This might be due to the impairment of the Ca^{2+} uptake ability of SR, caused by caffeine[7,8]; owing to the increased Ca^{2+} availability in the myoplasm, during relaxation, cross-bridges may be reformed, and the load dependence of tension decay disappears. The influence of the initial muscle length on isometric relaxation still remains in the presence of caffeine: the dependence of the rate and duration of isometric relaxation on the initial length seems to be more pronounced after caffeine addition (figure 2, A and B). No clear explanation of this caffeine effect is provided by this investigation.

These observations suggest that, in isometric twitches, performed under both conditions (control and caffeine), the time course of relaxation depends on the activation level (initial muscle length), while the load control of relaxation takes place only when activation has a very early decline.

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Effect of hormones on adenyl cyclase activity of rabbit mammary gland in vitro

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Summary. Ovarian hormones namely β-estradiol and progesterone were observed to stimulate the activity of adenyl cyclase of the mammary gland from pregnant rabbit in vitro, unlike the lactating tissue where it was inhibited. On the other hand, non-ovarian hormones like hydrocortisone, prolactin and insulin did not have a similar effect on this enzyme.

Hormones have been observed to be required for the mammary gland development and its metabolic function[2,3] during pregnancy and lactation. Cyclic AMP has been established to be the second messanger of hormonal stimulation at the cell surface[4]. Adenyl cyclase activity, which is responsible for the synthesis of cyclic AMP was observed to change during lactation cycle[5]. The present investigation was therefore undertaken to note the effect in vitro of hormones, both ovarian and non-ovarian, on the activity of this enzyme in the mammary gland from pregnant and lactating rabbits.

Materials and methods. Animals at various stages were obtained from the small animal house maintained at the institute. β-Estradiol, progesterone, hydrocortisone, caffeine, prolactin and insulin were purchased from Sigma Chemical Company, USA. The other chemicals used were of the analytical grade.

Mammary tissue was collected from rabbits at various stages of lactation and washed with cold 0.1% KCl solution. 5 g tissue was minced for 10 min and homogenized in 20 ml of 50 mM Tris-HCl buffer (pH 7.6). Homogenized tissue was strained through 4 layers of muslin cloth and the extract obtained was spun in a high speed centrifuge at 4000× g for 20 min. The pellet was washed twice with buffer and was resuspended in the 2.0 ml of buffer which was used as enzyme preparation[5].

Adenyl cyclase assay was carried out by the method of White[6] with certain modifications. Assay system contained 50 mM Tris-HCl buffer, pH 7.6, bovine serum albumin, 10 mM mercaptoethanol, 20 mM caffeine and ATP generating system. Final volume of the assay system was 0.15 ml containing 0.25 μCi 8-C^{14}-ATP (0.4 mM), steroid hormones (0.02 mM) and peptide hormones (0.3 units/ml). The enzyme was preincubated for 10 min at 37 °C with steroid hormones and peptide hormones. 50 μl of preincubated enzyme was added to the assay system and incubated for 20 min at 37 °C. Control sample was without the hormone addition. The reaction was terminated by keeping the mixture in boiling water bath for 3 min, cooled to room temperature and 1 ml of imidazole-HCl buffer (pH 7.0) was added.

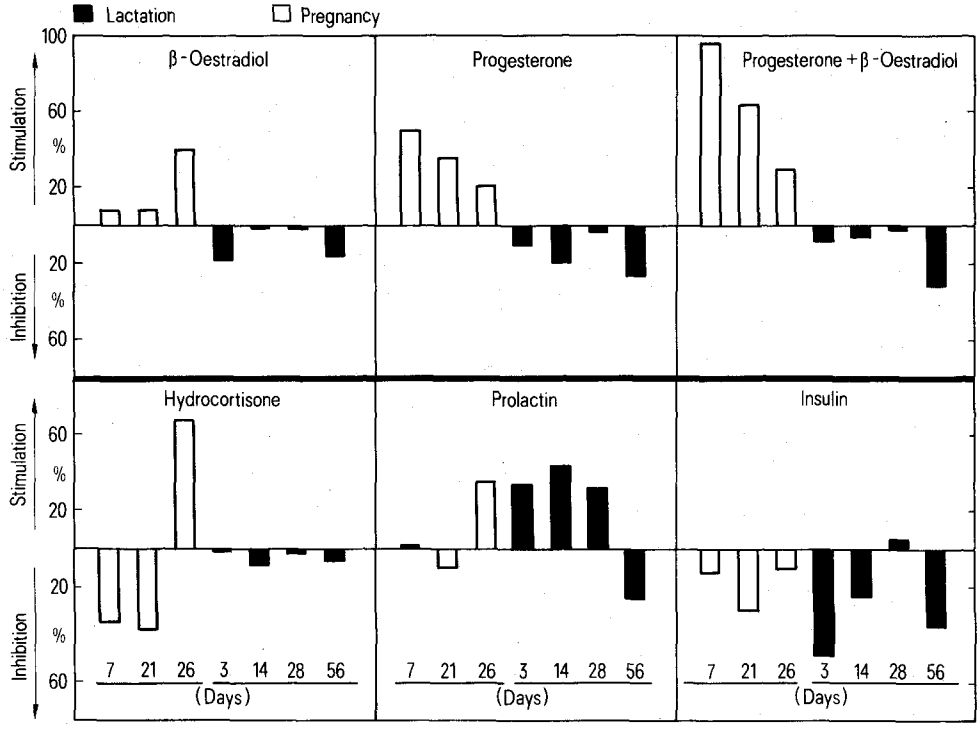
The rest of the procedure followed was as described earlier[5]. The specific activity of the enzyme was expressed as n mole of cAMP produced/mg protein/min at 37 °C.

Results and discussion. Ovarian hormones have a stimulatory effect on the enzyme isolated from pregnant animal as shown in the Figure. Progesterone and β-estradiol have a synergistic effect on the enzyme preparation from early and mid-pregnant rabbit, where the stimulation caused by the hormones put together in the in vitro system was higher than their individual effect. Whereas such effect was not

Adenyl cyclase of rabbit mammary gland as effected by hormones in vitro

Hormone	Adenyl cyclase specific activity (nmoles/mg protein/min)	
	Late pregnancy (26 days) Stimulation (%)	Late lactation (56 days) Inhibition (%)
β-Estradiol	39.1	15.7
Progesterone	21.3	25.5
β-Estradiol + progesterone	30.2	31.0
Hydrocortisone	68.3	5.9
Prolactin	35.1	25.5
Insulin	-9.8*	43.1

* Inhibition (%).



prominent in late pregnant mammary enzyme (figure and table). It is also evident that hydrocortisone stimulates the activity of the enzyme isolated from mammary gland of late pregnant animals only. Enzyme preparation from mammary gland of early and mid pregnant animals was strongly inhibited (38 and 41% respectively) while the post-partum animal showed almost no effect. It has been reported that during pregnancy, the secretion of hormones, such as β-estradiol[7], progesterone[8] and glucocorticoids[9] are markedly increased. Present findings suggest that hormone actions are mediated through the adenyl cyclase. Our findings also comply with the findings of Weist[8] who showed that progesterone is a more effective stimulator of adenyl cyclase than β-estradiol. Inhibition caused on the enzyme preparation from the post-partum animal can be explained on the basis of the findings of Cowie and Tindal[10] where they observed that estrogens in general suppress the mammary gland protein synthesis.

Prolactin was found to stimulate adenyl cyclase isolated from mammary gland of late pregnant and post-partum rabbit (figure) unlike the enzyme preparation from late lactation, where it was inhibited by this hormone (25%). On the other hand, insulin inhibited the enzyme from pregnant as well as lactating animals. The extent of inhibition was much higher in the post-partum enzyme preparation compared with that in the pregnant animal (table). McNeilly and Friesen[11] have reported that the level of prolactin in rabbit blood increases dramatically (3-25-fold) before parturition, which indirectly supports the present findings that the enzyme activity of 26-day pregnant rabbit mammary (figure and table) was observed to be strongly stimulated by prolactin. Insulin, on the contrary, has inhibitory or no effect on adenyl cyclase of mammary tissue from both lactating as well as pregnant animals.

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