Thus far there is no indication that a booster dose of virus is needed following the series of virus doses administered. Some neonatally infected hamsters treated with large doses of $SV_{40}$ have remained alive and well for over 400 days, and adenovirus-infected and treated hamsters have remained free of tumors for 275 days.

The $SV_{40}$ virus heated in the presence of magnesium chloride and used in treatment of $SV_{40}$-infected hamsters did not afford protection from tumor development. This may or may not indicate that protection can be achieved only by treatment with viable virus. It is possible that protection could be achieved by another means of inactivation. Immunogenic potency of other infective viruses are reported to be retained when heated with high concentrations of divalent cations(14).

*Summary.* The oncogenicity of adenovirus type 12 and $SV_{40}$ administered to hamsters when newborn could be inhibited or prevented by administration of large doses of the homologous virus at later dates. The protection achieved appeared to vary inversely with the concentration of virus in the initial infecting dose. The administration of greater initial infecting doses of virus resulted in fewer tumor-free animals in the treated groups. The size of the dose of virus used in treatment was a factor, and a large dose (1 ml) gave a little more protection than a 0.25 or 0.5 ml dose. The usual course of treatment was a total of 13 doses of virus administered at twice weekly intervals. Smaller numbers of doses used in treatment were sometimes effective. Treatment with virus heated to 50°C for an hour in the presence of magnesium chloride was not effective in preventing the induction of tumors.

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## Prolactin Secretion *in vitro*: Effects of Gonadal and Adrenal Cortical Steroids.* (29643)

CHARLES S. NICOLL† AND JOSEPH MEITES‡
*Department of Physiology, Michigan State University, East Lansing*

Several experimental observations indicate that gonadal and adrenal cortical steroids can influence prolactin secretion *in vivo*. Administration of estrogens (E) cause pronounced elevation of pituitary prolactin content(1,2), induces pseudopregnancy in rats (3,4) and initiates lactation in several spe-

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† Present address: Nat. Cancer Institute, Bethesda, Md.

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cies(1,2). Moderate increases in pituitary prolactin levels have been obtained with injections of relatively large doses of progesterone (P)(2,5), testosterone (T)(2,6) and adrenal glucocorticoids(7); and T(6,7,8,9,10), P(2,5) and corticoids(7) can initiate mammary secretion in rats. It has been concluded from these observations that all of these steroids can increase prolactin secretion by *in situ* adenohypophysis (AP).

We have recently reported that E increases prolactin production by rat AP *in vitro*(11). This observation, of a direct effect of E on prolactin secretion at the pituitary level, has since been confirmed by Kanematsu and Sawyer(12) in the rabbit, and by Ramirez and McCann(13) in rats. Intrahypophyseal implants of E promotes prolactin release in rabbits(12) and increases AP prolactin content and secretion in rats(13).

It has not been demonstrated whether nonestrogenic steroids, which influence prolactin secretion *in vivo,* accomplish this by a direct action on AP or by some other means. It was therefore of interest to investigate effects of these steroids on prolactin secretion by rat AP *in vitro*. In addition, effects of several concentrations of E were investigated since large doses of E are less effective in elevating AP prolactin content than small doses(2) and differential effects of different E concentrations are of significance in current theories on the initiation of lactation at parturition (14).

*Materials and methods.* Adenohypophyses from 3-month-old Carworth female rats (CFN strain; 180-200 g body weight) were used for all experiments. Explants of AP were incubated in synthetic medium "199" at 35°C in an atmosphere of 95% $O_2$-5% $CO_2$. Three-day culture periods were used for all experiments, except where otherwise indicated. Details of the paired culture and assay procedures used have been published(15). Medium samples from each culture pair were assayed individually for prolactin activity, in 3-5 White-King pigeons, by the intradermal "micro" method of Lyons(16) as modified by Reece and Turner(1). Standard preparations of NIH prolactin were administered concomitantly with the assays on culture media in most cases.

Steroid hormones were incorporated into the medium to desired concentration by adding 0.5 ml of ethanol "stock" solution of each steroid to 100 ml of medium. Medium used in all experiments contained 50 U penicillin G potassium and 0.1 mg streptomycin per ml. At the end of incubation, explants were weighed on a Mettler balance, then fixed in Bouin's fluid. Survival of explants was verified by histologic inspection of hematoxylin and eosin stained paraffin sections. Data were analyzed by the "2-sided" t test.

*Results.* Effects of gonadal steroids on prolactin secretion and explant weights are shown in Table I. E at 0.05 and 0.5 μg/ml, caused a significant increase in prolactin secretion *in vitro*. At 2.0 μg/ml, however, E had no effect. Although the stimulatory action of E at 0.5 μg/ml was greater than that of 0.05 μg/ml level, the increase in prolactin release produced by 0.5 μg/ml was not significantly greater than that produced by the lower concentration. Neither P nor T influenced prolactin secretion at 1.0 or 2.0 μg/ml. None of the gonadal steroids influenced explant weight.

It will be noted that average % difference of prolactin activities, in most cases, does not agree with difference of averages of control and steroid treated cultures. Also, algebraic sign of average % difference in prolactin activity of media in experiments with F at 0.5 μg/ml and with T at 1.0 μg/ml are reverse of what would be expected from average control and average experimental activities. This results from computation of average % difference from each individual culture pair, rather than from difference of averages of control and steroid treated cultures. The same reasoning applies to data on explant weights. Computation of prolactin activities in media on basis of IU/cultural AP, in all these experiments yielded same conclusion as computation on basis of IU/100 mg AP.

Results of the experiments with adrenal cortical steroids are shown in Table II. Neither cortisol (F) at 0.5 or 5.0 μg/ml, nor corticosterone (B), at 1.0 or 20.0 μg/ml, in-

TABLE I. Effects of Gonadal Steroids on Adenohypophysial Explant Weight and Prolactin Secretion *in vitro*.

| Steroids | μg/ml | No. of culture pairs | Explant wt (mg) | | | | I.U. Prolactin/100 mg AP* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control medium | Steroid medium | Avg wt difference‖ | t | Control medium | Steroid medium | Avg % difference‖ | t | p¶ |
| Estradiol† | .05 | 8 | .7 | .7 | —.04 ± .06 | .66 | 39.7 | 52.4 | +36.9 ± 14.8 | 2.49 | .05 |
| " ‡ | .5 | 8 | 1.7 | 1.7 | —.01 ± .09 | .11 | 13.8 | 27.7 | +56.0 ± 21.8 | 2.56 | .05 |
| " | 2.0 | 8 | 2.2 | 2.2 | +.06 ± .05 | 1.20 | 17.2 | 21.0 | +15.2 ± 8.1 | 1.87 | N.S. |
| Progesterone | 1.0 | 10 | 2.3 | 2.4 | +.1 ± .19 | .53 | 20.4 | 16.2 | —16.6 ± 16.5 | 1.00 | N.S. |
| " | 2.0 | 8 | 2.3 | 2.1 | —.15 ± .15 | 1.00 | 34.2 | 29.4 | — 2.9 ± 7.4 | .39 | N.S. |
| Testosterone | 1.0 | 11 | 2.8 | 2.8 | —.01 ± .22 | .05 | 13.1 | 11.0 | + 7.5 ± 32.0 | .23 | N.S. |
| " § | 2.0 | 6 | 2.0 | 2.3 | +.12 ± .08 | 1.45 | 24.3 | 21.3 | — 4.4 ± 5.2 | .85 | N.S. |

* AP = adenohypophysis. † One-sixth of an AP per culture. ‡ One-third of an AP per culture. § Control and experimental media used with these cultures contained 2 U of Zn free insulin per ml. ‖ Computed on basis of each individual culture pair. ¶ "2-sided" t test.
N.S. = not significant.

TABLE II. Effects of Adrenal Cortical Steroids on Adenohypophysial Explant Weight and Prolactin Secretion *in vitro*.

| Steroids | μg/ml | No. of culture pairs | Explant wt (mg) | | | | I.U. Prolactin/100 mg AP* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control medium | Steroid medium | Avg wt difference‡ | t | Control medium | Steroid medium | Avg % difference | t | p§ |
| Cortisol | .5 | 12 | 2.0 | 2.3 | +.33 ± .05 | 7.17 | 14.7 | 12.1 | +11.5 ± 36.0 | .32 | N.S. |
| " | 5.0 | 6 | 2.8 | 3.0 | +.17 ± .16 | 1.06 | 11.0 | 7.4 | — 9.5 ± 18.0 | .53 | N.S. |
| " † | 10.0 | 6 | 5.6 | 5.8 | +.25 ± .16 | 1.56 | 62.1 | 22.8 | —55.9 ± 6.9 | 8.2 | .001 |
| Corticosterone | 1.0 | 6 | 3.1 | 3.9 | +.83 ± .56 | 1.48 | 8.7 | 4.0 | —33.3 ± 16.7 | 1.99 | N.S. |
| " | 20.0 | 8 | 2.1 | 2.6 | +.53 ± .16 | 3.23 | 24.8 | 25.5 | + 2.7 ± 13.4 | .20 | N.S. |

* AP = adenohypophysis. † Explants from 1 AP per dish. 6-day cultures. ‡ Computed on basis of each individual culture pair. § "2-sided" t test.

fluenced prolactin secretion. Cortisol, however, at 10.0 μg/ml significantly depressed prolactin secretion. AP explants cultured with F (0.5 μg/ml) and B (20.0 μg/ml) were significantly heavier than corresponding control explants. Possibly these steroids, at these concentrations, promoted hydration of tissues. None of the other concentrations of adrenal cortical steroids influenced explant weight.

Survival of explants in all cultures was uniformly good. When difference in degree of survival between control and steroid treated tissues of a culture pair was found, the pair was not used in analysis of data.

*Discussion.* Of the steroid hormones tested, only E at lower concentrations, significantly increased prolactin production by rat AP *in vitro*. This observation confirms our previous finding(11) and is in agreement with observation that E implants in AP promote prolactin release in rabbits(12) and increase prolactin synthesis and release in rats(13). It has also been found that E (0.001 μg/ml) exerts pronounced stimulatory action on prolactin production *in vitro* by hamster AP (Nicoll, unpublished). Gala and Reece(17) have recently reported that they were unable to obtain stimulation of prolactin secretion by the rat AP *in vitro* when E was added to the culture medium. They concluded that *in vivo* E probably acts *via* the hypothalamus. However, our present and previous results(11) with E, and the reports of Kanematsu and Sawyer(12) and of Ramirez and McCann(13) establish that a direct action of E on the AP is part of the mechanism by which E influences prolactin secretion *in vivo*. Chowers and McCann(18) also found that implants of E in rat AP promote prolactin secretion.

Other evidence indicates that E can also influence AP prolactin synthesis and secretion *via* the hypothalamus. Implants of E in the posterior tuberal hypothalamus of rabbits apparently promote storage but not release of prolactin in the AP(12). In rats, implants of E in the median eminence increase AP prolactin content and secretion(13,18). Injections of E into intact rats can deplete the hypothalamus of prolactin inhibiting factor(19).

Failure of E at high concentration to influence prolactin secretion *in vitro* agrees with observation that large doses of E are less effective than small doses in promoting prolactin secretion *in vivo*(2). Although this observation does not support the belief that high concentrations of E depress prolactin secretion *in vivo*(20), it does indicate that high E levels fail to stimulate or are much less effective in promoting prolactin secretion than low concentrations.

The finding that F, at 10 μg/ml, had a pronounced depressing effect on prolactin secretion *in vitro* is of dubious physiologic significance since F at lower levels, and B (natural glucocorticoid of rats) at low or high concentrations, had no effect. In this connection, it is of interest that high concentrations of cortisone are reported to depress oxygen consumption by rat AP *in vitro*(21).

Since neither P nor T, at 1 or 2 μg/ml, influenced prolactin secretion *in vitro,* it seems unlikely that promotion of prolactin secretion by P and T, in rats(2,5,6,8,9) is accomplished by direct action of these steroids on the AP. The same conclusion applies to results with F, since F is also reported to promote prolactin secretion in intact rats(7). Possibly, T, P and F stimulate prolactin secretion *in vivo* by an action on the hypothalamus or other neural structures, by metabolic conversion to steroids with estrogenic potency or by influencing endogenous E secretion.

*Summary.* Effects of estradiol (E), progesterone (P), testosterone (T), cortisol (F) and corticosterone (B) on prolactin production by rat adenohypophysis *in vitro* were examined. Addition of P (1 and 2 μg/ml), T (1 and 2 μg/ml), B (1 and 20 μg/ml) or F (0.5 and 5.0 μg/ml to culture medium did not influence prolactin secretion; however, F at 10 μg/ml significantly depressed prolactin production *in vitro*. This depressant effect of F, however, cannot be regarded as physiological due to the high concentration used. Incorporation of E into medium at concentrations of 0.05 and 0.5 μg/ml significantly in-

creased prolactin secretion whereas E at 2.0 μg/ml was without effect. These observations indicate that only E, of all steroids tested, can act at the pituitary level to influence prolactin secretion. The effects of other steroid hormones on prolactin secretion *in vivo* are probably mediated *via* pathways other than by a direct effect on the adenohypophysis.

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## Sex Differences in a Progeria-Like Syndrome. (29644)

RALPH F. STREBEL, HUSHANG PAYAN, E. LAWRENCE HOUSE, BEN PANSKY AND MADELEINE BARATH (Introduced by Bernard Wagner)
*Departments of Pathology and Anatomy, New York Medical College, New York City*

It has been known for some time that arterial medial calcification is caused by excessive amounts of certain calcific agents such as irradiated ergosterol or vitamin $D_2$(1,2,3). Under short term conditions of heavy dihydrotachysterol ("DHT"—a vitamin D derivative) overdosage, the occurrence of arterial medial calcification in that rat is altered by certain metabolic conditions [pregnant females and intact males are protected while castrated males manifest lesions(4,5)]. It has further been reported that hydrocortisone administration to mice produces nonvascular myocardial calcification more easily in females than in males and that administration of testosterone to females prevents these calcific cardiac lesions(6).

Just recently it was demonstrated in the adult female rat that chronic treatment with relatively small amounts of DHT induces a unique syndrome which markedly resembles premature senility or progeria. As described elsewhere(7) this progeria-like model is reminiscent of senile changes as observed in animals and man. One exception to this similarity is that osteosclerosis is associated with the progeria-like model, while osteoporosis is the characteristic bone lesion associated with the aging process in man. Certain dissimilar substances such as ferric dextran (Fe-Dex), methyltestosterone and combinations of these agents have been found to be effective inhibitors of the progeria-like syndrome(8,9).

Since very little is known about the condi-