In conclusion, although this report confirms the large fraction of amiodarone bound to proteins found by other investigators[18], it also strongly supports the suggestion that the reduced doses of oral anticoagulant required when patients also receive amiodarone is not due to direct displacement from the protein binding sites[25]; it might result from increased catabolism of vitamin K-dependent clotting factors[13,26], as an effect of the metabolic changes and thyroid disorders induced by amiodarone.

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Effect of mycotoxin (T-2 toxin) on catecholamine and Na^+, K^+-ATPase levels in rat epididymis

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Summary. The effect of mycotoxin (T-2 toxin) on catecholamines and Na^+, K^+-ATPase activities in rat epididymis has been evaluated. Dopamine and norepinephrine levels were significantly elevated in the caput and corpus regions whereas their levels remained unchanged in the caudal part of the epididymis. Na^+, K^+-ATPase activity was significantly increased in all the three regions of rat epididymis as a result of the toxin treatment. These changes may suggest an adverse effect on epididymal functions in rats.
Key words. T-2 toxin; catecholamine; ATPase; rat epididymis.

The epididymis plays an important role in mammalian reproduction because spermatozoa acquire their ability to fertilize the ovum during their passage from the caput to the caudal region of the epididymis[1]. Thus, the epididymis provides a suitable biochemical environment for the maturation of spermatozoa[2]. The physiological functions of the epididymis are precisely regulated by androgenic hormones, intracellular enzymes and other biochemical constituents[3]. Amongst the trichothecene group of mycotoxins, T-2 toxin has been reported to cause a variety of changes such as reduced viability of spermatozoa, aspermia and degenerative changes in the testes of animals, which affect the male reproductive function adversely[4]. These changes have been reported in the testis, but the effect of T-2 toxin on the biochemistry of epididymis has not been described so far. In the present study, therefore, we investigated the effect of T-2 toxin on the levels of catecholamines, Na^+, K^+-ATPase activity in all the three anatomical regions of the rat epididymis.

Materials and methods. Norepinephrine (NE), dopamine (DA), opthalaldehyde, adenosine triphosphate (ATP), ouabain and 1,2,4-aminonaptholsulfonic acid (ANSA) were obtained from Sigma Chemicals Co., USA. All chemicals used were of analytical grade.

Male albino rats weighing about 150 g each from the Laboratory Animal Research Division of this institute were given T-2 toxin (1.25 mg/kg b.wt) by intubation for five consecutive days orally. Symptoms such as hair erection and loss of b.wt. were observed as a result of the toxin treatment. On the sixth day the animals were sacrificed by decapitation. Epididymes were collected and divided into different segments with scissors in the cold.

Na^+, K^+-ATPase assay. ATPase was assayed by the procedure

Table 1. Effect of T-2 toxin on Na^+, K^+-ATPase of rat epididymis

Treatment	n	Caput	Corpus	Cauda
Control	6	44.66 ± 3.41	51.80 ± 3.26	49.68 ± 3.70
Toxin-treated	6	61.84 ± 3.97***	73.00 ± 4.31***	61.50 ± 4.49

Values are mean ± SE expressed in μg/h/mg protein. ***$p < 0.01$.

Table 2. Effect of T-2 toxin on the catecholamine levels in rat epididymis

Treatment	n	Caput		Corpus		Cauda	
		DA	NE	DA	NE	DA	NE
Control	6	0.498 ± 0.080	0.391 ± 0.092	0.910 ± 0.096	0.372 ± 0.071	0.647 ± 0.051	0.368 ± 0.082
Toxin-treated	6	0.847** ± 0.087	0.754** ± 0.091	1.350*** ± 0.081	0.616* ± 0.069	0.830 ± 0.093	0.561 ± 0.058

Values are mean ± SE and expressed in μg/g tissue. *$p < 0.05$, **$p < 0.025$, ***$p < 0.01$.

used by Stajanoic et al.[5], with minor modifications. Tissue samples were homogenized in ice-cold 100 mM Tris HCl buffer, pH 7.4 using a Potter Elvehjem homogenizer. Triton X-100 (0.5% v/v, final concentration) was added and after 30 min standing in the cold, the pellet was removed by centrifugation at 12,000 × g for 30 min at 4 °C. The resulting supernatant was used. $Na^+ + K^+ + Mg^{2+}$ ATPase was estimated at 37 °C; the assay mixture (2 ml) contained 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$ and 100 mM Tris HCl buffer (pH 7.4). The reaction was initiated by the addition of ATP (final concentration 2.5 mM) and allowed to proceed for 25 min at 37 °C and terminated by the addition of 0.5 ml 1 M perchloric acid. The precipitated protein was removed an the inorganic phosphorus produced in the incubation mixture was assayed by the method of Fiske and Subbarow[6]. Mg^{2+} ATPase activity was measured in the presence of 0.5 mM ouabain (without NaCl and KCl). The difference in the activities between $Na^+ + K^+ + Mg^{2+}$-ATPase and Mg^{2+}-ATPase was taken as $Na^+ + K^+$-ATPase activity. Enzyme units were calculated as the amount of inorganic phosphorus produced per mg of protein per hour. Protein was estimated by the method of Lowry et al.[7].

Estimation of dopamine and norepinephrine. Dopamine (DA) and norepinephrine (NE) were estimated fluorometrically in the tissue by extraction in the acidified butanol by the method of Sadavongvivad[8]. Percent recovery was calculated from the amount of dopamine and norepinephrine recovered from the crude butanol extract containing a known amount of the amines. The values were expressed as μg per g fresh weight of tissue.

Results. It is evident from the data presented in table 1 that the enzyme Na^+, K^+-ATPase activity was significantly increased in all three segments of the epididymis in rats treated with T-2 toxin. An increase in the enzyme activity was also observed in the cauda but it was less than that in the caput and corpus regions of the epididymis. Dopamine and norepinephrine levels were markedly elevated due to the toxin treatment in the caput and corpus regions of the rat epididymis (table 2). The increase in dopamine was more prominent than the increase in norepinephrine in the corpus region. There was no significant change in the level of these biogenic amines in the cauda region of the epididymis.

Discussion. Mycotoxins, specially T-2 toxin have been reported to affect testicular function in animals. Aspermia and degenerative changes in the testes of goats due to the injestion of T-2 toxin are known[4] but the effect on the epididymis has not been elucidated so far. In the present study an increase in DA and NE levels of caput and corpus regions of rat epididymis were observed. It has been demonstrated earlier[9] that, in the rat, damage to the germinal epithelial leads to an increase in FSH, which triggers an increased turnover of amines which may lead to higher biogenic amine levels in the reproductive organs which causes a further increase in the germinal epithelial damage. It is likely that the increased levels of catecholamine due to T-2 toxin in epididymis adversely affect the maturation of sperm, and their fertilizing ability and transport, as the caput and corpus regions of the epididymis are the main sites of sperm maturation[10], and a marked increase in DA and NE levels was observed in them.

Our results on the values of NE in the different regions of epididymis are not in agreement with those reported by Eliasson and Risley[11] who observed that NE concentration in the cauda region is seven times higher than that of caput regions whereas in our experiments there was no significant change in the concentration of NE in caput and cauda epididymis.

Further evidence for damage to the epididymis is available through our observations on Na^+, K^+-ATPase levels in the present study. The properties of the membrane bound Na^+, K^+-ATPase are known to be a sensitive indicator of changes in the cell membrane[12]. Our results presented here indicate a significant increase in Na^+, K^+-ATPase levels in all three regions of the epididymis of T-2 toxin treated rats. Thus changes in ATPase levels indicate an alteration of the epididymal membrane surface due to T-2 toxin treatment.

The relationship between catecholamines and Na^+, K^+-ATPase has been established in tissues other than reproductive organs[13,14]. In rat brain microsomes, catecholamines have been shown to activate the Na^+, K^+-ATPase activity[15]. The elevated levels of catecholamines together with Na^+, K^+-ATPase level suggest a possibility of similar changes in the epididymis also. It may be concluded from the present study that T-2 toxin may cause degenerative changes in the rat epididymis which might impair the reproductive performance of the animals. Moreover, the biogenic amines are known to affect the androgenic hormones but their effect on the androgen receptors remains to be elucidated.

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