12057

Non-Pathogenicity of Gonococci for Larger Animals.*

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The need for an animal naturally susceptible to gonococcal infection is well known. Practically all common laboratory animals have been inoculated by natural and unnatural routes and the results have been almost uniformly discouraging. The only lower animal known to be susceptible to the gonococcus is the white mouse and this non-immunity can be shown only through intraäbdominal inoculation of relatively large doses suspended in mucin. The situation has been well summarized by Thomas and Bayne-Jones[1] and more recently by Thomas,[2] who attach considerable importance to the problem.

There appear to have been few efforts to study gonococcus inoculations in larger domestic animals. Stockmayer and Schmitz[3] inoculated young heifer calves intravaginally with fresh pus from cases of gonorrhea. Six of the 11 animals used developed mild vaginal reactions, but no intracellular cocci could be demonstrated by smears, no positive cultures were obtained and no positive complement-fixation reactions developed. In this connection it may be mentioned that many heifers will develop a mild non-specific discharge following intravaginal manipulation.

The data offered here summarize a series of attempts to infect 5 species of domestic animals by the inoculation of a pure culture of *Neisseria gonorrheæ* intravaginally or into the conjunctival sac.

Experimental. Three heifers, 5 female goats, 4 ewes, 2 sows, 1 mare, 2 bull calves, and 1 boar were utilized as experimental animals. The culture of gonococci used was derived from a case of acute pelvic inflammatory disease and had been maintained for one

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1 Thomas, R. B., and Bayne-Jones, S., *Am. J. Syph., Gonor., and Ven. Dis.*, **20**, Supplement, January, 1936.

2 Thomas, R. B., *The Gonococcus and Gonococcal Infections*, Supplement No. 8 to Venereal Disease Information, United States Public Health Service, 1939.

3 Stockmayer, W., and Schmitz, J., *Z. f. Immunitätsforsch. u. exp. Therap.*, 1935, **84**, 371.

month on chocolate-agar slants (Douglas agar + sheep's blood). Inoculations were made by saturating sterile swabs with a freshly prepared saline suspension of the organisms and inserting the swabs deep into the vaginas of the female animals or beneath one lower eyelid. The swabs were purposely applied vigorously and extensively to the mucous membranes. After the animals were inoculated the saline suspensions were cultured on chocolate-agar plates and all developed heavy growths of gonococci.

Cultures from the inoculated areas were made with sterile swabs every 2 to 3 days. Vaginal cultures from the larger animals were taken through glass or metal specula. Conjunctival cultures were secured by pulling down the lower eyelid and sweeping the swab the full extent of the sac.

The swabs were dropped into 1 cc of sterile broth which was protected from chilling and inoculated within one hour on freshly poured chocolate-agar plates. These were incubated 48 hours at 35-36°C under 10 to 12% CO_2. The plates were then examined for colonies morphologically suggestive of gonococci which gave a positive "oxydase" reaction. Suspicious colonies were isolated in pure culture for study.

Results. No gonococci were recovered from any of the inoculated animals. Many of the vaginal cultures from sows were badly overgrown with fecal "spreader" organisms. The sheep all developed bilateral purulent conjunctivitis associated with oxydase-positive organisms, but these bacteria formed more opaque colonies than gonococci and fermented all 4 test sugars. These animals had hair closely matted about the eyes and it is likely that the irritation produced by the hair and repeated swabbing produced the conjunctivitis. A few of the heifers developed a scant vaginal discharge, but no gonococci were recovered and the irritation probably resulted from the repeated insertions of the speculum.

Each animal was observed for 6 to 24 days after inoculation, during which interval from 4 to 7 cultures were made, except that one ewe was subjected to only one vaginal and to three conjunctival cultures. No smears were made since our experience[4] has paralleled that of others in that properly prepared cultures are superior. The culture-technics outlined by Carpenter[5] were used.

Summary. Vaginal and conjunctival inoculations of a pure culture of *Neisseria gonorrheæ* in cattle, sheep, swine, goats and the

[4] Tucker, W. W., Trussell, R. E., and Plass, E. D., *Am. J. Obst. and Gynec.*, 1939, **38**, 1055.

[5] Carpenter, C. M., *Am. Pub. Health Assn. Year Book*, p. 125, March, 1937.

horse have failed to produce any infection demonstrable by repeated cultures.

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12058

Passage of the Blue Dye T-1824 from the Blood Stream into the Lymph.*

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The fate of intravenously injected Brilliant Vital Red has been discussed by H. P. Smith with special reference to its use in blood volume determinations.[1, 2] The following observations on the blue dye T-1824 are reported at this time because of the interest in blood volume and shock which has resulted from the present preoccupation with problems of military importance. Our purpose is to record the fact that the blue dye T-1824 passes from the blood into the lymph and to discuss the bearing which this observation has upon estimations of plasma volume by the Blue Dye Method.[3]

Method. Normal dogs were fasted overnight, anesthetized with morphine and nembutal, and the thoracic and cervical lymphatic ducts cannulated following the method of White, Field, and Drinker.[4] Samples of venous blood and of thoracic and cervical lymph were collected to serve as blanks for the subsequent dye determinations. One cc of a 1% solution of the blue dye T-1824 was then injected into the external jugular vein. In each instance solutions of sodium thiocyanate and in 2 instances solutions of horse and rabbit globulins specific for type III pneumococcus were also injected. Observa-

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1 Smith, H. P., *Bull. Johns Hopkins Hosp.*, 1925, **36**, 325.

2 Smith, H. P., *J. Exp. Med.*, 1930, **51**, 379.

3 Gregersen, M. I., Gibson, J. G., 2d, and Stead, E. A., *Am. J. Physiol.*, 1935, **113**, 54.

4 White, J. C., Field, M. E., and Drinker, C. K., *Am. J. Physiol.*, 1933, **103**, 34.