

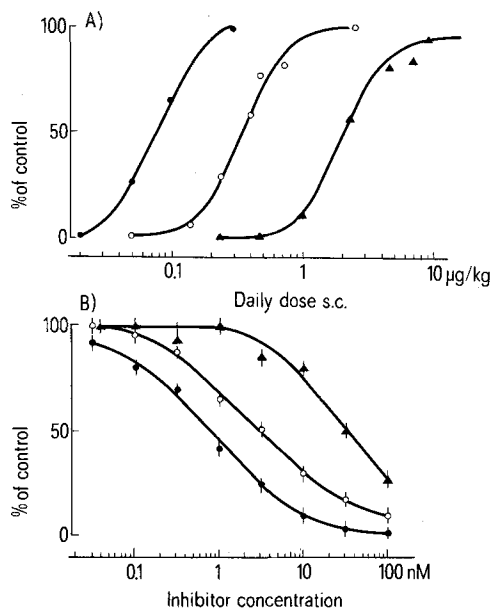
series, which in their turn were greater than those of the oestrone series. The relative potency of 17 $\alpha$ -oestradiol as compared with 17 $\beta$ -oestradiol was 0.4%.

Figure B shows the results of the in vitro tests with the same substances. As in the in vivo tests, activity declines from the normal to the D-homo substances, and further from these

to the D-homo- $\Delta^{16}$  series. The median inhibitory concentrations (IC<sub>50</sub>) are presented against the median effective in vivo doses (ED<sub>50</sub>) in the table. Statistical analysis of the values of both tests gave a correlation coefficient of  $r=0.85$  ( $p < 0.01$ ).

The oestriol results, not included in the foregoing analysis, show that this compound behaves differently from the others in both tests. Despite a good affinity for the receptor (IC<sub>50</sub>=10 nM), it has only a weak effect on the vaginal mucosa. This qualitative difference from oestradiol was also observed by Anderson et al.<sup>8</sup>, who attributed the low biological activity to a comparatively short period of stay of the oestriol-receptor complex in the nucleus.

The reduction of oestrogenic activity resulting from enlargement of the D rings is scarcely surprising in view of the fact that, in addition to 2- and 16 $\alpha$ -hydroxylation, production of a 6-membered D ring is a normal process in the metabolic inactivation of oestradiol in both rabbits and humans<sup>9</sup>.



A Number of oestrous events as per cent of all possible such events in a group of ovariectomized rats ( $n=10$ ) as a function of daily s.c. administered dose of test compound.

B Competition of test substances with <sup>3</sup>H-oestradiol for the uterine cytosol receptor expressed as per cent inhibition of oestradiol binding ( $n=5$ ). Results are mean values  $\pm$  SEM. Symbols: ●—● 17 $\alpha$ -ethinyloestradiol, ○—○ oestradiol, ▲—▲ oestrone. Note the differing abscissas.

- 1 Acknowledgment. I thank Dr R. Krause for his help and advice in preparation of the manuscript.
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## Effect of borax on testis of Indian desert gerbil, *Meriones hurriane* Jerdon

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**Summary.** Borax was injected at a dose level of 250 mg/kg b.wt for 16 days (total dose 4 g/kg b.wt) s.c. to active adult male gerbils. Borax caused several degenerative changes in the testes, of which giant cell formation, pyknosis and exfoliation are prominent. The increased activity of phosphatases was also noticed.

Several metallic salts have been said to evoke alterations in the testis, of which the compounds of lithium, molybdenum, thallium manganese, lead, cadmium seems to be the most effective<sup>2-4</sup>. Zinc and selenium salts have been reported to be antagonistic to these salts<sup>5-7</sup>. Borax, a compound of sodium, Boron and Oxygen (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>), has been reported to be effective on the female reproductive system in some ayurvedic literature. The aim of present study is to evaluate its effect on the testis.

Active adult male gerbils of approximately equal b.wt were collected locally and maintained under suitable laboratory conditions for a few days and were sorted out into 2 groups of 6 each. The animals of the 1st group were given 16 daily s.c. injections of borax in water at a dose level of 250 mg/kg

b.wt (total dose 4 g/kg b.wt). The 2nd group received the same volume of saline in same way and served as controls. The animals were sacrificed 24 h after the last injection. 1 testis was immediately processed for histological studies. Biochemical estimations for enzymes listed in the table were carried out using standard techniques.

**Result and discussion.** None of the animals showed sign of morbidity or mortality. There was no variation in the b.wt and the testicular weights in both the groups. The testis of the control animals exhibited normal spermatogenesis leading to the formation of motile spermatozoa. Testis of the treated animals showed degenerative changes in the seminiferous tubules. The reduction in the diameter of seminiferous tubules was noticed.

Exfoliation of the different stages of the germ cells was noticed. Some of the tubules showed all the representative stages of the spermatogenesis but in a disturbed form. Giant multinucleated cells with 2-6 nuclei were frequently observed. Pyknosis was also observed in a number of cells. There was no change in the structure and the population of the leydig cells.

The activity of acid and alkaline phosphatases, AtPase and glucose-1-phosphatase (G-1 pase) in the testes of the control and experimental animals have been recorded in the table.

Effect of borax (4 mg/kg b. wt) on testes of active adult gerbil

	Control animals	Experimental animals
B. wt in g before experiment	71.1 $\pm$ 3.9	73.7 $\pm$ 4.5 ( $p < 0.1$ )
B. wt in g after experiment	73.7 $\pm$ 2.5	72.8 $\pm$ 1.4 ( $p < 0.1$ )
Testicular wt in mg	227.8 $\pm$ 5.1	222.5 $\pm$ 21.1 ( $p < 0.1$ )
Alkaline phosphatase (Bodansky units)	30.57 $\pm$ 1.6	64.85 $\pm$ 1.16 ( $p < 0.001$ )
Acid phosphatase (Bodansky units)	2.66 $\pm$ 0.29	5.20 $\pm$ 0.11 ( $p < 0.001$ )
Adenosine triphosphatase (Bodansky units)	147.42 $\pm$ 1.2	165.42 $\pm$ 8.9 ( $p > 0.05$ )
Glucose-1-phosphatase (Bodansky units)	10.66 $\pm$ 0.47	17.33 $\pm$ 0.43 ( $p < 0.001$ )

Acid and alkaline phosphatases and G-1 pase showed significant increase in the treated animals when compared with controls. ATPase also showed an increase in the activity but this was not significant.

Metallic compounds have been known to exert their toxic effects by effecting cellular physiology in several ways<sup>6</sup>. Perhaps the most vital cellular constituent effected by metals are enzyme systems which are concerned with virtually every aspect of cellular activity. Besides acting on enzyme molecule itself, they also alter the normal functioning of the substrates co-factors and the activators, the factors which are responsible for the normal enzyme activity.

Results of the present study show that borax evokes several types of degenerative effects in the tests. In contrast to other metallic compounds, it does not evoke mass degeneration. However, the mechanism of the action of borax may be the same as that of other metallic compounds. The increased activity of acid phosphatase may be due to the release of nonspecific phosphatases from the lysosomes of the degenerating cells.

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### Effect of actinomycin D or puromycin on microsomal testosterone hydroxylase activity enhanced by testosterone in female rat liver

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**Summary.** The injection of testosterone propionate for 4 successive days into female rats resulted in an increase of the in vitro conversion of the hydroxylated testosterone from testosterone by the hepatic microsomal fraction, but no change in the content of microsomal cytochrome P-450 occurred. Actinomycin D or puromycin, which was administered for 4 days with injections of testosterone propionate, prevented the enzyme induction.

Androgen metabolism is sex-dependent in rats<sup>1-3</sup>. Testosterone is hydroxylated at 6 $\beta$ -, 7 $\alpha$ - and 16 $\alpha$ -positions by liver microsomes, but the conversion of testosterone to such polar products is low in females. Injection of testosterone into female rats tends to induce a male-type of steroid metabolism<sup>4</sup>, and injection of estrogen into male rats tends to induce a female-type of steroid metabolism<sup>5</sup>. The investigations described here were undertaken in order to clarify the mechanism of induction of microsomal testosterone hydroxylase by testosterone treatment in the liver of female rat.

**Material and methods.** 28 female rats of the Wistar strain, aged 9-11 weeks, were divided into 6 groups. In the experimental groups (groups B-F), testosterone propionate, dissolved in a small volume of ethanol and diluted with corn oil, was injected s.c. for 4 days. In group C or D, actinomycin D (Merck) or puromycin (Sigma), each dissolved in 0.2 ml of saline solution, was administered i.p. for

4 successive days whenever an injection of testosterone propionate was given. To group E or F, actinomycin D or puromycin was given i.p. only once when the final injection of testosterone propionate was performed. The control rats (group A) received the vehicle only. Details are described in the legend of figure 1. The animals were killed by decapitation 48 h after the final injection. The microsomal pellets which were prepared according to Ota et al.<sup>5</sup> were suspended in 0.25 M sucrose solution. The microsomal fraction, equivalent to 250 mg of the liver, was incubated with 50  $\mu$ g of testosterone containing 0.5  $\mu$ Ci of 4-<sup>14</sup>C-testosterone (New England Nuclear, sp. act. 57.5 mCi/mmole) which was purified by TLC on silica gel immediately before use, for 60 min at 37°C under the bubbling of O<sub>2</sub> and CO<sub>2</sub> in the presence of NADPH according to our previous method<sup>6</sup>. Immediately after incubation, the steroids were extracted twice with methylene dichloride. Isolation of the labelled metabolites from