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Penicillin in Treatment of Experimental *Clostridium welchii* Infection.LUCILE R. HAC* AND AGNES C. HUBERT.[†] (Introduced by H. Close Hesselstine.)

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Gas gangrene remains one of the most serious complications of war wounds in spite of present methods of chemotherapy, serum therapy, and surgery. The mortality is high and the rapidity with which the infection progresses makes treatment difficult. Several anaerobes or mixtures of anaerobes are known to cause the infection, but the one encountered most frequently is *Clostridium welchii*. Previously, we had made a study of the use of sulfonamides in the treatment of experimental *Cl. welchii* infection in mice.^{1,2} It was possible to protect over 50% of the infected animals with sulfadiazine or sulfathiazole, but all of them had a huge lesion at the site of the inoculation from which *Cl. welchii* could be isolated 4 to 5 weeks after inoculation. Penicillin has been used in the present study in an attempt to increase the survival rate of the infected animals as well as to study its effect upon the local lesion.

It has been only recently that penicillin has been used in the treatment of infections, and information concerning its use in *Cl. welchii* infection is very meager. Gardner³ found that in liquid medium penicillin completely inhibited the growth of *Cl. welchii* at a dilution of 1 in 6000. With highly purified penicillin, Florey and Jennings⁴ found the highest dilution which completely inhibited growth was 1 in 4 million, with partial inhibition at a dilution of 1 in 8 million. Recently,

McIntosh and Selbie⁵ stated that penicillin injected at the site of infection within 3 hours of infection was a powerful prophylactic against *Cl. welchii* infection in mice.

Mice and guinea pigs have been used in our study. A typical intramuscular infection was produced as previously described¹ by the inoculation into the inner aspect of the thigh of a whole, unwashed, 18-hour culture. No other necrotizing agent was used. Within an hour after inoculation, the leg had become inflamed. There was considerable swelling after 2 hours and some dark discoloration a few hours later. The amount of inoculum used was sufficient to kill 90-100% of the untreated animals. This amount had been determined by previous standardization (usu-

TABLE I.
Penicillin—Effect of Varying Dosage
Single Dose—Same Leg.

Units	No. animals	% survival
500	25	100
50	100	98
25	100	81
15	75	72
5	50	54

ally 0.25 ml of a 1:3 dilution of culture was used), and as a further check 10 control animals were inoculated with each group of animals studied.

The penicillin, 0.25 ml, containing the desired number of Florey units, was injected subcutaneously into the inguinal region of the infected leg, or of the opposite leg. The effect of immediate and delayed treatment (1, 2, and 3 hours) was determined.

The results (averages of repeated experiments) obtained in mice, when a single injection of various doses of penicillin was made at the same time as the inoculation with *Cl. welchii*, are given in Table I.

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¹ Hac, L. R., Eilert, M. L., and Adair, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 108.

² Hac, L. R., and Hubert, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1943, **53**, 58.

³ Gardner, A. D., *Nature*, London, 1940, **146**, 837.

⁴ Florey, H. W., and Jennings, M. A., *Brit. J. Exp. Path.*, 1942, **23**, 120.

⁵ McIntosh, J., and Selbie, F. R., *Lancet*, 1942, Dec. 26, **243**, 750.

Small doses of penicillin repeated every 4 hours over a period of 48 hours gave as good results as single large doses. (Table II.)

Better results were obtained when the drug was injected into the inguinal region of the infected leg than when similarly injected into the non-infected (opposite) leg. (Table III.)

TABLE II.
Penicillin—Effect of Repeated Doses q. 4 Hrs.

Units	No. animals	% survival	
		1 dose	8 doses
25	50	80	96
15	75	75	92
5	50	54	92

TABLE III.
Penicillin—Comparison of Same and Opposite Leg.

Units	No. animals	% survival	
		Same leg	Opposite leg
125	25	96	92
25	100	81	68
15	75	72	53
15 (8x)	25	92	88

Even when penicillin was administered before inoculation with *Cl. welchii*, the early inflammation, swelling, and discoloration which resulted in the local lesion was observed. Only a small lesion was produced, however, if a large dose of penicillin was used, and if repeated injections of the drug were made into the site of infection, the lesion healed completely within a period of 3 weeks. Direct smears from the lesion showed many of the organisms to be in long chains or filaments instead of single rods.

A comparison of the effect of penicillin and sulfonamide therapy (Table IV) begun before, at the same time, and at various intervals after infection, indicates that penicillin is superior to the sulfonamides. With penicillin,

TABLE IV.
Comparison of Effect of Time on Sulfonamide and Penicillin Therapy—Single Dose.

Time	% survival		
	Sulfa-thiazole*	Sulfa-diazine*	Penicillin†
3 hrs before <i>welchii</i>	—	97	—
Same time	45	53	100
1 hr after <i>welchii</i>	20	38	92
2 " "	14	16	80
3 " "	12	8	30

* 50 mg dose. See reference 2.

† 250 units—50 animals, same time, 1 hr and 2 hrs after; 10 animals, 3 hrs after.

the sharp drop in the survival rate does not occur until treatment has been delayed 3 hours. Deaths in animals treated with penicillin usually occurred 48 or more hours after inoculation while in the untreated and sulfonamide-treated animals, death occurred most frequently within 24 hours.

A few guinea pigs infected and treated in the same way as the mice have responded equally well to therapy.

No drug toxicity was observed in any of the animals.

Conclusions. 1. Penicillin is far superior to sulfonamides in the treatment of *Cl. welchii* infection in mice. A single injection of 50 Florey units of penicillin administered subcutaneously at the time of intramuscular inoculation with *Cl. welchii* protects 98% of the infected animals. 2. Repeated small doses of penicillin give as good protection as single large doses. 3. Delay in the institution of therapy lowers the survival rate, but not appreciably unless the delay is over 3 hours. 4. Local lesions were completely healed within 3 weeks if penicillin was injected repeatedly into the site of infection. 5. Response to the treatment of this infection in guinea pigs seems to be as good as that in mice.