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Experimental Male Genital Tuberculosis, the Possibility of Lymphatic Spread

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Summary. The spread of experimentally induced tuberculosis in the male genital organs was studied in guinea pigs. 40 animals were inoculated with $H_{37}Rv$ into the epididymis or seminal vesicle. Half of the animals in each series were prepared by resection of the ductus deferens before inoculation. - Canalicular, haematogenous and lymphatic spread are discussed. It is concluded that lymphatic spread is possible and often occurs.

Key words: Genital tuberculosis, male, experimental tuberculosis, spread of genital tuberculosis, experimental model - guinea pig.

Introduction

The mode of spread of tuberculous infection has long been unclear, and there have been a number of attempts to develop a suitable model for the study of this problem. Guinea pigs, which are susceptible to the classical human tuberculous strain, $H_{37}Rv$ (1) have proved to be suitable experimental animals. The large number of investigations performed around the turn of the century included considerations of the urogenital tract. However, there are few published studies on experimental tuberculosis of the male genitalia. Niclot (2), Baumgarten (3) and Kraemer (4) were pioneers, and in Scandinavia Hansen (5) was the first to describe such work.

The question of the routes of spread of tuberculosis between the urinary tract and the male genitalia, on the one hand, and within the genital system, on the other, was not settled with certainty. The problem has recently been actualized (6) and has been studied from the biochemical standpoint by Engel (7). In investigations to date, spread via the ductus deferens has been studied. To our knowledge no investigator has used an experimental technique which would permit an analysis of whether the infection may also spread by other routes. Nor have measures been taken to preclude leakage of inoculum after injection with consequent development of local tuberculous changes.

In a previous article (8) we described our experimental method in detail and presented preliminary evidence for lymphatic spread within the genital system. The results in their entirety are presented below.

Brief Review of Material and Methods

Sexually mature male guinea pigs were inoculated with 0.05 ml of a 14-day-old subculture of Mycobacterium Tuberculosis, varietas hominis ($H_{37}Rv$) diluted with physiological saline to a concentration of 0.1 mg per ml into the genital organs. 40 animals were divided into groups of 10 each which were treated as follows:

I. Inoculation into the left epididymis.
II. Inoculation into the left epididymis after resection of the vas.
III. Inoculation into the left seminal vesicle.
IV. Inoculation into the left seminal vesicle after resection of the vas.

The animals were killed after 3-8 weeks and the infection was evaluated macroscopically. The spread of tuberculosis in the genital organs, kidneys, spleen and in some iliac, paraaortic and renal lymph nodes was identified microscopically (haematoxylin-eosin, Ziehl-Neelsen and fluorescent staining) and with culture on Löwenstein-Jensen's medium. The combination of characteris-

tic histological changes and positive bacteriological test was taken to indicate specific infection in the genital organs. In the spleen and kidneys, where histological diagnosis could not be reliably based on minor changes, bacteriological criteria were decisive. Fluorescence microscopy proved to be highly valuable for demonstration of tubercle bacilli in tissue sections (9). Auramine-rhodamine fluorescent stain, which has been described by Matthaei (10), was used with potassium permanganate as counterstain (11). The tissue sections were examined using epi-illumination in a Zeiss Large Universal Fluorescence Microscope.

Table 1. Spread of tuberculous infection in the male genital system.
Inoculation into the left epididymis without vasoresection.

Animal	Weeks	LE	LSV	P	RSV	RE
1	8	+	-	-	-	-
2	†					
3	8	+	-	-	-	-
11	5	+	-	-	-	-
21	3	+	-	-	-	-
22	3	+	-	-	-	-
23	3	+	-	-	-	-
24	3	+	-	-	-	-
25	3	+	-	+	-	-
26	3	+	-	+	-	+
Total		9	0	2	0	1

LE left epididymis
LSV left seminal vesicle
P prostate
RSV right seminal vesicle
RE right epididymis

□ indicates the site of inoculation
\+ indicates tuberculous inflammation
\- indicates no tuberculous changes

Results

The results are shown in Tables 1-4. Most animals gained weight and none died of miliary tuberculosis. However one animal in group I died from an overdose of anaesthetic. Specific inflammation developed in all of the injected organs. Macroscopically, the inoculated epididymis had the appearance of an irregular tumor, difficult to distinguish from the testis, often containing visible caseous necrosis. Histologically, tuberculous changes were virtually confined to the epididymis. Tuberculous granulation tissue occasionally extended into the tunica testis, but usually did not perforate the tunica albuginea. Tubercle bacilli were never seen in the testicular tissue.

Inoculated seminal vesicles had the macroscopic appearance of stiff thickwalled tubes. Although the inoculum had been deposited in the lumen, the entire wall showed characteristic inflammatory changes when examined microscopically. In only a few instances were tubercle bacilli observed in the lumen. The prostates were enlarged and oedematous but not nodular. Tubercles containing bacilli were occasionally seen, near the walls of the vesicles.

A striking finding at autopsy, especially in animals in groups III and IV, was the presence of greyish white streaks joining the epididymis with the seminal vesicle (Fig. 1). These were as prominent in animals in which the ductus deferens had been resected as in those in which it had been left intact. These streaks, which even at the macroscopic level were suspected to be inflammatorily altered lymph vessels, followed the branches of the arteria deferentialis and continued to iliac lymph nodes which were clearly involved. These lymph nodes were infected in all animals, in contrast to the inguinal nodes, which remained free of tuberculosis. Tuberculous granulation tissue filled the lymphatics, but no changes were observed in the ductus deferens (Fig. 2).

In groups I and II, which were inoculated into the epididymis, changes in the prostate were noted in two animals. In one of them tuberculosis involved even the ipsilateral seminal vesicle. In the groups inoculated into a seminal vesicle (group

Table 2. Spread of tuberculous infection in the male genital system. Inoculation into the left epididymis after vasoresection

Animal	Weeks	LE	LSV	P	RSV	RE
4	8	+	-	-	-	-
5	8	+	-	+	-	-
6	8	+	-	-	-	-
12	5	+	-	-	-	-
13	5	+	-	-	-	-
14	5	+	-	-	-	+
27	3	+	-	-	-	-
28	3	+	-	-	-	-
29	3	+	-	-	-	-
30	3	+	+	+	-	-
Total		10	1	2	0	1

Table 3. Inoculation into the left seminal vesicle without vasoresection

Animal	Weeks	LE	LSV	P	RSV	RE
7	8	-	+	+	-	-
8	8	+	+	+	-	-
9	8	-	+	+	-	-
15	5	+	+	+	-	-
16	5	-	+	+	-	-
17	5	+	+	+	-	-
31	3	+	+	+	-	-
32	3	+	+	+	-	+
33	3	-	+	+	-	-
34	3	+	+	+	-	-
Total		6	10	10	0	1

III and IV) tuberculosis was demonstrated in the ipsilateral epididymis in all the animals subjected to vasoresection and in six of those with an intact deferent duct. Changes in the contralateral part of the genital tract were present in one animal in each group. In one of them tuberculosis involved both the epididymis and the seminal vesicle.

In all animals allowed to survive for 8 weeks the spleen was enlarged and clearly contained tubercles. In those animals that survived a shorter period of time the spleen appeared to be macroscopically normal. The histological findings usually consisted only of nonspecific inflammation with widened venous sinuses and proliferation

Table 4. Inoculation into the left seminal vesicle after vasoresection

Animal	Weeks	LE	LSV	P	RSV	RE
18	5	+	+	+	-	-
19	5	+	+	+	-	-
20	5	+	+	+	-	-
35	3	+	+	+	-	+
36	3	+	+	+	+	+
37	3	+	+	+	-	-
38	3	+	+	+	-	-
39	3	+	+	+	-	-
40	3	+	+	+	-	-
41	3	+	+	+	-	-
Total		10	10	10	1	2

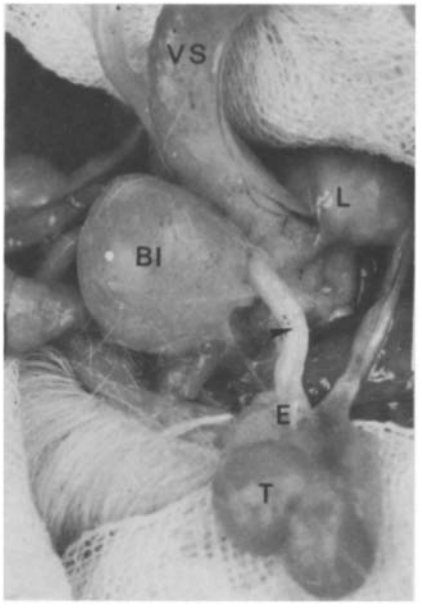

Fig. 1. Enlargement of seminal vesicle (VS) and epididymis (E) 35 days after injection of approximately 2500 tubercle bacilli into the seminal vesicle. Note the greyish white lymph vessels (arrow)

of reticuloendothelial cells. However tubercle bacilli were usually demonstrable both by microscopy and by culture.

In no case did the kidneys show macroscopic signs of tuberculous infection. A few isolated bacteria were seen in the kidneys of some of the animals, but there was no surrounding tissue reaction. In several animals the renal lymph nodes adjacent to the renal pelvis were clearly involved.

Discussion

The question of how tuberculous infection spreads in the male genital organs has been subject to considerable discussion. In the earlier literature it was usually claimed that infection occurs primarily in the epididymis and then spreads towards the prostate and the seminal vesicles. Spread can surely occur in either direction. More recent investigations as well as clinical experience indicate that the primary infection is usually in the pelvic genitalia and later spreads to the epididymis.

It has been generally believed that tuberculosis spreads mainly via the ductus deferens. There is no doubt that such canalicular spread occurs, in the same way as in nonspecific infections. However, Mycobacterium is nonmotile and must be transported by a medium. It has been shown (12) that urine can regurgitate into the ductus deferens and the seminal vesicle when there is increased pressure in the prostatic urethra. Experimentally induced antiperistalsis in the ductus deferens has also been described (13). Such a situation could arise in cases of advanced tuberculosis in which changes in the urethra may lead to stricture and there may be cavernous destruction of the prostate. If the urinary passage is intact and the pressure relationships in the urethra and ducti deferentes are normal, such canalicular spread would seem to be less likely.

In general, tuberculosis (for example, the pulmonary or gastrointestinal) is regarded as pri-

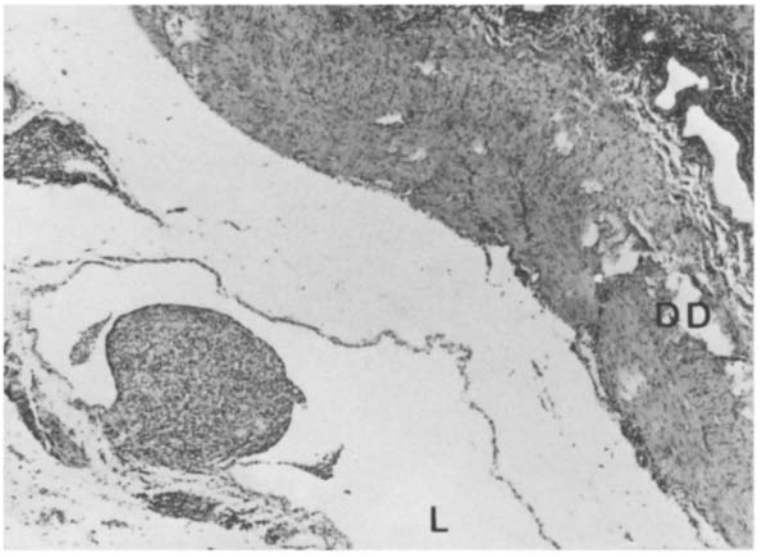

Fig. 2. Section of ductus deferens (DD) and adjacent tissue. In the lower left corner a dilated lymph vessel (L) with epithelioid granuloma. Htx. - eosin. x 50

marily an infection of the lymphatic system. Some authors (14, 15 and others) have expressed the opinion that lymphatic spread also occurs in tuberculosis of the urinary tract. The changes that are often present adjacent to the renal pelves and ureters in patients with urinary tract tuberculosis are suggestive of lymphatic spread.

It is however somewhat surprising that the possibility of lymphatic spread of tuberculosis between the organs comprising the male genitalia has been so little discussed. In man, as in most of the animal species used for experimental purposes, there is a dense network of lymphatics connecting the prostate, the seminal vesicles and the ampullae ductorum deferentium. The lymphatic vessels which accompany the ducti deferentes from the epididymis anastomose with this lymphatic network. The iliac nodes and/or the paraaortic nodes are the regional lymph nodes for both the pelvic and scrotal genitalia. Since there are direct lymphatic connections between the various genital organs, spread by the lymphatic route appears to be possible, and perhaps more likely, than spread via the ductus deferens.

In our material, tuberculous infection always occured at the site of inoculation. After inoculation into the seminal vesicle, inflammation was always encountered in the prostate as well. There was often spread to the genital organs of the same side, but occasionally to the contralateral side. Spread from the seminal vesicles and the prostate toward the epididymis was more frequent than spread in the opposite direction. The frequency of spread was largely independent of whether the ductus deferens was intact.

Tuberculous changes were always present in the regional lymph nodes - the iliac and paraaortic nodes. In the spleen, on the other hand, characteristic tuberculous changes were only seen fairly late in the course of the disease.

It has been shown that different organs of the guinea pig differ in their susceptibility to tuberculosis. The spleen, like the testis (16, 17, 7) is highly susceptible, while the kidney (18, 19) is much more resistant. The fact that characteristic changes were encountered in the spleen only late in the course of infection suggests that there was no hematogenous spread in connection with inoculation, but that generalized infection occured after the lymph barriers had been broken down. The testis is regarded by several authors as a favorable medium for tubercle bacilli. Some of them (20, 21) have recommended intratesticular injection in the guinea pig test as more reliable than inoculation into the traditional sites. In our animals there were sometimes superficial granulomas containing tubercle bacilli in the neighbourhood of the tunica testis. These changes were mainly unilateral (on the inoculated side), which further suggests that there was no primary hematogenous spread to the testis.

Our results support the current view that tuberculous infection spreads from the pelvic genital organs towards the scrotum more often than in the opposite direction. It is not clear why this is so. The epididymis is largely drained by lymphatics

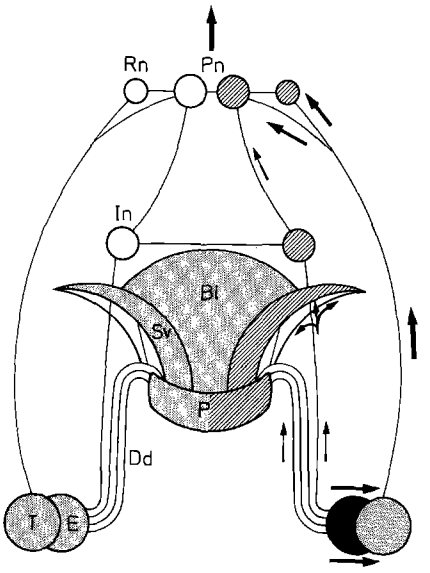

Fig. 3. The probable lymphatic route of spread of tuberculous infection from the epididymis.

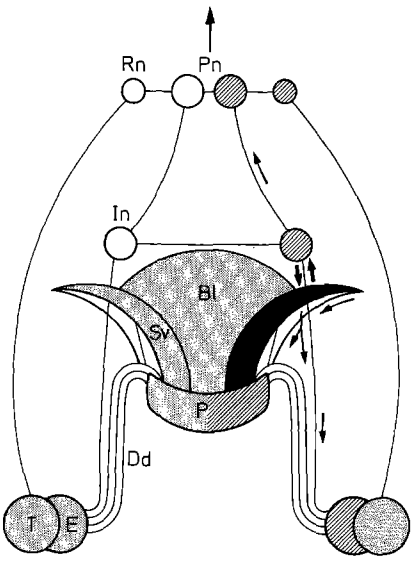

Fig. 4. The probable lymphatic route of spread of tuberculous infection from the seminal vesicle.

Bl ... urinary bladder
Dd ... ductus deferens
In ... iliac nodes
P ... prostate
Sv ... seminal vesicle
■ inoculated organ
□ spread of infection
Pn ... paraaortic nodes
Rn ... renal nodes
T ... testis

■ inoculated organ
□ spread of infection

which run along vasa spermatica and lead into the renal and paraaortic lymph nodes. A lesser proportion of the lymphatics follow the vasa deferentialia and drain into the iliac nodes. The latter lymphatics anastomose with lymphatics from the seminal vesicles and the prostate. Tubercle bacilli probably spread in the direction of lymphatic flow from the epididymis to the renal and paraaortic lymph nodes and only a minor proportion of them reach the pelvic genitalia and lead to tuberculous changes in the latter (Fig. 3).

That lymphatic spread occurs more readily in the retrograde direction may depend on successive blockade of the iliac nodes, which drain the seminal vesicles and the prostate. Following their blockade, there is a widening of the lymphatics that follow the ductus deferens. The lymphatic flow can be directed to the epididymis where tubercle bacilli can give rise to specific inflammation (Fig. 4). Infection may spread from these to the renal and/or paraaortic nodes, as described above. This is a possible explanation of the spread of infection in our guinea pigs. In addition, the infection may break through the lymphatic vessels and spread in the surrounding fat and connective tissue.

Our results strongly indicate that tuberculosis spreads in the male genitalia by way of the lymphatics. There is reason to believe that this lymphatic spread is as important as the canalicular and that it occurs considerably earlier in the course of the disease.

In recent years a large clinical experience of funicular tuberculosis has been accumulated (22). A number of these cases, such as that of a patient with secondary funicular tuberculosis which we have described (23), provide evidence that lymphatic spread also occurs in man.

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