

## THE AETIOLOGY OF RAM EPIDIDYMITIS

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### ABSTRACT

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A wide variety of organisms from the environment invade the preputial cavity of rams. Various of these organisms can be isolated from the deeper parts of the male genital tract, and especially from the accessory glands.

Some of the bacteria present in the sheath can be stimulated to migrate to the deeper parts of the genital tract by injections into the host animal of luteinizing-hormone-releasing-hormone and injections of pregnant mare serum gonadotrophin.

The increased levels of luteinizing hormone and follicle-stimulating hormone in surgically prepared cryptorchids also stimulate migration of the organisms.

The natural mode of development of genital infections in rams is formulated as follows: The preputial cavity of a ram becomes invaded by various organisms through contact with the environment. When, under the influence of systemic hormonal stimulation, the genitalia undergo development, suitable conditions are created for the migration of some of the bacteria in the sheath to the deeper-lying organs of the genital tract such as the vesiculae seminales, epididymides and testes. In these organs the bacteria can possibly initiate a pathological process.

### Résumé

#### L'AETIOLOGIE DE L'ÉPIDIDYMITITE DU BÉLIER

Une grande variété d'organismes de l'environnement envahissent la cavité préputiale des béliers. Certains de ces organismes peuvent être isolés des parties les plus profondes du conduit génital mâle et spécialement des glandes accessoires.

Certaines des bactéries présentes dans le fourreau peuvent être stimulées à émigrer vers les parties plus profondes du conduit génital par des injections d'hormones à la production d'hormones lutéinisantes à l'animal hôte et d'injections de serum gonadotrophine de la jument gestante.

Des niveaux accrus d'hormone lutéinisante et d'hormone folliculostimulante chez les cryptorchides chirurgicalement préparés stimulent également la migration des organismes.

La manière naturelle de développement des infections génitales chez les béliers est formulée comme suit: la cavité préputiale d'un bélier est envahie par des organismes variés par contact avec le milieu environnant. Quand, sous l'influence de la stimulation hormonale systémique, les genitalia se développent, des conditions favorables sont créées pour la migration de certaines de ces bactéries dans le fourreau jusqu'aux organes situés à un niveau plus profond du conduit génital, telle que la vésicule séminale, les epididymes et les testicules. Dans ces organes, les bactéries peuvent également initier un développement pathologique.

### INTRODUCTION

The best-known organism specifically responsible for epididymitis in rams is *Brucella ovis*. Since its first description in Australia (Simmons & Hall, 1953) and New Zealand (Buddle & Boyes, 1953), it has been found in several other sheep-breeding countries. No difficulty in transmitting it experimentally was experienced by any of the research workers who investigated its pathogenicity. Buddle & Boyes (1953), for instance, injected doses of  $100 \times 10^6$  and  $1000 \times 10^6$  organisms into ram hoggets by the intravenous, intratesticular and intrapreputial route and the animals later excreted the organisms in their semen. Buddle (1955) stated that rams are highly susceptible to experimental infection by intravenous, subcutaneous and intratesticular inoculations as well as by the oral route, and also by the application of infected material to mucous surfaces such as the conjunctiva and prepuce. He also showed that transmission of infection from infected to non-infected rams occurs readily when they are run together in isolation from ewes.

*Brucella ovis* has always been associated with diseases of the reproductive tract, with the exception of one recorded instance of pneumonia (Jebson, Hartley, McClure & McFarlane, 1955). In the light of this and since genital involvement can be accomplished by any number of non-venereal routes, a bacteraemic phase of the infection must be assumed before the bacteria finally settle in their predilection site, the reproductive tract.

Several bacteria other than *B. ovis* and different from it in many respects have also been described from lesions of the testes and epididymides of rams.

Jamieson & Soltys (1947) reported the occurrence of 10 cases of orchitis and epididymitis caused by *Pasteurella pseudotuberculosis* in one flock of sheep in Britain. Ekdahl, Money & Martin (1968) isolated a number of different organisms from the testes and epididymides of rams in New Zealand. The following bacteria are listed in their publication: *Brucella ovis*; Gram-negative pleomorph; *Actinobacillus*-like organisms; *Corynebacterium pyogenes*; *Corynebacterium pseudotuberculosis*; *Streptococcus* spp.; *Staphylococcus* spp.; *Pasteurella haemolytica*; *Pasteurella multocida*; *Pasteurella pseudotuberculosis*; *Bacteroides*; *Brucella abortus*; *Brucella abortus* (S19).

Baynes & Simmons (1960) isolated *Actinobacillus seminis* from the epididymides of 3 affected rams in Australia. *Histophilus ovis* was isolated from the testes of a ram by Claxton & Everett (1966).

Worthington & Bosman (1968) first reported the isolation of *Actinobacillus seminis* in South Africa. Subsequently, Van Tonder (1977) made many isolations of similar organisms and classified them into 2 groups, one group conforming to the description of *A. seminis* and the other group to the description of *H. ovis*.

Various organisms have been associated with epididymitis in rams from Idaho and Eastern Oregon. De Long, Waldhalm & Hall (1979) reported the following isolates: *Actinobacillus actinomycetem-comitans*; *Brucella ovis*; *Corynebacterium pyogenes*; *C. pseudotuberculosis*; *Pseudomonas maltophilia*.

As a group all these organisms differ from *B. ovis* in so far as they commonly cause pathological lesions in various sites in the body apart from the genitalia, and also affect species other than sheep. Watt, Bamford & Nairn (1970) reported the death of 25 out of 280 six-week-old lambs within 4 days after dipping. They had been castrated 2 weeks previously and showed a purulent polyarthritis before death. In another flock 4 uncastrated lambs died of purulent polyarthritis and severe myocarditis. Pure cultures of *A. seminis* were isolated from all these pathological lesions. The same organism was isolated from a ram with acute posthitis. Cultures of *A. seminis* injected into the teat canal of lactating ewes produced gangrenous mastitis.

A gram-negative pleomorphic organism has not infrequently been isolated in New Zealand from cases of polytenosynovitis in lambs (Kater, Marshall & Hartley, 1962). Similar bacteria have been recovered in Australia from cases of mastitis in ewes (Roberts, 1956).

Goss, Gutin & Dickhaus (1967) reported the isolation of *Actinobacillus actinomycetem-comitans* from endocarditis lesions in humans.

Organisms such as *C. pyogenes* and *C. pseudotuberculosis* are generally known as the cause of pyogenic conditions anywhere in the body of sheep, while *P. haemolytica* and *P. multocida* can be the cause of pneumonia and septicæmia.

Apart from some useful pointers to be found in some publications, there is a complete lack of definite information on how the above-mentioned organisms reach the sites in the male genital tract where they cause the reported lesions. Jamieson & Soltys (1947) stated that there was no evidence of tick bites or abrasion of the skin in the rams with epididymo-orchitis due to *P. pseudotuberculosis* examined by them. Ekdahl *et al.* (1968) found *Actinobacillus*-like organisms in the semen of several rams but not from any epididymal or testicular lesions found at autopsy. This might indicate that the lesions had been caused by organisms other than the *Actinobacillus*-like organisms and that the *Actinobacillus* organisms were limited to the prepuce or accessory glands without having reached the epididymides or testes.

Although attempts at causing orchitis and epididymitis by injecting cultures of *A. seminis* or infected semen into the testes or epididymides of rams have been successful in the hands of several workers (Van Tonder, 1977; Baynes & Simmons, 1960), this method can hardly be regarded as a means of imitating natural transmission. Van Tonder (1977) exposed 8-month-old rams to massive infection by the oral, rectal, conjunctival, preputial, nasal, intravenous and subcutaneous routes. Most of his animals excreted *A. seminis* in their semen at some time or another, but he states firmly that those experiments failed to establish the natural mode of transmission of *A. seminis*.

In assessing the assembled data with the object of arriving at a common predisposing factor for the localization of the variety of organisms in the genital tracts of rams, the following points seem relevant: Firstly, the preputial cavity of a ram is in direct contact with infected bedding and soil through the preputial orifice when the animal lies down. Secondly, it seems rational to conclude that, for such a diversity of organisms with the ability to set up lesions anywhere in the body without preference and to gain access to the accessory glands, epididymides and testes, a common, direct, non-haematogenous route must

exist. Thirdly, the development of the genital tract of a ram (which is under hormonal control) is aimed at providing a suitable environment for spermatogenesis and the survival of spermatozoa. The resulting milieu could possibly be suitable for the multiplication of bacteria and even their spread from the preputial cavity into the rest of the genital tract.

The sexual activity and development of the genitalia of a ram are dependent on the concentration of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) liberated by the pituitary gland. The pituitary in turn is stimulated to produce these hormones by the LH- and FSH-releasing hormone (LH-RH) which is formed in the hypothalamus.

Androgens are secreted by the Leydig cells in response to stimulation by LH. Although LH probably plays a dominant role, according to Cole & Cupps (1977) there is increasing evidence that FSH and prolactin also stimulate testicular steroidogenesis.

FSH localizes primarily in the Sertoli cells within the seminiferous tubules of the testes and is necessary for the final steps of spermatid maturation. FSH also stimulates the Sertoli cells to produce and secrete an androgen-binding protein (ABP) which increases the binding and accumulation of androgens within the seminiferous tubules. ABP might serve to concentrate androgens for the androgen-dependent germinal epithelium and also increase the amount of androgen transported within testicular fluid to the androgen-dependent caput epididymidis (Cole & Cupps, 1977).

The function of the accessory glands in the ram, namely, the Cowper's glands, prostate, seminal vesicles and the ampullae of the ductus deferens is dependent on the secretion of testosterone by the testes. Growth and function of the penis and epididymis are also dependent on testosterone. Testosterone acts via the seminal vesicles and prostate to increase the seminal fluid and its fructose, citrate and acidic phosphatase content and it has a trophic influence on the tubules of the testes.

There is therefore no doubt that hormones have a profound effect on the physical and physiological state of the genitalia of male animals. In support of this concept Moore & Bedford (1979) showed that in the rat the spermatozoa are dependent on the milieu of the normal epididymis for their maturation and survival and die within a few days after androgen support of the epididymal epithelium is withdrawn by removal of the testicles only. The principal epithelial cells of the caput and cauda epididymidis showed greater endocytosis, disappearance of the vesicles from the cell apex, reduction in rough endoplasmic reticulum, a drop in the volume of the Golgi's cisternae and an increase in lysosome content, changes indicative of inhibition of secretory function.

In the light of notions expressed thus far the following hypothesis seems worthy of experimental assessment: various bacteria from the environment in which rams live enter the preputial cavity while, for instance, the animals are lying down. Those that find the environment of the preputial cavity favourable, survive and multiply in it for a while. In rams experiencing hormonal stimulation of the genital tract, some of the bacterial species are able to migrate in a retrograde direction to the flow of the semen. Thus they can reach the accessory glands and afterwards the epididymides and testes. In this way organisms which have no specific predilection for the accessory glands, epididymides and testes can initiate a pathological process in these sites.

## MATERIALS AND METHODS

For routine cultivation of bacteria in this study tryptose-agar medium\* containing 10% horse blood was used. The cultures were incubated in a 10% CO<sub>2</sub> atmosphere for 48 h at 37 °C.

Rams for experimental purposes were obtained from flocks reputedly free of epididymitis. Their semen was obtained by electro-ejaculation and examined culturally for freedom from infection.

*Bacteriological examination of genital organs*

Rams clinically or culturally identified as infected were submitted for investigation from flocks from the whole of the Republic of South Africa. They were killed as soon after arrival as possible, their entire urinogenital system was removed and transferred to the laboratory for detailed cultural examination. Sheath washings were prepared by searing the preputial orifice with a hot spatula, introducing 2 ml of Hank's balanced salt solution (Kruse & Patterson, 1973) into the cavity by means of a sterile pipette, withdrawing the solution and preparing cultures from it.

Cultures were prepared from the urethra by dissecting out the penis, introducing Hank's solution into the urethra by means of a small syringe at about the level of the sigmoid flexure, withdrawing the solution and culturing it.

Cultures were prepared from the prostate, vesiculae seminales, ampullae, epididymides and testes by searing the surface of these organs with a hot spatula, incising the seared surface with a sterile scalpel and then introducing a sterile bacteriological loop into the tissue.

Sheath washings were prepared from live rams in a manner similar to the above except that the preputial opening was cleaned with alcohol and 5 ml of Hank's solution was used.

*Experimental infection of rams*

Artificial infection of rams was performed by injecting 2,0 ml of a culture suspension containing about  $2,3 \times 10^9$  organisms per ml into the urethra through a cat catheter introduced after amputation of the processus urethrae.

In the first experiment eight 2-year-old Dorper rams were kept on a spacious cement floor which was cleaned daily and provided with shelter against inclement weather. They were subdivided into 4 groups of 2 each and the different groups received the following treatment before being infected with *A. seminis* strain 0781\*\*.

Group A—no treatment before infection.

Group B—each ram received 0,5 ml Receptal\*\*\*/day intramuscularly for 5 consecutive days. This dose amounted to 2,1 µg Buserelin acetate, a synthetic LH-RH agonist, per day.

Group C—each ram received a daily intramuscular injection of 20 IU pregnant mare serum gonadotrophin (PMSG)\*\*\*\* per day for 5 consecutive days.

Group D—each ram received 2 intramuscular injections of 1,0 ml of Depo-testosterone\*\*\*\*\* containing 100 mg of testosterone cypionate at an interval of 7 days.

\* Biolab Chemicals, 4 Bernard St., Colbyn, Pretoria 0083

\*\* Obtained from Dr E. M. van Tonder, Veterinary Investigation Centre, Grootfontein

\*\*\* Hoechst, Frankfurt am Main

\*\*\*\* Prepared and standardized in own laboratory

\*\*\*\*\* Upjohn (Pty) Ltd, 44 Monteer Rd., Isando, Transvaal

The rams in groups B, C and D were infected 72 h after the last injection. Thirty-one days after infection all the rams were killed and the component parts of their genitalia examined for the presence of the organism in the manner described above.

In the second experiment twelve 1-year-old meat Merinos were kept in a cement-floored camp, 7 × 15 m, with the necessary protection against the sun and weather. The floor was not cleaned more than once/week. After they had been in the camp for 2 weeks a sheath washing was done on them and the washing solutions plated on culture media. After they had been in the camp for a month, they were subdivided and given the injections indicated below. The injections were given intramuscularly for 3 consecutive days; then the injections were stopped for 2 days and subsequently the injections were repeated daily for 3 more days. There was no particular reason for this schedule except that it suited our laboratory routine.

Group A: Two rams to serve as controls.

Group B: Two rams: 0,5 ml Receptal.

Group C: Two rams 1,0 ml Receptal.

Group D: Two rams 50 IU PMSG.

Group E: Two rams 150 IU PMSG.

One month after the beginning of the course of injections the rams were killed and the different parts of their genitalia subjected to a detailed bacteriological examination.

Since Shanbacher & Ford (1977) had shown that surgically-prepared cryptorchid rams showed increased levels of LH and FSH, the scrotal sacs of four 8-month-old Merino rams were amputated so that their testes were firmly positioned against the abdominal wall. They were kept under the same conditions as the rams in the 2nd experiment together with 2 controls. All the rams harboured a variety of contaminants in their sheaths.

After 3 months they were killed and the different sections of their genital tracts examined bacteriologically.

On account of the large variety of organisms recovered on culture in these studies, not all isolates were fully identified. Complete identification would have been unjustifiably laborious and expensive. Sufficient isolates were, however, identified to illustrate the points made in the text.

## RESULTS

*Results obtained with rams from outside flocks*

The results of the bacteriological examination of the subsections of the genital system of the affected rams submitted from different flocks are summarized in Table 1. Where more than one ram harboured the same organism(s) in the same organs, the results of only one were recorded.

In none of the rams examined could any of the bacteria isolated from their genitalia be seen to cause lesions in any other part of their bodies.

The entry "various organisms" signifies some of the members of a large group of contaminants. The following are only some of the representatives: *Streptobacillus* sp., *Micrococcus varians*, *Flavobacterium* sp., *Lactobacillus* sp., *Alcaligenes* sp., *Pseudomonas* sp., *Streptococcus bovis*, various Enterobacteriae, *Staphylococcus epidermidis*. It would have served little purpose to identify all the different isolates from all the animals examined.

THE AETIOLOGY OF RAM EPIDIDYMITIS

TABLE 1 The distribution of bacteria in the genital tracts of naturally infected rams

| Ram No. | Organ                          |                   |                   |   |                   |   |                  |                 |
|---------|--------------------------------|-------------------|-------------------|---|-------------------|---|------------------|-----------------|
|         | Sheath                         | Urethra           | Prostate          | Vesiculae sem.  | Ampulla           | Cauda epidid.   | Caput epidid.    | Testes          |
| 1       | <i>Pasteurella haemolytica</i> |                   |                   |   |                   |   |                  |                 |
| 2       | <i>P. haem.</i>                | <i>P. haem.</i>   |                   |   |                   |   |                  |                 |
| 3       | <i>P. haem.</i>                | <i>P. haem.</i>   | <i>P. haem.</i>   |   |                   |   |                  |                 |
| 4       | <i>P. haem.</i>                | <i>P. haem.</i>   |                   | <i>P. haem.</i>   |                   |   |                  |                 |
| 5       | <i>P. haem.</i>                | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>   |                  |                 |
| 6       | <i>P. haem.</i>                | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>  | <i>P. haem.</i> |
| 7       | <i>Staphylococcus</i> sp.      | <i>Staph.</i> sp. | <i>Staph.</i> sp. | <i>Staph.</i> sp.   | <i>Staph.</i> sp. | <i>Staph.</i> sp.   |                  |                 |
| 8       | Various organisms              |                   |                   |   |                   | <i>Corynebacterium ovis</i><br><i>C. paurometabolicum</i> |                  |                 |
| 9       | Various organisms              |                   |                   | <i>C. pseudo.</i><br><i>Micrococcus luteus</i><br><i>M. varians</i> |                   | <i>M. luteus</i>  | <i>M. luteus</i> |                 |
| 10      | Various organisms              | Various organisms | Various organisms | Various organisms   | Various organisms |   |                  |                 |

The results in Table 1 show that in all the animals the sheath was infected.

The results obtained in Rams 1-6 suggest that there is a stepwise progress of the infectious agent from the sheath to the testes.

Ram 7 shows that a *Staphylococcus* sp. can infect the whole series of organs from the sheath to the cauda epididymidis.

The results obtained from Rams 8, 9 and 10 show that not only does a variety of organisms infect the preputial cavity, but several of these can migrate to the more remote organs such as the cauda epididymidis, ampullae and the vesiculae seminales.

*Pasteurella haemolytica* was definitely the most common organism isolated. It was found that some of the *P. haemolytica* strains isolated were subject to variation with respect to their ability to produce acid from certain sugars. The classical examples of *P. haemolytica* produce acid from glucose and maltose.

From the strains obtained from the rams, single cell-derived colonies could be isolated that did not metabolize glucose and maltose and did not show haemolysis. These isolates resembled *A. seminis* in all respects.

*Results obtained with experimentally infected rams*

The results of the first experiment mentioned under Materials and Methods are recorded in Table 2. Since these animals were infected by introducing the organisms into the tip of the urethra, account was taken only of the spreading of the organisms further into the genital tract.

From the results in Table 2 it can be seen that only in the rams treated with LH-RH did the organisms spread beyond the point where they were originally deposited.

*Results obtained with rams treated with drugs without artificial infection*

The results of the second experiment are recorded in Table 3.

TABLE 2 The distribution of *Actinobacillus seminis* in the genital tracts of the rams in Groups A-D

| Group | Treatment            | Ram No. | Prostate | Ves. sem. | Ampulla | Cauda epidid. | Caput epidid. |
|-------|----------------------|---------|----------|-----------|---------|---------------|---------------|
| A     | Nil.....             | 1       | —        | —         | —       | —             | —             |
|       |                      | 2       | —        | —         | —       | —             | —             |
| B     | LH-RH.....           | 1       | +        | +         | +       | +             | —             |
|       |                      | 2       | +        | +         | +       | —             | —             |
| C     | PMSG.....            | 1       | —        | —         | —       | —             | —             |
|       |                      | 2       | —        | —         | —       | —             | —             |
| D     | Depo-testosterone... | 1       | —        | —         | —       | —             | —             |
|       |                      | 2       | —        | —         | —       | —             | —             |

TABLE 3 Bacteriological findings in rams kept on an irregularly cleaned floor and injected as indicated

| Ram No. | Injection       | Sheath  | Urethra  | Prostate  | Ves. sem.   | Ampulla   | Cauda epidid. |
|---------|-----------------|---|--|---|---|---|---------------|
| 1.....  | 0,5 ml Receptal | Various organisms<br><i>Pasteurella haemolytica</i> | <i>P. haem.</i>  |   |   | <i>P. haem.</i>   |               |
| 2.....  | 0,5 ml Receptal | Various organisms<br><i>P. multocida</i>            | <i>P. multo.</i>   |   | <i>P. multo.</i>  |   |               |
| 3.....  | 1,0 ml Receptal | Various organisms                                   |  |   |   |   |               |
| 4.....  | 1,0 ml Receptal | Various organisms                                   | <i>P. haem.</i><br><i>Acinetobactor</i> sp.                        |   |   |   |               |
| 5.....  | 50 IU PMSG...   | Various organisms                                   | <i>P. haem.</i>  | <i>P. haem.</i>   | <i>P. haem.</i>   | <i>P. haem.</i>   |               |
| 6.....  | 50 IU PMSG...   | Various organisms                                   | <i>C. pseudo.</i><br><i>C. pyogenes</i><br><i>Franciscella</i> sp. | <i>C. pseudo.</i><br><i>C. pyogenes</i><br><i>Fr. sp.</i> | <i>C. pseudo.</i><br><i>C. pyogenes</i><br><i>Fr. sp.</i> | <i>C. pseudo.</i><br><i>C. pyogenes</i><br><i>Fr. sp.</i> |               |
| 7.....  | 50 IU PMSG...   | Various organisms                                   | <i>P. haem.</i><br><i>C. pyog.</i>                                 | <i>P. haem.</i><br><i>C. pyog.</i>                        | <i>P. haem.</i><br><i>C. pyog.</i>                        | <i>P. haem.</i><br><i>C. pyog.</i>                        |               |
| 8.....  | 50 IU PMSG...   | Various organisms                                   |  |   |   |   |               |
| 9.....  | 150 IU PMSG..   | Various organisms                                   | Various Organisms  |   | <i>C. pyog.</i><br><i>C. pseudo.</i><br><i>P. haem.</i>   | <i>C. pyog.</i><br><i>C. pseudo.</i><br><i>P. haem.</i>   |               |
| 10..... | 150 IU PMSG..   | Various organisms                                   | Various organisms  |   |   |   |               |
| 11..... | Control.....    | Varous organisms                                    |  |   |   |   |               |
| 12..... | Control.....    | Various organisms                                   |  |   |   |   |               |

TABLE 4 Bacteriological findings in the genital tracts of rams with surgically shortened scrotal sacs

| Ram No.     | Sheath                | Urethra                                   | Ampulla           | Ves. sem.         | Prostate          | Epidid.           |
|-------------|-----------------------|---|-------------------|-------------------|-------------------|-------------------|
| 1.....      | Various organisms.... | <i>Corynebacterium pseudotuberculosis</i> | <i>C. pseudo.</i> | <i>C. pseudo.</i> | <i>C. pseudo.</i> | <i>C. pseudo.</i> |
| 2.....      | Various organisms.... | <i>C. pseudo</i> .....                    | <i>C. pseudo.</i> | <i>C. pseudo.</i> | <i>C. pseudo.</i> |                   |
| 3.....      | Various organisms.... | <i>C. pseudo</i> .....                    | <i>C. pseudo.</i> |                   |                   |                   |
| 4.....      | Various organisms.... | <i>C. pseudo</i> .....                    | <i>C. pseudo.</i> |                   | <i>C. pseudo.</i> |                   |
| Control.... | Various organisms     |   |                   |                   |                   |                   |
| Control.... | Various organisms     |   |                   |                   |                   |                   |

The findings recorded in Table 3 show that in all the rams kept on an irregularly cleaned concrete floor a wide variety of organisms invade the preputial cavity. In one of the rams that received 0,5 ml of Receptal, *P. haemolytica* migrated to the ampulla and in the other *P. multocida* reached the vesiculae seminales. In the 2 rams that received 1,0 ml of Receptal, the organisms hardly migrated—only in one of them did the urethra become infected.

In 3 of the 4 rams that received 50 IU of PMSG the organisms reached the prostate, vesiculae seminales and ampullae, while in the fourth they went no further than the sheath for no apparent reason. In one of the rams that received 150 IU of PMSG the organisms

moved as far as the vesiculae seminales and ampullae and in the others only to the urethra. In the control rams the bacteria did not leave the sheath.

#### Results obtained with rams with surgically shortened scrotal sacs

The results of the experiment conducted on the rams with surgically-shortened scrotal sacs are recorded in Table 4.

In addition, cultures prepared from the contents of the lymphatic vessels at the site where the vessels derived from the epididymitis and testis converge, were positive for *C. pseudotuberculosis* in the 4 experimental rams. This finding proves that the bac-

teria must have been present in either the testes or epididymides or both without having been detected by the method employed, except in Ram 1 where the organism could be recovered from the cauda epididymidis.

## DISCUSSION

A critical review of the results reflected in Table 1 leads one to doubt whether the term "ram epididymitis" is sufficiently descriptive of the condition affecting rams in this country. In many rams the urethra and accessory glands are infected without the presence of bacteria in their epididymides. The presence of bacteria in the accessory glands could account for the frequent finding of infected semen in rams without any clinical lesions in the epididymis.

From Table 1 it is also clear that in all the rams the sheath is infected and in all instances where a deeper part of the genitalia is infected, the same organisms are also present in the parts closer to the opening of the urethra. Furthermore, this table clearly shows that there is a stepwise progress in the infection from the opening of the urethra to the deeper parts. These findings together with the possibility of introducing a specific organism into the urethra and recovering it from the cauda epididymidis subsequently (see Ram 1, Group B, Table 2) leads one to the conclusion that the portal of entry for bacteria causing an infection of the genitalia in the ram is the tip of the urethra.

Above all, Table 1 shows that a multiplicity of bacteria can cause an infection of the different sections of the genital tract.

From the results recorded in Table 2 it is clear that only LH-RH stimulated a migration of the *A. seminis* from the site where it was deposited to the deeper tissues. In Ram 1 of this group the organism actually reached the cauda epididymidis. Both the PMSG and the Depo-testosterone, in the doses used, had no effect on the organism.

In the subsequent experiment, the results of which are given in Table 3, the bacterial flora of the sheath gaining entry through contact with the floor was stimulated to migrate by a dose of Receptal similar to that used in the previous experiment. Also in this experiment a dose of PMSG treble that of the previous experiment stimulated a definite migration of the organisms in 3 rams. This makes sense in so far as the active ingredients of PMSG, viz. LH and FSH, are essentially the same as the products stimulated by LH-RH, the active principle of Receptal.

On the other hand, Table 3 shows that doubling the dose of Receptal did not seem to produce the same stimulus to migration. This depressed response could possibly be the result of overdosing with LH-RH agonist, because Auclair, Kelly, Coy, Schally & Labrie (1977) have shown that the injection of high doses of LH-RH analogue lead to a reduction of the testicular hormone/human chorionic gonadotrophin receptor level of the testes in rats. Consequently, further studies are currently being conducted to determine the optimum dosage level and regimen for both Receptal and PMSG.

The results recorded in Table 4 provide further evidence that stimulation of the genital tract through LH and FSH provide circumstances suitable for the migration of bacteria in the genital tracts of rams. This stimulation, associated with amputation of the scrotal sac, has taken place in spite of the absence of an increased testosterone level in the blood (Schanbacher & Ford, 1977). According to the results

recorded in Table 2 it seems as if a therapeutic dose of testosterone in any case has no effect on the migration of the bacteria in the genital tract.

All the results achieved in the above series of experiments are consistent with the hypothesis formulated in the Introduction. This hypothesis thus provides a tenable explanation for the problem of how the wide variety of organisms reach the deepest parts of the male genital system without experiencing a bacteraemic phase.

Although many aspects of the relationship between the organisms present in the environment of sheep and the genital tracts of rams require to be elucidated, the conclusion seems justified that some organisms colonize the sheath and migrate in the genital tract in a direction against the flow of the semen. These 2 processes are profoundly influenced by the extent to which the genital tract is stimulated by the hormones normally occurring in the body. If this notion is accepted, some of the observations made by veterinarians in the field and practical stockmen can be explained. For instance, it is a common occurrence for the most vigorous and virile young rams showing the most rapid growth rate in a group of rams kept on an intensive system on a high plane of nutrition to develop epididymitis or orchitis. Usually these animals are exposed to faecal and other organic material on the floor which, for practical reasons, cannot be kept absolutely clean. The sheath of any ram living under such conditions must be exposed to a variety of bacteria and it seems reasonable that such bacteria can spread in the genital tract of those rams experiencing the greatest hormonal stimulation. Admittedly, this train of thought leads to many points to be solved by research.

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