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Action of Gramicidin on Streptococci of Bovine Mastitis.

R. B. LITTLE, R. J. DUBOS, AND R. D. HOTCHKISS

From the Department of Animal and Plant Pathology and the Department of the Hospital, The Rockefeller Institute for Medical Research, Princeton, N. J., and New York, N. Y.

Gramicidin—an alcohol-soluble, water-insoluble substance isolated from cultures of a sporulating bacillus—has been shown to exert a marked bactericidal effect against gram-positive microörganisms, both *in vitro* and *in vivo*. It has been found for instance that 0.002 mg of this substance injected intraäbdominally into white mice, exerts a therapeutic action against experimental peritonitis caused by pneumococci and streptococci; gramicidin, however, has proved almost completely ineffective when administered by the intravenous, intramuscular, or subcutaneous route.[1-3]

It is known that in the chronic form of bovine mastitis caused by *Streptococcus agalactiæ* (Lancefield group B), the infection is confined to the infected quarter of the udder and rarely results in a demonstrable systemic disturbance. It appeared of interest, therefore, to determine whether gramicidin, when injected into the infected quarter, would destroy the streptococci causing the mastitis.

A number of cases of chronic mastitis were selected for study and it was established by daily bacteriological examination of the milk that the numbers of streptococci remained high (over 100,000 per cc of milk) during a period of several weeks prior to treatment.

The toxic reactions which result from the injection of gramicidin into the bovine udder and a convenient method of administration of the substance, were determined on 2 cows suffering from chronic mastitis. These animals received repeated treatments with increasing amounts of gramicidin diluted in Ringer's solution, which proved very irritating, and later in distilled water, which was more satisfactory. The following technic was finally adopted for the treatment of each individual quarter. Gramicidin in amounts of 60 to 240 mg was diluted in 1000 cc of double distilled sterile water at 40° C. Following the morning milking, the residual milk in the cistern and in the teat was flushed out with 100 to 200 cc of a dilute solution of gramicidin; 800 to 900 cc of the preparation were then

1 Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 11.

2 Dubos, R. J., and Cattaneo, C., *J. Exp. Med.*, 1939, **70**, 249.

3 Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1940, **132**, 791.

injected under pressure into the quarter and allowed to remain until the next milking. Within one hour after the injection, the treated quarter became distended and the rectal temperature began to increase, reaching 41° C at the 5th or 6th hour. The temperature returned to nearly normal in about 3 hours thereafter, and the acute swelling had about subsided at the next milking.

Repeated treatments of the 2 animals mentioned above failed to eliminate permanently the streptococci from the infected quarters. This may be explained in part by the inadequacy of the method of administration of the bactericidal substance and also by the fact that these 2 animals were well advanced in their lactation periods and that the infected quarters were severely indurated.

Three cows less advanced in the lactation period were selected for the following tests. Nine infected quarters were treated, one of which had been inoculated artificially and allowed to carry an infection for 17 days before treatment. Repeated treatments failed to eliminate the streptococci from 2 quarters. Five treatments were required to sterilize one of the quarters which was moderately indurated. These repeated treatments stimulated the production of fibrosis and resulted in a decrease in milk-secretion. The streptococci disappeared from the other 6 quarters (in 5 cases after a single treatment) without an appreciable decrease in milk production. The fact that streptococci had been eliminated was established by daily bacteriological examination of the milk over periods ranging from 15 to 81 days.

Before the effectiveness of gramicidin in the control of bovine mastitis can be determined, a larger number of animals must be treated and observed over a longer period of time. The influence of fibrosis, the state of the lactation, the competency of the closing mechanism of the teat, and other factors will have to be considered. While the streptococci were not eliminated from all of the treated quarters, they were markedly decreased after each treatment, and the findings thus confirm the results obtained in mice, namely, that gramicidin, when injected directly into an infected focus, exhibits a definite bactericidal effect against streptococci.