated with increasing resistance *in vitro*.

## 16705

## Drug Sensitivity of Hemolytic Streptococci Isolated from Cases of Scarlet Fever Treated with Penicillin.*

THOMAS L. HARTMAN AND LOUIS WEINSTEIN.

*From the Haynes Memorial and Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, Boston, Mass.*

The development of resistance to penicillin has been observed to occur *in vivo* in strains of *Streptococcus viridans* and *Staphylococcus aureus* during therapy with this agent.[1,2]

Although penicillin has had very wide use in the treatment of group A hemolytic streptococcus infections there have been no reports of the development of strains with lack of sensitivity to this drug which constituted therapeutic problems.

Available information[3-5] would seem to indicate that *Strep. pyogenes* (group A) as encountered in the general population is almost uniformly highly susceptible to penicillin. Hirsch *et al.*,[6] however, have reported the isolation of a strain of this organism which was susceptible only to 0.625 units of penicillin from a patient ill with scarlet fever who had not received any antibiotic therapy. Recently, Milzer *et al.*,[7] reported the occurrence of penicillin-resistant beta hemolytic streptococci in the throat cultures of children with rheumatic fever treated prophylactically with 100,000 units of penicillin orally each day for at least 4 months. Although these strains were resistant to 10 units of penicillin following the period of prophylaxis, none of the streptococci present prior to chemotherapy were available for comparison; nor was the serologic group (group specific carbo-

* This study was aided by a grant from Schenley Laboratories, New York City.

1 Clark, W. H., Bryner, S., and Rantz, L. A., *Am. J. Med.*, 1948, **4**, 671.

2 Spink, W. W., and Ferris, V., *J. Clin. Invest.*, 1947, **26**, 379.

3 Watson, R. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 65.

4 Meads, M., Ory, E. M., Wilcox, C., and Finland, M., *J. Lab. and Clin. Med.*, 1945, **30**, 725.

5 Rantz, L. A., Randall, E., Spink, W. W., and Boisvert, P. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 54.

6 Hirsh, H. L., Rotman-Kavka, G., Dowling, H. F., and Sweet, L. K., *J.A.M.A.*, 1947, **133**, 657.

7 Milzer, A., Kohn, K. H., and Mae Sears, R., *J.A.M.A.*, 1948, **136**, 536.