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An Experimental Method for Studying the Spread of Genital Tuberculosis

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Summary. An experimental method is described for investigating the routes of spread of tuberculosis in the male genitalia. A human strain of tubercle bacilli ($H_{37}Rv$) was inoculated into the epididymis or seminal vesicle of guinea pigs (40 animals), half of whom had been prepared beforehand by resection of the ductus deferens with preservation of the surrounding vessels. The spread of infection was studied by histological and bacteriological methods. It was shown that there is an additional route of spread besides the lumen of the ductus deferens, probably via lymphatics.

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

Although almost 200 years have elapsed since genital tuberculosis first attracted attention (Morgagni, 1767; Bayle, 1803), there is still no unanimity of opinion as to how the infection reaches the genital tract, and how it spreads within it. Most conclusions have been drawn from studies on autopsy material (Teutschländer, 1906; Simmonds, 1914; Lehman, 1930; Auerbach, 1940; Medlar, Spain & Holliday, 1949; and others), some from clinical observations (Borthwick, 1945, -46, -47, -56; Gütgemann, 1951; Alyea, 1970; Albescu, 1970; and others), and only a few from experimental work (Niclot, 1898; Baumgartner, 1900; Kraemer, 1900; Hansen, 1903; Zádor, Baranyi, Földes & Csontai, 1967). Differences between the types of material studied have increased the difficulties of determining the most probable routes of spread.

There are two principal theories about the mode of spread of infection to the genitals: either via the haematogenous or the urinary (canalicular) routes.

Some authors (Berblinger, 1929; Lehman, 1930; Ljunggren, 1959; Neuwirth and Uhlir, 1965) have considered that infection reaches the genitalia, like the kidneys, via the bloodstream. Sussig's (1921) early work showed that any of the genital organs may be the site of primary infection. How rapidly the primary interstitial tubercules develop depends on a number of factors, both local and systemic. Engel (1968), in experimental studies of the development of infection in the testis and epididymis, clearly demonstrated the importance of the chemical milieu.

The theory that genital tuberculosis arises by urinary spread still has many adherents (Mazurek, 1963; Kühn & Unger, 1966; May, Hohenfellner & König, 1966). According to these authors, the bacteria reach the genitals from the posterior urethra, the so-called urogenital cross-roads. Most adherents of the theories either of haematogenous or of urinary dissemination agree that there is intracanalicular spread between the genital organs, via the ductus deferens. Certain clinical observations, however, suggest that this need not be the only route. For example, it is not uncommon for nonspecific epididymitis to occur in spite of previous resection of the vas, and similar cases of tuberculosis have been described (Zádor, Baranyi, Földes & Csontai, 1967).

If continuity of the ductus is broken, infection can reach the epididymis from the pelvic genitalia in two ways, either via the bloodstream or by way of lymphatics. The possibility of haematogenous spread has been extensively discussed. The possibility of lymphatic spread has only been implied in a few publications (Fey & Couvelaire, 1944; Borthwick, 1945-47; Alyea, 1970; Albescu, 1970), and it has not been studied experimentally. Connections between the lymphatics in the testes, prostate and the seminal vesicles in the guinea pig, rabbit, calf, pig, dog and in the human foetus has been con-

vincingly demonstrated by Hasumi (1931), and our studies on guinea pigs confirm his findings. It seemed of interest, to study whether lymphatics are of importance in the dissemination of tuberculosis, in addition to the more generally recognized intracanalicular route of spread.

Material and Methods

Experimental animals. Sexually mature male guinea pigs were employed, since they are sensitive to the strains of Mycobacterium tuberculosis varietas hominis used and have a suitable anatomy, with well-developed genitalia which are readily accessible for surgery. Each animal was isolated in an individual cage.

Inoculum. A 14-day-old culture of the classical human strain of tubercle bacillus, $H_{37}Rv$, diluted with physiological saline to a concentration of 0.1 mg/ml was used as inoculum. The number of bacteria was determined from a series of dilutions of the inoculum and varied between 15 000-75 000 per ml.

Grouping of animals. 40 guinea pigs were divided into groups of 10, each of which was treated as follows:

I. Inoculation into the epididymis.

II. Inoculation into the epididymis after resection of the vas.

III. Inoculation into the seminal vesicle.

IV. Inoculation into the seminal vesicle after resection of the vas.

Operative procedure. Mebumal sodium 0.05 mg/100 g body weight, was administered intraperitoneally; supplementory ether anesthesia was used on occasions. The abdomen was opened through a left-sided inguinal incision and the testis and epididymis, urinary bladder and seminal vesicles exposed. The ductus deferens was carefully dissected free from its vessels over a length of slightly more than 0.5 cm. It was ligated proximally and distally with 4/0 chromic catgut and the intervening portion of the ductus resected. 0.05 ml of inoculum was then injected into the epididymis (Fig. 1), or into the seminal vesicle (Fig. 2) from a disposable tuberculin syringe. Before the needle was withdrawn, a cotton-tipped applicator stick soaked with a strongly bactericidal plastic material, well tolerated by tissues, (Nobecutan^R, Bofors) was applied to the injection site (Fig. 3). This was held in place for about a minute after the completion of the injection. In preliminary experiments with this plastic film, the impermeability of the injection site was checked by using an inoculum stained with 0.1 ml bromthymol blue (Berlin blue) in a 1:1 000 dilution. The absence of leakage was shown by the lack of staining of the applicator. Following application of the plastic film, the organs were replaced in the abdominal cavity and the scrotum. The abdomen was closed in two layers. Initially the animals were killed after 8

Fig. 1. Injection of coloured inoculum into the epididymis.
T.....testis
E.....epididymis
The arrows indicate the ligated ends of the severed ductus deferens

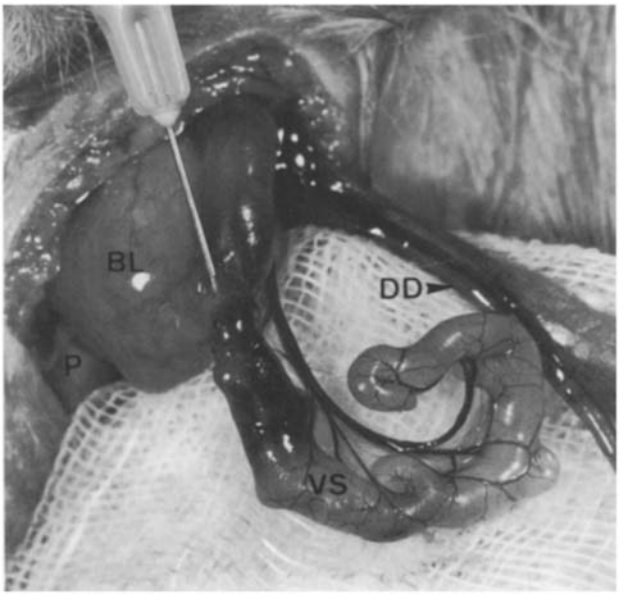

Fig. 2. Injection of coloured inoculum into the seminal vesicle.
BL..... urinary bladder
P ..... prostate
VS..... seminal vesicle
DD..... ductus deferens

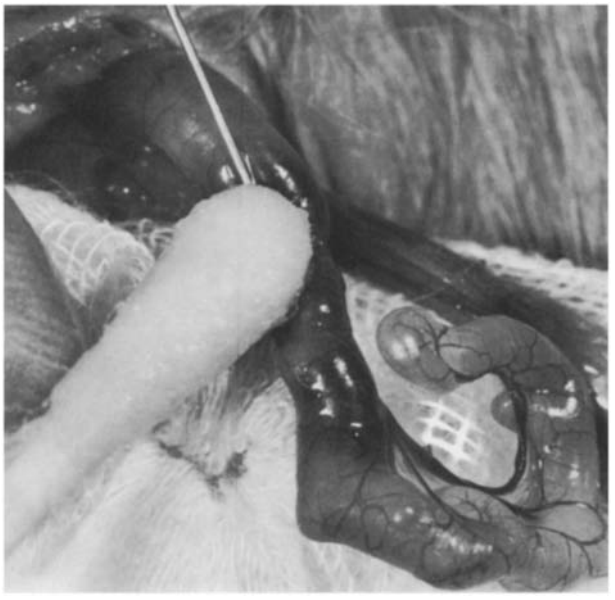

Fig. 3. As in Fig. 2. To prevent leakage, a cotton-tipped applicator stick soaked in a liquid plastic is applied to the injection site before withdrawal of the needle

weeks; this time was successively shortened to 3 weeks in order to confine the infection to the genital system.

Identification of tuberculous infection. The results were evaluated macroscopically, histologically and bacteriologically. Fresh instruments were used for the removal of each organ. One half of each organ investigated was used for culture, the other half for histological studies. The genital organs, kidneys, spleen and paraaortic lymph nodes were fixed in formalin and embedded in paraffin, histological sections were stained with haematoxylin-eosin and by the Ziehl-Neelsen method. It is hoped to employ a modified fluorescence technique in a future study. Culture on Löwenstein-Jensen's medium was performed after mincing the tissues, three cultures being taken from each organ. The features taken as indicative of tuberculous infection were characteristic histological changes, the demonstration of acid fast bacilli in Ziehl-Neelsen-stained slides and positive cultures. If two of these criteria were met, the organ was considered to show tuberculous infection.

Preliminary Results. (see table)

One animal in group I died from an overdose of anaesthetic. In all the surviving animals tuberculous changes were found at the site of inoculation. In the groups in whom the epididymis had been inoculated changes were noted in the prostates in 2 animals from each group. In one of them even the ipsilateral seminal vesicle was involved by tuberculosis. In the groups whose seminal vesicles had been inoculated, tuberculosis was demonstrated in the ipsilateral epididymis in all the animals subjected to resection of the vas, and in half of those with an intact ductus deferens. Lesions were found in the contralateral genital tract in one animal of each group. In one of them tuberculosis had affected both the epididymis and seminal vesicle.

The preliminary results are shown in the table:

	Number of animals	LE	LSV	P	RSV	RE
I Normal vas	10 (1+)	[9]	0	2	0	1
II Resection of vas	10	[10]	1	2	0	1
III Normal vas	10	5	[10]	7	0	1
IV Resection of vas	10	10	[10]	5	1	1

Tuberculous changes in the genital tract of male guinea pigs following inoculation in the epididymis or the seminal vesicle.

□ indicates the site of inoculation
LE ... left epididymis
LSV .. left seminal vesicle
P..... prostate
RSV... right seminal vesicle (vesicle)
RE ... right epididymis (epididymis)

Discussion

The choice of experimental animal was determined by the factors named above, plus the fact that guinea pigs are cheaper and more easily cared for than monkeys, a possible alternative.

Under modern conditions of life the human type of the tubercle bacillus is the only one of importance in man, and it was for this reason that the $H_{37}Rv$ strain was used.

To make it impossible for spread to occur via the ductus deferens it is necessary to resect the vas. Ligation alone is unreliable, since recanalization often occurs within a short time (9, 19); no instance was observed of reunion and recanalization following resection of 5 mm of the ductus deferens. In order to preserve most of the lymphatics, the dissection was performed taking every care not to damage the blood vessels around the ductus deferens.

Direct injection of bacilli into the epididymis or seminal vesicle is a better simulation of initial infection by the haematogenous route than would be injection into the lumen of the ductus deferens. Usually both the prostate and seminal vesicles are involved in cases of tuberculous infection. Injection into the seminal vesicles in guinea pigs is technically easier than injection into the prostate, which is why the former was chosen. Clear lateralization of the infection was obtained in this manner.

0.05 ml proved to be a suitable volume of inoculum for producing infection. In preliminary experiments, we found there was a greater risk of leakage if larger volumes were used. In earlier experiments, like other authors (29, 20, 11), tubercles were often found developing on the surface of injected organs and spreading to the peritoneum and the original incision. Leakage of this type has not occured since the procedure was adopted of covering the site of injection with a tuberculocidal plastic film.

The microscopical appearances of tuberculosis are characteristic and readily recognized, although it must always be remembered that histological diagnosis alone is inadequate as other conditions may produce a similar or even identical picture. Furthermore, sections may only show chronic inflammation without any of the features characteristic of tuberculosis, even though tubercle bacilli may be found on culture. Therefore a histological diagnosis alone was not accepted, and evaluation of the experimental results even based on additional bacteriological investigations.

The preliminary results indicate that tuberculous infection in the male genital tract may spread both within the ductus deferens and also via the lymphatics. In the guinea pig the spread more easily occurs toward the epididymis (testipetally) than from it (testifugally). More detailed analyses and further results will be presented in a subsequent report.

The method described should also be useful for studying the route of other infections than tuberculosis in the genitaltract of male.

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