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Prostaglandin Administration to Pregnant Rats: Effects on Pituitary-Target Gland Systems of Mother, Fetus and Neonate[1] (38512)

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It is well established that the prostaglandins (PGs) are among the most active natural substances known (1) and that these substances may exert profound effects on endocrine gland function (2, 3). Alterations in the functional activity of various pituitary-target gland systems are known to occur after treatment with prostaglandins. Administration of luteolytic doses of $PGF_{2\alpha}$ to pregnant rats is associated with large increases in pituitary LH stores, even exceeding those found in ovariectomized animals (4). The secretion of pituitary gonadotrophic hormones is differentially affected after infusion of PGE_1 and PGE_2 into the third ventricle of ovariectomized rats (5). PGE_1 reproduces most, if not all of the effects of TSH on the thyroid gland *in vitro*, possibly by interacting with the adenyl cyclase–cyclic AMP system (3, 6, 7). A stimulatory action of PGE_1 on the pituitary–adrenocortical system has also been described; the enhancing effect of PGE_1 on ACTH release is mediated by the median eminence (8) and is abolished in pentobarbital treated rats (9). Serum prolactin (PRL) levels increase substantially after injection of $PGF_{2\alpha}$ in late pregnancy (10), although this prostaglandin is alleged to be without effect on prolactin secretion in lactating rats (11).

Recent studies by D'Angelo and Wall (12) on maternal–fetal endocrine interrelations in the rat have demonstrated that the conjoint administration of goitrogens and adrenal inhibitors to the pregnant rat induces simultaneous hypersecretion of TSH and ACTH by the fetal hypophysis. Treatment with the dibutyryl derivative of 3′, 5′-cyclic adenosine monophosphate also enhances TSH secretion in mother, fetus and neonate but does not significantly affect functional activity of the pituitary–adrenal system (13). Although experimental research into the physiologic and pharmacologic role(s) of the prostaglandins in female reproductive processes has proliferated, the specific effects of these substances on anterior pituitary secretion and target endocrine function in pregnancy have received very little scrutiny. The spectrum of PGE_1 stimulating effects on the endocrine gland system prompted us to determine the morphologic and physiologic consequences of its administration in late pregnancy to the maternal and fetal pituitary-target gland systems.

Materials and Methods. Virgin female rats (CD strain: Charles River Breeding Laboratories, Wilmington, MA) were mated by the commercial supplier by placing males with females at 4 PM on the day of proestrus. The presence of sperm in the vagina at 7 AM the next morning was considered to indicate pregnancy (first day, "0" hr). The timed-pregnant rats arrived at the laboratory on the 15th or 16th day of gestation and were kept in separate breeding cages under controlled conditions of temperature (23 ± 1°) and artificial illumination (7 AM to 7 PM). Rats were given tap water and Purina Laboratory Chow in unrestricted amounts. Some pregnant rats were hypophysectomized (parapharyngeal approach) on the 14th or 15th day of gestation. Body weights of pregnant rats were recorded daily.

The response of the pituitary-target gland systems to PGE_1 was tested in the pregnant animal by subcutaneous administration (generous gifts from Dr. John Pike, The Upjohn Company, are gratefully acknowledged). PGE_1 was first dissolved in absolute ethanol (1 mg/0.1 ml) and alkalinized with 0.9 ml of sodium carbonate (0.2 mg/ml). Rats received injections of the drug (0.25 to 1.0 mg) at 90 or 120 min intervals beginning at 8:30 AM on the 21st day of gestation. Control ani-

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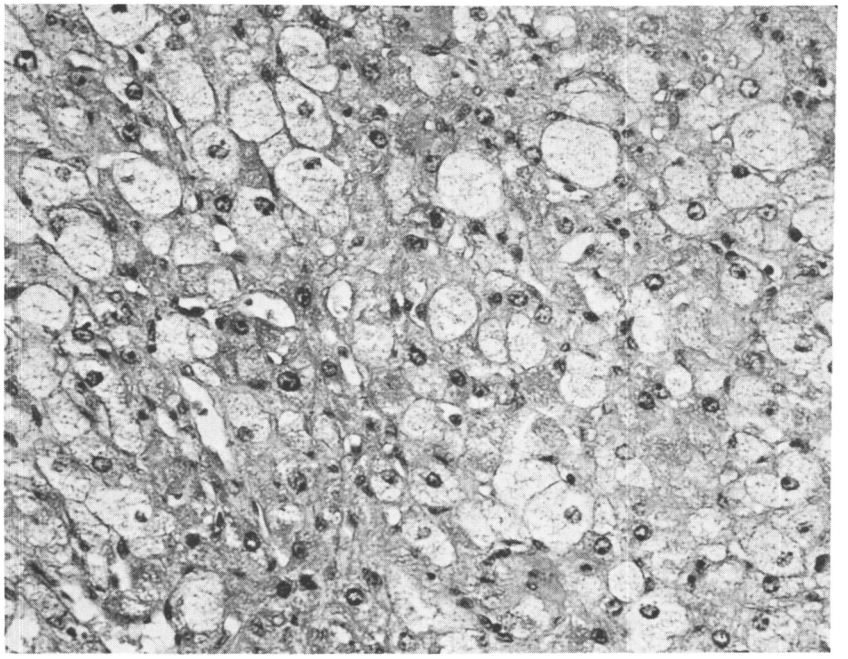
mals received the vehicle alone. Cesarean section was performed on the afternoon of the same day after three to five injections. Fetuses were quickly stripped from the uteri while the sectioned mothers were maintained under ether anesthesia (2–3 min), after which blood was withdrawn by direct cardiac puncture. Blood from the fetuses was obtained by decapitation and thoracic incision.

At autopsy, the endocrine organs were removed, weighed to the nearest 0.1 mg on a microtorsion balance, and prepared for histological, chemical or assay studies. Corticosteroid content of plasma and adrenals was determined by a fluorometric procedure, as previously described (14). Thyroid radioiodine uptakes were routinely determined in mothers and offspring 22–24 hr after ip administration of ^{125}I. Pregnant rats subjected to cesarean section received 10 μCi of ^{125}I on the 20th day of gestation. Mothers allowed to deliver spontaneously, were given ^{125}I immediately after delivery was completed. Their newborns were also injected at this time with 0.5 μCi of ^{125}I. Radioiodine accumulation in thyroids was measured after homogenizing the glands in vials containing 2% NaOH and determining their radioactivity in a well type scintillation counter. Radioactivity of heparinized blood (0.3 to 1 ml) was also determined. TSH and prolactin content of pooled plasma and acid saline pituitary extracts were estimated by radioimmunoassay technics using the Parlow kit. LH levels in plasma and pituitary were estmated by the radioimmunoassay method of Niswender (15). Endocrine gland weights were related to final body weight and are recorded herein as mg/100 g body. Statistical significance of the data was assessed using the Student's t test.

Results. 1. *Morphologic and physiologic consequences of PGE$_1$.* Mothers receiving PGE$_1$ displayed dramatic microscopic changes in their ovaries. The larger doses produced marked histologic changes in the corpora lutea, especially in peripheral cells. Parenchymal cells in normal corpora lutea were typically well defined by their clear cut borders, coarsely granular or vacuolated cytoplasm and by round vesicular nuclei (Fig. 1). Luteal cells in ovaries of PGE$_1$ treated mothers underwent marked transformation into enlarged polygonal cells characterized by clear or foamy cytoplasm and shrunken or shriveled nuclei (Fig. 2). Distension of sinusoidal capillaries was observed frequently. The 0.25 mg dose of PGE$_1$ induced only moderate microscopic change in luteal cells of intact mothers but marked histological alterations were seen in corpora lutea of hypophysectomized, pregnant rats with this dose. A moderate increase in ovarian weight (Table I) was accompanied by luteal cell changes (Figs. 3 and 4). Luteal cell borders were sharply delineated by retraction and clearing of cytoplasm from the cell periphery and by condensation around the deeply staining, round nuclei. Distension of the sinusoids with blood elements was frequently noted.

PGE$_1$ administration to intact, pregnant rats did not induce significant increases in thyroid, adrenal or ovarian weight. Thyroidal ^{125}I uptake and blood radioactivity were moderately diminished but the thyroid–blood radioactivity ratios increased from 6.88 to 7.88. PGE$_1$ treatment resulted in a moderate increase in plasma corticosteroids levels but corticosteroid content of the adrenal remained in the normal range. As expected, functional activity of the maternal pituitary-target gland systems was reduced by hypophysectomy. Ovarian, thyroid and adrenal gland weights and their functional indices were all substantially lowered. PGE$_1$ treatment of hypophysectomized mothers, however, effected some increase in ovarian weight to nearly normal values of intact mothers.

2. *Effects of PGE$_1$ on Fetus and Neonate.* There was no indication that PGE$_1$ treatment of intact mothers resulted in any substantial change in thyroid or adrenal function of the fetus. Adrenal and thyroid-body weight ratios remained in the normal control range and corticosteroid content of the fetal adrenal was not materially influenced by PGE$_1$ treatment. As demonstrated previously (14), fetal adrenal weight was consistently increased after maternal hypophysectomy. The adrenal hypertrophy was moderately accentuated with PGE$_1$ treatment although corticosteroid content of the gland was normal (Table II). Thyroidal radioiodine uptake in fetuses derived from intact or hypophysectomized mothers was not significantly altered.



FIGS. 1–4. All photomicrographs are of peripheral portions of corpora lutea in the maternal ovary (Bouin's, hemotoxylin, eosin, X 250: Polaroid Film No. 52).

FIG. 1. The effects of four injections of PGE$_1$ (0.5 mg) on luteal cells in the mature ovary are illustrated. There is cellular hypertrophy with clearing of cytoplasm and condensation of nuclei.

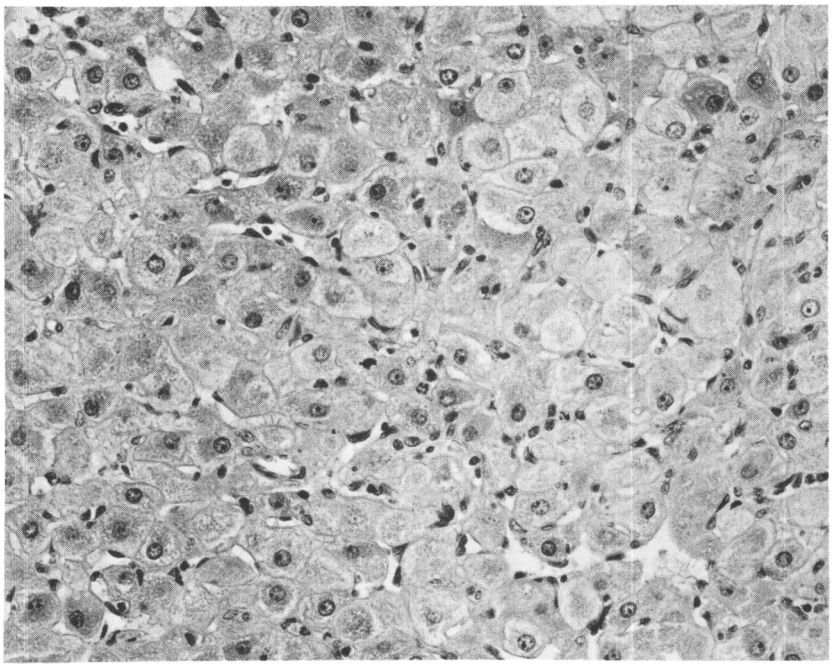


FIG. 2. The normal luteal cell histology is recorded for the ovary of mothers receiving saline injections.

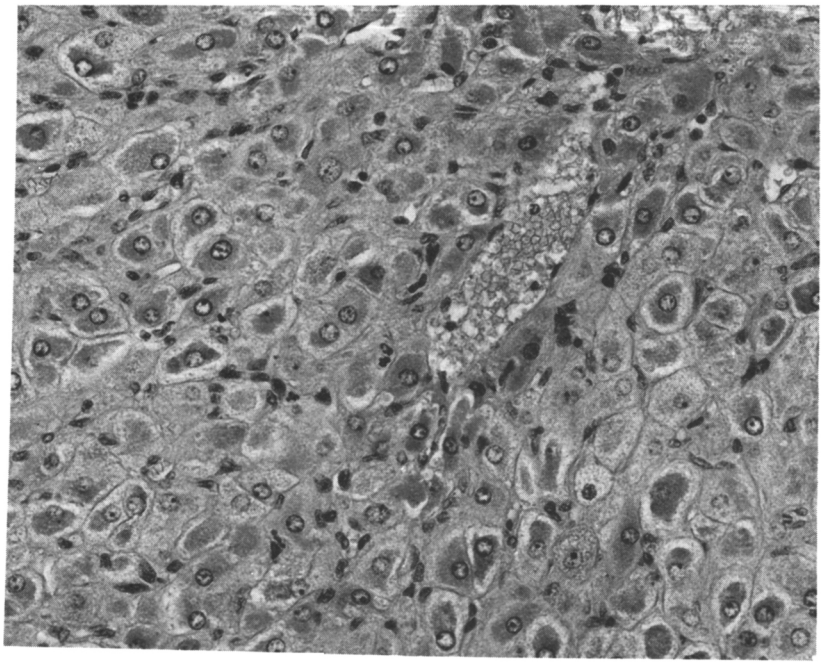


FIG. 3. The effects of four injections of PGE$_1$ (0.25 mg) on luteal cells in the ovary of the hypophysectomized mother is shown. Clearing and retraction of the cytoplasm from the periphery of luteal cells and its condensation around the nucleus are shown, as well as the sinusoidal distension.

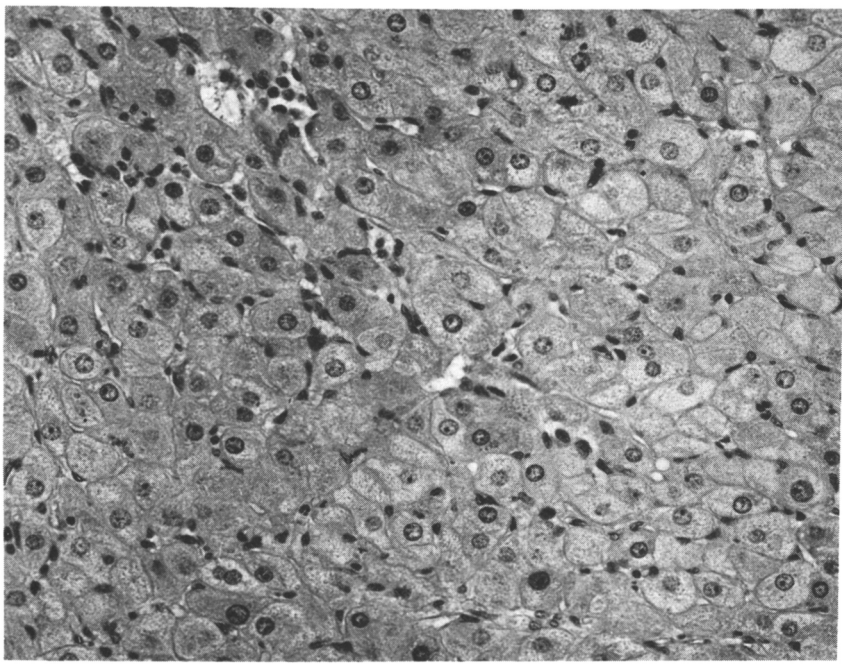


FIG. 4. Normal luteal cell histology is illustrated for the ovary in saline injected, hypophysectomized mother.

TABLE I. ENDOCRINE RESPONSE IN CESAREAN SECTIONED MOTHERS AFTER PGE_1 TREATMENT.

Group (No.)	Body weight g ± SE	Organ Weight mg/100 g Body ± SE			% ^{125}I Uptake × 10^{-2}		Corticosteroid	
		Thyroid	Adrenal	Ovary	Thyroid/mg ± SE	Blood/ml ± SE	Plasma μg/100 ml ± SE	Adrenal μg/g ± SE
Saline mothers[a] (8)	333 ± 13	5.2 ± 0.2	20.4 ± 0.7	30.2 ± 0.9	16.7 ± 3.0[c]	2.5 ± 0.4[c]	58.7 ± 4.4	41.3 ± 5.1
PGE_1 mothers (11)	329 ± 9	4.8 ± 0.3	21.3 ± 1.7	29.5 ± 1.0	13.4 ± 1.0	1.7 ± 0.1	84.6 ± 8.1*	44.6 ± 4.7
Hypx[b] saline mothers (5)	359 ± 13	3.8 ± 0.1	11.8 ± 1.4	23.6 ± 2.3	2.9 ± 0.1	8.2 ± 1.0	8.5 ± 0.1	3.5 ± 0.2
Hypx PGE_1 mothers (5)	350 ± 12	3.7 ± 0.1	11.1 ± 0.1	28.9 ± 1.8	3.1 ± 0.1	1.2 ± 0.1*	9.5 ± 0.8	6.4 ± 1.0

[a] All mothers received sc injections of saline or PGE_1 (0.25–1.0 mg) at 90 min intervals, beginning at 8:30 AM on the 21st day of gestation; cesarean section done following fourth or fifth injection.

[b] Hypophysectomy done on the 14th or 15th day of gestation.

[c] Radioiodine uptake measured 22–24 hr after ip injection of 10 μCi ^{125}I on the 20th day of gestation.

* Mean differs significantly ($P = < .025$) from that of the appropriate control group.

TABLE II. PGE_1 ADMINISTRATION TO PREGNANT RATS: EFFECTS ON THYROID AND ADRENAL IN FETUS AND NEONATE.

Group (No.)	Body weight g ± SE	Thyroid weight mg/100 g body ± SE	% ^{125}I Uptake ± SE[b]		Adrenal weight mg/100 g body ± SE	Adrenal corticosteroid μg/g ± SE
			Thyroid/mg	Blood/ml		
Saline mothers[a]						
Fetuses (56)	5.9 ± 0.08	18.6 ± 0.8	0.10 ± 0.01	0.04 ± 0.00	45.8 ± 1.4	32.5 ± 4.4
Neonates (32)	5.9 ± 0.06	21.3 ± 0.2	1.46 ± 0.21	4.71 ± 0.38*	44.2 ± 0.7	14.4 ± 2.5
PGE_1 mothers						
Fetuses (74)	5.9 ± 0.07	19.2 ± 0.9	0.10 ± 0.01	0.02 ± 0.00	48.6 ± 1.3	37.4 ± 0.7
Neonates (21)	5.7 ± 0.17	20.1 ± 0.5	1.21 ± 0.30	0.71 ± 0.24*	42.6 ± 0.8	12.4 ± 3.4
Saline hypx mothers						
Fetuses (28)	5.3 ± 0.08	19.0 ± 0.9	0.12 ± 0.02	0.16 ± 0.04	51.4 ± 0.9	35.8 ± 4.5
PGE_1 hypx mothers						
Fetuses (49)	4.7 ± 0.08	16.9 ± 0.8	0.12 ± 0.01	0.08 ± 0.01	57.3 ± 1.2	35.0 ± 1.2

[a] All mothers received sc injections of PGE_1 (0.25 or 0.5 mg) or saline at 90 min intervals beginning on the 21st day (8:30 AM) of gestation; cesarean section performed in the PM after four or five injections.

[b] Radioiodine uptake of fetal tissue measured 22–24 hr after injection of 10 μCi ^{125}I to mothers (on day 20); radioactivity in newborn determined 22–24 hr after injecting the neonate with 0.5 μCi of ^{125}I.

* Means differ significantly ($P = < .01$).

TABLE III. HORMONE LEVELS IN PLASMA AND PITUITARY OF MOTHER, FETUS AND NEONATE AFTER PGE$_1$ TREATMENT.

Treatment (No.)	Mean TSH ± SE		Mean prolactin ± SE		Mean LH ± SE	
	Plasma munit/100 ml	Pituitary[a] munit/mg	Plasma ng/ml	Pituitary[a] μg/mg	Plasma ng/ml	Pituitary μg/mg
Saline mothers (5)	23.0 ± 2.3 (<40)*	40.2 ± 4.5 (34.3)	79 ± 8.6	0.49 ± 0.10	4.1 ± 1.8	1.32 ± 0.4
Fetuses (46)	17.0 (<40)	2.8 (1.4)	6.7	0.05		
Neonates (32)	31.6 (<40)	6.9 (5.7)	32.0	<0.02		
PGE$_1$ mothers (13)						
1.0 mg (3)	152.5	37.0	426	1.06	23	1.05
0.50 mg (4)	103.6 (118.1)	49.9 (39.8)	127	0.77	36	0.77
0.50 mg (4)	93.3	55.3	130	1.05	36	1.38
0.25 mg (2)	81.6	29.8	135	0.42	4	0.44
Fetuses (74)	23.8 (<40)	2.9 (1.9)	5.6	0.06		
Neonates (22)	116.0 (200)	3.8 (4.1)	107.0	0.04		
Hypx mothers (3)	3.2		<4.0		1	
Fetuses (28)	22.9	2.6	5.3	<0.03		
PGE$_1$ hypx mothers (5)	5.3		<4.0		1	
Fetuses (44)	13.1	1.5	5.0	<0.03		

[a] Maternal values represent concentrations in the anterior pituitary; underlined values for fetuses and neonates are based on the whole hypophysis, and represent grand means of duplicate assay determinations on two or more separate pools of plasma and pituitary.

* Values in parentheses obtained by bioassay; all other values determined by radioimmunoassay.

Blood radioactivity was consistently lowered by PGE$_1$ treatment, however, and thyroid–blood radioactivity ratios doubled. A comparison of results observed in spontaneously delivered newborn from saline and PGE$_1$ treated mothers revealed some substantial differences from those in the fetus. Regardless of treatment, radioactivity in both blood and thyroid of the neonate was appreciably increased over fetal values. PGE$_1$ treatment markedly increased (from 0.3 to 1.7) thyroid–blood radioactivity ratios in the neonate. Adrenal weight in neonates moderately decreased and corticosteroid concentration of the neonatal adrenal was reduced to about a third of that in the fetal gland. PGE$_1$ treatment did not influence these indices of adrenal function in the newborn, however.

3. *PGE$_1$ and Pituitary Hormones in Mother, Fetus and Neonate.* Results from cesarean sectioned mothers indicate that sequential injections of PGE$_1$ on the last day of gestation caused marked release of hormones from the anterior pituitary. The combined mean assay results obtained from the several doses utilized demonstrate a fivefold increase over the control TSH plasma level (Table III); the 0.25 mg dose of PGE$_1$ still effected a significant rise in plasma TSH. Pituitary TSH stores remained in the normal range. Significant prolactin release from the maternal pituitary also occurred. Plasma prolactin levels rose sharply with the 1 mg does of PGE$_1$ and were still elevated with the 0.25 mg dose. The overall increase in plasma prolactin level after PGE$_1$ treatment was about three-fold. Prolactin concentration in the anterior pituitary was well maintained. The larger doses of PGE$_1$ also induced substantial LH release; the 0.25 mg dose ap-

parently was ineffectual in raising the plasma level of LH, although a sharp drop in pituitary LH content occurred. Circulating levels of these pituitary hormones fell sharply in hypophysectomized mothers, ranging from 5% or less of control values.

Despite the maternal rise in plasma levels of the pituitary hormones which attended PGE_1 treatment, there was no indication of any comparable hormone release from the fetal hypophysis. TSH and PRL concentrations in the fetal gland were only a small fraction of that demonstrated in the maternal pituitary. PRL was consistently detected in fetal blood but at levels usually one-tenth that of mothers. Substantial change in plasma hormone levels were recorded in the neonates, however. Plasma TSH and PRL concentrations were significantly increased over the fetal values in spontaneously delivered newborns from normal mothers. The elevation in blood levels of TSH and PRL in neonates of PGE_1 treated mothers far exceeded (500–1000%) that found in normal offspring (Table III).

Discussion. Results of this study clearly indicate that acute injections of PGE_1 in late pregnancy induce prompt and marked release of several trophic hormones from the maternal pituitary. Whether or not the stimulatory action of PGE_1 is mediated directly at the pituitary level or indirectly via the hypothalamus cannot be stated. Experiments utilizing prostaglandins in non-pregnant rats strongly suggest that hypothalamo-hypophysial mechanisms are involved. Harms *et al.* (5) infused prostaglandins into the third ventricle of ovariectomized rats and described rapid elevations (15–30 min) in plasma levels of prolactin and LH with PGE_1 and PGE_2, respectively. Neither substance had any gonadotrophin releasing effect when infused directly into the pituitary. It was inferred that these prostaglandins acted primarily to alter secretory rates of the corresponding hypothalamic releasing or inhibiting hormones into portal blood. Peng *et al.* (9) demonstrated that intravenous injections of PGE_1 stimulated release of ACTH in rats, but not in animals anesthesized with pentobarbital. A primary action on the central nervous system, presumably the hypothalamus, was postulated. Hedge and Hanson (8) confirmed this view by demonstrating that microinjections of PGE_1 into the rat's median eminence released ACTH but were not effective when made directly into the pituitary. Electrolytic lesioning or deafferentation of the medial-basal hypothalamus of the pregnant rat prior to PGE_1 administration would be helpful in elucidating its site of action but such operative procedures are rarely successful in pregnancy; microinjection technics would seem more promising.

The histologic changes observed in corpora lutea of the maternal ovary after PGE_1 treatment are of particular interest. The luteolytic effects of $PGF_{2\alpha}$ were not considered to be dependent upon a primary action on gonadotrophin secretion (16, 17) but Labhsetwar (4) associated luteolysis following administration of this prostaglandin with marked increase in stores of pituitary LH, and postulated an action mediated in part by augmented synthesis and release of LH. Vermouth and Dies (10) were able to inhibit partially the $PGF_{2\alpha}$ induced rise in serum prolactin in late pregnancy by progesterone administration. It was suggested that $PGF_{2\alpha}$ releases prolactin by decreasing plasma progesterone level. Without direct estimation of progesterone secretory rates by the maternal ovaries, interpretation of the luteal histologic changes in our study can only be speculative. It is plausible that elevated circulating levels of prolactin and/or LH may have stimulated luteal cell hypertrophy but the significant increase in pituitary LH stores found earlier (4) was not observed. Prolactin administration in high doses has been found to decrease ovarian weight and reduce the size and numbers of cells in corpora lutea of hypophysectomized rats (18). Such regressive morphologic changes were not observed in ovaries of intact or hypophysectomized mothers regardless of prolactin or LH blood levels; a direct, nonhormonal effect of PGE_1 on the ovary of the pregnant rat seems likely.

Although PGE_1 is known to mimic most of the primary actions of TSH on physiologic and morphologic concomitants of thyroid hormone secretion (3, 6, 7, 21), it is equally clear that there are basic differences in mechanisms of action between TSH and PGE_1 (6, 7, 19, 20). Burke *et al.* (22) have postulated that an intracellular cyclic AMP–

PGE system exists which serves to modulate TSH effects on thyroid function. Others deny that the action of TSH on a thyroidal adenyl cyclase–cyclic AMP system is mediated by endogenous PGE$_1$ (6). It is of singular interest that in our *in vivo* study thyroidal radioiodine accumulation was reduced rather than enhanced, despite a fivefold increase in the maternal level of circulating TSH. The The consistent lowering of the blood radioactivity, and corresponding increases in thyroid–blood radioactivity ratios in mother and offspring very likely indicate some change in the dynamics of iodide transport. Altered thyroid hormone secretory rate and disposal at the periphery may also have occurred. A recent study by Shenkman *et al.* (23) demonstrated that PGF$_{2\alpha}$ had a direct action on the thyroid of pregnant women; T_4 and T_3 serum levels were significantly increased without change in basal TSH levels. Anomalous effects of nucleotides on radiometric functions of the thyroid have already been described for the rat's perinatal period (13). It is felt that prostaglandins, like nucleosides, may have separate and independent *in vivo* actions on thyroid and adenohypophysis.

The release of maternal pituitary hormones induced by PGE$_1$ treatment was accompanied by comparable release of TSH and PRL from the pituitary of the neonate. The dramatic increase in blood levels of these hormones in the day-old newborn strongly suggests that the response of the fetal hypophysis to PGE$_1$ was actually delayed. The reason for this latency is not clearly apparent but it may reflect diflerences in sensitivity of the maternal and fetal pituitary to the administered PGE$_1$. The process of spontaneous delivery itself has been correlated with significant shifts in parameters of pituitary-thyroid function in the rat's perinatal period (13). The delayed pattern of TSH release from the fetal pituitary observed in the present study holds for prolactin secretion as well. How much of the PGE$_1$ administered to the mother actually traverses the placental to enter the fetus, and to persist in the neonate cannot be stated. There is no reason to suspect, however, that the fetal pituitary-adrenocortical system was materially influenced by PGE$_1$ treatment. The reduction in weight and corticosteroid content which occurs in the neonatal adrenal is well known and has been attributed to prolonged feedback action of fetal and maternal corticosteroids on ACTH secretion in the late fetus (14). PGE$_1$ administered to the mother apparently fails to influence this process. We have preliminary evidence (unpublished) to indicate that postnatal administration of PGE$_1$ causes marked stimulation of pituitary TSH secretion in newborn rats. The functional activity of pituitary-target gland systems after parturition and during postnatal development are now being studied.

Summary and Conclusions. Acute administration of PGE$_1$ in graded doses (0.25–1.0 mg) to pregnant rats in late gestation (21st day) induced significant release of TSH, prolactin and LH from the maternal pituitary. Plasma levels of PRL and TSH were elevated (three- to fivefold) in cesarean sectioned mothers given 4–5 sc injections of PGE$_1$; higher doses also stimulated LH release. Comparable increases in blood TSH and PRL levels were also found in their newborn but no indication of enhanced hormone release from the fetal hypophysis was noted. Histologic alterations were observed in the ovary (luteal cell hypertrophy, cytoplasmic clearing, nuclear condensation, sinusoidal distension) of mothers receiving PGE$_1$ treatment. Thyroidal radioiodine accumulation in mother, fetus and neonate were usually decreased whereas thyroid:blood radioactivity ratios were consistently increased.

It is concluded that PGE$_1$ treatment of the pregnant rat near term stimulates release of hormones from the pituitary in mothers (TSH, LH, PRL) and in their newborn (TSH, PRL). The results also strongly suggest that PGE$_1$ administration may induce morphologic and functional changes in target endocrines (ovary, thyroid) by direct and separate actions.

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1. Weeks, J. R., Fed. Proc. **33,** 37, 1974.
2. Labhsetwar, A. P., Fed. Proc. **33,** 61, 1974.
3. Mashiter, K., and Field, J. B., Fed. Proc. **33,** 78, 1974.
4. Labhsetwar, A. P., J. Reprod. Fert. **23,** 155, 1970.
5. Harms, P. G., Ojeda, S. R., and McCann, S. M., Science **181,** 760, 1973.
6. Mashiter, K., Mashiter, G. D., and Field, J. B., Endocrinology **94,** 370, 1974.
7. Burke, G., Amer. J. Physiol. **218,** 1445, 1970.
8. Hedge, G. A., and Hanson, S. D., Endocrinology **91,** 925, 1972.
9. Peng, T. C., Six, M. K., and Munson, P. L., Endocrinology **86,** 202, 1970.
10. Vermouth, N. T., and Dies, R. P., Nature New Biol. **238,** 248, 1972.
11. Guthknecht, G. D., Cornette, J. C., and Pharriss, B. B., Biol. Reprod. **1,** 367, 1969.
12. D'Angelo, S. A., and Wall, N. R., Endocrinology **89,** 591, 1971.
13. D'Angelo, S. A., and Wall, N. R., Proc. Soc. Exp. Biol. Med. **143,** 941, 1973.
14. D'Angelo, S. A., Paul, D. H., and Wall, N. R., Amer. J. Physiol. **224,** 543, 1973.
15. Niswender, G. N., Midgley, Jr., A. R., Monroe, S. E., and Reichert, Jr., L. E., Proc. Soc. Exp. Biol. Med. **128,** 807, 1968.
16. Pharriss, B. B., Tillson, S. A., and Erickson, R. R., Recent Progr. Horm. Res. **28,** 51, 1972.
17. Pharriss, B. B., and Wyngarden, L. J., *in* "Gonadotropins 1968" (E. Rosemberg, ed.), p. 121, Geron-X, Inc., Los Altos, CA (1968).
18. MacDonald, G. J., Yoshinaga, K., and Greep, R. O., Proc. Soc. Exp. Biol. Med. **143,** 1031, 1973.
19. Kendall-Taylor, P., J. Endocrinology **54,** 137, 1972.
20. Dekker, A., and Field, J. B., Metabolism **19,** 453, 1970.
21. Onaya, T., and Solomon, D. H., Endocrinology **86,** 423, 1970.
22. Burke, G., Chang, L. L., and Szabo, M., Science **180,** 872, 1973.
23. Shenkman, L., Imai, Y., Kataoka, K., Hollander, C. S., Wan, L., Tang, S. C., and AvRuskin, T., Science **184,** 81, 1974.

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