Testicular Δ^5-3β-hydroxysteroid dehydrogenase, ascorbic acid and dehydroascorbic acid in actinomycin D-treated toads

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Summary. Unilaterally intratesticular injection of actinomycin D in toad inhibited Δ^5-3β-hydroxysteroid dehydrogenase activity with significant increase of dehydroascorbic acid and decrease of ascorbic acid level in the testis.

The previous studies of Biswas and Mukherji[3] have demonstrated that spermatogenesis in toad is stimulated under the condition of increased testicular ascorbic acid (AsA). Again AsA in combination with gonadotropins increase the activity of testicular Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD), concerned with steroid hormone synthesis in the toad[4,5]. This vitamin in the form of dehydroascorbic acid (DHA) stimulates Δ^5-3β-HSD activity by oxidation of reduced diphosphopyridine nucleotide (DPNH)[6], while gonadotropins promote synthesis of Δ^5-3β-HSD[7] by increasing protein synthesis[8]. Since the antibiotic actinomycin D (AMD) inhibits testicular protein biosynthesis in response to follicle stimulating hormone (FSH)[9-12], reduces the rate of progesterone synthesis in the follicles and inhibits spermatogenesis in the toad[13], the present investigation has been undertaken to explore the effects of AMD on Δ^5-3β-HSD activity and relationship with AsA and DHA content in toad testis.

Material and methods. 16 adult male toads *(Bufo melanostictus)* weighing 60 g were used in the present investigation during the breeding season (June-August). AMD (obtained from Merk, Sharp, and Dohme, Rahway, New Jersey) was dissolved in amphibian saline. Intratesticular injections of 0.02 ml were administered unilaterally through a midventral incision under ether anesthesia. 10 animals were injected with 0.4 µg of AMD in amphibian saline into the right testis and with amphibian saline (0.02 ml) into the left testis. 6 control animals which were not exposed to AMD, received intratesticular injections of saline (0.02 ml/testis); 72 h after treatment, all the animals were sacrificed together with controls. The testes from all the animals were removed and fresh frozen cryostat sections of 20 µm were mounted on coverslips. Δ^5-3β-HSD activity was determined by incubation at 37 °C in a substrate (dehydroepiandrosterone) medium described by Dean et al.[14]. Parallel sections incubated for 60 min at 37 °C in a medium containing no substrate served as controls. After incubation, sections were fixed and mounted in glycerine jelly. AsA and DHA content of the testes were estimated by DNPH method developed by Roe and Kuether[15].

Results. Histochemical preparations showed Δ^5-3β-HSD activity in both the tubular and Leydig cells of saline-injected control animals (figure 1). Enzyme activity in the AMD-treated right and saline-treated left testis appeared to decrease markedly in both the tubular and Leydig cells, compared with controls (figure 2). Δ^5-3β-HSD activity of the AMD-treated right testis showed no significant difference with the saline-treated testis. Section of the testes incubated in a substrate-free medium showed no activity of the enzyme. Biochemically, DHA content in the AMD and saline-treated testes appeared to increase, while AsA and total ascorbis acid (AsA + DHA) were decreased significantly in both the testes over the controls (table).

Discussion. The present study shows a marked fall of Δ^5-

Effect of actinomycin D on testicular ascorbic acid and dehydroascorbic acid of toads (mg/100 g of testis)

Treatment	Ascorbic acid (AsA)	Dehydroascorbic acid (DHA)	Total ascorbic acid (AsA + DHA)
Control	77.14 ± 3.60*	10.29 ± 2.47	86.92 ± 1.99
Saline (left testis)	51.58 ± 5.40	19.92 ± 2.92	68.83 ± 5.82
AMD (right testis)	44.90 ± 4.63	27.60 ± 3.13	62.31 ± 4.66
Control vs saline	$p < 0.01$	$p < 0.05$	$p < 0.01$
Control vs AMD	$p < 0.001$	$p < 0.01$	$p < 0.001$

*Mean ± SE.

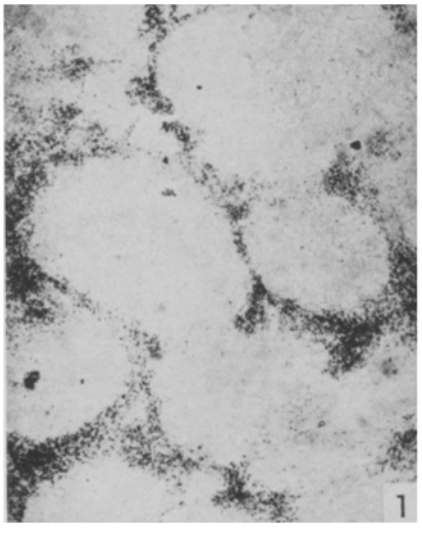

Fig. 1. Δ^5-3β-hydroxysteroid dehydrogenase in the testis of the control toad. × 96.

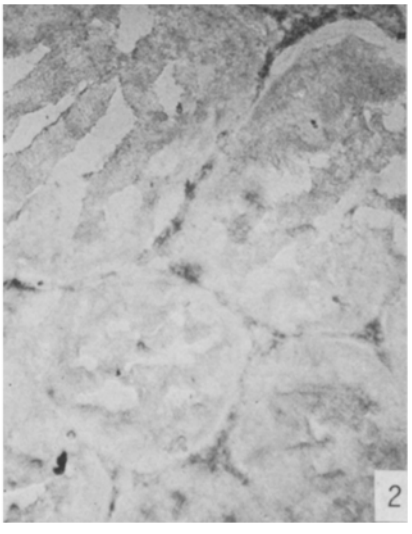

Fig. 2. A marked decrease in Δ^5-3β-hydroxysteroid dehydrogenase in the AMD-treated testis. × 96.

3β-HSD activity and AsA content, while DHA is significantly elevated in both testes after unilateral AMD injection. The enzyme Δ^5-3β-HSD is present in both the tubular and Leydig cells, as was observed previously[6,16]. Testicular Δ^5-3β-HSD activity in toad is known to be stimulated by AsA and DHA[4,6]. AsA is converted to DHA in the testis of the toad[17] and DHA possibly stimulates Δ^5-3β-HSD activity by oxidizing reduced DPN[6]. Thus the oxidized form of AsA (DHA) is reduced to AsA reversibly by steroid dehydrogenase. It seems reasonable to speculate that the increase in DHA and decrease in AsA in the testes after AMD injection may be due to decreased Δ^5-3β-HSD activity. On the other hand, testicular steroid hormones, related to the enzyme Δ^5-3β-HSD, stimulate the synthesis of ascorbic acid in the kidney of toad[18]. So the fall of total ascorbic acid is possibly the result of less ascorbic acid synthesis due to the inhibition of Δ^5-3β-HSD activity in AMD-treated toads.

The mechanism by which AMD decreases Δ^5-3β-HSD activity in the toad testis has not been established. AMD is known to inhibit DNA-dependent RNA synthesis[19,20]. This antibiotic also reduces the basal rate of progesterone synthesis and luteinizing hormone-induced progesterone secretion in the follicles[12]. Savard et al.[21] have reported that AMD, as well as puromycin, inhibits steroidgenesis in the bovine corpora lutea. The present investigation shows that AMD possibly decreases Δ^5-3β-HSD activity by inhibiting protein synthesis in the toad testis, and the effect of AMD, unlike in the hamster[22], is not confined to the unilateral site of injection. This could be explained by the fact that the blood-testis barrier responsible for permeability restrictions in the higher vertebrates[23] is not well developed in toad.

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2 Acknowledgments. Thanks are due to Prof. C. Deb, Department of Physiology, Calcutta University, for his suggestions and constant encouragment. Thanks are also extended to Mr R.K. Bhattacharya, Microphotographer, Department of Physiology, for his kind cooperation.

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DISPUTANDUM

Comments on the significance of the quasi-valence number for chemical carcinogenesis

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Summary. A quasi-valence number of less than 3.20 was reported to be significant in correlating carcinogenicity. This criterion has no meaningful relationship since such a large proportion of organic compounds fall in this group that it provides no selectivity.

The recent correlation of the quasi-valence number with carcinogenicity appeared to provide a convenient and simple method of identifying those compounds which might be chemical carcinogens[2]. This concept, to be significant, would have to provide a structural screen which would eliminate a large number of structures which are not carcinogenic while identifying most of the structures of active compounds. The quasi-valence number in our opinion does not meet either of these important requirements. The quasi-valence number Z* is defined by the equation:

$$Z^* = \frac{\sum_{i=1}^{m} N_i Z_i}{\sum_{i=1}^{m} N_i}$$

N, number of atoms of *i*th type, Z, number of valence electrons on atoms of the *i*th type (halogens = 1).

The maximal limit of 3.20 for Z* does not exclude a significant number of organic structures which are not carcinogens. All saturated compounds of the general formula C_nX_{2n+2} where X is univalent (hydrogen or halogen) will fall in the category of potential carcinogenic compounds. Any unsaturated or cyclic organic compound with greater than (0.35)X for every carbon will have Z* values below 3.2 and may be presumed to be carcinogenic. Saturated compounds which would give the largest Z* values are the oxygenated compounds such as sugars which contain large numbers of atoms with many valence electrons. The structures $C_nX_{2n+2}M_n$ and $C_nX_{2n}M_n$ where X is univalent and M is oxygen or sulfur all fall in the category of potential carcinogenic compounds. Only if more than one-third of the oxygens are in the carbonyl form ($C_nX_{2n-2}M_n$) or if the hydroxyl groups are converted to methanesulfonate esters, do compounds fall in the non-carcinogenic category with $Z^* > 3.2$. Ironically, some of these compounds, mannitol myleran, for example, are carcinogens.