Effect of retinyl acetate feeding on the activity of testicular enzymes in young rats

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Summary. Feeding of excess vitamin A significantly increased the activity of β -galactosidase and β -glucuronidase without effecting the activity of acid and alkaline phosphatase in rat testicular homogenates. It is suggested that imbalance of vitamin A affects the normal process of spermatogenesis by altering the activity of lysosomal enzymes.

The importance of vitamin A in testicular development, maintenance of spermatogenesis and regulation of epithelial differentiation is well recognized2. However, little is known about the mode of action of vitamin A in these processes. Spermatogenesis involves a complex process of cell differentiation involving biochemical changes in the cellular composition of the testis and leading to the formation of highly specialized cell-spermatozoon. It has been earlier reported that imbalance of vitamin A; hypo- or hyper-vitaminosis A, resulted in alterations in the activity of various testicular enzymes thereby causing impairement of spermatogenesis³⁻⁵. Lysosomal enzymes are considered to play an important role in the process of spermatogenesis^{6,7}. Therefore, the present investigation was undertaken to study the effect of excess of vitamin A on lysosomal and other enzymes in the testes of weanling rats in 2 age groups. Materials and methods. Male albino rats (28 days old) of the Institute colony were used and were divided into 2 groups: group A received orally 24,000 RE of retinyl acetate (1200 RE/day/rat for 20 days) and group B received 28,800 RE (1200 RE/day/rat for 24 days) in groundnut oil. Control rats received an equivalent amount of groundnut oil. Rats were killed at the age of 48 days (group A) and 68 days (group B) by decapitation. The testes were removed, decapsulated and homogenized in cold saline. Activities of acid phosphatase, alkaline phosphatase, β -glucuronidase and β galactosidase were assayed according to methods described earlier⁶⁻⁸ and expressed as nmoles of p-nitrophenol liberated/min/mg of protein for acid and alkaline phosphatase and β -galactosidase and absorbance at 540 nm/min/mg of protein for β -glucuronidase. All the substrates used were obtained from Sigma Chemical Co., USA. Protein was estimated in homogenates by the method of Lowry et al.9. Results and discussion. Feeding of vitamin A did not change the general condition of the animals. However, rats showed typical signs of hypervitaminosis A at the later stage of

feeding. Results obtained showed a significant increase in the activity of β -galactosidase and β -glucuronidase in retinol fed-rats as compared to controls in both groups A and B, while no difference was observed in the activity of acid and alkaline phosphatases under similar conditions, as shown in the table.

It has been reported that the activity of β -galactosidase and β -glucuronidase is highest during the period of spermatocyte formation and decreased markedly during the further development of germinal epithelium. On the other hand the activity of acid phosphatase was lower at the initial stages of spermatogenesis and highest at maturity of spermatozoa^{6,7}. In the present study, a significant increase in the activity of β -galactosidase and β -glucuronidase in retinyl acetate-fed rats has been observed, suggesting an alteration in the normal course of enzymatic activity. It may be further emphasized that excess of vitamin A selectively affected the activity of β -galactosidase and β -glucuronidase and not the activity of acid phosphatase which is also considered to play an important role in the process of spermatogenesis. Moreover, the effect of excess of vitamin A is more pronounced in younger rats and seems to be long lasting, as in group B, the vitamin A feeding was discontinued for 15 days after 24 days of feeding to see the restoration of the enzyme activities, but the alterations in the activity of enzymes continued to exist.

Results of the present study, along with those reported earlier³⁻⁵, are compatible with the view that the excess of vitamin A caused pronounced biochemical changes in rat testes and alterations in the activity of testicular lysosomal enzymes thereby causing testicular degeneration and delay in spermatogenesis as demonstrated by Maddock et al. ¹⁰ and Lopes et al. ¹¹. This may be of great importance in further understanding of the biochemistry of spermatogenesis, the mode of action of vitamin A and the regulatory mechanisms involved.

Activity of various enzymes in testes homogenates of rats fed retinyl acetate

Enzymes	Group A Control	Experimental	Group B Control	Experimental
Alkaline phosphatase Acid phosphatase β-galactosidase β-glucuronidase	32.0±6.30* 57.0±5.30 2.0±0.18 5.5±0.86	41.0 ± 8.60 61.0 ± 6.00 $3.0 \pm 0.32***$ $8.9 \pm 2.40**$	67.0 ± 16.00 51.0 ± 11.00 1.8 ± 0.34 1.5 ± 0.22	61.0±11.50 57.0±18.00 2.5± 0.28*** 4.5± 1.50***

^{*} Each figure is the mean \pm SD from 5-6 experiments. ** p < 0.01; *** p < 0.005.

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