single, or the first few exposures, showed no significant alterations from that of the controls; the same was true of those which, while they were subjected to more exposures, were killed shortly after the occurrence of motor disability. It would appear therefore that animals must survive the C.N.S. injury induced by successive exposure to OHP for some time in order to permit the development of demonstrable cytological changes. This supports the explanation, mentioned above, for the failure of earlier investigations to demonstrate parenchymal damage to the C.N.S. in poisoning by OHP in more acute exposures. Further detailed examination of C.N.S. changes in a longer series of experimental animals now in progress provides confirmatory evidence that successive exposures to OHP cause irreversible damage of degenerative character in tissue of the C.N.S., especially in nerve fiber tracts, and that it is this damage which is responsible for the chronic motor disability observed in animals following successive exposure to OHP.

It has long been known that exposure to OHP causes congestion in various organs of the body, but the finding of especial interest here is that of the injury to the parenchyma of the C.N.S. The occurrence of this tissue damage in animals suffering chronic neuromuscular disabilities as a result of exposure to OHP together with the likelihood that such tissue injury is an extension of some of those etiological factors responsible for the acute after-effects suggest that some of the acute reactions themselves are manifestations of less severe and reversible tissue injury.

The cause of the tissue injury, regardless of its severity and degree of reversibility, must lie in some transient influence. This may well be a combination of the increased CO_2 tension in the tissues, the accompanying increased tissue Ch and a direct action of OHP on cellular enzyme mechanisms, all of which conditions obtain in exposures to O_2 at high pressure. The postulation of a special toxic substance and its persistence after the return of the animal to atmospheric pressure not only lacks experimental support[2,4,5] but also appears to be unessential to explain the reactions which occur during and after exposure to O_2 at high pressure. But in any case the observation that exposure to OHP induces demonstrable tissue injury within the C.N.S. seriously questions the contention that the nervous manifestations of poisoning by OHP are entirely "functional."

The author wishes to express his appreciation to Drs. Wanstrom and Parsons, Department of Pathology; Dr. Loewenberg of the Neuropathological Institute, and to Dr. Crosby of the Neuro Anatomy Department for their help in the examination of the tissues concerned in these experiments.

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5 Bert, P., *La Pression Barometrique*, G. Masson, Paris, 1878.

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Development *in vitro* of Penicillin-Resistant Strains of the Gonococcus.*

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It has been demonstrated that certain strains of the staphylococcus, the streptococcus, and the pneumococcus acquire resistance to penicillin when grown in broth containing increasing concentrations of the drug. Abraham and his colleagues[1] were able in 16 weeks to adapt a strain of *Staphylococcus*

* This study was financed by a grant from the Ortho Research Foundation, Linden, N.J.

1 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.

aureus to grow in a concentration of penicillin one thousand times greater than the original inhibitory concentration. McKee and Rake[2] doubled the penicillin-resistance of 2 cultures of sulfonamide-resistant pneumococci after a few daily subcultures. McKee and Houck[3] markedly increased *in vitro* the resistance to penicillin of 3 strains of staphylococci, a pneumococcus, type III, and a strain of *Streptococcus pyogenes*. Schmidt and Sesler[4] were able by serial mouse passage and by serial transfer in liquid medium to develop a penicillin-resistant strain of the pneumococcus. *In vivo* resistance to the drug was also reported by Rammelkamp and Maxon[5] who recovered resistant strains of staphylococci from patients treated with penicillin. With regard to the gonococcus, Frisch[6] has recently reported that by employing chocolate agar as a basic medium he was unable to develop penicillin-resistant strains.

Although penicillin is an effective chemotherapeutic agent in gonococcal infections, a few cases resist treatment. This evidence suggests that either penicillin-resistant strains of the gonococcus occur in nature or the organism acquires resistance to penicillin *in vivo*. The present study was undertaken to determine if the gonococcus when grown in the presence of gradually increased concentrations of penicillin acquires resistance *in vitro*.

Technic. Five strains of *Neisseria gonorrhoeae*, non-resistant to sulfanilamide and sulfathiazole, were selected for the study. They are identified as No. 515, No. 3215, No. 5609, No. 5723, and No. 8071. Three of the strains were isolated from patients with cervicitis and 2 from urethritis in the male. Douglas's broth with 0.05% potassium nitrate, 0.04% potassium dihydrogen phosphate, and 5% lapine blood was used as the basic medium. From 0.005 to 0.01 Oxford unit of penicillin† per ml of the medium was then added because previous tests demonstrated that growth of the selected strains was not inhibited in these concentrations. The cultures were transferred to the same and to a greater concentration of the drug every 48 hours, and sub-cultures for viability were made on chocolate agar plates at the same interval but on alternate days. At approximately weekly intervals, each strain was tested on a suitable carbohydrate medium to determine if any change in fermentation reactions had occurred. Two of the strains which were most resistant to penicillin *in vitro* were then maintained on penicillin-free chocolate agar and also lyophilized by the technic of Flosdorf and Mudd[7] to determine if the acquired resistance to the drug was transient or a permanent factor.

Results. After 32 weeks' exposure to gradually increased concentrations of penicillin in blood broth, 5 strains of *Neisseria gonorrhoeae* grew readily in concentrations 8, 64, 128, 200, and 400 times greater, respectively, than the initial concentrations permitting growth (Fig. 1). The degree of resistance of the two most resistant strains was maintained after cultivation on penicillin-free chocolate agar for 3 weeks. These same strains likewise maintained their resistance after lyophilization for 2 months.

Of special interest were changes in the morphology of the gonococcal cells which were most marked when the organisms were first exposed to the lower concentrations of penicillin. Films from 2 of the strains stained by Gram's method showed groups of enlarged, indistinct, irregularly stained, pleomorphic cells. The cells of the remaining 3 strains were typically biscuit-shaped, but from 4 to 5 times larger than the normal cell. After resistance was acquired, cells conforming in size and shape to a typical gonococcus reappeared. The fermentation of glucose by strains showing pleomorphic cells was delayed.

2 McKee, C. M., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 275.

3 McKee, C. M., and Houck, C. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 33.

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

5 Rammelkamp, C. H., and Maxon, T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 386.

6 Frisch, M. C., Behr, B., Edwards, R. B., and Edwards, M. W., *Am. J. Syph., Gonor., and Ven. Dis.*, 1944, **28**, 627.

† Penicillin (Pfizer) was supplied by the Committee on Medical Research of the National Research Foundation, Washington, D.C.

7 Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

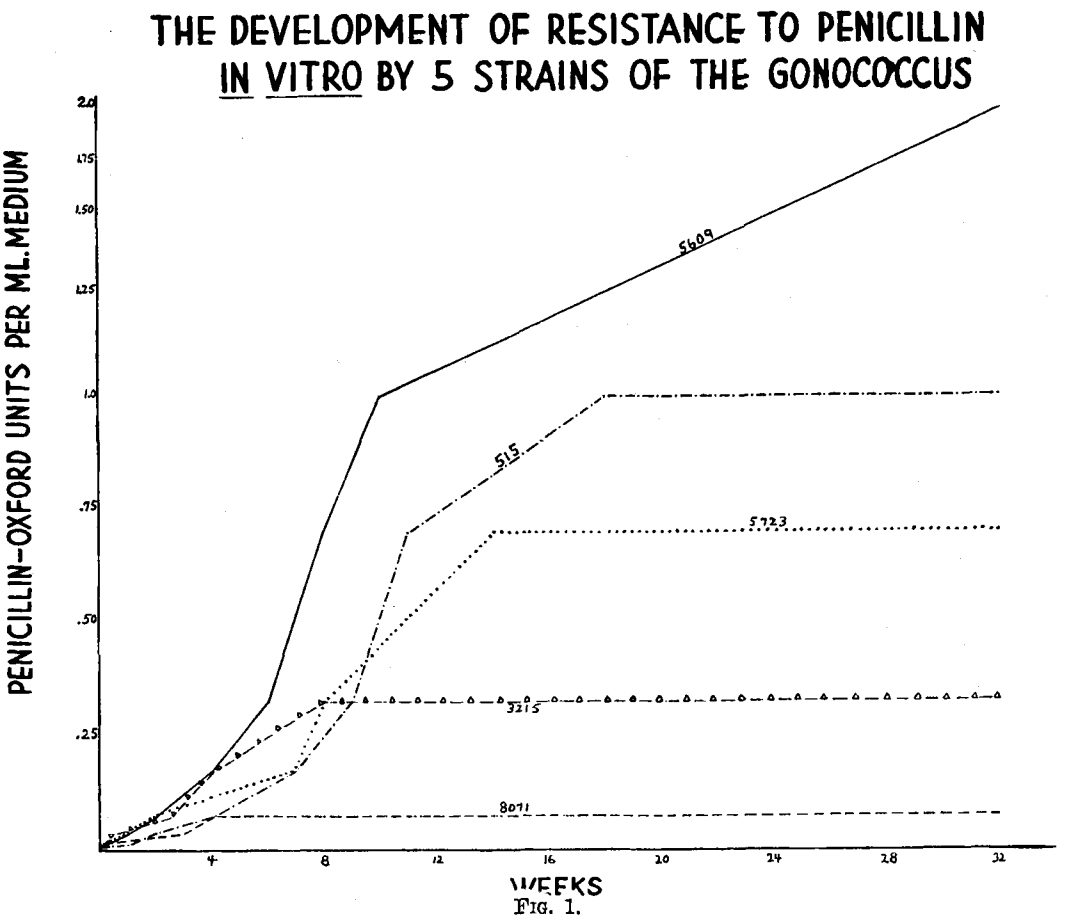

FIG. 1.

The reaction took place from 24 to 48 hours after that observed in the control cultures.

Discussion. The gonococcus is unusually sensitive to penicillin *in vitro*. Cohn and Seijo[8] reported that a concentration of 0.008 Oxford unit per ml of medium was bactericidal for 45.5% of 82 sulfonamide-resistant and non-resistant strains of the gonococcus and that none of the strains would grow in 0.16 unit per ml of medium. The 5 strains which we have studied varied considerably in their adaptability to growth in penicillin. Only comparatively low concentrations of the drug were tolerated. To date the maximum amount of penicillin in which the most resistant strain will grow is approximately 2 units per ml of blood broth. The acquisition of resistance to the drug by the gonococcus was anticipated, however, especially since development of resistance to penicillin by other species of bacteria has been reported.

Morphological changes in bacteria produced by the action of penicillin have been noted by Gardner,[9] who observed an enlargement of cocci in the presence of a bacteriostatic concentration of penicillin. Spink, Ferris, and Vivino[10] reported as permanent characteristics peculiar morphological forms in stained films of *Staphylococcus aureus* during the stage of acquiring resistance to penicillin. Our studies with the gonococcus indicate these morphological changes are temporary.

In their original report on penicillin, Abraham and his colleagues[1] observed that a strain of *Staphylococcus aureus* having developed

8 Cohn, A., and Seijo, I. H., *J. A. M. A.*, 1944, **124**, 1125.

9 Gardner, A. D., *Nature*, 1940, **146**, 837.

10 Spink, W. W., Ferris, V., and Vivino, J. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 210.

resistance to penicillin required 48 hours to complete the fermentation of lactose, whereas the control strain effected the same result in 24 hours. A similar delay was noticed in the fermentation of glucose by the penicillin-resistant strains of the gonococcus.

Far more difficulty was encountered in developing penicillin-resistant strains than sulfathiazole-fast strains of the gonococcus *in vitro*. These observations suggest that penicillin-resistant strains of the gonococcus are less likely to become established in the population at large than sulfathiazole-resistant strains, although this possibility should be given consideration.

Summary. Five strains of ***Neisseria gonorrhoeae*** were made resistant *in vitro* to concentrations of penicillin 8, 64, 128, 200, and 400 times greater than the initial concentration permitting growth. Morphological changes of the gonococcus and a delayed fermentation of glucose were observed in the strains during the development of resistance to the drug.

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Action of Certain Sulfonamides on Anaerobic Activity of Respiratory Center of Young Animals.

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Earlier reports have shown that the anaerobic activity of the respiratory center of young animals, as indicated by the gasping pattern of the ischemic or completely isolated head, is altered by various anesthetic agents and tissue poisons[1] and by the carbohydrate stores of the nervous tissue.[2,3] Glucose greatly prolongs survival and the number of gasps of the second series. Insulin and iodoacetic acid diminish or completely abolish the second series: cyanide diminishes the first.[4] These results suggest that the prolonged survival of the respiratory center of young animals is due to an anaerobic source of energy resulting from the breakdown of carbohydrates by enzymes.

Because the sulfa drugs are known to interfere with certain enzymatic processes, it was thought desirable to study the action of these on the gasping pattern and survival of the unperfused isolated head of the young animal. The respiratory center of such a preparation, unlike that of the whole animal with circulation intact, cannot be influenced secondarily by changes in other parts of the body.

Methods. Employing a technic previously described,[1,4] rats 12-15 days of age and weighing 18-26 g were used as test animals. The gasping pattern of the isolated head of rats of this age consists of 28-35 mandibular movements lasting approximately 5 minutes and is composed of an initial or aerobic series having a relatively constant number of gasps, averaging about 9, and a second or anaerobic series having a somewhat variable number (19-26) averaging about 23. A pause or period of complete inactivity between the end of the first series and the beginning of the second (the interseries interval) lasts 35 to 50 seconds. In previous experiments on 65 litters comprising 445 animals, the standard deviation of the mean number of gasps for untreated littermates was found to be slightly less than 2; the average variation for survival was 8.4% and that for number of gasps of the second series was 6.1%.

The drugs employed were sulfanilamide and the sodium salts of sulfathiazole, sulfadiazine, and sulfamerazine. These were injected intraperitoneally in doses of 3, 4.5, and 6 mg. Toxic

1 Selle, W. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 50.

2 Selle, W. A., *Am. J. Physiol.*, 1944, **141**, 297.

3 Hiestand, W. A., Tschirgi, R. D., and Miller, H. R., *Am. J. Physiol.*, 1944, **142**, 153.

4 Selle, W. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 291.