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 ±3.9	73.7 ±4.5 ($p<0.1$)
B. wt in g after experiment	73.7 ±2.5	72.8 ±1.4 ($p<0.1$)
Testicular wt in mg	227.8 ±5.1	222.5 ±21.1 ($p<0.1$)
Alkaline phosphatase (Bodansky units)	30.57±1.6	64.85±1.16 ($p<0.001$)
Acid phosphatase (Bodansky units)	2.66±0.29	5.20±0.11 ($p<0.001$)
Adenosine triphosphatase (Bodansky units)	147.42±1.2	165.42±8.9 ($p>0.05$)
Glucose-1-phosphatase (Bodansky units)	10.66±0.47	17.33±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[6]. 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 testosterones 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[1-3]. Testosterone is hydroxylated at 6β-, 7α- and 16α-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[4], and injection of estrogen into male rats tends to induce a female-type of steroid metabolism[5]. 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.[5] were suspended in 0.25 M sucrose solution. The microsomal fraction, equivalent to 250 mg of the liver, was incubated with 50 µg of testosterone containing 0.5 µCi of 4-^{14}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_2 and CO_2 in the presence of NADPH according to our previous method[6]. Immediately after incubation, the steroids were extracted twice with methylene dichloride. Isolation of the labelled metabolites from

the pooled extract and identification of these steroids were performed according to Ota et al.[5] and Sato et al.[7]. The radioactivity was measured with a liquid scintillation counter (Aloka LSC601, Tokyo). The efficiency of counting ^{14}C was about 74%. The activity of testosterone hydroxylase was tentatively expressed as the sum of 6β-, 7α- and 16α-hydroxytestosterones formed from the labelled testosterone after 60 min incubation with the microsomal fraction. The protein content of the microsomal fraction was measured by the copper-Folin method[8] and the cytochrome P-450 content was measured according to Omura and Sato[9].

Results and discussion. As shown in figure 1, no significant difference was observed in the cytochrome P-450 contents among the experimental groups except group D. Figure 2 shows testosterone-hydroxylase in hepatic microsomes of testosterone-treated rats with or without antibiotics. Cytochrome P-450 is involved in steroid hydroxylation, and the sex difference in the binding capacity of cytochrome P-450 with testosterone is suggested as a major factor responsible for the sex difference in the hydroxylating activities of steroid hormones in rat liver microsomes[10,11]. In this study, therefore, the hydroxylase activity was expressed per nmole of microsomal cytochrome P-450 as well as per mg of microsomal protein. Although administration of testosterone into female rats did not increase the contents of cytochrome P-450 per mg protein, it increased the activity of hydroxylase per mg protein (female: testosterone-treated female = 1:1.3) and per nmole of cytochrome P-450 (female: testosterone-treated female = 1:1.5). These findings suggested that the activity of hydroxylation of testosterone in female rats was influenced without change in the cytochrome P-450 contents.

Testosterone hydroxylase activity enhanced by testosterone was blocked by actinomycin D, an inhibitor of mRNA synthesis, and puromycin, an inhibitor of protein synthesis, both of which were given for 4 days with testosterone propionate to female rats. However, the content of cytochrome P-450 per protein in group C or D receiving actinomycin D or puromycin for 4 days was similar to that in group B receiving only testosterone. These findings indicate that the action of these antibiotics on the enzymatic induction is probably not related to the decrease of microsomal cytochrome P-450 content. It was observed that the enhancement of hydroxylase activity was maintained for at least 48 h after discontinuation of testosterone-treatment. Neither actinomycin D nor puromycin showed a suppressive effect on the induced activity of hydroxylase, when injected only once simultaneously with the final injection of testosterone. The finding suggested the possibility that the induced enzymes persisted for at least 3 days. The observations obtained here may suggest that DNA-mediated RNA synthesis and also protein synthesis are involved in the induction of the enzymes by testosterone propionate, the former being predominate. Therefore, the induction of the microsomal hydroxylase by testosterone might be due to promotion of the synthesis of the enzyme.

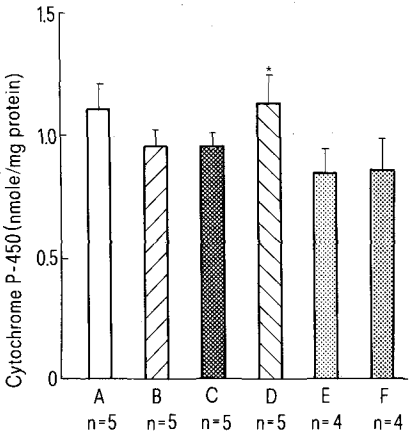

Fig. 1. Effects of actinomycin D or puromycin on the cytochrome P-450 in hepatic microsomes of testosterone-treated female rats. Group A was the control receiving the vehicles only. Groups B–F were treated with daily s.c. injections of testosterone propionate (2.5 mg/100 g b.wt) for 4 days. In addition, group C received 10 µg (8 nmoles) of actinomycin D and group D received 50 µg (106 nmoles) of puromycin for 4 days, respectively. Group E received 10 µg of actinomycin D and group F received 50 µg of puromycin, once with the final injection of testosterone propionate, respectively. n, Number of rats in each group. Each bar represents the means ±SD. *Different from group B by Student's t-test, $p < 0.01$.

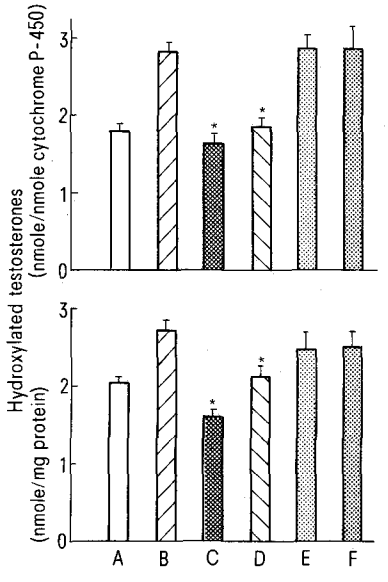

Fig. 2. Effects of actinomycin D or puromycin on hydroxylation of testosterone in hepatic microsomes of testosterone-treated female rats. Treatment details are given in the legend for figure 1. 4-^{14}C-testosterone was incubated with microsomal fraction (equivalent to 250 mg liver) at 37 °C for 60 min under bubbling of O_2 and CO_2 (95:5, by vol.) in the presence of NADPH (500 µg). * Different from group B by Student's t-test, $p < 0.001$.

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