

# EFFECT OF PITUITARY HOMOGRAFT ON ACCESSORY SEX ORGANS IN YOUNG MALE RATS

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( Received on April 14, 1987 )

**Summary :** The effect of prolactin secreted from pituitary homografts under renal capsule of castrated young male rats (30 days old) either alone or in combination with testosterone propionate (TP) on accessory sex organs were studied. Fresh weights of seminal vesicle, prostate coagulating glands and preputial glands were recorded as well as the total protein content and acid phosphatase activity in prostate and seminal vesicle was determined. Prolactin secreted from grafted tissue along or in combination with TP significantly increased the weights of sex accessories but no clear synergism was evident. However, synergism between prolactin and TP was observed in stimulating protein content and acid phosphatase activity of prostate and seminal vesicle. The results indicate that prolactin can act directly and also synergistically with androgen to maintain certain aspects of the functions of accessory sex glands in male rats.

**Key words :** prolactin                              pituitary homograft                              testosterone propionate  
prostate    seminal vesicle                                      acid phosphatase  
protein content                                      coagulating gland                                      preputial gland  
young rats

## INTRODUCTION

The effects of prolactin on growth and maintenance of accessory male sex organs are well documented. Receptors for prolactin have been demonstrated in testis, prostate and seminal Vesicle (1, 3, 14). The reports regarding the effects of prolactin on the weights of seminal Vesicles and the different portions of prostate glands in castrated and testosterone treated animals are contradictory (12, 18, 20, 22, 26). Although a number of reports indicate an increase in the growth of accessory sex organs and activity in terms of nucleic acid levels (23, 29), protein synthesis (29) and enzyme activity (10), whereas

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other investigations indicate the inhibitory actions of prolactin on male reproductive functions (8, 30). Moreover, whether prolactin can act on its own or the effects are mediated through the actions of testosterone is still unsettled (10, 12, 17, 26).

The aim of the present investigation was to study the effects of prolactin secreting graft independently and in combination with testosterone on the growth of male accessory sex organs.

### MATERIAL AND METHODS

Inbred male rats of Charles Foster strain (30 days old, 40-50 g body wt.) were used. Animals had free access to water and standard diet. They were housed (4 rats/cage) in a well ventilated animal house under similar husbandry conditions.

Animals were divided into Group I (sham control) ; Group II (castrated) ; Group III (grafted and castrated) ; Group IV (castrated and TP treated) and Group V (grafted, castrated and TP treated).

Animals first received pituitary grafts (Group III & V) and within 24 hours were castrated. Pituitaries from two adult female rats were transplanted under right renal capsule of anaesthetised male rats. Pituitary glands were dissected out and posterior pituitary was removed before transplantation. The sham control rats (Group I) received no grafts but only surgical exposure of kidney. Castrations (Group II, III, IV & V) were performed via scrotal route. Each of the rats of Group IV and V received 25  $\mu$ g of TP (dissolved in olive oil) subcutaneously on every alternate days from 7th day following castration till 21st day. All the animals of the various groups were sacrificed on 21st day following castration.

Prostate, seminal Vesicle, coagulating glands, preputial glands, adrenal glands and spleen were dissected out. Fresh tissue weights were recorded after removing fat and other adhesive tissues. The organ weights were expressed in mg/100 g of body weight.

Prostate and seminal Vesicle was minced and homogenized in 0.1M Tris-HCL buffer, pH 7.4 (1.0 ml/g tissue) using precooled elvehjem type of homogenizer. An aliquot of 0.1-0.2 ml was used for the estimation of total homogenate protein. Total protein was estimated according to Lowry *et al.* (19). Rest of the homogenate was centrifuged at 12000 x g at - 4°C for 15 minutes. The supernatant obtained was used for enzyme assay. The optimum substrate concentration was determined for prostate and seminal Vesicle. Maximum enzymatic activity was obtained at a substrate concentration

of  $0.56 \mu\text{mol}$ . Therefore, all assay mixture contained  $0.56 \mu\text{mol}$  of p-nitrophenol phosphate. The standard incubation mixture contained in a total volume of  $1 \text{ ml}$  ( $0.56 \mu\text{mol}$  of p-nitrophenol phosphate,  $0.4 \text{ ml}$  of  $0.5 \text{ M}$  acetate buffer,  $\text{pH} = 5$ ) and requisite amount of distilled water and tissue homogenate (usually equivalent to around  $200 \mu\text{g}$  protein). Incubation was carried out at  $37^\circ\text{C}$  for 1 hour in water bath with constant shaking. Reaction was stopped by adding  $4 \text{ ml}$  of  $0.1 \text{ N}$   $\text{NaOH}$  and colour developed was measured by determining absorbance at  $415 \text{ nm}$  in spectro colorimeter. Enzyme activity was expressed as  $\mu\text{mol}$  p-nitrophenol liberated total tissue protein/hour.

Kidneys bearing pituitary grafts were fixed in  $10\%$  formaldehyde solution and routine histological procedures were carried out to check viability of the graft. All the results were analysed using student 't' test.

## RESULTS

Weights of prostate, seminal vesicle, coagulating glands and preputial glands in various experimental groups are shown in Fig. 1. The weights of all sex accessories in castrated rats (Gr. II) increased significantly following graft placement (Gr. III). Prolactin secreted from graft in combination with exogenously administered TP (Gr. V) caused a

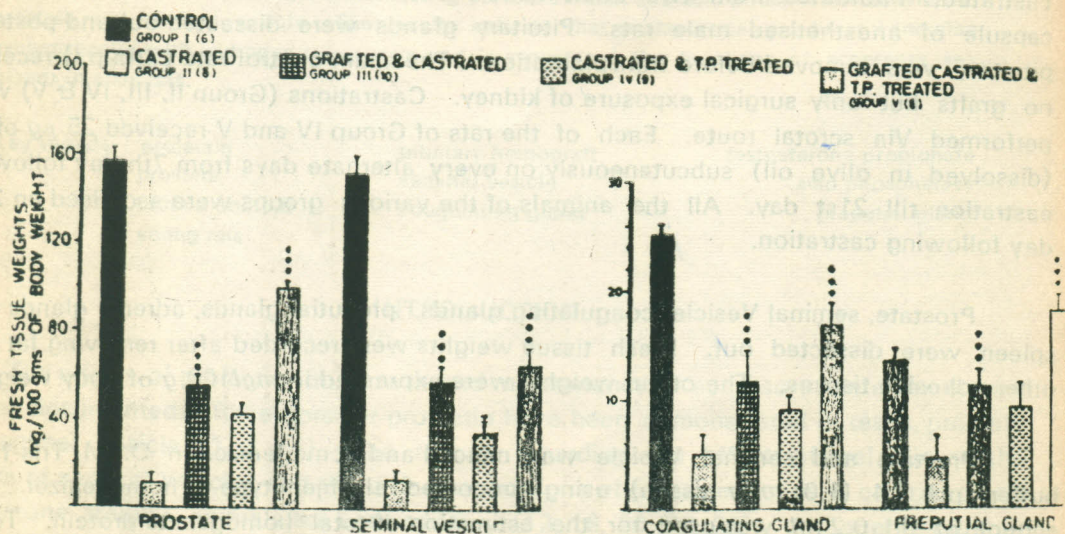


Fig. 1: Organ weights of accessory sex organs following various experimental treatments. (Values are Mean  $\pm$  SD). Figures in paranthesis indicate numbers of rats used. Comparisons were made between Gr. II & III, Gr. IV & V.

\*\*\* shows  $P < 0.001$ .

significant increase in the weights of all four accessory sex organs over that obtained with TP treatment alone (Gr. IV). The growth of coagulating glands and preputial glands were more pronounced. the weight of later became even higher than that of intact control.

Fig. 2. shows the weights of adrenal gland and spleen. No significant change in the weight of adrenal gland was observed in different experimental groups. However, spleen recorded significant increase in weight only in group V.

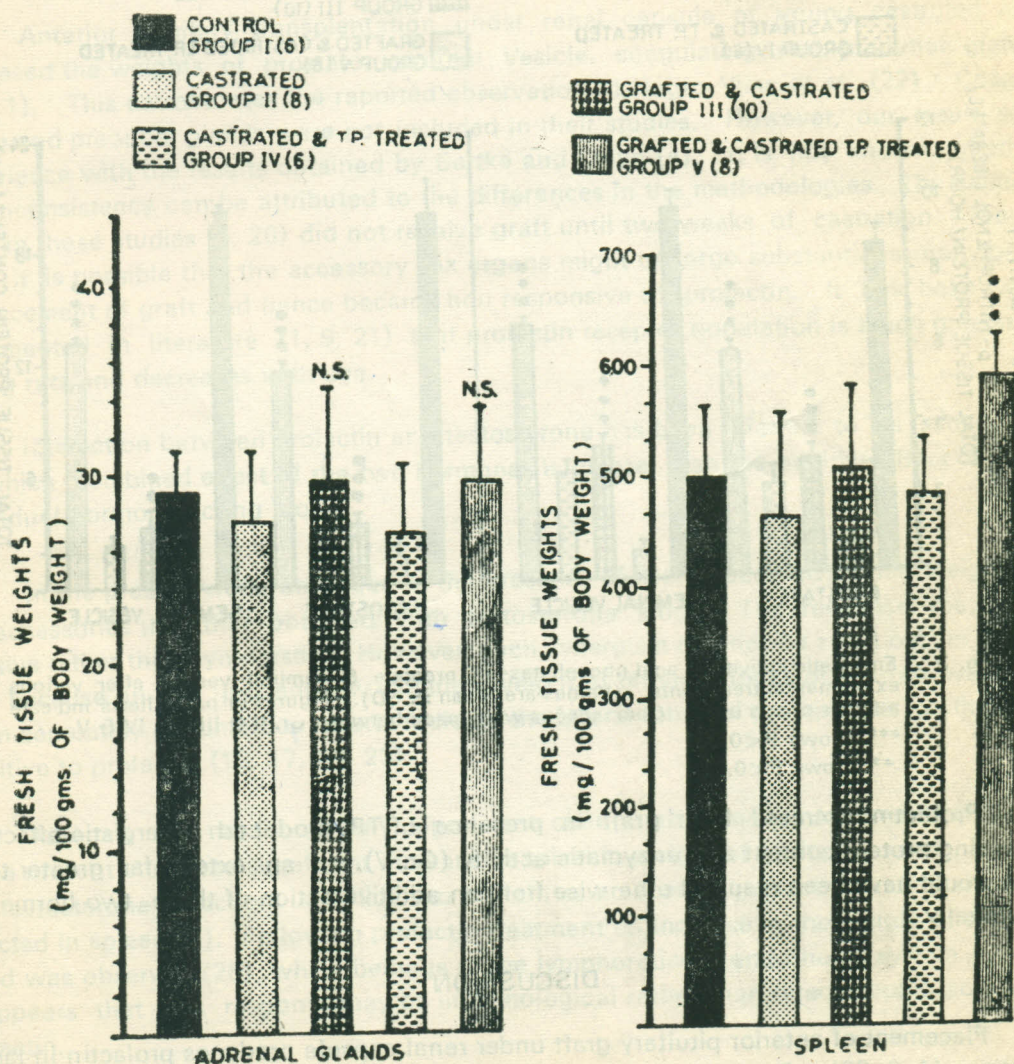


Fig. 2 : Effect of pituitary homograft on non-target tissues. (Values are Mean  $\pm$  SD). Figures in parenthesis indicate number of rats used. Comparisons were made between Gr. II & III, Gr. IV & V. N.S. : Not significant. \*\*\* shows  $P < 0.001$ .

It is evident from Fig 3 that prolactin secreted from graft stimulated the protein content and enzymatic activity of these two glands (Gr. III), significantly.

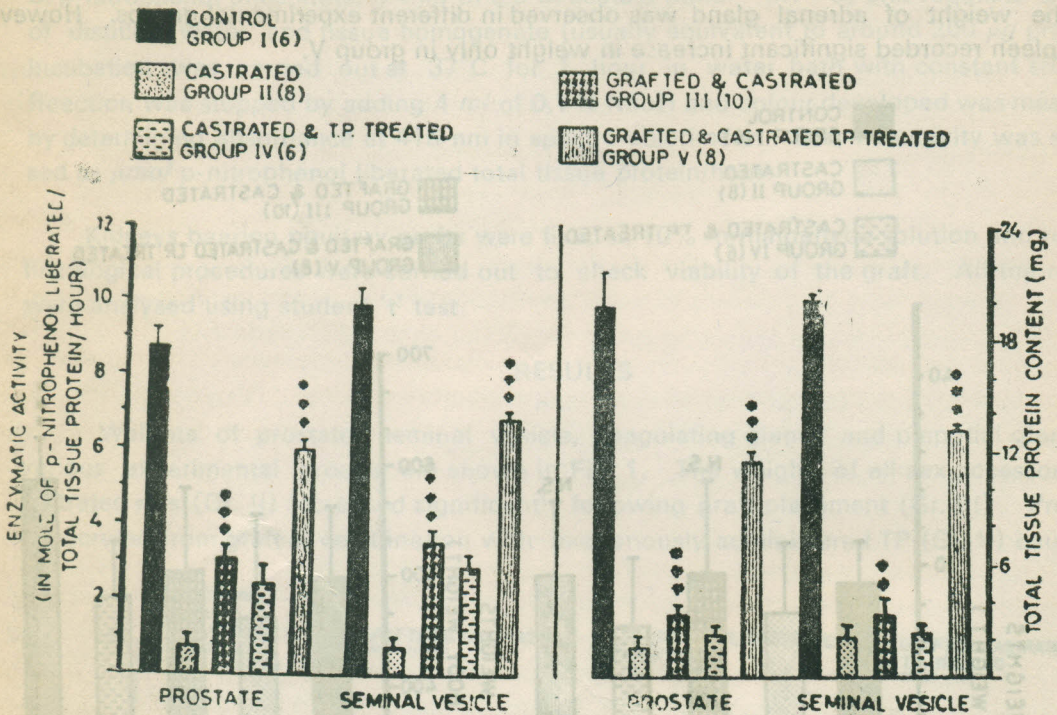


Fig. 3 : Enzymatic activity of acid phosphatase in prostate & seminal vesicle after various experimental treatments. (Values are Mean  $\pm$  SD). Figures in paranthesis indicate number of rats used. Comparisons were made between Gr. II & III, Gr. IV & V.  
\*\*\* Shows P<0.001  
\*\* Shows P<0.01

Prolactin secreted from graft in presence of TP, produced synergistic effect by increasing protein content and enzymatic activity (Gr. V), to an extent far greater than what would have been resulted otherwise from an additive action of these two hormones individually.

### DISCUSSION

Placement of anterior pituitary graft under renal capsule produces prolactin in large quantities (4, 6, 22). Production of LH and FSH diminishes under these conditions (5). It is unclear that other pituitary hormones such as growth hormone, ACTH and TSH are produced in significant amount from grafted pituitary (17). However, it is unlikely that

other hormones and adrenal steroids have any significant effect on the growth of accessory sex organs (5, 17, 28). Prolactin secretion from grafted pituitary increases because the negative influence of prolactin inhibiting hormone, dopamine is removed (11). Therefore, in the present study sustained release of prolactin was maintained by placement of pituitary homograft under renal capsule of orchidectomized rats.

Anterior pituitary transplantation under renal capsule of young castrated rats increased the weights of prostate, seminal Vesicle, coagulating and preputial glands (Fig. 1). This corroborates the reported observation of Negro-Vilar *et al.* (22). Coagulating and preputial glands were not included in their studies. However, our result was at variance with the results obtained by Bartke and Lloyd (4). It is not known whether this inconsistency can be attributed to the differences in the methodologies. The animals used in these studies (4, 26) did not receive graft until two weeks of castration. Therefore, it is possible that the accessory sex organs might undergo substantial atrophy prior to placement of graft and hence became non responsive to prolactin. It has been well documented in literature (1, 9, 21) that prolactin receptor population is much greater in young rats and decreases with age.

Interaction between prolactin and testosterone has been referred to as synergism, in which combined effect of the two hormones is greater than the additive effect of each individual hormone acting alone.

In the present work, androgen in the presence of grafts recorded higher weight of sex accessories than that obtained with testosterone alone. The response seen was additive rather than synergistic. However, such synergism can not be ruled out in prostate because in the present investigation weight of different lobes of prostate were not taken separately. Numerous reports indicate the lateral lobes of prostate are particularly sensitive to prolactin (13, 17, 29, 23).

Adrenals did not show any significant change after various treatments (Fig. 2). In the present study the significant increase in splenic weight of grafted animals receiving testosterone injection is difficult to explain as no specific prolactin binding site was detected in spleen (7). Following prolactin treatment an increase in the weight of thymus gland was observed (26), which belongs to the lymphoreticulo-endothelial system. Thus it appears that this response may be immunological rather than direct trophic action of prolactin.

It is distinct from results that prolactin alone secreted from grafted pituitary appreciably increased the total protein content of prostate and seminal Vesicle (Fig. 3).

Thomas & Manadhar (29) also reported that prolactin treatment enhances the protein synthesis in the prostate gland. Polyamine levels, which is considered to be a criterion for the rate of protein synthesis, has been reported to increase in the prostatic tissue following prolactin treatment (15, 16).

Acid phosphatase activity of male accessory sex glands which is known to be under androgenic control (27) also increased considerably in grafted rats (Fig. 3). But it was earlier reported that prolactin treatment alone had little effect on the enzymatic activity of prostate and seminal Vesicle (10). This could be due to difference in the source of prolactin and age of the animals used. These authors employed adult rats and an exogenous source of ovine prolactin in contrast with the young rats and an endogenous prolactin secreted from homograft in the present study.

Synergism between prolactin and testosterone was seen in increasing total protein content and acid phosphatase activity of prostate and seminal Vesicle (Fig. 3). This is in accordance with other well documented studies (10, 29). Evidences of complex interaction between these two hormones on several parameters of prostatic activity has also been reported (25).

Thus, the present study shows that prolactin does play an important role in the growth of prostate, & seminal Vesicle. Other sex accessories like coagulating gland and preputial glands are particularly sensitive to the action of prolactin. However, precise mechanism of action of prolactin may involve several possibilities, such as, (i) promotion of growth by direct action of prolactin, (ii) through an androgen mediated action or (iii) by stimulating output of testicular androgen which in turn acts on sex accessories.

In normal conditions it would appear all the three mechanisms would operate and complement each other in promoting growth and maturation of male sex accessories. However, in the present model the last possibility could not be tested as animals were castrated. There are evidences supporting the other two possibilities. Prolactin can influence the number of androgen receptor complex in cytosol of accessory sex organs (2). It is also possible that prolactin might influence the androgenic action by affecting androgen binding protein (10). Recent reports indicate that prolactin might act on accessory sex organs without any involvement of androgens (24). The present investigation suggests both androgen mediated as well as direct action of prolactin on male sex accessories.

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