system may induce a shift in autonomic predominance resulting in a decreased or increased threshold in the other. Such a thesis would be in conformity with that proposed by Gellhorn(11,12). It should be emphasized, however, that alteration in self-stimulation rates as effected by steroids may be a result of effects which are independent of those involved in the regulation of ACTH release. Indeed, the effects of corticosteroids on changes in brain excitability, behavior, and numerous physiological phenomena have been amply reviewed by Woodbury(13).

Summary. Self-stimulation rates in rats were altered 2 to 24 hours after a single subcutaneous injection of steroid in doses reported to inhibit acute release of ACTH. Dexamethasone exerted more profound effects than hydrocortisone. Self-stimulation rates were consistently increased in rats stimulating to electrodes in the LHA and/or MFB. Bilateral adrenalectomy produced increased rates of self-stimulation by the 5th postoperative week.

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Effect of Different Steroids on Lactating Rats.* (30607)

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We have recently reported(1) the effect of different steroids on prolactin secretion in organ co-culture. We found that estradiol and hydrocortisone stimulate prolactin release in doses of 1 and 10 μg/cc and were depressive at doses of 10 and 100 μg/cc while progesterone and its derivatives in doses higher than 1 μg/cc depress prolactin release. Nicoll and Meites(2) also observed an increase in prolactin production by rat pituitary cultured *in vitro* when estrogen was added to the medium.

There is no doubt that prolactin is necessary for milk secretion and maintenance(3,4), but estrogen, which promotes lactation when used at low doses for priming, was found to be a potent inhibitor of milk production in many species, including the rat, when the optimal dosage was surpassed(5,6).

Since various doses of estradiol showed a different influence on prolactin production by rat pituitary *in vitro,* we set out to test whether different doses of estradiol and other steroids act similarly on milk yield in lactating rats.

Materials and methods. Lactating rats of the "Sabra" Hebrew University strain, weighing 250 ± 20 g, each nursing 6 litter mates, were kept in individual cages and received Purina Lab Chow and water *ad libitum.*

Daily weights of dams and litters were recorded from day 7 to day 20 postpartum. Beginning on the 7th day, s.c. injections of different steroids dissolved in disacidified olive oil were given daily until the 20th day. With one dose of estradiol, (0.01 mg/kg/d) injec-

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tions were deliberately interrupted on the 16th day.

On days 14, 16, 18 and 20 post-partum, milk yield was estimated from the litters' weight gain during one hour's nursing period and controlled by weight loss of the mothers. Ten hours before suckling, litters were isolated from their mothers without food supply. One minute prior to suckling 1 I.U. of synthetic oxytocin (Syntocinon—Sandoz) was injected s.c. to all dams to achieve complete let down of milk.

Results. Estradiol (0.01-0.3 mg/kg/d s.c.) inhibits milk production partially or wholly, while a lesser dose (0.001 mg/kg/d s.c.) increases milk secretion by 10% especially during the period of the 7th-18th days of suckling (Table I).

Administration of 0.01 mg/kg/d of estradiol from day 7 to day 21 suppressed milk yield to 1.7 g on the 18th day and to 0.3 g on the 20th day. On the other hand interruption of the same treatment on the 16th day reestablished milk yield up to 5.0 g on day 18 and to 7.0 g on day 20. Litter weight gains were in accordance with milk yield in all estradiol treated animals.

Progesterone (100 mg/kg/d s.c.) showed no inhibitory influence on milk yield and only a slight decrease in the litter weight gain was observed, a finding to be discussed.

Testosterone (1 mg/kg/d s.c.) given on the 7th day caused milk secretion to cease on day 16, so that the litters lost weight considerably. On the other hand a lower dose of 0.1 mg/kg/d s.c. failed to inhibit milk yield.

Like testosterone, but to a lesser degree, dehydroepiandrosterone (1 mg/kg/d s.c.) started on day 7 depressed milk secretion down to zero on day 20 while at a low dose of 0.1 mg/kg/d s.c., it failed to depress milk production.

Discussion. The inhibitory effect of estradiol on litter growth of intact lactating rats has been well established by Barsantini and Masson(6) and others. Whether lack of milk or presence of estrogens or their metabolites in the milk or a possible failure of the milk-ejection reflex is responsible for the inhibition in litter growth has not been established. Our results clearly indicate that lack of milk is the limiting factor, since no milk was found in the mammary glands of the estradiol-overdosed mother animals even though they always received oxytocin prior to nursing. Proof that estradiol treatment alone is responsible for the decrease in milk yield of

TABLE I. Effects of Different Steroids on Milk Yield (Mean ± S.E.) and Litter Weight Gains in Normal Lactating Rats from Day 7 to 20 Post Partum.

Treatment	Dose (mg/kg)	No. of animals	Milk yield after 10 hr separation* (g) Day 14	Day 16	Day 18	Day 20	Litter wt gain (g), mean ± S.E. Day 14	Day 16	Day 18	Day 20
Control (oil)	.3 ml	12	6.0 ± .5	7.1 ± .8	8.8 ± .6	7.4 ± .6	72 ± 2.6	78 ± 3.3	82 ± 2.7	97 ± 2.2
Estradiol	.300	6	1.5 ± .5	0	(—)†	(—)	44 ± 3.5	12 ± 2.7	(—)	(—)
	.100	6	3.0 ± .5	0	(—)	(—)	65 ± 1.7	44 ± 1.8	(—)	(—)
	.030	6	5.3 ± .4	4.7 ± .5	2.0 ± .3	0	70 ± 6.5	56 ± 5.3	26 ± 4.2	8 ± 2.1
	.010	6	3.7 ± .8	2.7 ± .7	1.7 ± .3	.3 ± .3	69 ± 3.1	66 ± 4.2	64 ± 8.1	58 ± 3.5
	.010‡	6	4.0 ± .6	3.0 ± .4	5.0 ± .7	7.0 ± .6	69 ± 4	67 ± 3.7	67 ± 3.8	80 ± 4.1
	.003	5	6.7 ± .7	8.0 ±1.1	7.6 ± .7	7.5 ± .8	78 ± 3.3	78 ± 2.8	83 ± 3	88 ± 3.5
	.001	6	8.3 ± .3	10.3 ± .9	11.2 ± .9	8.4 ± .7	79 ± 4.1	82 ± 4.2	91 ± 5.2	110 ± 6.4
Progesterone	100	6	6.5 ± .6	6.5 ± .7	9.3 ±1.1	9.2 ± .9	63 ± 3.5	73 ± 3.7	77 ± 4.1	88 ± 5.1
Dehydroepian-drosterone	1	6	6.6 ± .4	2.6 ± .7	2.6 ± .7	.6 ± .6	58 ± 2.8	70 ± 1.8	57 ± 3.1	53 ± 2.9
	.1	6	5.3 ± .2	7 ±1	10 ±1.1	8.7 ± .8	65 ± 3.0	73 ± 3.4	81 ± 3.5	95 ± 3.2
Testosterone	1	6	3 ±1.3	0	0	0	66 ± 2.1	64 ± 3.1	39 ± 6.5	12 ± 2.1
	.1	6	6.5 ± .5	7.7 ± .65	9.7 ±1.3	10 ±1.4	75 ± 2.1	80 ± 2.3	89 ± 3.1	100 ± 2.5

* 1 I.U. of oxytocin (Syntocinon—Sandoz) was given to all animals immediately before nursing.
† Death of litters induced by lack of milk in the mammary glands.
‡ Treatment was interrupted on the 16th day post partum to study the reestablishment of lactation.

intact lactating rats was furnished when we showed that interruption of estradiol administration on the 16th day reinitiated milk secretion and gain of body weight in the litters, thus indicating that the inhibitory effect of estradiol on milk yield is reversible.

A direct correlation exists between estradiol dosage and its influence on milk secretion. This was revealed by "titration" at different dose levels: the inhibitory effect of estradiol became evident at the higher dosage and decreased as the dose was diminished until a slight increase of milk secretion was achieved by a dose as low as 0.001 mg/kg/d estradiol. This fact was postulated by Folley and Malpress(7) when they suggested that large doses of estrogen may inhibit prolactin secretion, while small doses may be stimulatory. The action of estradiol is most probably achieved through alteration of prolactin secretion by the anterior pituitary either directly(2) or indirectly *via* the hypothalamus(8), as we have shown in organ co-culture(1).

Whereas progesterone decreased prolactin production in the anterior pituitary(1), it did not significantly affect milk yield in lactating rats. Similar results were achieved by Walker and Matthews(9). This may point to the possibility of progesterone being transferred *in vivo* into other steroids, as in organ co-culture (*in vitro*) it tended to depress prolactin production(1).

Testosterone suppressed milk yield at higher doses (1 mg/kg/d) and failed to decrease it at low dose (0.1 mg/kg/d). Does the inhibitory action of testosterone take place at the hypothalamus-hypophysis level by blocking the secretion of lactogenic hormones or does it occur in the mammary gland? There is evidence to suggest that testosterone acts at least in part on the mammary gland itself since it did not affect prolactin secretion in pituitary-hypothalamus organ co-culture(1).

Dehydroepiandrosterone was also tested in order to establish whether the inhibitory action of testosterone was due to its androgenic effect operating *via* the hypothalamus or pituitary. Results indicated that the action of testosterone was not due to its inhibitory androgenic effect(10), since a weak androgen such as dehydroepiandrosterone was just as active an inhibitor as testosterone. We would, therefore, assume that dehydroepiandrosterone acts directly on the mammary gland rendering it insensitive to prolactin.

No parallel can be drawn between the effect of the steroids injected on milk yield in lactating rats and their effect on prolactin secretion in pituitary-hypothalamus co-culture reported by us(1).

Summary. The effect of different doses of estradiol, progesterone, testosterone and dehydroepiandrosterone on milk yield of normal lactating rats was assayed by daily s.c. injections of the steroids from day 7 to day 20 postpartum. It was found that high doses of estradiol (0.01-0.3 mg/kg/d) decrease milk secretion while a small dose of 0.001 mg/kg/d slightly increases it. Interruption of estradiol treatment on day 16 reestablished increased milk yield on day 20. Progesterone showed no significant decrease in milk yield at a dose of up to 100 mg/kg/d. Testosterone, and to a lesser degree dehydroepiandrosterone, were found to be potent inhibitors of milk secretion at a dose of 1 mg/kg/d while a low dose of 0.1 mg/kg/d failed to suppress milk production. The target organs of the above steroids are discussed.

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Spontaneous Virus Carrier Cultures and Postmortem Isolation of Virus from Infants with Congenital Rubella.* (30608)

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Infants born to mothers contracting rubella in the first trimester of pregnancy often have congenital anomalies. These anomalies may be due to the effect of the virus upon the fetus during a critical period of organogenesis or they may result from the effect of the persistence of rubella virus in the developing tissues. The critical period of infection which results in a persistent viral infection also seems to be the first trimester of pregnancy. Studies of the products of conception obtained by therapeutic or spontaneous abortions from women who experienced clinical rubella early in pregnancy have demonstrated the presence of virus in the placenta as well as in multiple organs(1-5). Postnatally, infants born with the rubella syndrome may continue to excrete virus for many months(3,6,7). A study of postmortem material was therefore undertaken to determine the extent of tissue involvement of infants who had been born with the rubella syndrome and who had subsequently expired. In addition, the investigation was designed to compare two techniques for isolating viruses from postmortem tissues. These included the *in vitro* propagation of tissues obtained at autopsy and the direct inoculation of suspensions of postmortem tissues into appropriate tissue culture systems.

An epidemic of rubella occurred in the Houston, Texas area in the spring of 1964(8). Seventy infants born with anomalies consisting of low birth weight, cataracts, congenital heart defects, thrombocytopenic purpura, hepatosplenomegaly and evident dysfunction of the central nervous system, came to the attention of Baylor investigators in the winter of 1964(6). Ten of these infants expired and comprise the basis of the present study. Postmortem material from 6 infants without the above mentioned complex of anomalies was also studied.

Materials and methods. Procurement of postmortem material. Portions of bone marrow, brain, heart, kidney, liver, lung, lymph node, spleen, thymus and thyroid were obtained at autopsy with care taken to avoid cross-contamination. Tissues were placed in separate sterile jars containing Melnick's lactalbumin hydrolysate medium and antibiotics (penicillin 500 U/ml, streptomycin 500 μg/ml). The tissues were processed within several hours in most cases; however, occasional specimens were held at 4°C for 24-48 hours before being processed.

Virus isolation from tissue suspensions. 10-20% suspensions of tissues in Eagle's basal medium were prepared by grinding with alundum followed by centrifugation at 3000 rpm for 30 minutes. The supernate was inoculated into 4 tubes of African green monkey kidney cell cultures (GMK) prepared as previously described(9). Each tube received 0.2 ml of supernate. Following adsorption for 1 hour, 1 ml of Eagle's basal medium with 2% fetal bovine serum and 1.5 g per liter of bicarbonate was added. After one week of incubation at 36°C, 100 TCD_{50} echovirus 11 was added to one-half of the tubes. When control tubes were found to have developed 2-3+ CPE (*i.e.*, 50 to 75% of the cells in

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