Journal of Steroid Biochemistry, Vol. 11, pp. 1485 to 1489
Pergamon Press Ltd 1979. Printed in Great Britain

STUDIES CONCERNING THE HORMONAL INDUCTION OF LACTOGENESIS BY PROSTAGLANDIN $F_2\alpha$ IN PREGNANT RATS

LEONARDO E. BUSSMANN and RICARDO P. DEIS
Laboratorio de Reproducción y Lactancia, C.O.N.I.C.E.T., Casilla de Correo 855, 5500 Mendoza, Argentina

(*Received* 6 *March* 1979)

SUMMARY

Factors involved in the induction of lactogenesis by prostaglandin $F_2\alpha$ in pregnant rats have been studied. $PGF_2\alpha$-tromethamine salt (150 μg × 2) injected on day 18 of pregnancy, induced a significant increase in ovarian 20α-hydroxysteroid dehydrogenase activity with maximal values 24 h after treatment. Simultaneously, serum progesterone concentration starts to decrease significantly 4 h after $PGF_2\alpha$ administration. These ovarian changes are related to the significant increase in serum prolactin which occur between 12 and 24 h after prostaglandin treatment.

At mammary gland level, specific prolactin binding sites were significantly higher 4 and 8 h after $PGF_2\alpha$ administration followed by a rapid decrease to normal values at 12 and 24 h. The effect on placental function was determined by measurement of the chorionic mammotrophin in plasma. No modification was found in chorionic mammotrophin levels in the first 24 h after $PGF_2\alpha$ treatment, being the values similar to those in the control rats.

Lactogenesis visualized by an oxytocin test, occurred in the pregnant $PGF_2\alpha$ treated rats 24 h after the administration of the drug.

The described hormonal changes occurring after $PGF_2\alpha$ treatment, resemble the physiological events that take place in the control rats before parturition. These results suggest that prostaglandin may play a physiological role in the induction of lactogenesis in pregnant rats.

INTRODUCTION

Many years ago an association of the ovarian hormones with lactogenesis and parturition was postulated in women [1]. The rapid disappearance of progesterone (Pg) on the last days of pregnancy appears to trigger the iniciation of both lactogenesis and parturition in rabbits [2] and rats [3, 4]. A physiological decrease in plasma Pg concentration at the end of pregnancy has been observed in rats [5, 6] but the mechanism responsible for the regression of the corpus luteum remains unknown. Also in the rat, Morishige, Pepe and Rothchild[7] showed a correlation between serum Pg decrease and serum prolactin (PRL) concentration on day 21 of pregnancy.

It has recently been reported [8, 9] that prostaglandin $F_2\alpha$ ($PGF_2\alpha$) induced lactogenesis and abortion in pregnant rats, and that Pg prevented the abortive action of $PGF_2\alpha$. Thus lactogenesis and abortion may be the consequence of the luteolytic effect of $PGF_2\alpha$ [10–12].

The following experiments were undertaken to obtain more evidence on the physiological participation of $PGF_2\alpha$ in the induction of lactogenesis.

MATERIALS AND METHODS

General laboratory conditions. White primiparous rats, 3–4 months old, were used. They were placed with a male during the night following proestrus, the next morning was designated day 0 of pregnancy if spermatozoa were found in the vaginal smears. In our colony, rats usually deliver on day 22 of pregnancy. Rats were kept in groups of six in special wire cages in a constant temperature room (22°C) in which the lighting schedule was 14 h of light (06.00 h to 20.00 h) and 10 h of darkness.

$PGF_2\alpha$ treatment of pregnant rats. Groups of pregnant rats were injected intraperitoneally with $PGF_2\alpha$-tromethamine salt (Upjohn) in two doses of 150 μg each (equivalent to 112 μg of free acid) on day 18 of pregnancy at 08.00 h and 12.00 h. Control rats were injected with 0.9% NaCl solution. Control and treated rats were sacrificed at different times until the onset of lactogenesis. The rats treated with $PGF_2\alpha$ were killed 4, 8, 12 and 24 h after the last dose. The control groups were killed at 4, 8, 12, 24, 48, 72 and 78 h after the second injection of normal saline solution.

Hormone assay. Blood samples obtained immediately after decapitation were allowed to clot at 4°C overnight and centrifuged. The serum was kept frozen at −20°C until assay.

Serum PRL concentration was assessed by radioimmunoassay [13] at two dilution levels using the kit provided by the NIAMDD program. The results are expressed in terms of the NIAMDD-Rat prolactin RP-1 standard.

Pg serum levels were measured by radioimmunoassay [14] using an antiserum raised by us in rabbits

against progesterone-11-BSA. The cross reaction at 50% inhibition, with the other steroids which could interfere with the measurement of Pg in serum was less than 1%. Steroids extraction from serum was performed using petroleum ether 40–70°C. Separation of free and bound Pg after overnight incubation at 4°C with the antiserum and 25 pg of [1, 2, 6, 7-^{3}H]-Pg (New England Nuclear, NEN) was achieved by incubation during 10 min at 0°C with charcoal–dextran (0.5%–0.05%). The samples were then centrifuged at 1,200 *g* × 20 min at 4°C. The supernatant was decanted into a counting vial and counted in a liquid scintillation counter, after standing for 12 to 15 h with the LSC cocktail.

Rat chorionic mammotrophin (rCM) was assayed by radioreceptor assay (RRA) according to Shiu *et al.*[15], using a membrane preparation of liver from female rats. The tracer used was ^{125}I ovine PRL (NIH-oPRL S12, 35 IU/mg), iodinated by the lactoperoxidase method [16] and purified as described in the PRL binding sites assay. For the standard curve the same oPRL preparation was used plus 0.025 ml of male rat serum. The sensitivity of the assay ranged from 16 ng to 512 ng and a 85% of binding inhibition was obtained with 2 μg of oPRL. The specific binding was proportional to the amount of membrane used; serum dilutions of 18th day pregnant rats inhibited the binding of the tracer in a way that paralleled the inhibition by dilutions of the oPRL standard to which male rat serum was added.

Determination of 20α-hydroxysteroid dehydrogenase activity in corpora lutea. Both ovaries were removed, trimmed of surrounding fat and transferred to liquid nitrogen for storage before analysis. Corpora lutea 20 α-hydroxysteroid dehydrogenase (20α-OH-SDH) enzymic activity was assayed spectrophotometrically in a Unican SP500 with a SP505 cell programmer using the technique described by Wilcox and Wiest[17], but with the omission of cysteine. Isolated corpora lutea were homogenized in buffer Tris 0.1 M, EDTA 1 mM ph 8.0, centrifuged at 15,000 *g* × 20 min and the supernatant (0.2–0.3 ml) assayed for 20α-OH-SDH activity in a 3 ml cuvette, 1 cm light path, containing 2.5–2.6 ml of homogenizing buffer and 1 μmol of NADP in 0.1 ml of the same buffer. Cuvettes were preincubated for 10 min at 37°C before the reaction was started by the addition of 0.316 μmol of 20α-hydroxypregn-4-en-3-one (20-OHPg) in 0.1 ml of ethanol. Change in extinction at 340 nm was recorded for 5 min.

Prolactin binding sites determination. The inguinal mammary gland of each side was dissected, wrapped in aluminum foil and transferred to liquid nitrogen until assay.

One gram of mammary tissue was cut into small pieces and homogenized in buffer Tris 25 mM, EDTA 1 mM, DTT 0.5 mM PH 7.4, with an Ultra-Turrax homogenizer. The homogenate was centrifuged at 10,000 *g* × 10 min and the supernatant centrifuged again for 60 min at 105,000 *g*. The resultant pellet was resuspended in Dulbecco buffer and kept at −70°C until the determination of the PRL binding sites.

Ovine PRL (NIH-oPRL S12, 35 IU/mg) was used as tracer and for displacement. Labelling of the oPRL with ^{125}INa (NEN) was performed by the lactoperoxidase method described by Thorell and Johansson[16]. The reaction was stopped by the addition of 0.2 ml phosphate buffer 50 mM with 0.02% Na_3N ph 7.0, and the free iodine was separated by means of a Sephadex G75 (Pharmacia Fine Chemicals) column. The PRL peak was further purified in an acrylamide-agarose column (Ultrogel AcA 54, LKB).

Samples of 0.1 ml membrane preparation (0.1 mg of protein) were incubated with ^{125}I-oPRL 125,000 cpm (S.A. 22 μCi/μg, counting efficiency 68%) in a final volume of 0.25 ml of Dulbecco buffer containing 0.1% BSA and 0.01% Neomicin sulfate. After 16 h of incubation at room temperature, 3 ml of ice cold buffer was added and the separation of bound and free PRL was done by centrifugation. The difference between the tubes incubated with an excess of PRL (2.5 μg) and those with the tracer alone was referred as specific PRL binding to the membranes, and expressed as percentage of the total amount of ^{125}I-oPRL added.

Protein determination was done as described by Lowry *et al.*[18].

Lactogenesis determination. In a small group of $PGF_2\alpha$ treated rats, determination of lactogenesis was performed by the *in vivo* oxytocin test [3]. To avoid any interference of oxytocin with our studies in all the other groups of control and $PGF_2\alpha$ treated rats, a simple *in vitro* oxytocin test was used for the determination of presence of milk in the mammary gland. A small piece of mammary tissue (50 mg approx.), obtained immediately after killing the rats, was placed in a test tube containing 10 mU of oxytocin (Syntocinon, Sandoz) in 1 ml of normal saline. According with previous compared test, the liquid will become opalescend by one minute when milk is present in the mammary tissue and "ejected" by the oxytocin.

RESULTS

20 α-OH-SDH Activity and serum progesterone concentration in $PGF_2\alpha$ treated and control pregnant rats

In the rats treated with $PGF_2\alpha$-OH-SDH enzymic activity could be detected 4 h after the last dose of prostaglandin (Fig. 1 upper panel). The activity dramatically increased when determination was done 24 h after $PGF_2\alpha$ treatment. In the control rats treated with saline, detectable activity was determined 48 h after treatment corresponding to day 20 of pregnancy and the maximal enzymic activity was obtained 72 h (day 21 of pregnancy) after last dose of saline.

Serum Pg values (Fig. 1, lower panel) started to decrease significantly ($P < 0.05$) 4 h after the last dose of $PGF_2\alpha$. A sharp decrease, compared to the control animals, occurred between 4 and 12 h after treatment. In the control rats a progressive but significant de-

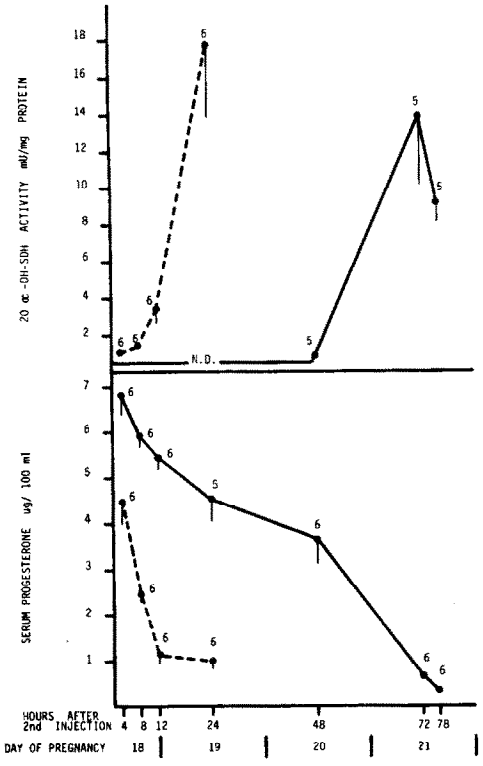

Fig. 1. (Upper panel) Corpora lutea 20α-hydroxysteroid dehydrogenase activity in milliunits per mg of protein, one unit being equal to the formation of 1 µmol of NADPH/min at 37°C. ND = not detectable activity (4,8,12 and 24 h control groups, six rats each group). (Lower panel) Serum progesterone levels expressed as µg per 100 ml. ●---● $PGF_2\alpha$ injected rats. ●——● Vehicle injected rats. Number of rats accompanies each mean value. Vertical lines represent SEM. Numbers in the abscissa depict the hours after the second injection of either $PGF_2\alpha$ or vehicle (12.00 h), and the day of pregnancy respectively.

crease of serum Pg concentration started 24 h after saline treatment and the lowest Pg level was observed 78 h after (21 day of pregnancy).

Serum PRL, PRL binding sites in mammary gland and serum rCM

As Fig. 2 shows (upper panel), serum PRL concentration increased significantly ($P < 0.05$) 8 h after prostaglandin treatment when compared with the appropriate control. Maximum serum PRL concentration was reached 24 h after treatment, a difference being highly significant ($P < 0.001$) when compared to the control rats.

In the control rats 72 h after normal saline treatment, serum PRL levels were not significantly different to that measured at 24 h in the group treated with $PGF_2\alpha$.

The profile in specific binding of PRL to mammary gland membranes obtained in $PGF_2\alpha$ treated and control rats, is shown in Fig. 2, lower panel. The specific PRL binding increased significantly ($P < 0.05$) at 4 and 8 h after the last dose of prostaglandin, but a rapid decrease took place at 12 and 24 h with similar values to the control rats.

In the mammary gland of the control rats a barely detectable amount of PRL binding sites was found at 4, 8, 12 and 24 h after saline treatment (days 18 and 19 of pregnancy) with a gradual increase on days 20 and 21 of pregnancy. Thus 72 h after saline treatment (day 21 of pregnancy) PRL binding sites reached a value not different to that obtained at 8 h in the $PGF_2\alpha$ treated rats.

A progressive decrease in serum rCM was observed in control rats (Table 1). On day 21 of pregnancy the mean serum concentrations were significantly lower at 72 and 78 h ($P < 0.01$) after vehicle administration, when compared to the values observed at 48 h. In the $PGF_2\alpha$ treated rats the mean serum rCM concentrations were similar at 4, 8, 12 and 24 h and were not different to the values in the control rats. It should be noted that the rCM levels are overestimated in those groups that had high serum PRL values (72, 78 h control and 12, 24 h $PGF_2\alpha$ treated group) which cross react in the assay of lactogenics hormones with the rCM.

Lactogenesis according to the oxytocin test

In a group of 3 $PGF_2\alpha$ treated rats on which only lactogenesis by the *in vivo* oxytocin test was per-

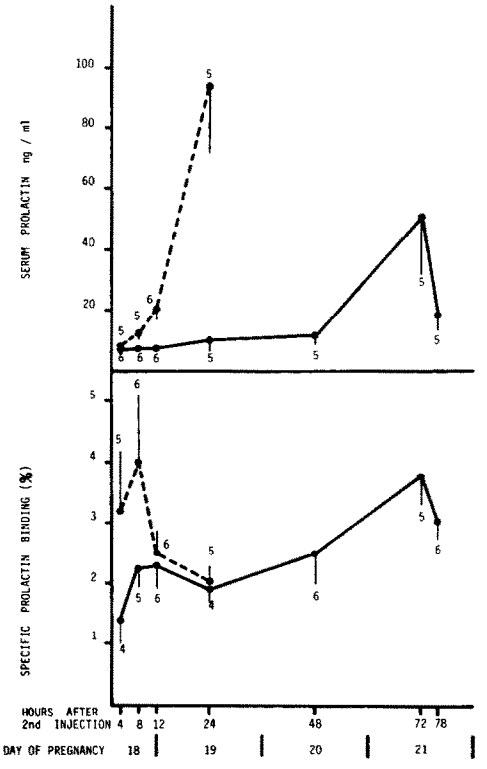

Fig. 2. (Upper panel) Serum prolactin (ng/ml) expressed in terms of the reference preparation NIH-RP 1. (Lower panel) ^{125}I-oPRL specific binding to membranes from mammary gland (100 µg of protein) expressed as percentage of the total counts added. ●---● $PGF_2\alpha$ treated rats. ●——● Vehicle treated rats. Number of rats accompanies each mean value. Vertical lines represent SEM. Numbers in the abscissa depict the hours after the second injection of either $PGF_2\alpha$ or vehicle (12.00 h), and the day of pregnancy respectively.

Table 1. rCM serum concentration in pregnant rats

Day of pregnancy	Hours after 2nd injection	Vehicle	$PGF_2\alpha$
18	4	502 ± 64 (6)	471 ± 34 (5)
18	8	501 ± 74 (6)	541 ± 49 (6)
18	12	533 ± 80 (5)	534 ± 40 (6)
19	24	450 ± 43 (6)	516 ± 40 (6)
20	48	444 ± 29 (6)	
21	72	356 ± 39 (6)	
21	78	374 ± 30 (6)	

Vehicle and $PGF_2\alpha$ were injected intraperitoneally on day 18 of pregnancy at 08.00 and 12.00 h. The mean values ± SEM are expressed in terms of ovine PRL,NIH-P-S12.

Parentheses enclose the number of rats tested.

formed, milk ejection occurred 24 h after prostaglandin treatment. In all the others $PGF_2\alpha$ treated rats killed 24 h after treatment, a positive response to the *in vitro* oxytocin test was obtained. In control vehicle treated rats, none of the animals responded to the oxytocin when the mammary tissue was taken 48 h after normal saline administration. At 72 h, 3 out of 6 rats, gave a weak response and at 78 h, from 6 rats, one gave a clear positive response, in four only a weak response was obtained and the other one did not respond to oxytocin.

DISCUSSION

Lactogenesis has been adscribed to a decrease in ovarian progestin output in several species, including the human and the rat [1, 3, 4, 20, 22]. We confirm and extend those studies in which we found a dramatic increase in luteal 20-OH-SDH activity associated with an accelerated decrease in circulating progesterone concentration during the hours preceeding lactogenesis.

In previous works [8, 9, 19] it was postulated that the lactogenic and abortive action of $PGF_2\alpha$ in pregnant rats may also be dependent on a decrease in Pg secretion by the ovaries. In fact, a rapid withdrawal of Pg by ovariectomy at the end of pregnancy induced lactose accumulation in mammary tissue [4, 22] and visible milk, in response to oxytocin [3], appeared 24 h after the removal of the ovaries. Both events could be prevented by Pg administration [3, 4, Deis and Delouis, unpublished results].

The results reported in this paper perfectly agree with those earlier findings and interpretations. Both, the decrease in circulating Pg concentration and the increase in luteal 20 α-OH-SDH activity, that normally occur near the end of pregnancy, were advanced by $PGF_2\alpha$ administration.

$PGF_2\alpha$ administration did not produce any significant change in the slowly declining levels of serum rCM at the end of pregnancy, indicating that placental function was not affected by treatment. On the other hand it is interesting to point out that in spite of high serum rCM concentration, when $PGF_2\alpha$ was injected, the corpus luteum was not protected against this luteolytic action of prostaglandin. Thus the effect of $PGF_2\alpha$ on corpus luteum is independent of the rCM circulating levels but according to previous results [19] may be dependent on Pg levels.

These, and previous results [9, 19] indicate that $PGF_2\alpha$ administration is followed by increased circulating levels of PRL which may account for the lactogenic action of $PGF_2\alpha$ [8]. Comparing the rapid decrease in circulating Pg observed in Fig. 1 with the PRL curve (Fig. 2) after $PGF_2\alpha$, it is evident that one event, PRL increase, is mediated by the former. Progesterone is known to prevent PRL release and lactogenesis, induced by $PGF_2\alpha$ in pregnant rats [9].

Strauss and Stambaugh[23] found that ovine PRL prevented the effect of $PGF_2\alpha$ on 20 α-OH-SDH activation on days 8 and 9 of pregnancy but had no effect when PRL and prostaglandin were injected on days 14 and 15. In our report the significant amount of endogenous PRL released by prostaglandin treatment did not affect whatsoever the rapid increase in 20 α-OH-SDH activity observed. Behrman *et al.*[24] have shown that $PGF_2\alpha$ may antagonize the action of PRL on the rat corpus luteum. Vermouth and Deis [19] showed that the administration of ovine PRL simultaneously with $PGF_2\alpha$ on day 18 of pregnancy did not prevent the lactogenic and abortive action of prostaglandin.

The fact that the increase in PRL secretion is followed by lactogenesis, both in control rats and in rats treated with $PGF_2\alpha$, could be considered as an indication of an action of PRL at the mammary gland level. Accordingly, lactogenesis is always preceeded by an increase in PRL membrane receptors both in control and in treated rats. However it should be remarked that these increases were not associated with any previous increase in the circulating levels of the lactogenic hormones (PRL and rCM). This might be surprising if it is born in mind that PRL seems to induce the synthesis of its own receptors [25]. However, an alternative possibility can be considered, that $PGF_2\alpha$ induces the synthesis of lactogenic membrane receptors in the mammary gland, either through a direct or an indirect mechanism. In fact, the sharp increase in mammary prolactin binding which followed $PGF_2\alpha$ treatment, is not due to an effect of

"unmasking" the binding sites, since rCM levels are high simultaneously with rise in specific PRL binding sites.

Prostaglandin may also facilitate the action of PRL and other lactogenic hormones at the mammary gland level. Rillema[26] has recently described a possible participation of $PGF_2\alpha$ as a mediator of PRL on RNA metabolism and casein synthesis in mammary gland of mice. In pregnant rabbits $PGF_2\alpha$ induced lactogenesis correlated with a significant increase in casein synthesis, lactose and lactosamine synthetase activities (R. Deis, L. Houdebine and C. Delouis, unpublished results).

In conclusion, prostaglandin $F_2\alpha$ may have a physiological role in the establishment of lactogenesis inducing a decrease in progesterone secretion which allows prolactin to be released and to occupy the PRL receptor previously synthesized.

Acknowledgements—This work was supported by PLA-MIRH research grant 126.224.2.78 and the Consejo Nacional de Investigaciones Cientificas y Técnicas of which L.E.B. is a post-doctoral fellow and R.P.D. is a career scientist.

We are grateful to the National Institue of Arthritis, Metabolism and Digestive Diseases for the radioimmunoassay kit for prolactin and the ovine prolactin, and to Dr. J. E. Pike of the Upjohn Co. for the gift of $PGF_2\alpha$. We thank Mr. A. Stati for his skillful technical assistance.

REFERENCES

1. Halban J.: Die innere Secretion von Ovarum und Placenta und ihre Bedeutung für die Function der Milchdrüsen. *Arch. Gynaek* **75** (1905) 353–441.
2. Csapo A. I.: Progesterone "block". *Am. J. Anat.* **98** (1956) 273–291.
3. Deis R. P.: Oxytocin test to demonstrate the initiation and end of lactation in rats. *J. Endocr.* **40** (1968) 133–134.
4. Khun N. J.: Progesterone withdrawal as the lactogenic trigger in the rat. *J. Endocr.* **44** (1969) 39–54.
5. Eto T., Masuda H., Susuki Y. and Hosi T.: Progesterone and pregn-4-ene-20α-ol-3-one in rat ovarian venous blood at different stages in the reproductive cycle. *Jap. J. Anim. Reprod.* **8** (1962) 34–40.
6. Grota L. J. and Eik-Nes K. B.: Plasma progesterone concentration during pregnancy and lactation in rat. *J. Reprod. Fert.* **13** (1967) 83–91.
7. Morishige W. K., Pepe G. J. and Rothchild I.: Serum luteinizing hormone, prolactin and progesterone levels during pregnancy in the rat. *Endocrinology* **92** (1973) 1527–1530.
8. Deis R. P.: Induction of lactogenesis and abortion by prostaglandin $F_2\alpha$ in pregnant rats. *Nature* **229** (1971) 568.
9. Vermouth N. T. and Deis R. P.: Prolactin release induced by prostaglandin $F_2\alpha$ in pregnant rats. *Nature New Biol.* **238** (1972) 248–250.
10. Gutknecht G. D., Cornette J. C. and Pharris B. B.: Antifertility properties of $PGF_2\alpha$. *Biol. Reprod.* **1** (1969) 367–371.
11. Pharris B. B. and Wyngarden L. J.: The effect of prostaglandin $F_2\alpha$ on the progestogen content of ovaries from pseudopregnant rats. *Proc. Soc. Exp. Biol Med.* **130** (1969) 92–94.
12. Behrman H. R., Yoshinaga K., Wyman H. and Greep R. O.: Effects of prostaglandin on ovarian steroid secretion and biosynthesis during pregnancy. *Am. J. Phys.* **221** (1971) 189–193.
13. Niswender G. D., Chen C. L., Midgley A. R. Jr, Meites J. and Ellis S.: Radioimmunoassay for rat prolactin. *Proc. Soc. Exp. Biol. Med.* **130** (1969) 793–797.
14. Abraham G. E., Swerdloff R., Tulchinsky C. and Odell W.: Radioimmunoassay of progesterone. *J. clin. Endocr. Metab.* **32** (1971) 619–625.
15. Shiu R. P. C., Kelly P. A. and Friesen H. G.: Radioreceptor assay for prolactin and other Lactogenic hormones. *Science* **180** (1973) 968–971.
16. Thorell J. I. and Johansson B. G.: Enzymatic iodination of polypeptides with ^{125}I to high specific activity. *Biochim. biophys. Acta* **251** (1971) 363–369.
17. Wilcox R. B. and Wiest W. G.: Further studies of rat ovarian 20 α-hydroxysteroid dehydrogenase. *Steroids* **7** (1966) 395–413.
18. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–276.
19. Vermouth N. T. and Deis, R. P.: Inhibitory effect of progesterone on the lactogenic and abortive action of prostaglandin $F_2\alpha$. *J. Endocr.* **66** (1975) 21–29.
20. Wiest W. G., Kidwell W. R. and Balogh K. Jr.: Progesterone catabolism in the rat ovary: A regulatory mechanism for progestational potency during prenancy. *Endocrinology* **32** (1968) 844–859.
21. Khun N. J. and Briley M. S.: The roles of pregn-5-ene-3-β, 20α-diol and 20α-Hydroxy steroid dehydrogenase in the control of progesterone synthesis preceding parturition and lactogenesis in the rat. *Biochem. J.* **117** (1970) 193–201.
22. Shinde Y., Ota K. and Yokoyama A.: Lactose content of mammary glands of pregnant rats near term; effect of removal of ovary, placenta and foetus. *J. Endocr.* **31** (1965) 105–114.
23. Strauss J. F. and Stambaugh R. L.: Induction of 20 α-hydroxysteroid dehydrogenase in rat corpora lutea of pregnancy by prostaglandin $F_2\alpha$. *Prostaglandins* **5** (1974) 73–85.
24. Behrman H. R., MacDonald G. and Greep R. O.: Regulation of ovarian cholesterol esters. Evidence for the enzymatic sites of prostaglandin-induced loss of corpus luteum function. *Lipids* **6** (1971) 791–796.
25. Bohnet H. G., Gómez F. and Friesen H. G.: Prolactin and estrogen binding sites in the mammary gland of the lactating and not lactating rat. *Endocrinology* **101** (1977) 1111–1121.
26. Rillema J. A.: Effects of prostaglandins on RNA and casein synthesis in mammary gland explants of mice. *Endocrinology* **99** (1976) 490–495.