

In the rabbits the process in the lungs was diffuse and involved all the lobes. The surfaces were purple red except at the margins where the tissue was pink and crepitant. The lobular markings were distinct. The bronchi and bronchioles contained a pink frothy fluid and the cut surfaces were wet. Scattered throughout were numerous granular raised areas. Microscopically the lung preparations showed extensive congestion and edema with numerous foci of pneumonia.

In the monkeys the process was patchy and far less extensive. The greater portion of the lungs were pink and air-containing. The cut surfaces, however, were moist and the bronchioles contained some pink frothy fluid. Some granular areas were also noted, particularly in the lower lobes. Microscopically the lungs showed congestion with some edema and a few small foci of pneumonia. There were also some small areas of emphysema.

Summary and Conclusions. Ten normal Rhesus Monkeys continuously exposed to 90+ % oxygen appeared to maintain good

health for a 5-day period. Then the reaction was variable. Anorexia first appeared, then apathy, drowsiness, cough and dyspnea. Survival periods ranged from 6 to 18 days in 8 monkeys. Two were alive at the end of 20 days and remained in good health. Pairs of rabbits used as controls died after 4 days and were replaced by new controls who again died after 4 days. Autopsies were performed and showed a slight amount of congestion and exudate in the lungs resembling a mild pneumonia in the monkeys, while the rabbits showed extensive pneumonia. The marked difference in reaction of the two sets of monkeys suggests that there may be a difference of resistance to 90+ % oxygen in different strains, or that lowering the tent temperature to 60° may have played a role in diminishing their resistance. The reaction of the Rhesus Monkeys suggests the possibility that it may be safe for human beings to be exposed continuously, if necessary, to a 90-95% oxygen concentration for periods up to 5 days.

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Studies on the Action of Penicillin. III. Bactericidal Action of Penicillin on *Meningococcus in vitro*.

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The meningococcus is highly susceptible to penicillin although it is gram-negative. In this respect as in its reaction to certain germicides, it resembles the gram-positive bacteria.¹ A preceding communication² reports a study of the action of penicillin on meningococcus *in vivo*.

Methods. Suspensions of meningococci grown for 6 hours on agar slants were made up in gelatin-Locke's solution or broth to contain approximately 10^8 microorganisms per

ml. After the addition of other ingredients the tubes were slanted on a rack and incubated in that position to expose the maximum surface to the air. Samples were withdrawn at frequent intervals and 10-fold dilutions made in gelatin-Locke's solution. Loopfuls were cultured from appropriate dilutions by careful streaking onto the surface of blood agar plates. Each bacterial count was based on 3 platings. Repeated checks on the accuracy of this method showed it to be adequate for the purposes at hand and better for meningococci than the ordinary methods involving the use of poured plates. Plate cultures were examined after 24 hours' incubation in

¹ Miller, C. P., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49**, 197.

² Miller, C. P., and Foster, A. Z., *Proc. Soc. Exp. Biol. and Med.*, in press.

a candle jar and again after 48 hours.

Preliminary experiments had demonstrated that 10 units of penicillin* per ml of broth could reduce the number of meningococci from 10,000,000 to 100,000 per ml in $2\frac{1}{2}$ -3 hours. Within the range at which we worked (10 units per ml) 10-fold differences in concentration of penicillin made but unimportant changes in the results.

Effect of Fresh or Inactivated Serum on the Activity of Penicillin in Broth. Serum was obtained from normal snuffle-free rabbits and a part of it was inactivated for 30 minutes at 56° . The fresh serum added in concentrations of 10-50% to broth containing 10 units of penicillin per ml speeded up the rate of decrease in the number of viable meningococci. That this gain in meningococidal action was due to a heat-labile substance (complement) was demonstrated by comparing the effect of inactivated serum, which not only failed to accelerate the decrease in numbers of meningococci as compared with the broth, but in some experiments slowed it down to an appreciable degree. (Fig. 1 and 2.)

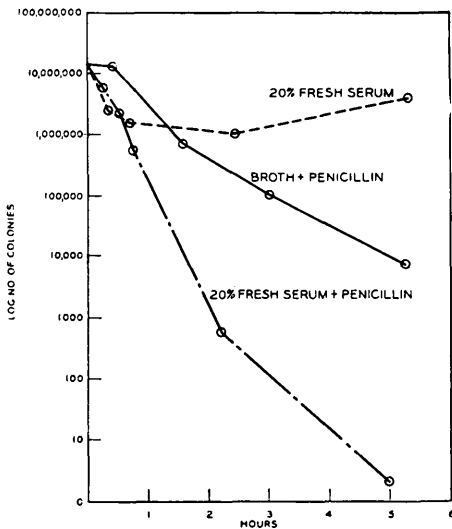


FIG. 1.

Effect of fresh normal rabbit serum on the meningococidal action of penicillin.

* 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.

Broth containing fresh rabbit serum without penicillin caused an abrupt but transitory decrease in the population which was followed by a rise. It seemed most likely, therefore, that the greater activity of penicillin in fresh normal serum was a summation of 2 meningococidal agents rather than an interaction of penicillin with some heat-labile substance in the serum.

Within certain limits the extent of the meningococidal action of fresh normal rabbit serum was proportional to its concentration. The results with sera from different rabbits, while not identical, fell within limits which might be expected from individual variation. The fresh and inactivated serum of man, guinea pigs, and mice gave results similar to those with rabbit serum. Not all strains of meningococcus were equally susceptible to fresh serum.³

The Effect of Penicillin in Non-Nutrient Medium. Preliminary observations demonstrated that the viable population of meningococci suspended in gelatin-Locke's solution

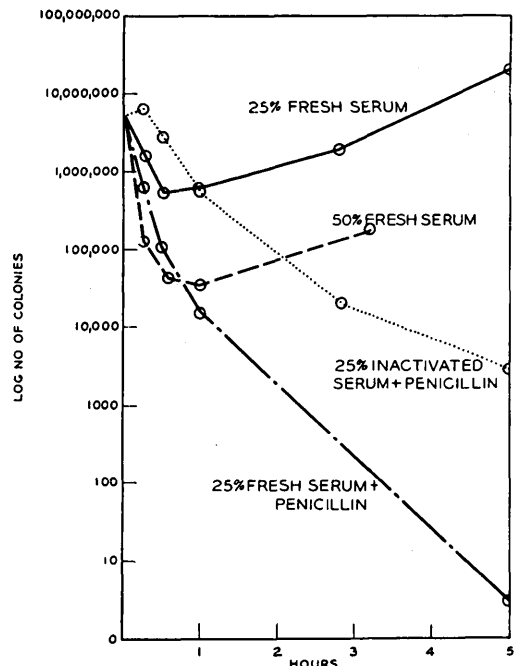


FIG. 2.

Effect of fresh human serum on the meningococidal action of penicillin.

³ Thomas, L., and Dingle, J. H., *J. Clin. Invest.*, 1943, **22**, 375.

TABLE I.
Effect of Temperature on Action of Penicillin.
Colonies per ml developing in subcultures from broth suspensions.

	Broth	Broth containing 10 units penicillin per ml
After 24 hr in ice box	10,000,000	11,000,000
After 48 hr in ice box	5,300,000	6,300,000
Transferred to incubator, examined 18 hr thereafter	∞	0
	Washed sediment from 2 ml	

remained practically constant (within the limits of accuracy of the method) for a period of 6 hours at 37°. The addition of penicillin to such suspensions was almost without effect on the bacterial population. (Fig. 3.)

When inactivated rabbit serum was added in a concentration (10%) which was able to promote some multiplication of meningococcus in the otherwise non-nutrient medium, penicillin reduced the population about as rapidly as it did in broth.

This observation supports the contention of Hobby, Meyer, and Chaffee,⁴ that penicillin acts only under conditions favorable to bacterial multiplication.

Effect of Temperature on the Action of Penicillin in Broth. Ten units of penicillin per ml were added to one half of a broth suspension of meningococci and both tubes placed in the ice box. Samples were withdrawn after 24 and 48 hours, diluted and plated as in the previous experiment. The results of a typical experiment are presented in Table I and show that exposure to this concentration of penicillin for 48 hours caused no decrease in numbers of meningococci. When these same suspensions were transferred to the incubator for 18 hours, heavy growth occurred in the broth control and the penicillin suspension became sterile.

Bacteriolysis. Lysis by penicillin could be observed if the suspensions contained approximately 2.5×10^8 per ml meningococci which gave the solution a distinct haziness, but not if the bacterial suspensions were much heavier than this. Ten units of penicillin per ml produced definite, but incomplete clearing which could be increased only by great increases in concentration.

Lysis occurred at a rate which was barely

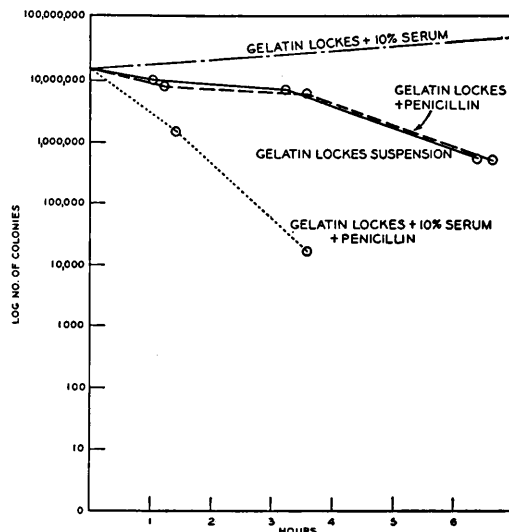


FIG. 3.
Ineffectiveness of penicillin upon meningococci in a non-nutrient medium.

detectable from hour to hour, but was quite distinct at the end of 24 hours after which little or no change could be noted. It was most pronounced at 37°, although it progressed more rapidly at 45° for the first hour, and then stopped altogether. Lysis did not occur at ice box temperature.

Meningococci killed by heating at 45° for 15 minutes were not lysed by penicillin, nor were meningococci recovered from old (48-hr) cultures.

These observations on meningococcus do not agree with the findings of several investigators who report failure to demonstrate lysis of other bacteria.^{4, 5}

Morphological Changes. In dilutions of penicillin beyond the concentration which inhibited the growth of meningococcus, changes occurred in the morphology of some cells which could be plainly seen in ordinary micro-

⁴ Hobby, G. L., Meyer, K., and Chaffee, E., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50**, 281.

⁵ Waksman, S. A., *J. Bact.*, 1943, **45**, 64.

scopic preparations. Enlargement and distortion from normal shape were the most frequent abnormalities. Such changes were not found by Gardner⁶ in meningococci.

Summary and Conclusions. Meningococci are readily killed by penicillin in nutrient broth at 37°. This meningococcidal action is markedly increased by fresh serum of man, rabbit, guinea pig and mouse, due to a heat-labile component, presumably complement. The meningococcus therefore is an unsuitable microorganism on which to test human sera for their content of penicillin by the bacteriostatic method commonly employed. The presence of inactivated serum, however, fre-

quently reduces the activity of penicillin on meningococci in broth.

Penicillin fails to kill meningococci in a non-nutrient medium (gelatin-Locke's solution) at 37° and in nutrient broth at ice box temperature, two conditions unfavorable for their growth. These findings lend support to the notion, already expressed by others, that penicillin acts on bacteria only in an environment which promotes their multiplication.

Penicillin causes a certain degree of lysis of meningococci actively growing in broth.

Meningococci in broth containing concentrations of penicillin just below the level of bacteriostasis develop enlarged forms which suggest an inability to complete the normal process of fission.

⁶ Gardner, A. D., *Nature*, 1940, **146**, 837.

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Effect of High Protein (Meat) Diet on Mortality from Surgical Shock Due to Repeated Hemorrhage.*

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A high protein diet consisting of horse meat *ad libitum* was given to dogs later subjected to repeated hemorrhage according to a standardized technic previously described.¹ A definitely beneficial influence was observed as compared with animals not so prepared. Although there is a considerable literature on the effect of diet on the regeneration of serum protein from the low concentrations produced by non-fatal hemorrhage, we could find no studies on the influence of the preceding dietary regime on an acute fatal bleeding.

Experimental Procedure. Fatal surgical shock was produced by the removal of 10 cc per kilogram of body weight each hour through puncture or cannulization of the femoral artery, under local anesthesia. The supine position was maintained during the entire

procedure. Mongrel dogs were used weighing from 7 to 12 kg. Four groups of experiments were performed as follows:

Group 1 consisted of 14 animals who had been on a non-protein diet² for 4 of 5 weeks; during the fourth week various forms of protein were given by mouth to determine their effects on serum protein regeneration. The average caloric intake was 50 per kilo per day.

Group 2 consisted of 10 animals who had been on a "normal" ration of Purina chow for periods of 1 to 3 weeks before the experiment. The approximate proportions of protein, fat, and carbohydrate in the Purina chow are 4, 1, and 9 respectively. The average caloric intake was not measured in the group.

Group 3 consisted of 10 animals given an *ad libitum* horse meat diet for a period of 3 weeks before the experiment was carried out. The amount of meat consumed daily in this

* Aided by a grant from the Commonwealth Fund.

¹ Elman, R., and Lischer, C. E., *Ann. Surg.*, 1943, **118**, 225.

² Sachar, L. A., Horvitz, A., and Elman, R., *J. Exp. Med.*, 1942, **75**, 453.