It is of interest to note that the sensitivity of *H. ducreyi* to penicillin *in vitro* would seem to differentiate it somewhat from other organisms of the same group, such as *H. influenzae* and *H. pertussis*, which are penicillin resistant.

Summary and Conclusions. The activity of penicillin on the growth of 5 strains of *H. ducreyi* was tested *in vitro* by the serial dilution method. *H. ducreyi* was found to be penicillin sensitive. This sensitivity was less than that of *Streptococcus hemolyticus* and paralleled that of the test strain of *Staphylococcus aureus*.

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Studies on the Action of Penicillin. II. Therapeutic Action of Penicillin on Experimental Meningococcal Infection in Mice.

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The effect of penicillin on several experimental infections in mice has been investigated[1-4] but even though it has been found to be highly satisfactory in the treatment of cerebrospinal fever, its application to experimental meningococcal infection in a laboratory animal has not as yet been reported. The present study undertook to fill a gap in our knowledge about this valuable chemotherapeutic agent.

Methods. Meningococci were grown for 6 hours on agar slants, suspended in Gelatin-Locke's solution to a turbidity known to approximate 1 billion meningococci per ml and then titrated in 4% mucin* by 10-fold dilution for intraperitoneal injection in 1 ml volumes.[5] Penicillin† in aqueous or saline solution was administered in the following volumes: Subcutaneous—.2 ml, intramuscular—.1 ml, intravenous—.2 ml.

Experiment I. Relation of the Time of Administration of Penicillin to Its Effect on Experimental Meningococcal Infection. The first experiments were made with a stock strain of meningococcus which had been carried for a number of months on artificial media with frequent mouse-passages. It possessed maximal virulence for mice by ordinary standards, *i.e.* fewer than 10 meningococci in 1 ml of 4% mucin regularly initiated a lethal infection.

Mice were infected with 100,000 MLD (10^{-4} of the standard suspension which at 10^{-9} killed the controls). At intervals of ½, 2, and 4 hours thereafter, groups of 4 mice were injected intravenously or subcutaneously with penicillin in a single dose of 100 Oxford units each.

As shown in Table I, the route of administration of penicillin made practically no difference in the results. Of the 8 treated ½ hour after inoculation, only one survived; whereas among those treated at 2 and 4 hours, 8 of 12 survived.

In a series of 13 mice infected with the next lower inoculum of meningococci (10,000 MLD) and treated at the same intervals, only one, which was treated at ½ hour, failed to survive.

* Granular mucin, Type 1701-W, supplied by the Wilson Laboratories, Chicago, Ill.

† 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.

1 Hobby, G. L. Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 285.

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

3 McIntosh, J., and Selbie, F. R., *Lancet*, 1943, **2**, 224.

4 Hac, L. R., *J. Infect. Dis.*, 1944, **74**, 164.

5 Miller, C. P., and Castles, Ruth, *J. Inf. Dis.*, 1936, **58**, 263.

TABLE I.
Penicillin Treatment of Mice Infected with a Stock Strain of Meningococci.
Each dose of penicillin—100 Oxford units.

Inocula	Penicillin intravenous Time after inoculation ½ hr	2 hr	4 hr	Penicillin subcutaneous Time after inoculation ½ hr	2 hr	4 hr
100,000 MLD*	23	22	46	22	72	
	23	46	S	22	S	
	27	S	S	26	S	
	46	S	S	S	S	
10,000 MLD				23	S	S
				S	S	S
				S	S	S
				S	S	S

* Mice inoculated intraperitoneally with 1 ml of a 4% mucin suspension (100,000-1,000,000 meningococci) of which less than 10 sufficed to initiate a lethal infection in controls.
Numbers represent hour of death.
S = survival for 72 hours.

TABLE II.
Penicillin Treatment of Mice Infected with a Recently Isolated Strain of Meningococcus.

Dose	No. of injections Time after inoculation	1 ½ hr	1 3 hr	2 3 and 6 hr	3 1½, 4, 6 hr
	Mice Inoculated with 100,000 MLD.*				
50 Oxford units		22	22	26	25
		22	22	46	S
		22	22	46	S
		22	22	S	S
					S
100 " "		22	22	22	
		22	22	22	
		22	22	46	
		22	22	S	
				S	
	Mice Inoculated with 10,000 MLD				
50 " "		22	22	S	
		22	22	S	
		22	22	S	
		25	53	S	
				S	
100 " "		22	22		
		22	25		
		22	30		
		26	S		

These results showed that mice infected with very large doses of meningococci could be treated less successfully immediately after inoculation than a few hours later. These unexpected observations were repeatedly confirmed.

Experiment 2. Influence of Virulence of the Infecting Strain. When the foregoing experiment was repeated with a recently isolated strain the following differences were observed. None of the mice inoculated with 100,000 MLD and treated with 1 dose of 100 units of penicillin, survived and only one of those inoculated with 10,000 MLD.

Two injections of 50 units each sufficed to save all the mice inoculated with 10,000 MLD, but only one of 4 inoculated with 100,000 MLD. Two injections of 100 units saved 2 of 5 mice inoculated with 100,000 MLD and three injections of 50 units saved 4 of 5.

These results indicate that mice infected with a recently isolated strain did not respond to penicillin as readily as mice inoculated with a stock strain, even though the MLD of all strains used was less than 10 meningococci by

this method.[5] Whereas mice infected with the stock strain recovered after a single dose of penicillin, those inoculated with comparable numbers of recently isolated meningococci required repeated doses of penicillin.

Experiment III. To determine the effect of penicillin on the progress of the experimental infection, mice were inoculated intraperitoneally with 10,000 MLD of a recently isolated strain, as in the foregoing experiment, and one and 3 hours thereafter, groups of mice (designated first and second groups) were injected with penicillin intramuscularly. A group of infected, untreated mice served as controls. At intervals a pair of mice from each group was sacrificed and cultured by inoculating a drop of heart's blood and a loopful of peritoneal fluid onto a blood agar plate. The results of the blood cultures showed that meningococci did not invade the blood stream until about 3 hours after inoculation and disappeared immediately after the intramuscular administration of penicillin. The results of the cultures of peritoneal fluid combined from 2 experiments are plotted in Fig. 1 and show: (a) that the administration of penicillin at one hour (1st group) did not cause as rapid a reduction in numbers of meningococci in the peritoneal cavity as did its administration at 3 hours. (2nd group) (b) The penicillin appeared to have been active for 2-3 hours. (c) The peritoneal cavity was not sterilized. (d) The surviving meningococci in the first group multiplied rapidly after the effect of penicillin had worn off.

Experiment IV. As the foregoing experi-

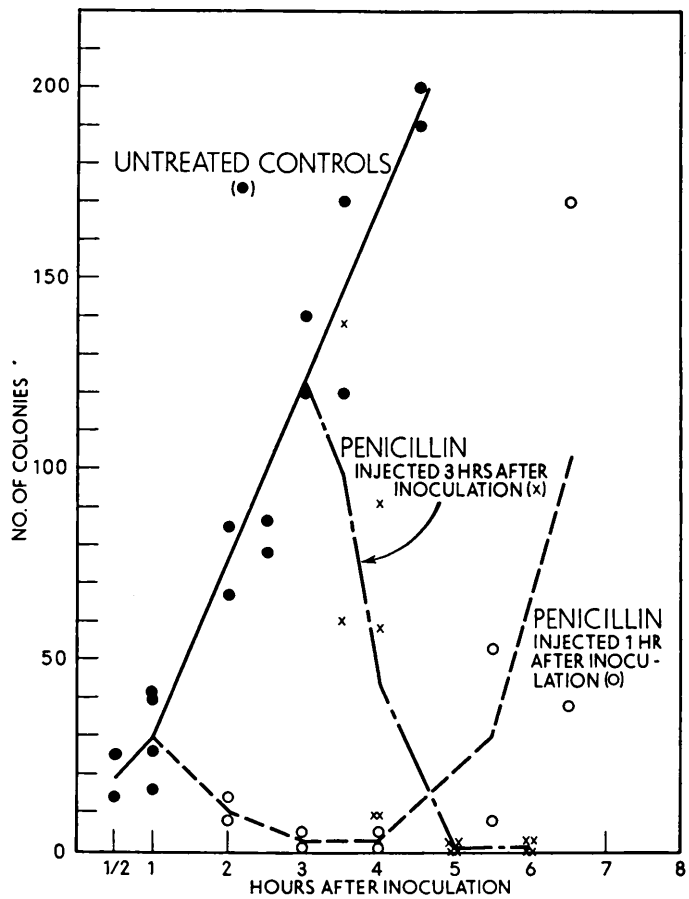

FIG. 1.

Effect of penicillin on progress of infection as indicated by the recovery of meningococci from the peritoneal fluid.

ment showed that the activity of penicillin lasted only 2 or 3 hours (see Fig. 1), the following experiment was designed to determine the time required for penicillin administered intramuscularly to reach the blood and peritoneal fluid in detectable amounts and the length of time it persisted.

Mice were injected intraperitoneally with 1 ml of sterile, 4% mucin and intramuscularly with 100 Oxford units of penicillin, and sacrificed at stated intervals thereafter. The heart's blood and peritoneal fluid obtained (.25 to 0.5 ml) were added to 1.5 ml of broth, titrated by 5-fold dilution and inoculated with 2×10^7 meningococci. The highest dilutions which inhibited growth showed that penicillin was detectable in the blood within 5 minutes (the first observation) and for about 2 hours thereafter. In the peritoneal fluid penicillin appeared within 5 minutes, rose for ½ hour to a somewhat higher level than in the blood and fell off about the end of the second hour. The penicillin determinations on the blood are subject to the limitations brought out in a later communication[6] which describes meningococcidal action of fresh serum.

Discussion and Summary. It should be remembered that the method of producing experimental meningococcal infection in the mouse involves the use of mucin, without which meningococci, even the most virulent strains, will not initiate a genuine infection. The method, nevertheless, lends itself to investigation of the action of penicillin *in vivo*. These studies have so far brought out the following points: Penicillin was equally effective if injected intravenously, subcutaneously, or intramuscularly. When given intramuscularly, it was detectable in the heart's blood within 5 minutes and persisted there for about 2 hours. Its diffusion into the peritoneal cavity occurred simultaneously and its concentration there rose to a relatively higher level than in the blood. (Cf. the clinical observations of Rammelkamp and Keefer[7] on its diffusion out of serous cavities). The penetration of penicillin into the peritoneal cavity may or may not have been affected by the presence of mucin.

The effect of penicillin on the bacterial population in the peritoneal fluid was an immediate decrease in numbers which was more striking if the infection had progressed for 3 hours than if the inoculum had been introduced only one hour before.

Infection with a stock strain of meningococcus, although fully virulent by ordinary standards, was more easily brought under control than infection with strains recently isolated from human cases of meningococcal infection. The latter required repeated doses of penicillin.

[6] Miller, C. P., and Foster, Alice Zimmerman, PROC. SOC. EXP. BIOL. AND MED., in press.

[7] Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.

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Effect on Blood Agglutinins of a Polysaccharide Isolated from *Ascaris suum*.

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The inhibitory effect of a polysaccharide isolated from the pig roundworm, *Ascaris suum*, on the α and β agglutinins of human

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