Indian J Physiol Pharmacol 2001; 45 (1) : 87–94

INDUCTION OF SEMINIFEROUS TUBULAR ATROPHY BY SINGLE DOSE OF 5-FLUOROURACIL (5-FU) IN WISTAR RATS

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(Received on August 14, 2000)

Abstract : Antimetabolite, 5-fluorouracil (5-FU) is known to cause testicular damage by epithelial sloughing and cell killing. However, it is not known whether 5-FU induces tubular atrophy and the fate of exfoliated germ cells. Present study was conducted to evaluate these effects of 5-FU on rat testis. Animals were injected, single dose of 5-FU (10.50 & 100 mg/kg, i.p.) and sampled at 1, 3, 15 and 30 day following the treatment. The testes were perfusion fixed by Bouin's fluid. Five micron thick paraffin sections of testes and epididymis were stained with haematoxylin and eosin. Slides were examined for the incidence of abnormal tubules (per 200 tubules), tubular diameter (STD), epithelial height (SEH) and for the presence of germ cells in the epididymes. Data were analysed by Mann-Whitney 'U' test. The testes weight, STD, SEH were decreased ($P<0.05$–0.01) in treated animals. The abnormal tubules were increased in a dose dependent manner with atrophic tubules seen on 30 d. The exfoliated germ cells have not blocked the post testicular ductal system and found in the epididymis in a dose dependent manner. The present study concludes that 5-FU causes tubular shrinkage and atrophy. Further, epididymis is involved in the phagocytosis of germ cells.

Key words : atrophy rat epididymis testis 5–fluorouracil

INTRODUCTION

The antimetabolite, 5-fluorouracil (5-FU) has been effective against tumours of head and neck, breast and gastrointestinal system (1). Combination therapy of 5-FU with other drugs found to be effective and this approach has been reviewed previously (2). Despite its efficacy in cancer chemotherapy it was found to be a mutagen inducing chromosome aberrations and micronuclei (3). Besides, it was also known to cause testicular damage by hampering the cellular maturation, forming the abnormal spermatids with acrosomal and head shape defects (4), sloughing and formation of giant cells (5). The epithelial sloughing caused by some chemicals known to produce tubular atrophy (6). However, it is unknown whether 5-FU induces tubular atrophy eventhough it causes

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sloughing. It is also not known whether the exfoliated cells block the efferent ductules or flow freely into the epididymes. Although, previous studies have demonstrated the 5-FU toxicity on testis, they have not uncovered the permanent effects such as tubular atrophy and the fate of exfoliated germ cells. Hence, this study was aimed to address on these effects of 5-FU on rat testis.

METHODS

Inbred adult male albino rats of Wistar strain were used. Animals were housed in plastic cages and maintained according to the ethical guidelines under standard laboratory conditions with access to food and water ad libitum.

Rats were segregated into 13 groups of 5 animals each. A single injection of 0.1 mL distilled water was given (i.p.) to one group which served as control. Four groups received single dose of 10 mg, another four groups 50 mg and remaining four groups 100 mg/kg body weight of 5-FU (Fluracil, Biochem) intraperitoneally. Four sample times on 1, 3, 15 and 30 d were selected following the exposure. On the day of experiment, one group each from three different dose study was selected. The animals were anaesthetised using sodium pentabarbitol (40 mg/kg) and the testes were perfusion fixed. The mediastinum was opened and perfusion fixation was done by injection of heparinised (1 mL/L) normal saline followed by Bouin's fluid until the testes turned yellowish (5, 7, 8). Laparatomy was conducted, testes were removed along with the epididymes, separated and weighed. Tunica vaginalis was removed and the testes were immersed in fresh Bouin's fluid for 10 h. The testes were cut into 3–4 mm thick blocks and processed for paraffin embedding (9). Five micron thick sections were obtained and stained with haematoxylin and eosin (10).

Slides were coded and analysed. Seminiferous tubular diameter (STD) and seminiferous epithelial height (SEH) of ten transversely cut seminiferous tubules/ section were measured by ocular micrometer calibrated with stage micrometer (Erma opticals, Japan). For each animal, 10 sections were examined and the average was taken. Two hundred tubules per animal were examined and classified into normal and abnormal tubules according to the method of Hess et al (11), with slight modification. The abnormal tubules were the one in which disruption of normal cell association in seminiferous epithelium was seen. The epididymal sections were screened for the incidence of germ cells in the lumina and graded as follows. The sections without any germ cell in the lumina were rated as '0', very few cells as '+', moderate number as '++' and many cells as '+++'.

The data were analysed by Mann-Whitney 'U' test. $P<0.05$ was considered as the level of significance.

RESULTS

Following the treatment of 5-FU, we have observed a decline in the testis weight compared to the control group (Table I). This difference in animals treated with 10 mg

TABLE I: Effects of 5-FU on rat testis.

Parameter	Control	10 mg/kg			50 mg/kg			100 mg/kg		
		1 d	3 d	15 d	30 d	1 d	3 d	15 d	30 d	1 d	3 d	15 d	30 d
Testis weight (g)	1.47 ± 0.01	1.47 ± 0.01	1.45 ± 0.01	1.44 ± 0.02	1.45 ± 0.01	1.46 ± 0.01	1.33 ± 0.02$^{*\bullet\bullet}$	1.36 ± 0.01$^{*\$\$}$	1.24 ± 0.02*††	1.30 ± 0.00$^{*\circledcirc\circledcirc}$	1.27 ± 0.00$^{*\bullet\bullet}$	1.24 ± 0.01**	1.12 ± 0.01**††
STD (µm)	322.66 ± 2.50	314.36 ± 1.31*	315.58 ± 1.06*	314.03 ± 1.19*	308.59 ± 1.37**	314.86 ± 1.49*	309.32 ± 1.26***	292.66 ± 1.18$^{*\$\$}$	282.59 ± 0.88*††	306.13 ± 1.29$^{*\circledcirc\circledcirc}$	295.49 ± 1.05$^{*\bullet\bullet}$	275.15 ± 1.30$^{*\$\$}$	263.01 ± 0.73**††
SEH (µm)	78.13 ± 1.59	76.95 ± 0.36	74.69 ± 0.77	75.31 ± 0.33	72.22 ± 0.34**	74.22 ± 0.28$^{\circledcirc\circledcirc}$	71.90 ± 0.13***	71.60 ± 0.24**	68.74 ± 0.20*††	70.54 ± 0.30$^{*\circledcirc\circledcirc}$	67.95 ± 0.41$^{*\bullet\bullet}$	65.30 ± 0.12$^{*\$\$}$	62.88 ± 0.27**††
No. of Abnormal tubules	3.20 ± 0.37	4.80 ± 0.37*	10.00 ± 0.71**	16.00 ± 1.05**	27.40 ± 0.81**	50.20 ± 0.58$^{*\circledcirc\circledcirc}$	57.20 ± 1.46$^{*\bullet\bullet}$	72.80 ± 1.39$^{*\$\$}$	73.60 ± 1.17*††	55.00 ± 1.41$^{*\circledcirc\circledcirc}$	77.80 ± 0.58$^{*\bullet\bullet}$	79.20 ± 0.37$^{*\$\$}$	82.40 ± 0.68**††

Values are Mean ± SEM, n = 5 each group
*P<0.05; **P<0.01-Control versus treated; $^{\circledcirc}$P<0.05-10 mg 1 d versus 50 mg 1 d, $^{\circledcirc\circledcirc}$P<0.01-10 mg 1 d versus 100 mg 1 d; $^{\bullet}$P<0.05-10 mg 3 d versus 50 mg 3 d, $^{\bullet\bullet}$P<0.01-10 mg 3 d versus 100 mg 3 d; ††P<0.01-10 mg 30 d versus 50 mg 30 d & 10 mg 30 d versus 100 mg 30 d; $^{\$\$}$P<0.01-10 mg 15 d versus 50 mg 15 d & 10 mg 15 d versus 100 mg 15 d.

was not significant (P>0.05) but in other two doses; 50 and 100 mg/kg, the testis weight was reduced (P<0.01) at all sample times except on first day in 50 mg.

The STD was decreased in all the groups over the control (P<0.05 – 0.01). In 10 mg/kg the effect was maximum on 30 d, whereas in 50 mg/kg reduction in STD was from 3 d through 30 d. But 100 mg caused reduction on first day itself and the trend was continued in other sample periods. The SEH was decreased (P<0.01) only on 30 d in 10 mg and in other two doses (50 and 100 mg) at all sample times (P<0.01) except on first day in 50 mg/kg. The testicular sections from control rats (Fig. 1) showed normal cellular and tubular integrity. The number of abnormal tubules were increased (P<0.05 – 0.01) in all groups. Tubular atrophy (Fig. 2) was seen in animals exposed to two higher doses on 30 d.

TABLE II: Grading of number of exfoliated cells.

Drug dose mg/kg	Sample time (days) 1 d	3 d	15 d	30 d
10	0	+++	+	0
50	0	+++	+	+
100	+++	+++	++	++

There were no exfoliated germ cells in the lumina of epididymal tubular cross sections from control rats (Fig. 3). Observation of epididymal sections from treated groups revealed the presence of exfoliated germ cells in

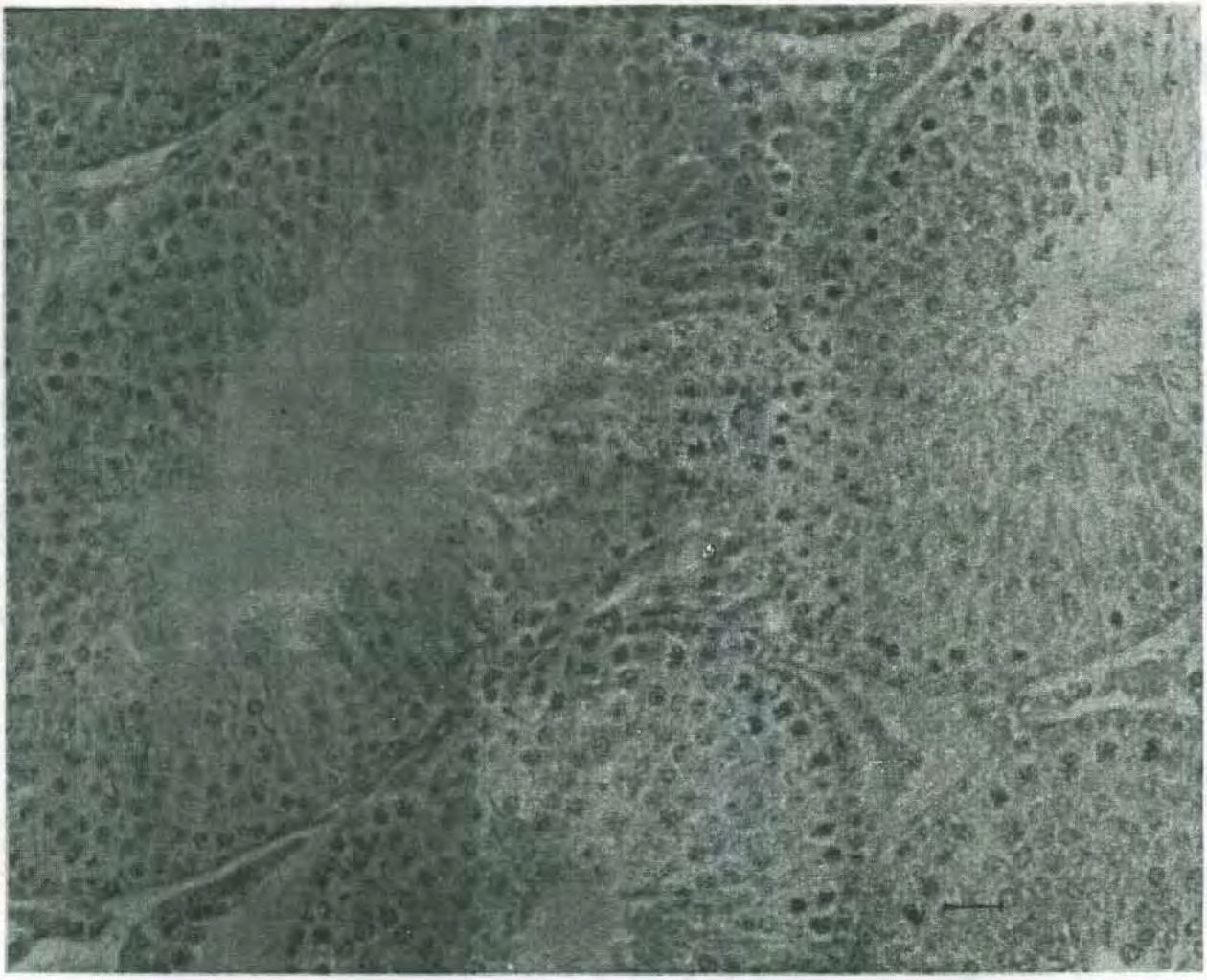

Fig. 1 : Testis section showing the normal seminiferous tubules (Control)

the lumina (Fig. 4). The number of germ cells was maximum on 3 d in all the dose levels (Table II). However, in the case of animals treated with 100 mg, the cells were found even on first day itself. In animals exposed to 10 mg and 50 mg, the germ cells were first seen only on 3 d but disappeared by 30 d only in 10 mg group. Treatment of 100 mg, induced the appearance of germ cells on first day itself, and the trend was continued through day 30 (Table II). In general the effect of 5-FU was dose and time dependent.

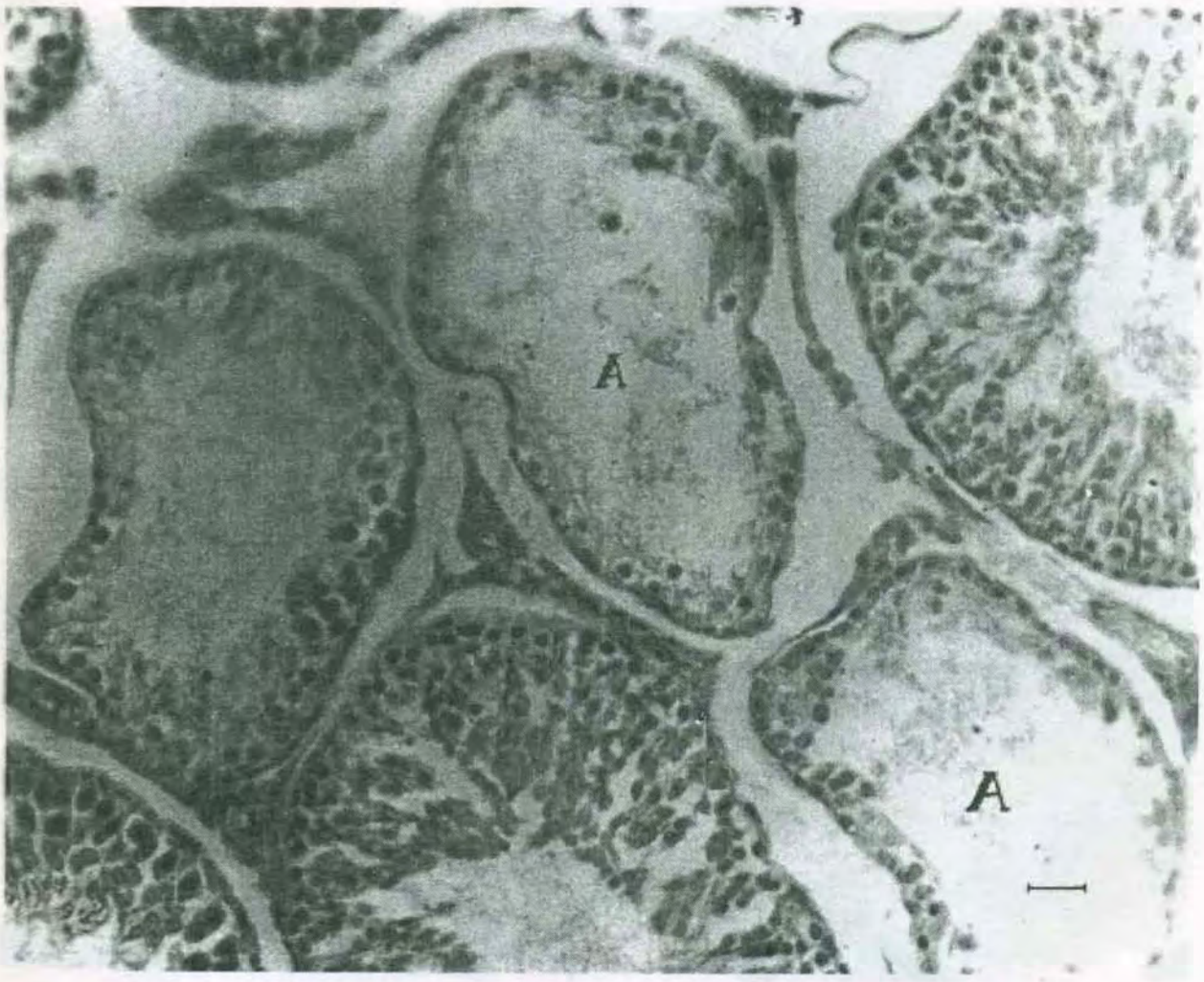

Fig. 2 : Section of testis from 5-FU (100 mg/kg) treated rat showing atrophied tubules (A) at 30 d post exposure. Other tubules show the process of degeneration.

DISCUSSION

The gonadotoxic effects of chemotherapeutic drugs are of extreme concern since they affect the fertility parameters. Primarily, the anticancer drugs are cytotoxic to spermatogonial cells (12), but their effects also extend to other somatic and germ cells in the testis (13). In the present study, 5-FU has been evaluated for its potential to induce seminiferous tubular atrophy in the rat testis. The drug has affected the testis reducing the weight in a dose dependent manner. The reduction in testis weight can be attributed for the loss of germ cells due to cell killing (14) or exfoliation. Sustained decrease of weight indicates that the testis

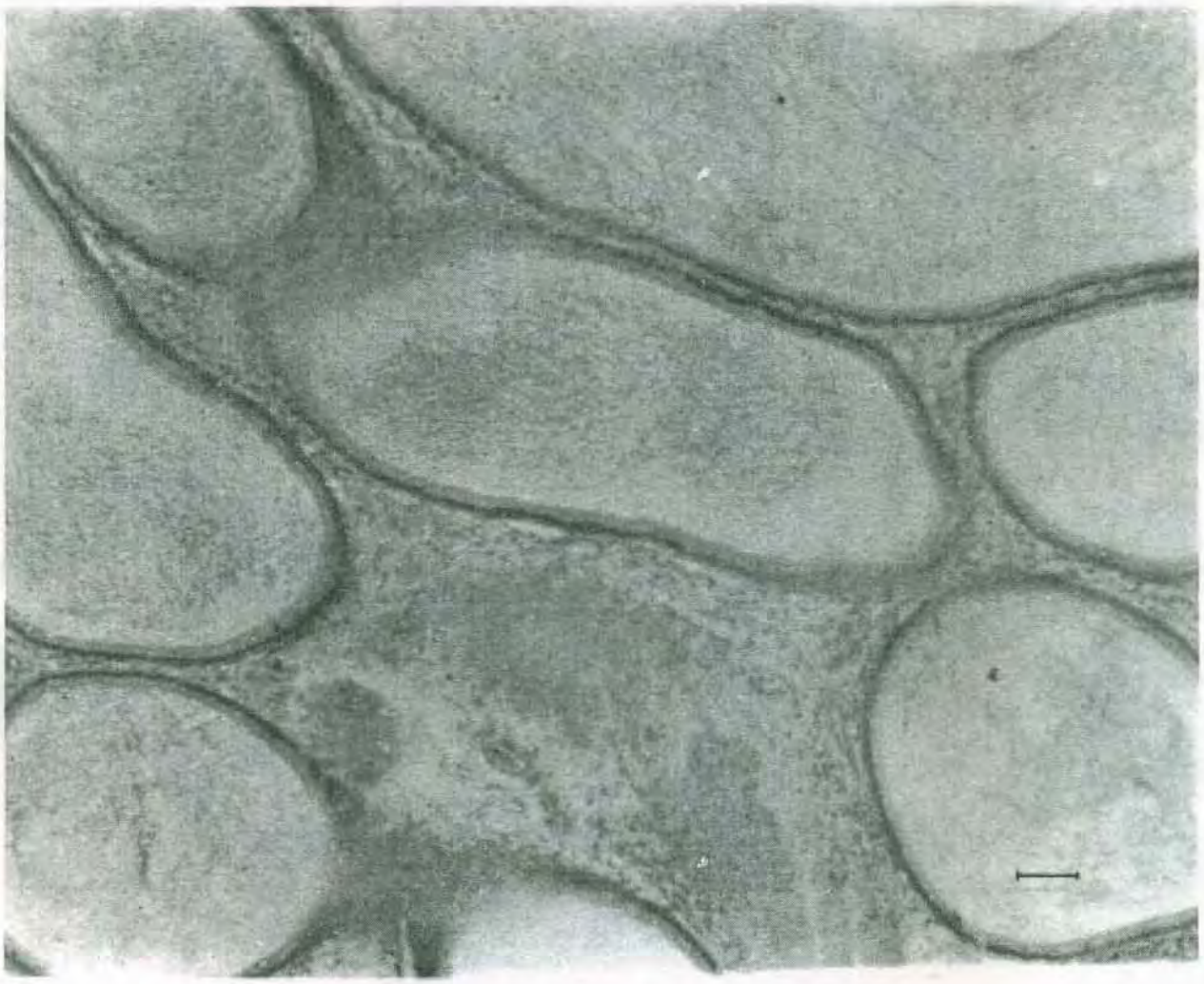

Fig. 3 : Epididymal section from control animal, showing normal appearance. Fig. 1–3, scale bar = 10 micron, H & E.

is becoming progressively atrophic. Similar reports were given previously for vepeside (15) and benomyl treated rats (16).

Decreased STD in higher doses reflects the tubular shrinkage because of the cell loss. Tubular shrinkage occurs due to the cell death (15) or sloughing of epithelial cells. Similar effects were noticed in benomyl (17) and dinitrobenzene (11) treated rats. Decreased STD also indicates that the initiation of backflow of sloughed cells does not take place in this case; hence, cells flow out of the testis and efferent ductules. If backflow takes place, STD should increase, similarly the testis weight (6). This did not happen in 5-FU treated rats indicating that 5-FU induced tubular atrophy is simply because of removal of

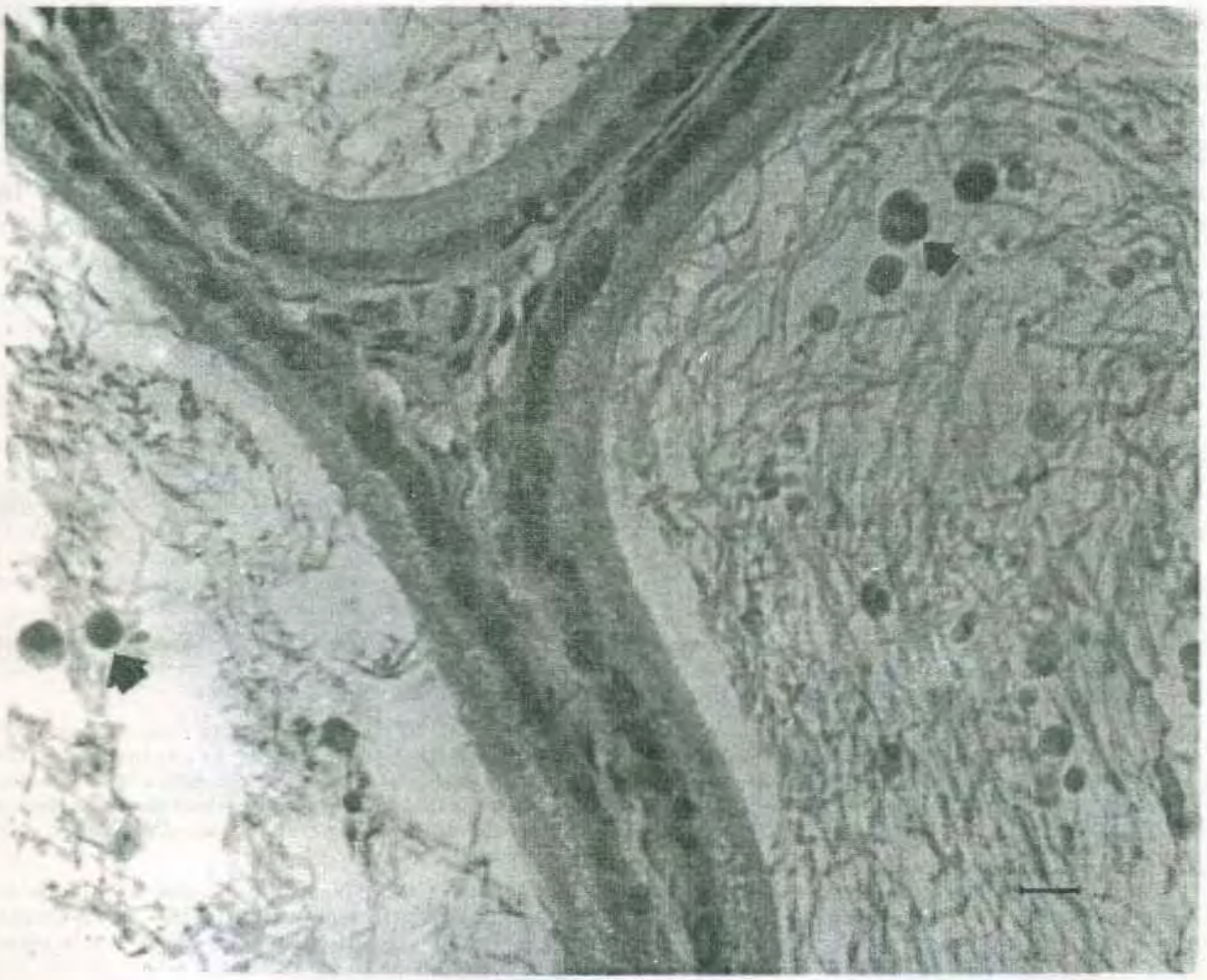

Fig. 4 : Section of epididymis from 5-FU treated animal on 3 d showing exfoliated germ cells in the lumina of epididymal tubule (arrows). Scale bar = 23 micron, H & E.

germ cells as reflected by the reduced SEH in higher doses.

Quantification of abnormal tubules also revealed that 5-FU induces tubular lesions leading to atrophy in a dose dependent manner. However, atrophied tubules were first appeared at 30 d in two higher doses. The mechanism of induction of tubular atrophy is probably due to the effect of 5-FU on Sertoli cells (4). This initiates the germ cells to undergo cell death as seen in 5-FU treated mice (12) and sloughing in rats. This is the first report on 5-FU induced tubular atrophy in rats hence comparison of our data at present is not possible.

Another hitherto unreported property of 5-FU on male reproductive system was

the dose dependent drainage of germ cells into the epididymes. It also provides an evidence that the efferent ductules were unblocked by the exfoliated germ cells. Hence, it is clear that seminiferous tubular atrophy concomitantly occurs with the drainage of germ cells into the epididymes. The germ cells disappeared in a dose dependent pattern probably due to the phagocytic activity of epididymis as seen in phosphamidon treated animals (18).

In conclusion, 5-FU causes seminiferous tubular atrophy. This effect does not depend on the post testicular ductal blockage but, depends on outflow of germ cells. In addition, 5-FU also causes the appearance of germ cells in a dose dependent manner in the epididymes, which subsequently disappear due to the phagocytosis.

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