CHEMICALLY INDUCED SPERM RETENTION CYSTS IN THE RAT

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Summary. The development and fate of spermatocoeles induced in the rat epididymal pathway by giving a single dose of ethylenedimethanesulphonate has been followed histologically for almost 5 months. Many of the retention cysts, which have intact walls, resolve completely but large spermatocoeles may persist. Their content of dead spermatozoa is removed and replaced by viable spermatozoa as the testis recovers from the antispermatogenic action of the compound. The presence of such residual cysts is then compatible with normal fertility. Rupture of the cyst walls with the formation of permanent spermgranulomata sometimes occurs.

No definite relationship to the loss of androgen support induced by the compound has been found, since concurrent treatment with testosterone did not prevent the formation of lesions.

INTRODUCTION

Sperm retention cysts or spermatocoeles occur in the sperm-conducting system in man. They are uni- or multilocular and may be found in the ductuli efferentes (Wakeley, 1943–44; Ackerman, 1964), caput epididymidis (Bailey & Love, 1971), epididymis or rete testis (Lapides & Schroeder, 1965). Escape of spermatozoa from the epididymal duct may follow direct or surgical trauma, lower urinary tract infection or obstruction, or through the intact epithelium (King, 1955), when stroma cell reaction occurs, creating a spermgranuloma (Collins & Pugh, 1964) which is extratubal.

In Wistar rats, a certain dosage of the alkylating agent, ethylenedimethanesulphonate (EDS), exerts reversible antispermatogenic effects on the testis. It frequently induces pathological changes in the epididymis, culminating in cyst formation in this duct on one or both sides (Cooper & Jackson, 1970). Ericsson (1970) found that α -chlorhydrin (U-5897) caused cysts in the rat, specifically in the ductuli efferentes and initial part of the caput epididymidis, which led to permanent sterility. Recently, Ericsson (1971) reported that in rats another compound (U-29409, 2,3-dihydro-2-(1-naphthyl)-4(1H)-quinazolinone) caused mass exfoliation of cells of the germinal epithelium into the epididymis. Spermatocoeles and spermgranulomata developed in a few rats.

The present study was undertaken to investigate the pathological changes involved in the development and resolution of EDS-induced spermatocoeles and to assess their possible rôle in the infertility produced by this compound.

MATERIALS AND METHODS

Proven fertile male Wistar rats received a single intraperitoneal dose of EDS (75 mg/kg) and the effects were examined between 12 hr and 140 days after the dose. In the entire study, 120 animals, weighing 270 to 320 g, were used. At autopsy, the testis, epididymis and ductus deferens, including the genital fat containing the ductuli efferentes, were removed, fixed in Bouin's fluid and serial longitudinal sections were prepared.

To determine the relationship between the fate of the spermatocoeles and the restoration of spermatogenesis, the location of the cysts was recorded during laparotomy in a series of thirteen EDS-treated rats. In unilateral cases, the unaffected epididymis along with the testis was excised. Ten weeks later, the rats were paired weekly until the return of fertility was established. The males were then killed and the reproductive tract was examined histologically. To investigate the development of the spermatocoeles, specimens were prepared from 12 hr up to 7 days after treatment.

RESULTS

At autopsy, the first visible feature in the epididymis was hyperaemia at the corpus-cauda junction, 12 to 24 hr after injection of EDS. In corresponding longitudinal sections, this region appeared as a 'wedge'-shaped area in which hypertrophy of the lining epithelium was characteristic (Pl. 1, Fig. 1). By Days 2 and 3, the change had spread to the corpus, caput and ductuli efferentes. During Days 3 to 5, small dilatations arose which soon 'opened up' into each other, thus creating the familiar spermatocoeles (Pl. 1, Figs 2 and 3). Mitotic figures were observed among the epithelial cells, a rare occurrence in the normal adult rat (Clermont & Flannery, 1970).

Spermatocoeles were first visible to the naked eye between Days 5 and 7 and approached a maximum size about Day 14. At this time, the seminiferous tubules were devoid of post-meiotic elements and pre-meiotic cells were depleted

EXPLANATION OF PLATE 1

FIG. 1. Typical 'wedge'-shaped area at the corpus-cauda junction of the epididymis of a rat 24 hr after treatment with EDS, showing hypertrophy of the lining and restriction of the lumen of the epididymal tubules. The superficial vein (5) lies at the base of the 'wedge'. $\times 30$. Inset shows one tubule. $\times 100$.

FIG. 2. Spermatocoeles (1 and 1A) in middle and distal region of the caput epididymidis (2) of a rat 6 days after treatment with EDS. Adjacent tubules are becoming empty. Note the 'opening up' of the small dilatations at 1A. $\times 12$.

FIG. 3. Spermatocoele (1) at corpus-cauda junction of the epididymis of a rat 9 days after treatment with EDS. Epididymal tubules are shrunk and empty. 3 = corpus; 4 = cauda. $\times 12$.

FIG. 4. Recanalized spermatocoele (1) at corpus-cauda junction of the epididymis of a rat 105 days after treatment with EDS. Epididymal tubules refilled with spermatozoa. 3 = corpus; 4 = cauda. $\times 12$.

PLATE 1

FIG. 1

FIG. 2

FIG. 3

FIG. 4

(Facing p. 446)

PLATE 2

FIG. 5

FIG. 6

FIG. 7

FIG. 8

(Facing p. 447)

(Cooper & Jackson, 1970). The associated aspermia lasted about 8 weeks (Jackson, 1971). During the first half of this period, immediate *post-mortem* examination of the spermatocoele content revealed clumps of fragmented spermatozoa, scattered macrophages and polymorphs. By Weeks 7 and 8, motile spermatozoa were recovered from cysts still present at the caput-corpus and corpus-cauda junctional regions and in Week 9, fluid expressed from the commencement of the ductus deferens contained motile spermatozoa.

Thus resolution and/or canalization of the spermatocoeles had progressed concurrently with recovery of spermatogenic activity (Pl. 1, Fig. 4 and Pl. 2, Fig. 5). It is apparent that, during the period of testicular aspermia, disposal of the contents of spermatocoeles had occurred. In the larger cysts, as the volume of contents diminished, the wall appeared to contract down to more normal proportions. By the time spermatogenesis was restored, the majority of the cysts had either resolved or recanalized, the epididymis was patent and compatible with the transport of spermatozoa. Two rats showing unilateral spermatocoeles at exploration, and ten of eleven with bilateral cysts, recovered their fertility (Pl. 1, Fig. 4). The eleventh rat in this series is referred to on page 448 (Pl. 2, Figs 6 to 8).

Spermatocoeles were round or oval and varied in size up to 6 mm in length. They occurred most frequently at the corpus-cauda junction (Pl. 1, Figs 3 and 4; Pl. 2, Figs 6 and 7), a region bounded on the surface by a dilated U-shaped vein. The epididymis adjacent to the lesions was shrunken and practically empty in the early stages (Pl. 1, Fig. 3). Cysts also occurred in the caput (Pl. 1, Fig. 2) and corpus epididymidis, while those in the ductuli efferentes were microscopic (Cooper & Jackson, 1972).

Polymorph, lymphocyte and macrophage reaction in the epididymal stroma was evident in the vicinity of the cyst. Structurally, the spermatocoele was a local distension by coagulum and clumped spermatozoa. The wall consisted of the basement membrane supporting an epithelium of two or three layers (Pl. 2, Fig. 5). In some instances, sperm heads were present among the lining cells and extended into the supporting stroma causing local tissue reaction. Stromal cells were observed among the epithelial cells and within the sperm-laden content. On occasions, an intensive invasion by these reactionary cells changed the intratubal spermatocoele into a spermgranuloma (Pl. 2, Figs 6 to 8). Histologically, this differed from the spermatocoele in that the epithelial lining and basement membrane were disrupted and obscured by

EXPLANATION OF PLATE 2

FIG. 5. Recanalized spermatocoele (1) in a rat 111 days after treatment with EDS. The lining epithelium is hypertrophic. The cyst and epididymal tubules are filled with spermatozoa. $\times 110$.

FIG. 6. Dissection showing the testes and epididymides *in situ* in a rat 111 days after treatment with EDS. Spermatocoeles (1) and spermgranulomata (9) are indicated. 6 = ductus deferens; 7 = testis.

FIG. 7. Left testis and epididymis of the specimen shown in Fig. 6. Spermatocoeles (1) lie in the corpus and corpus-cauda junction and a spermgranuloma (9) occupies the cauda epididymidis. $\times 4$.

FIG. 8. Detail of spermatocoele (1) and spermgranuloma (9) shown in Fig. 7. Invasion of the granuloma by cells of the stroma (8) is apparent. The testis has fully recovered and the epididymis contains spermatozoa, but the rat was infertile. $\times 15$.

macrophages and other reactionary cells, the formation becoming extratubal and not restricted in its spread (Pl. 2, Fig. 8).

DISCUSSION

Lesions within the epididymis induced by EDS in the rat may be single or multiple, uni- or bilateral and involve a variety of sites. The action of the chemical is associated with a general epididymal reaction with visible hyperaemia. In some rats, the primary effect subsides completely but progression to scattered bilateral lesions is common.

The present research has shown that spermatocoeles so induced undergo resolution and recanalization. Small cysts may disappear completely while larger ones contract down, as the arrested contents are cleared, permitting sperm transport when the testis recovers from the concurrent antispermato-genic action of the compound. Among many rats, permanent sterility occurred in only one animal and was associated with the presence of both spermatocoeles and spermgranulomata although there was evidence that spermatogenesis had returned since the epididymis was full of spermatozoa (Pl. 2, Figs 6 to 8).

In the rat, the epididymis is androgen-dependent and the action of EDS is accompanied by considerable reduction of androgen support from the testis (Cooper & Jackson, 1970; Bu'Lock & Jackson, 1971-72). Recent experiments have shown that both testosterone and HCG could counteract the antispermato-genic effects and maintain the fertility of EDS-treated rats, but the development of spermatocoeles was not prevented (H. Jackson and C. M. Jackson, unpublished data).

In immature male rats (6 weeks old), EDS (75 mg/kg) caused antispermato-genic effects but neither visible nor microscopic cysts developed. This finding might be associated with the lack of physiological secretory activity of the epididymis at this time of life (Setchell, 1970).

Ethylenedimethanesulphonate is an alkylating sulphonic ester and, surprisingly, its close homologues, Myleran and propylene dimethanesulphonate, did not induce similar pathological changes (Cooper & Jackson, 1970). As yet, there is no definite clue as to the biochemical-pharmacological mechanisms underlying the remarkable actions of this compound.

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