spayed or non-spayed females injected with testosterone propionate, coagulating gland transplants contained 102 to 191 mg % fructose; here too, the level in the transplants fell to 3 to 12 mg % fructose when testosterone was withheld for 3 weeks.

*Summary.* Subcutaneous transplants of coagulating gland and seminal vesicle in rats, were capable of producing fructose and citric acid when grown a) in normal males, b) castrated testosterone-treated males, and c) testosterone-treated non-spayed or spayed females.

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## Therapeutic Activity of Bacitracin in Rabbit Syphilis, and Its Synergistic Action with Penicillin.*

HARRY EAGLE AND RALPH FLEISCHMAN. (Introduced by J. A. Shannon.)

*From the Laboratory of Experimental Therapeutics, U. S. Public Health Service, and the Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.*

Bacitracin is an antibiotic derived from culture filtrates of certain strains of *Bacillus subtilis*, and first described by Johnson, Anker, and Meleney.[1] As shown in a previous concentration from this laboratory,[2] it has a low renal clearance which approximates the glomerular filtration rate, in contrast to penicillin which has a renal clearance approximating the total renal plasma flow.[3] In consequence, bacitracin is excreted more slowly than penicillin, the rate of excretion remaining at a reasonably constant level for a period of several hours; and the blood level also falls more slowly than does that of penicillin G injected in similar gravimetric dosage.

*In vitro*, bacitracin has been shown to be actively treponemicidal against the cultured Reiter strain of *Treponema pallidum*.[4] Concentrations of 0.004 units per cc inhibited the growth of the organisms, and 0.1 unit per cc killed 99.9% in 48 hours. Against the pathogenic *T. pallidum in vivo*, doses of 72 units/kg given intramuscularly caused the temporary disappearance of the organisms from rabbit testicular chancres.[4] It may be noted that in both rabbits and man, this dosage provides plasma concentrations in excess of 0.1 unit per cc for approximately 2 to 3 hours, and in excess of 0.01 unit per cc for approximately 6 to 8 hours.[2] This period of exposure was apparently enough to render the chancre temporarily dark-field negative, but was not enough to kill all the organisms. The organisms regularly reappeared in the lesions days, weeks or even months after the injection; and it required single doses of 5,000 units/kg and more to effect the permanent healing of the primary lesion.

The present paper is a report on the therapeutic activity of bacitracin in rabbit syphilis when aqueous solutions were injected intramuscularly once daily for 4 days. Further, data are given which suggest a remarkable synergistic action between penicillin and

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1 Johnson, B., Anker, H., and Meleney, F., *Science*, 1945, **102**, 376.

2 Eagle, H., Newman, E. V., Greif, R., Burkholder, L. M., and Goodman, S. C., *J. Clin. Invest.*, 1947, **26**, 919.

3 Eagle, H., and Newman, E., *J. Clin. Invest.*, 1947, **26**, 903.

4 Eagle, H., Musselman, A. D., and Fleischman, R., *J. Bact.*, 1948, **55**, 347.

bacitracin. The relatively small numbers of animals so far tested make the results of qualitative rather than quantitative significance. The synergistic action was nevertheless striking; and the incomplete data are reported at this time because they indicate the desirability of a clinical trial of bacitracin and penicillin used in combination in the treatment of human syphilis.

*Methods and materials.* The method of evaluating cure in the treated rabbits by lymph node transfers to normal animals 4 to 6 months after treatment has been adequately described in previous reports from this laboratory.[5,6]

The bacitracin was obtained from the Ben Venue Laboratories, Bedford, Ohio, and the various lots assayed at 18 to 41 units per mg. The penicillin used was a commercial preparation of crystalline penicillin G, generously supplied by the Commercial Solvents Company (Lot No. 46042605), assaying at 1,500 units/mg. The dosages of penicillin indicated in Fig. 1 and Table I were adjusted for 1,667 units/mg (1.11 mg administered instead of 1, etc.).

Aqueous solutions of the two drugs were injected into the muscles of the thigh, on opposite legs, within a minute of each other; and

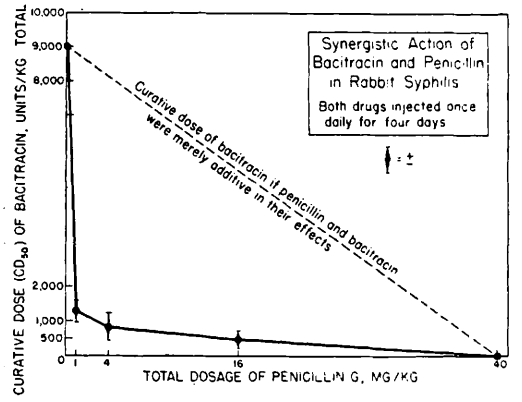


FIG. 1.
The synergism between bacitracin and penicillin with respect to the curative dose in rabbit syphilis. (From data of Table I.)

TABLE I.
Approximate Curative Dose of Bacitracin in Rabbit Syphilis, and its Synergistic Action with Penicillin. (Both drugs injected intramuscularly in aqueous solution, once daily for 4 days.)

| Total penicillin dosage, mg/kg | Proportion of rabbits cured by total bacitracin dosages (units/kg) of | | | | | | | | | | Approximate $CD_{50}$ dosage of bacitracin, units/kg* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 40,000 | 20,000 | 10,240 | 5120 | 2560 | 1280 | 640 | 320 | 160 | 80 | 40 | |
| 0 | 6/6 | 5/5 | 3/5 | 0/9 | 0/5 | | | | | | | 9200 ± 2200 |
| 1 | 3/3 | 5/6 | 6/6 | 5/5 | 5/6 | 1/3 | 1/4 | 0/5 | 0/2 | 0/4 | 0/2 | 1280 ± 310 |
| 4 | | 4/4 | 4/4 | 4/5 | 6/6 | 1/3 | 0/4 | 2/5 | 1/3 | 0/1 | 0/3 | 840 ± 380 |
| 16 | | 6/6 | 3/3 | 5/5 | 5/5 | 2/4 | 1/3 | 3/5 | 1/5 | 0/3 | 0/3 | 480 ± 240 |

* Calculated by the method of Miller and Tainter[6]; ± = standard error.†

[5] Eagle, H., and Hogan, R. B., *Ven. Dis. Inf.*, 1943, **24**, 69.

[6] Eagle, H., and Magnuson, H. J., *Bull. Johns Hopkins Hosp.*, 1946, **79**, 168.

the injections were repeated once daily for 4 days.

*Experimental results.* Table I lists the proportion of animals cured by various dosages of bacitracin used either alone or in combination with 1, 4 or 16 mg/kg of penicillin G. The last vertical column of the table gives the $CD_{50}$ dosages of bacitracin, calculated by the Miller-Tainter method.[7] Approximately 9,000 units/kg of bacitracin was necessary to cure 50% of the animals ($CD_{50}$), and the similarly curative dose of penicillin on the same schedule was on the order of approximately 30-40 mg/kg.[6] When as little as 1 mg/kg of penicillin was administered simultaneously with bacitracin, the curative dose of bacitracin was reduced from 9,200 ($\pm$ 2,200)† to 1,280 ($\pm$ 310)† units/kg. In other words, approximately 1/40th of the $CD_{50}$ dose of penicillin and 1/7th of the curative dose of bacitracin were curative when used in conjunction. Further increase in the dosage of penicillin from 1 to 4 to 16 mg/kg, caused a further progressive decrease in the $CD_{50}$ dose of bacitracin from 1,280 to 840 to 480 units/kg.

In Fig. 1, the top dotted line represents what the curative dose of bacitracin would have been were bacitracin and penicillin merely additive in their therapeutic action. The degree to which these experimental data deviate from that line is a measure of the striking synergistic action of the 2 drugs used in conjunction.

*Discussion.* Although both penicillin and bacitracin are curative in rabbit syphilis, the 2 drugs were as much as 7 times more effective when they were used in combination, judged by the total amounts of each necessary to effect cure. It is possible that in the treatment of human syphilis also, the synergistic use of the 2 drugs may permit a significant reduction in dosage, a significant decrease in the minimum time necessary for cure, or perhaps in both. The primary problem in the treatment of early human syphilis is, however, not the amount of treatment necessary for cure, or even the length of time for which treatment must be continued, but the fact that a significant proportion of cases are not cured by penicillin alone in whatever dosage it has yet been used.[8] Only the actual trial in man will determine whether penicillin and bacitracin are synergistic in the sense that patients not cured by penicillin or bacitracin alone may perhaps be cured if penicillin and bacitracin are used in conjunction. Pilot studies in these directions are now in progress.

*Summary.* Bacitracin has been shown to be effective in the treatment of rabbit syphilis. An intramuscular dose of 2,300 units/kg repeated once daily for 4 days, cured approximately half the animals tested. A striking synergism was noted in the therapeutic effect of penicillin and bacitracin. As little as 1 mg/kg of penicillin (approximately 1/40th of the $CD_{50}$ dosage of penicillin alone) and 1,280 units/kg of bacitracin (approximately 1/7th of the $CD_{50}$ value) were curative if used in conjunction. These data are reported because of their possible significance in relation to the treatment of the human disease.

[7] Miller, L. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 261.

† The standard deviation of these $CD_{50}$ levels is large primarily because of the small number of animals so far used: the trend is nevertheless clear. The assistance of Mr. Nathan Mantel of the Statistical Section of the National Cancer Institute in calculating the $CD_{50}$ values and their standard error is gratefully acknowledged.

[8] Merrill, M., Syphilis Symposium, Syphilis Study Section, Washington, D.C., April 8, 1948