

clinically sensitive to grass-pollens showed significantly larger skin reactions with the more highly purified extracts than with the less highly purified or with the untreated extracts.

Summary. 1. Fractionation of aqueous grass-pollen extracts with water-miscible organic liquids promotes grass-purification of these extracts. 2. Precipitation of extracts purified by the above treatment with high concentrations of sulfates affords further purification.

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Effect of Gramicidin Suspended in Mineral Oil on Streptococci of Bovine Mastitis.

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Gramicidin, an alcohol-soluble, water-insoluble substance extracted from cultures of an aerobic sporulating bacillus, has been found to exert a marked bactericidal effect against Gram positive microorganisms, both *in vitro* and *in vivo*.¹ When injected by way of the cistern into infected quarters of cows suffering from mastitis caused by *Streptococcus agalactiae* (Lancefield serological group B), gramicidin apparently cured a number of the infections.²

The method originally used for the administration of gramicidin in the treatment of bovine mastitis consisted in diluting 60-240 mg of the bactericidal substance in 1000 cc of aqueous media and injecting this material into the cistern of the infected quarter. As reported earlier, this method of administration caused an intense swelling of the treated quarter, accompanied by a marked elevation of temperature. Although this reaction lasted only a few hours, it was severe enough to prevent repeated treatments on successive days.

It has now been found that sterile mineral oil is a suitable, non-irritating vehicle for the administration of gramicidin. Of 42 quarters treated with gramicidin-oil mixtures, only 4, which had received 120-160 mg of bactericidal substance, exhibited a severe reaction. On the second and third day following treatment, the fore milk in 3 of these quarters was tinged with blood, whereas the secretion of milk was suppressed for 4 days in a fourth quarter. The reaction in

¹ Dubos, R. J., *Ann. Int. Med.*, 1940, **13**, 2025.

² Little, R. B., Dubos, R. J., and Hotchkiss, R. D., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 444.

the remaining 38 quarters was milder than in those injected with gramicidin in water.

On the basis of present experience, the following technique of treatment appears the most satisfactory. 2-3 cc of an alcoholic solution of gramicidin, containing 40 mg per cc, are emulsified in 15 cc of sterile double distilled water and the emulsion mixed thoroughly in 25 cc of heavy mineral oil. The gramicidin-oil mixture is injected into the cistern shortly after the morning milking and allowed to remain until the evening milking. In usual cases, the mildness of the reaction makes it possible—if necessary—to repeat the treatment for several days in succession or to treat more than one quarter at the same time.

The effect of the treatment has been determined by a combination of bacteriological tests including plating the fore milk on blood agar, direct incubation of the milk sample, and the Hotis test. These tests were made repeatedly over periods of more than 30 days and attempts were made to recover and identify the cultural and serological types of streptococci responsible for the mastitis.

Of 31 quarters naturally infected with *Streptococcus agalactiae* and treated by the gramicidin-oil mixture, 26 appear to have responded by a complete disappearance of the streptococci. The infection in some of the cured cases was of a severe chronic nature. In 20 of the quarters a single treatment sufficed, while 6 required additional treatments. The infection was not eliminated in 5 quarters.*

Surprisingly enough, less satisfactory results have been obtained in the treatment of bovine mastitis caused by *Streptococcus uberis*; of four cases of this condition which were treated with gramicidin-oil mixtures, only one responded satisfactorily. The nature of this discrepancy is under investigation.

* In the case of 8 infected quarters which were treated with mineral oil alone, without the addition of gramicidin, we have observed a temporary reduction or even disappearance of the streptococci present in the milk. Nevertheless, in these 8 cases the number of bacteria returned to the original level 2-3 days after the last injection of oil alone; it is possible however that this treatment may be sufficient at times to cure an infection of a very mild character.