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Acute Experimental *E. Coli* Epididymitis in the Rat and Its Consequences on Spermatogenesis

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Summary. The injection of *E. Coli* into the tail of the epididymis in the rat caused epididymitis. In 20% of the cases, the inflammation is spread through the lymphatic system to the testis. The germinal cells are destroyed in 50% of the cases by the second day, and are not reestablished for several months. Signs of testicular inflammation are not found. The destruction seems to be caused by *E. Coli* toxins, as shown by injections of killed bacteria.

Key words: *E. coli,* Epididymis, Epididymitis, Dymphatic system, Testicular inflammation.

Introduction

Until 1970, in more than 50% of all cases, inflammation of the epididymis was considered as idiopathic in origin. When a bacteria was identified, it was most often *E. Coli.* Recently Berger et al. [1], studying 50 cases of acute epididymitis in detail, found an aetiology in 80% of the cases. Bacteriological examination of urine, urethral smears and epididymal aspirations were carried out. In men over 35 years of age, *E. Coli* was found to be the bacteria most frequently responsible, whereas *Chlamydia trachomatis* was the principal cause implicated in men less than 35 years old.

When properly treated, acute epidymitis has a rapidly favourable evolution locally. The long-term consequences on fertility are less certain. It is now known that one of the complications of epididymitis, particularly when it occurs in the tail, is obstruction of the epididymal duct. This must have an effect on fecundity.

Another possible explanation of post-epididymitis fertility problems is attributed to the presence of testicular lesions following acute epididymitis, although, no direct anatomical relation has, as yet, been found between the epididymis and testis [3]. A number of authors have attempted to demonstrate these testicular lesions which occur without obvious clinical signs: by testicular function at the time of, and after the acute phase of epididymitis [4]: by a study of the spermogram [6] and by surgical biopsy [7].

In order to study these lesions more precisely and their repercussions on the male reproductive organs, an animal model that closely resembles acute epididymitis in man was developed and is discussed in the present article.

Materials and Methods

Experiments were carried out on white Wistar rats weighing 150 grams. General anaesthesia was obtained with chloroform, after which the right testicle and epididymis were exposed by a low inguinal incision. A total of 0.2 ml of a bacterial solution was injected into the tail of the right epididymis with the aid of an intradermal needle. The bacterial solutions injected were of decreasing dilutions (1/1, 1/10, 1/100, 1/1000) in a culture medium containing 500,000 germs/0.1 ml. Injections of equal amounts of sterile saline solution, sterile culture medium and solutions of killed bacteria were also made for comparison. Rats were sacrificed every day during the first week of the experiment, then every week for the following month, and finally every other week until 4 1/2 months. The epididymis and testicle from both the treated and untreated side (considered as control) were removed and fixed in Bouin's solution. Five micron sections were made and stained with Hematoxylin, Eosin and Masson's trichromal stain.

A preliminary study was first carried out with a variety of different germs. Equivalent solutions (0.1 ml containing a total of approximately 500,000 germs) of *E. Coli,* non-hemolytic Staphylococcus aureus and Streptococcus veridans were injected into the tail of the epididymis of eight rats. Half of the rats were sacrificed on the 8th day and the rest on the 15th day. *E. Coli* was found to be the most pathogenic germ for the epididymis and the testicle. For this reason it was chosen for the continuation of this study.

Results

The results are summarised in Table 1. The effect of the experimental surgical method is demonstrated by the results of those animals injected with sterile saline solution or culture medium (13 cases). No lesions, neither testicular nor epididymal were found.

Table 1

	Cases	Epididymitis	Testicular Inflammation	Non-Inflammatory Testicular Lesions
Saline Solution + Culture medium	13	0	0	0
Staphylococcus aureus	8	8	0	5
Streptococcus viridans	4	3	0	3
Escherichia coli				
1/1	10	9	2	7
1/10	14	9	2	7
1/100	26	23	3	14
1/1000	12	9	3	0
Killed escherichia coli	7	0	0	3

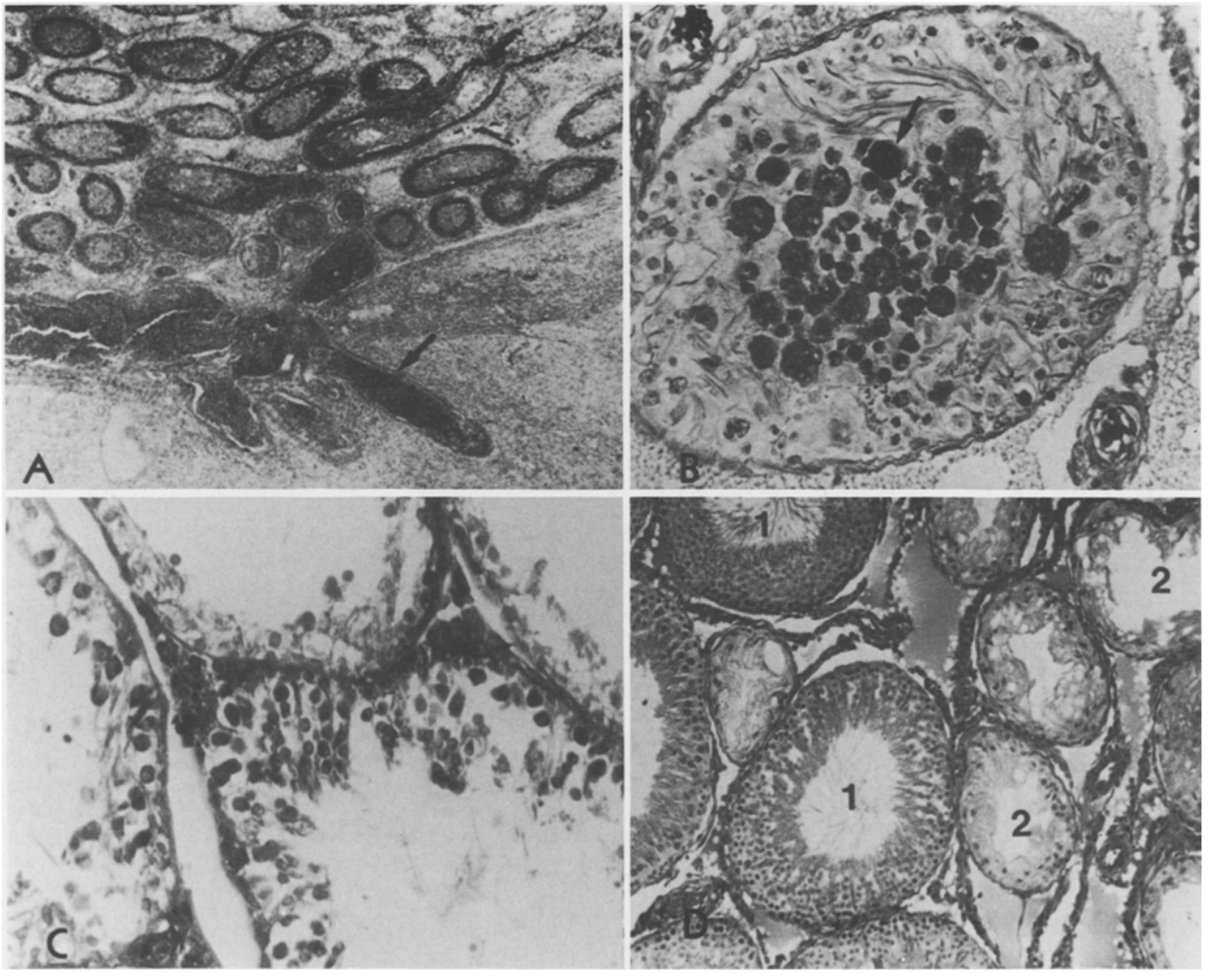

Fig. 1A–D. Testicular Inflammation (A). There is a peritubular infiltration of leucocytes with semeniferous tubule necrosis. Lymphatics in albuginea (*arrow*) are invaded by the infection process. This lymphatic invasion is seen distal to the epididymal lesion. B Non-inflammatory testicular lesions. There is no infiltration of lymphocytes two days after treatment. Germinal cells undergo necrosis and are transformed into multinucleolar cells and dense corpuscules, found in the lumen (*arrow*). C Non-inflammatory testicular lesions. Eight days after treatment: the germinal cells are almost entierely destroyed. Only Sertoli cells are present. D Non-inflammatory testicular lesions. 4 months after treatment: some seminiferous tubules are normal in appearence (*1*) while others remain empty (*2*)

Types of Lesions Observed After Injection of E. Coli

All lesions were unilateral and confined to the side of injection. No contralateral lesions, either epididymal of testicular, were observed.

– in 28.8% of cases no lesions were found

– in 12.9% of cases the inflammation remained localised in the tail of the epididymis

– in 16.2% of cases the inflammation spread to the testicle via lymphatic vessels without evidence of diffusion through the head of the epididymis (Fig. 1A)

– in 45.1% of cases testicular lesions were seen without other signs of inflammation.

A more detailed histological study revealed: (1) the absence of a leucocytic infiltration, (2) normal interstitial tissue, (3) the formation of multinucleated cells and a precocious desquamation of the germinal cells, beginning on the second day following epididymal injection (Fig. 1B) and almost completely disappearing by the 8th day (Fig. 1C). A progressive and more or less complete regeneration of these cells in most seminiferous tubes with persistance of a certain number of tubes remaining without germinal cells 3 or 4 months after treatment (Fig. 1D) was observed.

Dose Related Effects

Epididymal or testicular inflammation are not dependent on the concentration of injected bacteria. There exists, however, a relation between the number of bacteria injected and the frequency of testicular lesions of non-inflammatory type.

Lesions Observed After Injection of a Solution of Killed Bacteria

When killed bacteria were injected and the animals sacrificed 3 months later, testicular lesions were present in 40% of the cases. These lesions were of the non-inflammatory type described earlier. No histological evidence of epididymal damage was seen.

Discussion

Critical Study of the Experimental Model

Effects due to general anaesthesia in the production of lesions described above can be discounted by the fact that no lesions were seen in the contralateral organs. Similarly, the lesions observed cannot be accounted for by the surgical experimental manipulation as neither macroscopic nor histological lesions were noted when either sterile saline or fresh sterile culture medium were injected. When compared to Streptococcus or Staphylococcus, *E. Coli* seems to be the germ with the greatest pathogenicity in the rat. In man it is the most frequently identified germ in cases of acute epididymitis. This experimental model may serve as an authentic model for human epididymitis.

Mechanism of Spread of Inflammation

As no lesions were observed in the genital organs contralateral to the site of injection, it is possible to exclude the diffusion of the inflammatory process through the bloodstream. When epididymal lesions were present, these always remained localised in the tail without propagation to the head. Thus, an ascending canalicular diffusion does not occur.

Inflammatory spread occurs by means of lymphatic propagation between the albuginea and tunica of the seminiferous tubules (Fig. 1). This lymphatic communication has been described in the rabbit [2]. But it is not possible to exclude the role of urethro-deferential reflux as the origin of the initial epididymal inflammation.

Pathology of Non-inflammatory Testicular Lesions

Non-inflammatory testicular lesions are frequently seen, occurring in almost 50% of the cases studied. They appear very rapidly and on the second day the desquamation of the germinal cell is already observable. The exact mechanism of this testicular damage is unknown. Two hypotheses can be made:

1. action of bacterial toxin
2. the effect of sudden stop of the liquid flow

in the seminiferous tubules [5]. The fact that testicular damage with permanent lesions of the germinal cells was observed in 40% of the cases after injection of a solution containing dead bacteria seems to allow the first hypothesis.

At the present time, the second hypothesis cannot be excluded.

Conclusion

The results of this study show that the most frequent sequela and also the most important lesion that occur following acute *E. Coli* epididymitis is a non-inflammatory lesion of the germinal cells. It is highly probable that bacterial toxins are responsible for definitive problems of spermatogenesis in the rat.

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