Effect of Hydrocortisone Acetate on Mammary Gland Nucleic Content of Pregnant Rats (33757)

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Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content of rat mammary glands increases following parturition (1, 2). The rise in RNA reflects the increase in protein synthesis associated with lactation (1). The DNA levels suggest a significant degree of cell division during early lactation (3).

It has been suggested (4, 5) that an increase in corticosteroid activity during late pregnancy is important for the initiation of lactation. Gala and Westphal (4) have observed a significant elevation in corticosteroid activity in the rat during late pregnancy. Talwalker *et al.* (5) found evidence of alveolar secretion in mammary glands of pregnant rats injected with hydrocortisone acetate (HCA) for 5 days. Our investigation was undertaken to see if pregnant rats similarly treated with HCA would show an increase in RNA and DNA comparable to that which occurs in mammary glands during early lactation.

Methods and Materials. Sprague-Dawley-Rolfsmeyer rats were kept in a temperature controlled (25° ± 3), artificially lighted room (8 a.m.–10 p.m.). Wayne Lab Blox and water were provided *ad libitum*. Mature virgin females were placed in cages with males; vaginal smears were made each morning. Presence of sperm was considered as day 1 of pregnancy. Hydrocortisone acetate (Nutritional Biochemicals Corporation) and a placebo without the hormone were prepared as described by Nandi (6). Injections of 0.1 ml were made subcutaneously. One group received 2 mg of HCA each day from days 9–13 of pregnancy; another group was given the same treatment from days 16–20 of pregnancy. Control groups received placebo injections during the same periods. The day after the final injection each rat was killed by an overdose of ether; abdominal inquinal glands were removed and immediately frozen. Frozen tissue was minced and defatted in 2:1 chloroform–methanol for 24 hr, then in ether for 24 hr. The tissue was dried, weighed, and ground to a powder in a Wiley mill. Total nucleic acid was extracted from 25-mg samples of ground tissue with hot trichloroacetic acid, as described by Schneider (7). The DNA content was estimated by Burton's diphenylamine method (8), and RNA was estimated using the orcinol procedure (9). To eliminate variability resulting from size differences, DNA per 100 g of body weight was used as an index of mammary growth, and the ratio of total RNA to total DNA was considered a more accurate index of metabolic activity than total RNA alone. Data were analyzed for statistical significance using Student's t test.

Results. The HCA administered during midpregnancy, as well as during late pregnancy (Table I), produced no difference in DNA content from that found in the mammary glands of control rats. The RNA was not affected by HCA given during midpregnancy, but during late pregnancy HCA did induce an increase in RNA. The RNA/DNA ratio was significantly higher ($p<0.05$) than values obtained from control rats, indicating elevated cellular RNA content.

Discussion. The increase in RNA resulting from HCA administration during late pregnancy is evidence that preparturient elevation of corticosteroid may, in fact, play an important role in the initiation of lactation. However, the same treatment with HCA during midpregnancy failed to promote the rise in cellular RNA normally associated with the initiation of lactation. This may simply indicate that the mammary gland is not sufficiently differentiated by midpregnancy to be affected by the increased corticosteroid level. The inability of exogenous HCA to influence

RNA metabolism during midpregnancy might also result from a difference in the hormonal condition of the rat at this time. Prolactin levels remain low during most of the gestation period, but begin to rise during late pregnancy, and rapidly increase following parturition (10). If the action of glucocorticoids is to synergise with prolactin to induce lactation, the difference in prolactin levels during midpregnancy and late pregnancy might explain why RNA increased only during late pregnancy. It is interesting to note that the preparturient increase in corticosteroid activity described by Gala and Westphal (4), did not result from changes in costicosteroid-binding globulin or to any major change in corticosteroid levels. Gala and Westphal suggested that this increased activity might be due to an increase in the biological effectiveness of corticosteroids. If some condition does potentiate the biological effect of the glucocorticoids during late pregnancy, it may be responsible for the ability of exogenous HCA to stimulate an increase in RNA at this time.

Our results show that HCA treatment of rats during pregnancy has no effect on mammary growth, as measured by DNA content. Kumaresan *et al.* (11) gave corticosterone to pregnant rats for a period of 20 days and did observe an increase in mammary DNA. Conflicting results concerning the effects of glucocorticoids on mammary glands are not unusual, and most likely result from differences in treatment, dose level, time and length of injection, type of hormone, or the endocrine and metabolic condition of the experimental animals. Our data, however, show that increasing the glucocorticoid level during late pregnancy sufficiently to stimulate an increase in mammary RNA does not produce a corresponding rise in DNA as occurs in normal mammary glands following the initiation of lactation.

Summary. The RNA and DNA content of mammary glands from pregnant rats treated with HCA was determined. Rats injected with 2 mg of HCA per day for 5 days during midpregnancy exhibited no difference in mammary DNA or RNA from that of mam-

TABLE I. Mammary Gland Nucleic Acid Content.

Treatment	No. of rats	Body wt.[a] (g; mean)	DFFT[b] (mg; mean)	Total DNA (mg; mean)	Total RNA (mg; mean)	DNA (mg/100 g of body wt.; mean)	RNA/DNA (mean)
HCA, 2 mg/day 9–13 days	10	242	481 ± 20[c]	13.55 ± 0.65	10.43 ± 0.22	5.62 ± 0.15	0.765 ± 0.05
Placebo 9–13 days	10	257	512 ± 26	14.59 ± 1.21	11.39 ± 0.17	5.69 ± 0.15	0.783 ± 0.04
HCA, 2 mg/day 16–20 days	12	256	680 ± 32	19.68 ± 0.38	20.82 ± 1.20	7.67 ± 0.39	1.085 ± 0.04[d]
Placebo 16–20 days	8	272	604 ± 38	21.37 ± 1.72	18.51 ± 2.30	7.84 ± 0.47	0.864 ± 0.07

[a] Corrected for weight of fetuses.
[b] Dry fat-free tissue.
[c] Mean and SE.
[d] Significantly larger than placebo (16–20 day) $p < 0.05$.

mary glands from control rats. Rats treated during late pregnancy also showed no change in DNA, but an increase in cellular RNA content was observed. These results are interpreted as indicating that a preparturient increase in corticosteroid activity may be involved in the normal initiation of lactation, but does not appear to be related to the increase in mammary DNA normally associated with the initiation of lactation.

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Effect of Reserpine on Sympathetic Vasoconstrictor Responses (33758)

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The majority of the antihypertensive agents in common usage today impair sympathetic reflex activity to a greater or lesser extent. Investigations of the effects of reserpine on vasoconstrictor responses have produced variable results. While some studies reported partial or complete inhibition of the Valsalva overshoot (1, 2) and mild orthostatic hypotension (3) this has not been confirmed by other investigators (4–6). The present study was undertaken to assess the extent of inhibition of therapeutic doses of reserpine in man to a variety of tests for sympathetic vasomotor activity.

Methods. Ten male patients on the general medical wards or from the Hypertension Clinic of the Veterans Administration Hospital were studied before and at the end of a period of reserpine administration. All subjects except two were hypertensive. Five patients received 0.5 mg of reserpine daily for at least 5 weeks and the other 5 received 2.0 mg for 6–10 days. All studies were performed in the hemodynamic laboratory. Arterial pressure was monitored by means of a Teflon "long dwell" needle inserted into the brachial artery and connected to a Statham P23Db Strain gauge transducer. The digital plethysmograph, which consisted of a plastic cup sealed around the finger with silicone grease, was connected to a Statham PM5 pressure transducer. Sanborn carrier preamplifiers and multichannel direct writing recorder were used for amplification and recording.

The following tests of reflex sympathetic activity were carried out. The Valsalva maneuver was performed by asking the subjects to take a deep breath and then to expel the air forcefully into a closed system for 10 sec. The blowing pressure, which was maintained at approximately 40 mm Hg, was recorded via a Statham P23AA pressure transducer. The highest intra-arterial pressure recorded within a few seconds after release of the expiratory effort was taken as the overshoot. The pressor response to cold was determined by immersion of one hand in ice water for 1 min. The orthostatic effect on the arterial pressure of quiet standing at 90° tilt for 5 min was recorded. An intravenous in-

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