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Oral Use of Penicillin in Treatment of Experimental *Erysipelothrix rhusiopathiae* Infection in Mice.

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The development of an effective treatment for swine erysipelas becomes of considerable importance because of the increasing prevalence of this disease in the United States and in foreign countries. The present study was therefore undertaken to evaluate the use of penicillin in the control of experimental infection. Heilman and Herrell[1] have reported successful treatment of infected mice by frequent injections of penicillin, totaling 1000 units per day per mouse, over a 7-day period. Since simplicity of treatment often becomes a prime factor in the field of animal disease control, we were particularly interested in the possibility of oral administration rather than the injection method.

Procedures. Mice: Test animals throughout were 16 to 18 g mice. For the major portion of tests, Rockland female mice were used.† At one stage it was necessary to substitute a Dilute Brown Agouti, tumor-susceptible strain due to scarcity of the Rocklands. Comparative tests using the 2 strains showed no difference in response.

Penicillin-in-feed: In early trials penicillin was suspended in corn oil in a concentration of 1000 units per cc of oil. This was then mixed with a cereal type feed to give a final concentration of either 100 or 1000 units of penicillin per gram of feed. In later tests, the dried penicillin was ground with dried feed in varying concentrations and fed with no protective coating of oil. Test mice were placed in individual cages and given weighed amounts of penicillin-containing food. After 24 hours, animals were challenged with an injection of 10 MLD of test culture. Feeding with treated material was continued for the

* American Cyanamid Research Laboratories, Stamford, Connecticut.

1 Heilman, F. R., and Herrell, W. E., *Proc. Staff Meetings of the Mayo Clinic*, 1944, **19**, 340.

† Obtained from Rockland Farms, New City, N.Y.

TABLE I.
Effectiveness of Penicillin in Feed.

Method of administration	10 units/g	100 units/g	1000 units/g	2500 units/g
Oil	0/10*	0/10	0/10	0/10
Dry	0/10	0/10	10/10	10/10

* Survivals/mice injected.

full time of the experiment. A like number of animals were fed a normal diet and served as control animals.

Penicillin-Drinking Water: Penicillin was diluted with tap water in concentrations ranging from 10 to 1000 units per cc of water. Fifty cc amounts of final dilution were supplied to each cage of 5 mice. This amount was enough for 48 hours in most instances. The penicillin-water was replaced every 48 hours with fresh material, regardless of any amount which might remain. In original trials, animals were given the treated drinking water for 24 hours to develop a blood level prior to challenging. In later experiments the challenge dose and beginning of treatment were simultaneous. In final trials, the challenge took place 24 or 48 hours prior to the beginning of treatment. The length of time for continuance of treatment varied from 24 hours to 10 days—the duration of tests.

Culture: The test culture used throughout was *Erysipelothrix rhusiopathiæ,* Lederle No. 358. This strain is used routinely for protection tests against anti-swine erysipelas serum and is of extremely high virulence for mice. Subcutaneous injection of 0.5 cc of a 10^{-8} dilution of culture will consistently kill 100% of unprotected mice, and a similar injection of a 10^{-9} dilution usually kills at least 2 out of 3 mice.

Results. Effectiveness of penicillin-in-feed: The oil-coated penicillin appeared to be very unpalatable and was eaten in smaller amounts than the control feed. For this reason, the mice failed to develop a protective blood level and all animals died within 4 days from the date of challenge. When the dried material was ground with the feed in a concentration of 1000 units per gram, or more, a satisfactory level was obtained and 100% protection was afforded, while all control animals died within 4 days of the date of challenge. (Table I shows sample experiment.)

Effectiveness of penicillin-in-water: Addition of 1000 units of penicillin per cc of water provided complete protection when begun in advance of or simultaneously with the injection of the challenge dose. When the concentration was cut to 500 units per cc, the protective rate dropped to 20% of the test animals. Concentrations lower than 500 units per cc were of no protective value even when supplied for 48 hours prior to the date of challenge. (Table II.)

Tests were then carried out to determine whether or not the 1000-unit concentration would be effective against more than the 10 MLD challenge dose. Injections were made from serial dilutions prepared to give 100, 1000, and 10,000 MLD and treatment was begun simultaneously. Complete protection was provided against even the highest number of MLD used for challenging.

When challenge injections were made prior to the beginning of treatment, results were variable. Treatment begun 24 hours after challenge in some instances gave almost complete protection, while in duplicate trials only 20% survival resulted. After 48 hours higher

TABLE II.
Effectiveness of Penicillin in Drinking Water.

Treatment begun	10 units/cc	100 units/cc	500 units/cc	1000 units/cc	5000 units/cc	10,000 units/cc
24 hr before challenge	0/10	0/10	2/10	10/10	10/10	10/10
Simultaneously with challenge	—	—	0/10	10/10	10/10	10/10
24 hr after challenge	—	—	—	4/10	5/10	5/10
48 hr after challenge	—	—	—	0/10	0/10	0/10

dosages, even up to 10,000 units per cc, were of no value and all animals died.

Discussion. The evidence presented here indicates that penicillin, with no protective coating or buffering materials, may be successfully administered to mice by mouth and, provided that sufficiently large quantities are given, protection is afforded against *E. rhusiopathiæ*. Since 50 cc of water containing 1000 units/cc was consumed by 5 mice in 48 hours, the effective average intake per mouse was 5000 units/day. This is approximately 5 times the amount used by Heilman and Herrell[1] in injecting the material. Penicillin is effective in the treatment of *Erysipelothrix rhusiopathiæ* infection if given early enough after exposure. Our results on this point are in accord with the findings of Heilman and Herrell[1] who showed protective action by the injection method of administration when begun 21 to 24 hours after challenge. Under our experimental conditions the effective time interval was 24 hours or less, but in field conditions institution of therapy at such an early stage of infection may be unattainable. It would seem, therefore, that penicillin may be of considerable value only in the control of the outbreak of this infection in domestic animals. The ease of oral administration makes its use feasible on a far greater scale than would be possible where injections are necessary.

Summary. Penicillin is shown to be of value in the protection of mice against experimental *Erysipelothrix rhusiopathiæ* infection.

Oral administration is successful in the treatment of mice even without any protective action from oil coating, or buffers, provided it is instituted within 24 hours after exposure. The use of penicillin in drinking water is found to be as effective as is its addition to the feed. Oral administration compares favorably with parenteral injection although larger amounts are required.

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Preparation of Human Typing Sera by Iso-immunization of Human Donors with Group Specific Substances.

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Typing sera with extremely high agglutination titers have been prepared by Witebsky and his coworkers[1] in human donors following the intravenous injection of solutions containing minute amounts of group A and B specific substances. Wiener,[2] employing intramuscular injections of autoclaved saliva from group A and B individuals (secretors) instead of isolated group specific substances, confirmed Witebsky's observations. Pillemer *et al.*[3] have prepared as by-products of plasma fraction, concentrated isohemagglutinin globulins that were characterized not only by their high titer, but by their high avidity as well, *i.e.*, by the speed with which the serum agglutinated red cells. To the best of our knowledge, an investigation of the change in the avidity of typing sera following isoimmunization of human donors with group specific substances has not appeared in the literature. In the course of studies on blood group specific substances, we have had the opportunity to make such observations.

Nine subjects, 3 belonging to each of the following groups, O, A, and B, received intravenously 0.5 cc of a solution of group A and B

1 Witebsky, E., Klendshoj, N. C., and McNeil, C., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 167.

2 Wiener, A. S., Soble, R., and Polivka, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 310.

3 Pillemer, L., Oncley, J. L., Melin, M., Elliot, J., and Hutchison, M. C., *J. Clin. Invest.*, 1944, **23**, 550.