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Methyl Isocyanate Induced Morphological Changes in the Seminiferous Epithelium of Rats Maintained on Normal or Protein Deficient Diets

M. Bose,[1,*] K. D. Vachhrajani,[1] B. S. Jha,[2] K. K. Dutta[1]

[1]Industrial Toxicology Research Centre, Post Box No. 80, Mahatma Gandhi Marg, Lucknow 226 001, India
[2]Department of Zoology, L. N. Mithila University, Darbhanga, India

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Methyl isocyanate (MIC), a pulmonary toxicant, is also reported to cause reproductive anomalies (ICMR 1986; Varma et al., 1987) MIC exposure decreased mating performance and fertility of mice and rats (Varma et al., 1987; Agarwal and Bose 1992). However, the target cell type responsible for the decreased fertility could not be identified. Therefore, present studies are undertaken to analyze the action of MIC on spermatogenesis during one cycle of seminiferous epithelium.

Protein deficiency, an important aspect of malnourishment, alter toxic potentials of various chemicals (Shakman, 1974). About 90% of the affected people of Bhopal gas calamity belonged to low income group and were categorized as malnourished (Gupta et al., 1988). Nutritional deficiency is also known to impair spermatogenesis and fertility (Vawda and Mandlwana 1990). Therefore, experiments were designed to evaluate whether the protein deficiency influence the toxic potentials of MIC on testicular tissues.

MATERIALS AND METHODS

Male albino rats, collected from ITRC, Gheru Campus breeding colony after weaning, were randomly divided into four groups (n=24 per group). Animals of two groups were fed 20% protein diet while, animals of two other groups were fed 8% protein diet (isocaloric) (Wetherholtz et al., 1969) till the sacrifice of the animals. The grouping of animals was as below :

Group I : 20% dietary, unexposed animals (control),
Group II : 20% dietary, MIC exposed animals,
Group III : 8% dietary, unexposed animals (control),
Group IV : 8% dietary, MIC exposed animals,

When the rats gained 150±10g body weight, animals of

Present address: Laboratory of Pathology, Wadsworth Centre for Laboratories & Research, N.Y. State Department of Health, Empire State Plaza, P.O. Box 509, Albany, NY 12201-0509
Correspondence to: K. K. Dutta

Groups II and IV were exposed to single dose of 1.60mg MIC/L of air for 8 min in all glass whole body inhalation chamber (21 lit capacity) in static conditions (Dutta et al., 1988). MIC (99% pure) was synthesized fresh in our laboratory by modified curtius method (Bose et al., 1990). One of the ports of the chamber was connected with a compressed air tube and through this the required amount of MIC (1.60mg/L of air) was injected into the chamber with a Hamilton microsyringe. Standardization of the doses and selection of present doses were based on our previous toxicological studies (Dutta et al., 1988). Animals of Groups I and III were given fresh air maintaining other conditions as above.

The body weight and food and water intake of the animals were recorded regularly. Animals from both the dietary treated groups and respective controls were sacrificed immediately after exposure and on days 2,5,8,10 and 12 post exposure. The duration of the seminiferous epithelial cycle (SE) is around 12 days in the rats. Therefore, the duration of 12 days selected for this experiment. Testes were cleanly dissected out and fixed in Bouin's fluid. Paraffin sections of 5 um were stained with hematoxylin and eosin..

Qualitative analysis of spermatogenesis was done at different stage groups viz., stages I-IV, V-VI, VII-VIII, IX-XII and XIII-XIV of SE. The status of seminiferous peritubular membrane and vacuolation of spermatogenic cells were critically looked into. Ten tubules were analyzed for each group on different durations. On the basis of stage-wise analysis data, quantitation of spermatogenesis was carried out at stage XI of SE. Total spermatogonia, leptotene & pachytene spermatocytes and Sertoli cells were counted in ten round tubular cross sections. The data were analyzed using student's 't' test and p value at 0.05% level was considered significant.

RESULTS AND DISCUSSION

The animals upon exposure to MIC exhibited typical symptoms like irritation, oral discharge, difficulty in breathing and severe dyspnea. The growth rate of animals maintained on 8% protein diet was comparatively slow. In 20% and 8% dietary groups weight gain was to the extent of 17g and 9g per week, during the pre exposure period. Hence, the animals of 20% dietary group reached 150g when they were around 3wks younger than low protein dietary group. MIC exposure effect on body weight was noted upto day 5 in both the dietary groups. The changes in body weight of MIC exposed rats are presented in Table 1.

In the control rats (Group 1, 20% protein diet) testicular tissues exhibited active spermatogenesis. The

Table 1. Effect of MIC exposure on the body weight (g) of rats.

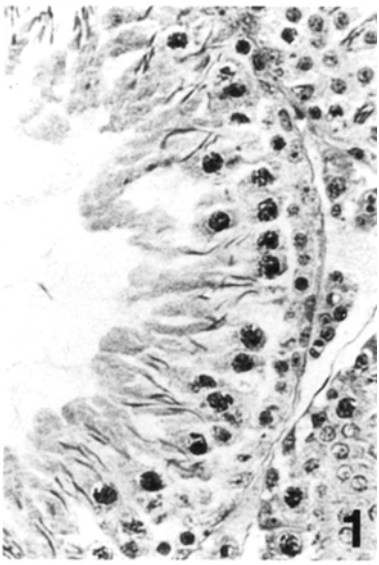
	Observation duration (days)					
	0	2	5	8	10	12
20% dietary group						
Group I	156.7 ± 6.7*	158.4 ± 6.7	164.0 ± 7.0	175.0 ±10.4	182.4 ± 8.5	188.3 ±10.2
Group II	150.0 ± 5.8	145.0 ± 2.9	146.6 ± 4.0	153.6 ± 8.3	158.0 ± 7.5	164.0 ±12.7
8% dietary group						
Group III	150.0 ± 8.2	152.1 ± 5.8	157.4 ± 6.7	164.7 ±12.1	170.8 ±10.5	174.0 ±10.9
Group IV	151.7 ± 6.0	145.7 ± 6.7	146.1 ± 8.0	150.3 ±10.4	151.6 ± 8.7	155.6 ± 9.0

*Values are Mean ± SE of 4 observations.

Table 2. Effect of MIC on contents of spermatogenic cells per tubular cross section, at stage XI of seminiferous epithelium in rats.

	SC	SG	LEPT	PACH
20% dietary group				
Group I	18.8±0.9	24.3±1.2	51.1±1.9	62.5±2.0
Group II, day 8	20.3±1.0	25.4±0.9	57.0±1.4	64.5±1.5
Group II, day 10	21.0±0.7	24.7±0.7	54.5±1.2	64.8±1.3
8% dietary group				
Group III	20.2±0.7	25.5±0.8	45.9±1.3	56.0±1.6
Group IV, day 8	20.6±1.0	24.2±1.0	63.8±1.8*	75.2±1.6*
Group IV, day 10	20.3±1.8	22.2±2.7	52.2±2.1*	64.3±2.2*

Values are Mean ± SE of 10 observations.
Sc, Sertoli cell: SG, Spermatogonia; LEPT, Leptotene spermatocytes; PACH, pachytene spermatocyte.
Significance calculated against respective controls *$p<0.05$



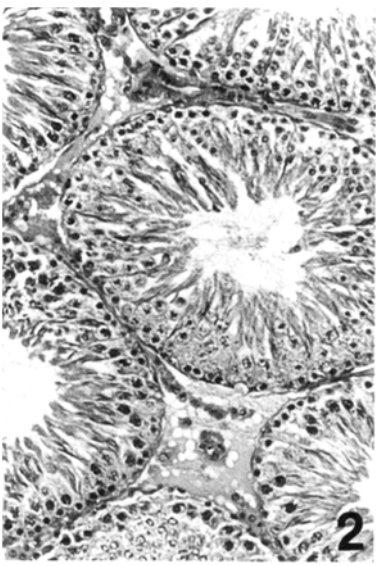


Figure 1: Transection of testis from control rat. X 250.

Figure 2: Group II testis on day 5 exhibiting interotitial fluid accumulation. X 125.

spermatogenic cell counts were within the normal range (Table 1). The testis from low protein diet control rats (Group III) apparently looked normal, however, leptotene and pachytene spermatocytes contents were lesser as compared to 20% dietary control group (Fig. 1, Table 2).

MIC exposure led to accumulation of fluid in the interstitium in group II rats on day 5 (Fig. 2). Progressive degenerative changes including disintegration of spermatogenic cell nuclei, cytoplasmic blebbing, vacuolation of some spermatid nuclei and cell loss were observed till day 8 in the same group, but the elongate spermatids at different stages were normal. The changes were more profound in Group IV, where the tubular architecture was distorted. Enormous vacuolation of Sertoli cells and stage VII-VIII round spermatids was observed on day 8 in Group IV (Fig. 3).

Stage-wise qualitative analysis showed that, in group IV tubules at stages IX-XIV were more damaged. At stage XI, increase in leptotene & pachytene spermatocytes on day 8 then after a decline towards control on day 10 (Table 2) is suggestive of delay in progression of spermatogenesis during conversion of leptotenes to pachytenes and between meiotically dividing pachytene and secondary spermatocytes. Necrosis of pachytenes was observed at later stages on day 8. By back calculation to day 1, these cells at stages XI-XIII on day 8, would have been the early pachytenes at

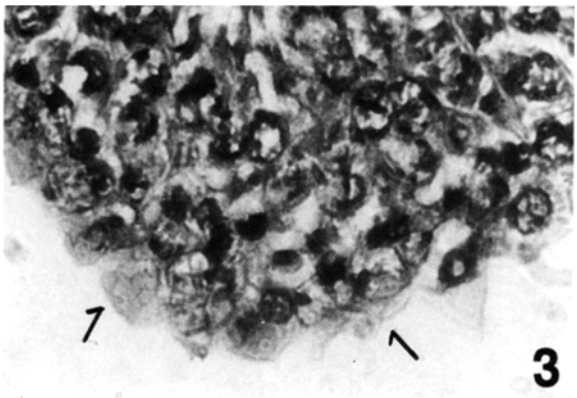


Figure 3: Testis from Group IV rat on day 8. Arrow indicated distorted peritubular membrane. X 500.

stages I-IV which after MIC exposure could develop further till stage XI and gradually degenerate beyond stage XI. Probably, the barrier system was more damaged at stages IX-XIV, therefore, MIC or its active metabolites might influence the spermatogenic cells at these stages.

Stage specific damage to spermatogenic cells may be mediated through Sertoli cells. The Sertoli cells are known to undergo morphologic and metabolic cyclic changes during SE cycle (Wright et al., 1983). Therefore, some interference with specific metabolic processes of Sertoli cells, important for particular cell type, may lead to specific cell damage.

Earlier studies from our laboratory showed impairment of mating performance and fertility of male rats during first week of MIC exposure (3.20 mg/L for 8 min) (Agarwal and Bose 1992). The epididymal spermatozoa were normal. Arora and Vijayaraghavan (1989) reported deformation of elongated spermatid in mice exposed to vapours (134 mg/L for 30 min). Large variations in the doses and duration of exposure may be responsible for such differences in observations. In present studies also we did not observe any alterations of elongated spermatids. Daniel et al. (1987) reported that MIC exposure had no effect on semen parameters after six months of exposure, an acute effect was not ruled out.

The MIC induced damage to peritubular membrane was in the order of stage IX-XII, XIII-XIV, VII-VIII, V-VI. The peritubular membrane of tubules at stages I-IV was normal. Recent studies demonstrated that MIC binds to proteins and that it crosses the blood tissue barrier (Bhattacharya et al., 1988). Possibly, MIC might alter the integrity of extra and intra tubular blood testis barrier, by binding with specific membrane proteins. However, at the moment, the variability of MIC action at different stages of SE cannot be explained.

Present studies suggested that MIC exposure cause reversible testicular damage and deficiency of protein may potentiate the effect of MIC within the frame work of these experiments.

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