

EFFECT OF TESTOSTERONE PROPIONATE ON GLYCOPROTEINS FROM SUBMANDIBULAR AND SUBLINGUAL GLANDS OF MALE MICE

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(Received 12th December 1998)

SUMMARY

The effect of testosterone on glycoprotein synthesis in salivary glands has been worked out. When salivary gland sections were stained for Periodic acid (PAS) and Alcian blue (AB) techniques, it was observed that glycoproteins were mainly located in the secretory acini of salivary glands. The staining intensity was reduced in castrated mice and increased after the injection of testosterone (4mg / 250g body wt.). Soluble glycoproteins from salivary glands were isolated from defatted powder of the glands with 1 M CaCl₂. In juveniles, concentrations of hexoses and sialic acid were low, increased in juvenile adults and were maximum in adults. In adult castrated mice, these concentrations decreased and upon testosterone propionate administration, they increased. Soluble glycoproteins were separated on 5% polyacrylamide gel electrophoresis. With AB pH 2.5 and AB pH 1 there were 2 bands for the submandibular gland for glycoproteins and 8 and 9 bands for AB pH 2.5 and pH 1.0, respectively. In both the glands some bands completely disappeared and some of them were reduced in their intensity in castrated mice and intensity was regained after the injection of testosterone. The results suggest that testosterone plays an important role in the synthesis of glycoproteins in acinar cells of salivary glands.

Key words : Acinar cells; Glycoproteins; Sublingual gland; Submandibular gland; Testosterone propionate.

INTRODUCTION

The granular convoluted tubules (GCTs) of the submandibular gland are known to be trophically influenced by hormones like, testosterone, thyroxine and adrenocortical steroids (1-11). The submandibular gland contains a number of biologically active polypeptides like NGF (12-14), EGF (9,15), renin (16), kallikreins and proteases (17-19). Most, if not all polypeptides are localized in and synthesised by cells of GCTs (20), and their concentration is increased in the presence of androgen (6,9,15, 21-23).

Activities of proteases (17,21, 24-29), amylase (25, 29-31), G-6-P dehydrogenase (32)

and concentrations of proteins (5) sialic and (33-37) and m-RNAs (23) are influenced by testosterone in salivary glands. However, on the sulfated glycoproteins which are part of acid glycoproteins and are synthesized in submandibular and sublingual glands. Secondly, though sublingual gland has its major share of glycoproteins in the saliva, it has been totally neglected for investigation, perhaps due to its small size. Therefore, the present study is aimed to know the effect of testosterone propionate on acid glycoprotein synthesis in mouse submandibular and sublingual glands.

MATERIALS AND METHODS

Ten male mice (*Mus musculus*) from each of the following group were used for the present investigation. They were supplied with Gold-Mohur mouse feed (Lipton India Ltd.) and drinking water *ad-libitum*.

They were grouped into five.

Group 1. Juvenile group : (3 to 5 weeks)

Group 2. Juvenile adult group : (6 to 12 weeks)

Group 3. Adult group : (16 to 28 weeks)

Group 4. Adult castrated (control) : They were 16 to 28 weeks old and received olive oil (0.8 ml/250 gm body wt.), subcutaneously for three days from the 4th day after castration.

Group 5. Adult castrated mice treated with testosterone propionate : This group consisted of 16 to 28 weeks old mice and received testosterone propionate (4 mg in 0.8 ml olive oil/250 gm body wt.), subcutaneously from the 4th day after castration for three days. On the 4th day after the first injection, the animals were sacrificed.

The mice from all the groups were sacrificed between 9.0 to 11.0 am., by cervical dislocation and salivary glands were dissected, pulled and used for the isolation and histochemical demonstration of glycoproteins.

Histochemical demonstration of glycoproteins : Salivary glands were fixed in 2% calcium acetate formalin at 8°C for 24 hrs. Paraffin embedded sections were used for the demonstration of glycoproteins.

- a. Periodic Acid - Schiff Reaction (PAS) : This method was used for the identification of glycoproteins. A +ve staining reaction is given by all neutral glycoproteins and acid mucopolysaccharides (38,39).
- b. Alcian blue (AB) at pH 2.5 : This technique was carried out for the demonstration of acidic glycoproteins in the salivary glands (40)

- c. AB at pH 1.0 : This method was used for the identification of sulfated acidic glycoproteins (40).

Isolation of glycoproteins : Soluble glycoproteins were isolated from the salivary glands as described by Smith *et al* (41). The residue was dissolved in a small amount of water and used for the estimation of carbohydrate components and for the electrophoretic separation of glycoproteins

- a. Determination of Hexoses : Estimations of hexoses were carried out using isolated glycoprotein using D-glucuronic acid as standard (42).
- b. Estimation of sialic acid : Estimation of sialic acid was carried out using thiobarbituric acid reagent and N-acetylneuraminic acid as standard (43).

Electrophoretic separation of glycoproteins (44) : Glycoproteins were separated on polyacrylamide gel electrophoresis (PAGE) and were stained with AB pH 2.5 and AB pH 1.0 (45). Gels were fixed and stored in 5% glacial acetic acid. Stained gels were photographed and the gels for glycoproteins were scanned using Shimadzu UV-240 spectrophotometer at 520 nm.

RESULTS

- a. *Histochemical demonstration of glycoproteins* : In PAS stained sections of submandibular and sublingual glands, acini from both the glands (Plate No. 1, figs 1 to 6) and granular convoluted tubules from submandibular glands (figs. 1 to 3) revealed magenta colour. Acini were intensely stained in adult glands (figs. 1 & 4). In castrated mice, PAS + vity was reduced in acini (figs. 2 & 5) and GCT cells (fig. 2), whereas on testosterone administration, the normal intensity of staining was obtained (figs. 3 & 6).

Submandibular acini (Plate No. 2, fig. 7) exhibited intense staining reactivity with AB pH 2.5. Number of AB pH 2.5 positive acini was reduced compared to PAS +ve acini. It was reduced in castrated (Plate No. 2 fig. 8) and increased after the injection of testosterone to castrated mice (Plate No. 2, fig. 9). All PAS +ve acini of sublingual glands were stained for Ab pH 2.5. Staining intensity was maximum in adult, reduced in castrated and regained after testosterone injection (Plate No. 2 figs. 10, 11 & 12).

When sections of submandibular (Plate No. 3 fig 13) and sublingual (Plate No. 3 fig. 16) glands were stained with AB pH 1.0 they exhibited intense staining reactivity, which is partly lost in both submandibular (Plate No. 3 fig. 14) and sublingual (Plate No. 3 fig. 17) gland sections of castrated mice but normal staining intensity was obtained after testosterone injection (Plate No. 3 figs. 15 & 18).

- b. *Biochemical estimations of glycoproteins* :

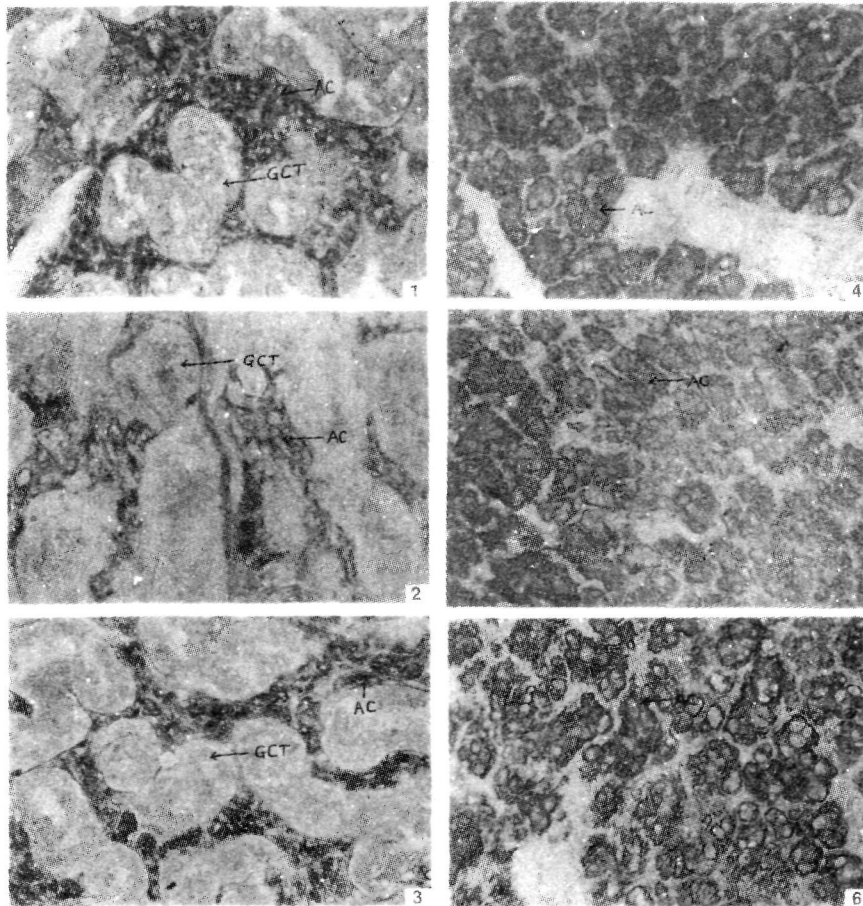


PLATE NO. 1

- Fig. 1 :** The section of submandibular gland of adult male mice stained with PAS X 128.
- Fig. 2 :** The section of submandibular gland of adult castrated mice stained with PAS X 128
- Fig. 3 :** The section of submandibular gland of adult castrated mice supplemented testosterone propionate stained with PAS x 128.
- Fig. 4 :** The section of sublingual gland of adult male mice stained with PAS x 128.
- Fig. 5 :** The section of sublingual gland of adult castrated male mice stained with PAS x 128.
- Fig. 6 :** The section of sublingual gland of adult castrated mice supplemented testosterone propionate stained with PAS X 128.

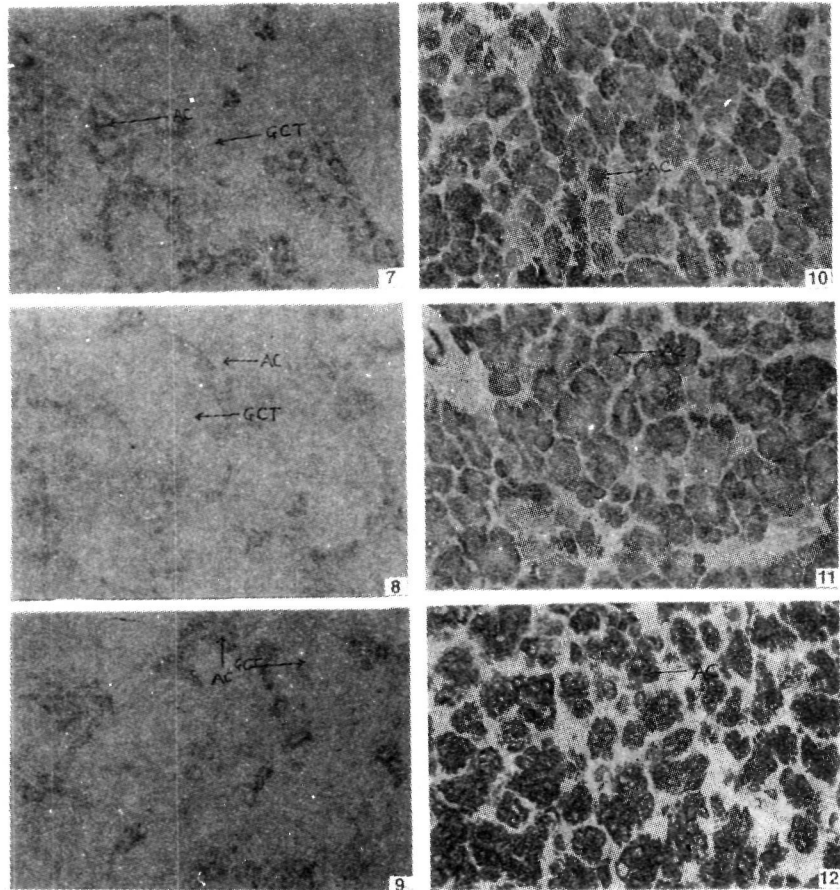
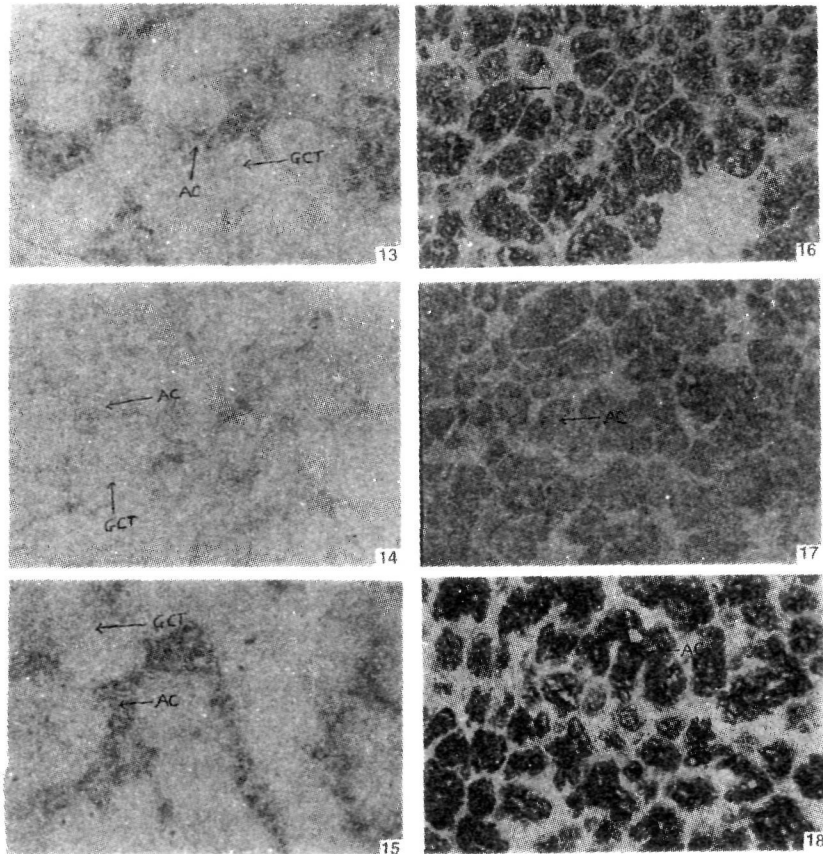


PLATE NO. 2

- Fig. 7 : The section of submandibular gland of adult male mice stained with AB pH 2.5 x 128.
- Fig. 8 : The section of submandibular gland of adult castrated male mice stained with AB pH 2.5 x 128.
- Fig. 9 : The section of submandibular gland of adult castrated injected male mice testosterone propionate stained with AB pH 2.5 x 128
- Fig. 10 : The section of submandibular gland of adult male stained with AB pH 2.5 x 128.
- Fig. 11 : The section of submandibular gland of adult castrated male mice stained with AB pH 2.5 x 128.
- Fig. 12 : The section of submandibular gland of adult castrated but testosterone propionate injected male mice stained with AB pH 2.5 x 128.

GCT - Granular convoluted tubules. AC - Acinar cells.



PLAT NO. 3

- Fig. 13 :** The section of submandibular gland of adult male mice stained with AB pH 1.0 x 128.
- Fig. 14 :** The section of submandibular gland of adult castrated male mice stained with AB pH 1.0 x 128.
- Fig 15 :** The section of submandibular gland of adult castrated but testosterone propionate injected male mice stained with AB pH 1.0 x 128.
- Fig .16 :** The section of submandibular gland of adult male mice stained with AB pH 1.0 x 128.
- Fig. 17 :** The section of submandibular gland of adult castrated male mice stained with AB pH 1.0 x 128
- Fig. 18 :** The section of submandibular gland of adult castrated but testosterone propionate injected male mice stained with AB pH 1.0 x 128.
- GCT - Granular convoluted tubules. AC - Acinar cells.

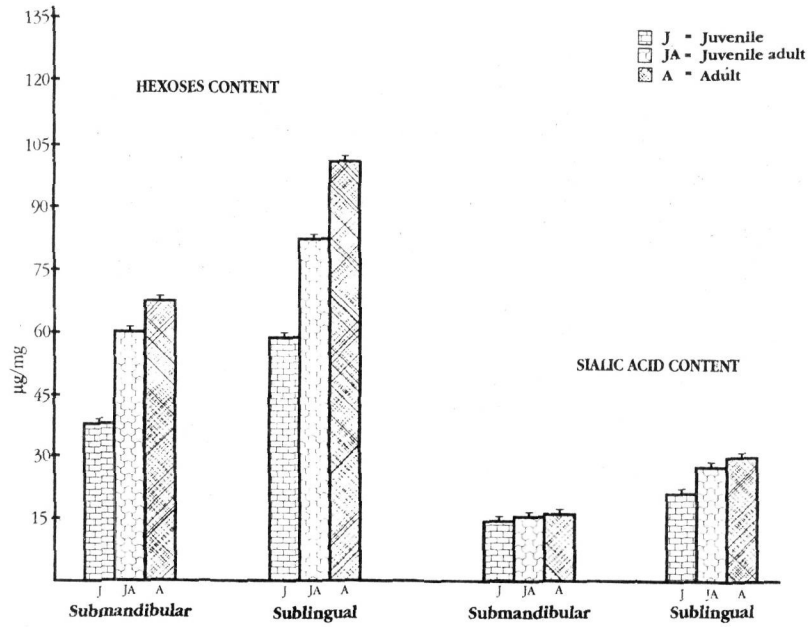


Fig. 19 : Changes in salivary glycoproteins during development

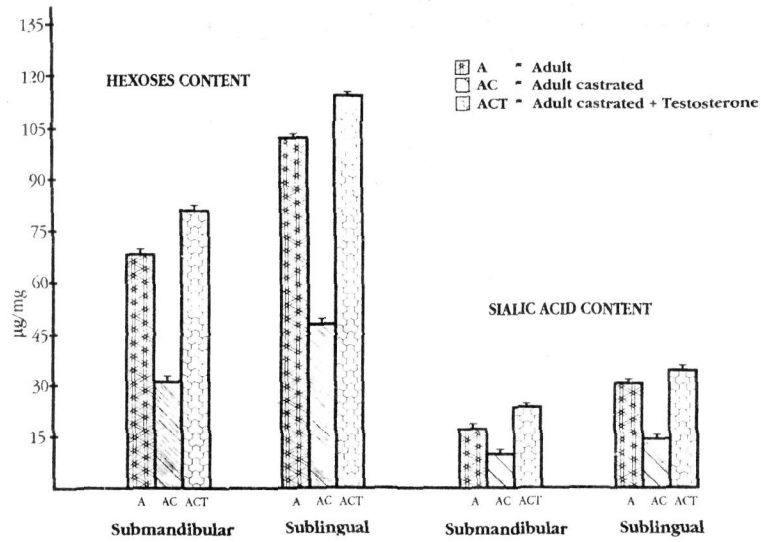


Fig. 20 : Effect of testosterone propionate on glycoproteins from salivary glands of male mice

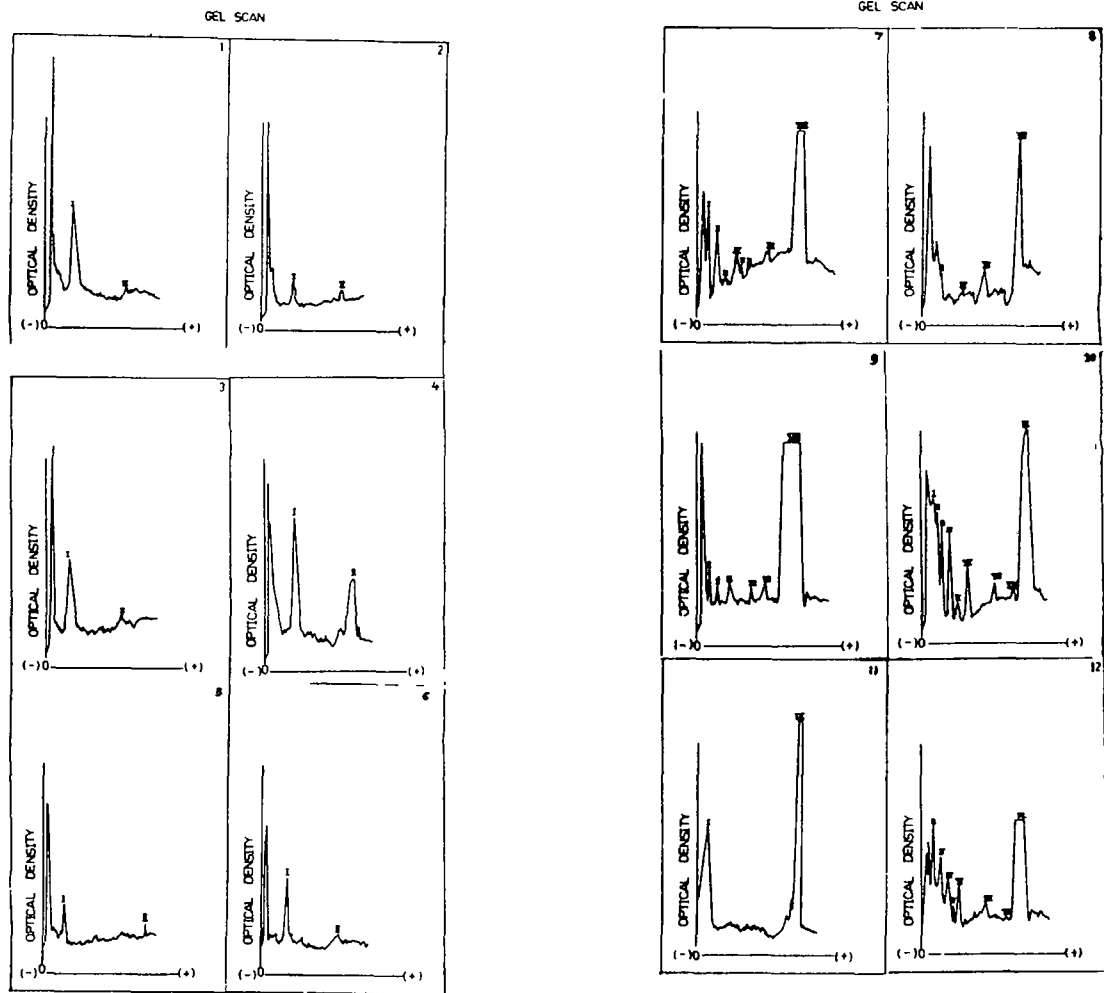


Fig. 21 : Electropherogram of submandibular gland glycoprotein.

- 1 : Adult male mice (AB pH 2.5)
- 2 : Adult castrated male mice (AB pH 2.5)
- 3 : Adult castrated mice supplemented testosterone propionate (AB pH 2.5)
- 4 : Adult male mice (AB pH 1.0)
- 5 : Adult castrated male mice (AB pH 1.0)
- 6 : Adult castrated mice supplemented testosterone propionate (AB pH 1.0)
- 7 : Adult male mice (AB pH 2.5)
- 8 : Adult castrated male mice stained with (AB pH 2.5)
- 9 : Adult castrated but testosterone propionate injected male mice stained with (AB pH 2.5)
- 10 : Adult male mice stained with AB pH 1.0
- 11 : Adult castrated male mice stained with (AB pH 1.0)
- 12 : Adult castrated but testosterone propionate injected male mice stained with AB pH 1.0

In the submandibular gland of juveniles, the hexoses content was less. Compared to juvenile, in juvenile adult and adult the hexoses content (fig. 19) significantly increased (1:2, $P < 0.001$; 1:3 $P < 0.001$). In castrated mice, the hexoses content decreased, whereas testosterone propionate treatment to castrated mice, increased the hexoses content significantly (4:5 $P < 0.001$).

Concentration of hexoses was more in sublingual gland which significantly decreased in castrated mice and was restored to normality after testosterone administration.

Sialic acid content in the submandibular gland of juvenile mice was less which increased significantly in adult male mice (fig. 20). While castration decreased the concentration of sialic acid, administration of testosterone propionate to castrates increased the same significantly ($P < 0.001$): Similar alterations were observed in sublingual gland in all groups of animals studied.

c) Electrophoretic separation of glycoproteins :

When electrophoretically separated, glycoproteins of submandibular adult gland stained for AB pH 2.5, two bands were seen and both were moderately stained. (fig 21:1) The intensity of these bands were reduced considerably in castrated mice (fig 21:2) and reappeared in testosterone injected mice (fig 21:3). Same bands were intensely stained in adult for AB pH 1.0 (fig 21:4), reduced in castrated mice (fig 21:5) and reappeared upon the injection of testosterone propionate to castrated mice (fig 21:6).

When electrophoretically separated, glycoproteins from sublingual gland were stained with AB pH 2.5, it was observed that in adult mice the glycoproteins were separated into 8 bands (fig. 21.7), whereas, in castrated mice glycoproteins were separated into 5 bands but their intensity was not like adult, and remaining bands were abolished (fig. 21.8) but after the administration of testosterone to castrated mice, 6 bands reappeared with full intensity (fig 21.9).

When proteins were stained with AB pH 1.0, it was observed that glycoproteins were separated into 9 bands (fig 21.10) in adult male mice. In castrated mice the number of bands was reduced into two bands (fig. 21:11) but number and intensity of bands were recovered upon the administration of testosterone propionate to castrated mice (fig. 21.12).

DISCUSSION

The synthesis of glycoproteins in salivary glands is under the control of testosterone, as evident from the sections of submandibular and sublingual glands stained with PAS, AB pH 2.5 and AB pH 1.0 for the demonstration of neutral glycoproteins, acidic glycoproteins and sulfated glycoproteins, respectively in castrated and testosterone treated mice. It has been observed that neutral glycoproteins are synthesized in the secretory and granular convoluted tubules of submandibular gland and acini of sublingual gland, whereas acid mucopolysaccharides are

synthesized only in acinar cells and sulfated glycoproteins in acinar cells of sublingual glands. Synthesis of glycoproteins in acinar cells has already been described by many workers (35, 46-50). Autoradiography following injection of $\text{Na}_2\text{S}^{35}\text{O}_4$ provided means of localization of sulfated glycoproteins in salivary glands (51-54). The presence of sialic acid in salivary glands is also described by some workers (54-56). Though there is vast literature on the presence of various glycoproteins in salivary glands, the effect of testosterone on them have not been worked out but the relationship between the salivary gland and the endocrine system has been under investigation for more than five decades. It has also been shown that testosterone increases the size and number of granular convoluted tubules when given to gonadectomized glycoproteins male rats (7, 57-60). Androgen receptors in the cytosol fraction of male and female mouse submandibular gland have been well characterized by some workers (3, 8, 11, 61). In past studies the effect of testosterone on sublingual gland has also been totally neglected. In the present study we have shown that the testosterone influences not only the synthesis of in GCT cells but also in acinar cells of submandibular as well as sublingual glands.

It has been observed that the quantity of glycoproteins in juvenile mice is comparatively less but on adulthood it is elevated. This indicates that with the onset of testosterone biosynthesis there is gradual increase in adult. The synthesis of hexoses and sialic acid in the submandibular and sublingual gland is regulated by testosterone. It is shown that castration induced decrease in glycoprotein can be corrected after testosterone propionate administration. Except Devalle *et. al* (62), some workers have shown the role of androgen on the synthesis of sialic acid in the submandibular glands, where the injections of testosterone increased sialic acid contents in submandibular gland. Electrophoretic separation of glycoproteins also supports the role of testosterone propionate in glycoprotein synthesis in salivary glands. Electrophoretically glycoproteins are separated into 2 bands in submandibular and 8 and 9 bands in sublingual glands for AB pH 2.5 and AB pH 1.0 respectively. In castrated adults number of bands were reduced which reappeared when castrated mice were injected with testosterone propionate.

Therefore, it is seen that testosterone has a direct action on neutral glycoproteins, synthesized in GCT and acinar cells of submandibular and acid glycoproteins synthesized in acinar cells of submandibular and sublingual glands.

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