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RESEARCH COMMUNICATION

A SURGICAL TECHNIQUE FOR THE EXPERIMENTAL REPRODUCTION OF EPIDIDYMITIS IN RAMS

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ABSTRACT

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A surgical technique is described for introducing a bacterial culture into the vas deferens of a ram close to the epididymis in such a manner that the infective material spreads to the lumen of the ductus epididymidis.

Résumé

UNE TECHNIQUE CHIRURGICALE POUR LA REPRODUCTION EXPÉRIMENTALE D'UNE ÉPIDIDYMITE CHEZ LE BÉLIER

Une technique chirurgicale pour l'introduction d'une culture de bactéries dans le vas diferens d'un bélier à proximité de l'épididyme, de telle manière que le matériel infectieux se propage dans le lumen du ductus épididymis, est décrite.

INTRODUCTION

An investigation into the aetiology and pathogenesis of ram epididymitis calls for the reproduction of the condition by a method which resembles the natural evolution of the disease. This implies that the infectious agent has to be introduced into the lumen of the ductus epididymidis to conform to the pathogenesis of epididymitis in nature as explained by Jansen (1980). He showed that bacteria from the environment entering the preputial cavity migrate up the genital canal in a direction retrograde to the flow of the semen, ultimately to land in the lumen of the ductus epididymidis. At this site they can initiate a pathological process.

Although attempts at causing orchitis and epididymitis by injecting cultures of *Actinobacillus seminis* or infected semen into the testes or epididymides of rams have been successful in causing an inflammation of these organs (Baynes & Simmons, 1960; Van Tonder, 1977), this method can hardly be regarded as a means of imitating the natural route of infection. The organisms would be deposited by this method in the interstitial tissue rather than in the lumen of the tubuli.

In the present study the author aimed at introducing bacterial cultures into the vas deferens in such a way that the organisms would reach the lumen of the ductus epididymidis.

MATERIALS AND METHODS

Rams with clinically normal genitalia as determined by inspection and palpation were selected. Furthermore, semen specimens were obtained by electro-ejaculation and only animals yielding semen from which no bacteria could be isolated were used in the experiments.

After 20 h without food and water, the rams were anaesthetized by a slow intravenous injection of a freshly prepared 10% solution of chloral hydrate until all palpebral reflexes disappeared. Then the scrotal sac was shaved and thoroughly cleaned with 70% ethyl alcohol.

The ram was placed on its back on the operating table with its head in a lower position than its body and with its mouth pointing downwards to avoid the inhalation of rumen fluid if it happened to be regurgitated.

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With the application of standard surgical techniques the posterior aspect of the scrotal sac was opened by a vertical incision on the midline at the level of the caput epididymidis. Subsequently, the tunica vaginalis of the left testis was reached by blunt dissection and opened by a vertical incision over the vas deferens which lies anterior to the body of the epididymis on the medial aspect of the testis. The vas deferens, which can be felt as a string-like structure, together with its blood-vessels, is contained in a fold. The identification of the vas deferens can be further facilitated if one closes it gently with one's fingers at the body end of the incision and has an assistant massage the semen from the cauda epididymidis into the lumen of the vas deferens.

The left vas deferens was selected to enable a right-handed surgeon to grasp the free end of the fold containing the vas deferens between the thumb and index finger of his left hand while the injection is done with the right hand. The right epididymis consequently served as a control.

A No. 26 Luer-Lock hypodermic needle attached to a 1 mℓ tuberculin syringe containing the infective material was gently introduced into the lumen of the vas deferens as close to the cauda epididymidis as possible. A volume of 0,1 mℓ of culture suspension containing about 1×10^3 organisms per mℓ was injected in a direction against the natural flow of the sperm cells.

To prevent leakage of the contents of the vas deferens through the puncture caused by the injection, a small area around the point where the needle was introduced was lightly cauterized with an electrocautery apparatus immediately after withdrawal of the needle and before release of the hold on the vas deferens.

The incision in the tunica vaginalis was then closed with interrupted stitches. The cavity left in the subcutaneous tissue between the testes was subsequently eliminated by appropriate stitching, and finally the skin was closed with interrupted stitches.

To establish whether a substance in suspension injected into the vas deferens at the site described does in fact spread to the lumen of the tubuli epididymidis, 0,1 mℓ of India ink was injected into the vas deferens of one of the rams. After 24 h the ram was killed and its epididymis examined histologically for the presence of carbon particles in the lumen of its tubules.

A culture of *Pasteurella haemolytica* was injected into the vas deferens of another ram which was killed 8 days afterwards. Cultures were prepared from its epididymides on blood-tryptose-agar and incubated in 10% CO_2 for 48 h at 37 °C. Histological sections were also prepared from the epididymides and stained with haematoxylin-eosin.

Results

The operation as described in Materials and Methods was carried out successfully on a number of rams using various bacterial species. As an example of the results obtained, the following is presented:

The histological sections of the epididymis of the ram injected with India ink showed the distribution of carbon particles among the spermatozoa in the lumen of the tubuli epididymidis, as can be seen in Fig. 1 & 2.

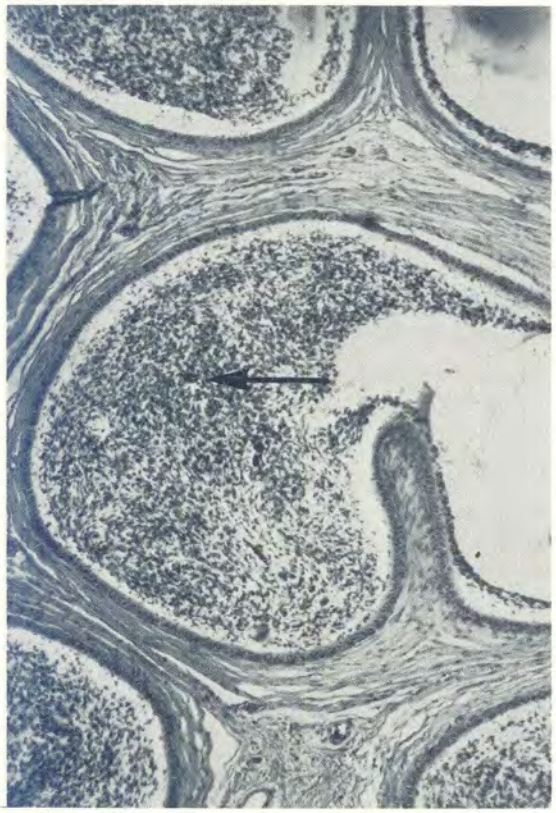

FIG. 1 The presence of carbon particle accumulations in the lumen of the epididymal tubule: ×120

P. haemolytica was isolated from the left epididymis of the ram injected with the culture but not from its right counterpart. In the histological sections from the left epididymis accumulations of polymorphonuclear leukocytes could be seen under the basal membrane of the epithelium of the ductus epididymidis. Lymphocytes and leukocytes also infiltrated the epithelium itself and were also present inside the lumen of the tubules. These changes can be seen in Fig. 3. The right epididymis showed nothing abnormal.

The operation wounds healed without any complications and no pathological changes were evident outside the vas deferens.

FIG. 2 Carbon particles among spermatozoa in lumen of epididymal tubule: ×500

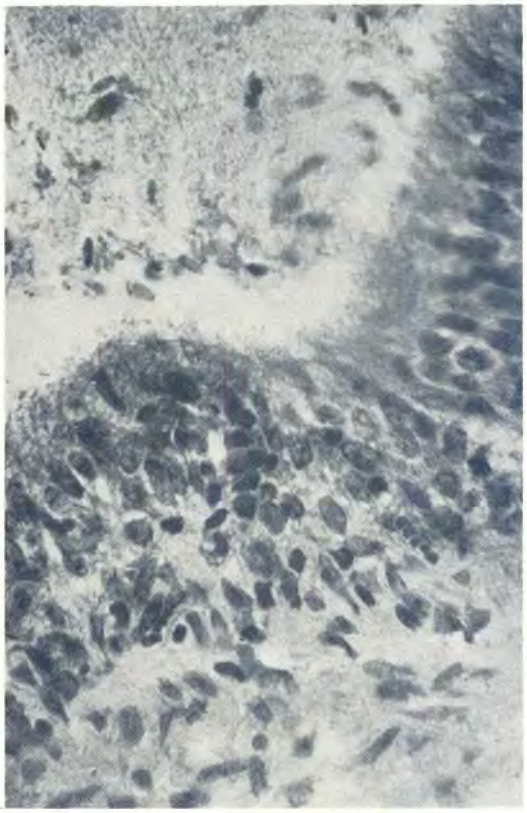

FIG. 3 Polymorphonuclear neutrophils and lymphocytes in the epithelium of the tubule of the epididymis. Neutrophils and lymphocytes in the lumen of the tubule: ×500

Discussion

The results obtained with the injection of India ink and the culture of *P. haemolytica* leave no doubt that a surgical technique has been applied for introducing a bacterial culture into the lumen of the tubuli epididymidis. This technique affords an opportunity for studying both the pathogenicity of various bacterial species for the epididymal tissue and the pathogenesis of ram epididymitis.

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