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The Effects of Adrenalectomy on the Onset of Puberty in the Female Golden Hamster

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by

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VITA

The author, Gina Marie Micaletti, is the daughter of Louis and Eleanor Micaletti. She was born on December 13, 1960, in Chicago, Illinois.

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CHAPTER 1

INTRODUCTION

The sexual maturation of the female golden hamster (Mesocricetus auratus) represents a number of developmental processes which culminate in fertility. Gorski and Lawton (1973) studied the involvement of the adrenal gland in the onset of puberty in the female rat. Their data suggest that, during a certain period of time (between 25 and 35 days of age), adrenal secretions play a role in the maturation of the hypothalamic-pituitary-ovarian axis. The adrenals may play a role in the timing of the onset of puberty in the female hamster as well. The purpose of this thesis is to determine the effect of adrenalectomy on the onset of puberty in the female hamster. Since the adrenal cortex, and not the adrenal medulla, has been shown to be responsible for the effects of adrenalectomy (Gorski and Lawton, 1973), it was of interest to determine whether or not it is the lack of glucocorticoids that affects puberty onset in the hamster. The characteristic estrous discharge in the four-day estrous cycle of the hamster was utilized as the external indicator of the onset of puberty. Internal markers of puberty such as increased uterine weight and the presence of corpora lutea, hemorrhagic follicles, and ova in the oviducts were also helpful in identifying puberty onset.

The mechanism of puberty onset in hamsters is not known at present. To help identify this mechanism, the level at which adrenalectomy has its effect was examined. To accomplish this, the effect of adrenalectomy on induced ovulation was tested. If ovulation can be induced in adrenalectomized hamsters, then it can be determined that adrenalectomy does not have its effect at the follicular level, but at some other level.

CHAPTER II

REVIEW OF RELATED LITERATURE

What is Puberty?

Puberty, as described by Ramaley (1979), refers to a period of secondary sexual development which culminates in fertility. Ramaley divides the prepubertal period of the rat into 4 phases: (1) the neonatal phase, during the first week of life in which the ovaries are unresponsive to gonadotropin stimulation and are not yet actively secreting steroids; (2) the early juvenile phase beginning within the second week of life, during which follicular maturation and gonadotropin sensitivity are initiated; (3) the late juvenile phase, which occurs at around age 21 days of age, in which ovulation can be induced by extraordinary stimuli; and finally, (4) the peripubertal phase (around days 26-28) when ovulation can begin spontaneously. In the hamster, the phases can be divided as follows: (1) the neonatal phase, during the first 10 days of life, in which the ovaries are unresponsive to gonadotropin secretion and are not actively secreting steroids; (2) the early juvenile phase, during which primary interstitial development (at 14-21 days of age) (Greenwald and Peppler, 1968) and gonadotropin sensitivity (beginning at 16 days of age) (Smith and Stetson, 1980) are initiated; (3) the late juvenile phase

(which occurs at around 26-27 days of age), in which ovulation can first be induced by extraordinary means (Greenwald and Peppler, 1968; Bodemer et al., 1959); and finally, (4) the peripubertal phase around days 29-33 when ovulation can begin spontaneously (Greenwald and Peppler, 1968). The experiments outlined in this thesis deal with the latter two phases of pubertal development.

Previous studies have indicated that puberty occurs at around 29-33 days of age in the intact hamster (Greenwald and Peppler, 1968; Bex and Goldman, 1977). For Greenwald and Peppler (1968), the best criteria for the onset of puberty in the female hamster are the initiation of ovulation, as well as the ability of the animal to become pregnant. They found that the earliest spontaneous ovulation occurs at 29 and 30 days of age in the hamster. When 28 day old females were placed with males, the earliest mating occurred on day 31. Diamond and Yanagimachi (1970) assessed the pubertal maturation of the female hamster with respect to spontaneous estrus, mating behavior during this period, and the ability of the hamster to conceive. They found that spontaneous estrus was demonstrated over a span of 17 days (26-43 days of age). The coincidence of spontaneous estrus, ovulation, and reproductive maturity which could result in successful pregnancy occurred consistently by 35 days of age. Diamond and Yanagimachi (1970) also studied the relationship between puberty onset

and body weight, since these variables have been shown in the past to be positively correlated in rats (Kennedy and Mitra, 1963; Glass et al., 1976, 1979; Frisch et al., 1977; Wilson et al., 1983) and mice (Hansen et al., 1983). No significant correlation between body weight and day of first estrus was found in hamsters (Diamond and Yanagimachi, 1970).

Hormonal Events Surrounding Puberty

Puberty represents not a single event, but a number of developmental changes occurring within an organism. The transition of the female hamster from sexual immaturity to maturity represents a period of rapid change in serum hormone levels. The continued rise of plasma estradiol to maximum concentration at proestrus in the adult female is critical in synchronizing the hormonal signals of the ovulatory cycle (Brinkley, 1981). In the hamster, plasma levels of estradiol peak from 10-18 days of age, followed by a drop in serum estradiol between days 18-21, with a subsequent peak (similar in magnitude) from 21 to 30 days of age (Vomachka and Greenwald, 1979). Vomachka and Greenwald (1979) reported that an increase in serum estradiol accompanies the increase in gonadotropins (viz. luteinizing hormone, LH, and follicle stimulating hormone, FSH) around day 15 or 16 in the hamster. LH and FSH are gonadotropins secreted by the anterior pituitary, which stimulate the

ovaries to produce estrogen and progesterone; LH also causes ovulation. The FSH rise precedes the LH rise by about 5-6 days. The FSH rise begins at 10-12 days of age and peaks at 22-24 days of age; LH begins rising sharply at 16-18 days of age and peaks at 19-21 days of age (Vomachka and Greenwald, 1979). Just prior to puberty in hamsters, the second peak of estradiol is associated with decreasing levels of LH and FSH. In female hamsters, a daily rise in gonadotropin release begins at 16 days of age, while ovulation occurs from 26-32 days of age (Smith and Stetson, 1980). Thus, the onset of this daily rise in gonadotropin release is not synchronized with first ovulation in hamsters, yet in rats and mice, a daily surge of gonadotropin release begins immediately before the time of first ovulation (Smith and Stetson, 1980).

During the peripubertal period, there is a rapid change in hypothalamic gonadotropin releasing hormone (GNRH) content (Ojeda, et al., 1983). In addition, it is believed that the ability of alpha-fetoprotein to recognize and bind circulating estradiol has a significant role in the timing of the onset of puberty in rats (Andrews et al., 1981). This binding protein, as opposed to GNRH content and release, seems to be the "rate-limiting" step in the maturation process in rats (Chappel et al., 1983).

What roles do these hormonal changes play in the onset of puberty? The rising LH levels may act to precipitate an

estrogen-progesterone rise which, in turn, may be the cause of, or at least be associated with, the onset of follicular maturation. This estrogen stimulates an LH surge which is the preovulatory signal for ovulation (Brinkley, 1981). The initial rise in FSH secretion may act to condition ovarian folliculogenesis and follicular maturation for the next cycle (Bast and Greenwald, 1974; Ramaley, 1979). Thus, at puberty, changes in hormone levels are essential for the development of cyclic phenomena such as egg maturation, ovulation, and the estrous cycle.

Follicular Maturation

The process of follicular maturation is defined as the development of the primordial follicle to maturation (Austin and Short, 1982). In every estrous cycle in the hamster, including the first, follicular maturation begins with the primary follicle. The primary follicle is an oocyte with one layer of cells around it. Cell layers in the developing follicle increase to form an inner layer of granulosa cells and an outer layer of thecal cells. The mature follicle, also called the antral follicle, or Graafian follicle, is characterized by an antrum, or fluid-filled cavity. Development of this antral follicle coincides with ovulation (Greenwald and Peppler, 1968). Only antral follicles can ovulate (Austin and Short, 1982).

According to Greenwald and Peppler (1968), in intact hamsters, follicular maturation is completed by approximately 28 days of age. Antral follicles first appear on day 26. On day 26, there is a sudden increase in the size of the largest follicles in the ovary-- follicles measuring 185-219 u in diameter increase in size to approximately 325-359 u. Yet, between days 26 and 30, there is no significant difference in the size distribution of the follicles in the ovary. Follicular maturation can culminate in spontaneous ovulation as early as 29 days of age, according to this study.

External Indicators of Puberty

In female rats, "puberty" actually begins around the second week of life with the onset of follicular maturation and hormonal changes; yet, the first external sign of impending ovulation at puberty is vaginal opening. Ovulation occurs within one or two days following vaginal opening in the rat (Ramaley, 1974). In female hamsters, vaginal opening cannot be used as a marker for first ovulation, since vaginal opening, occurring by 9-12 days of age, is dissociated from ovulation in the hamster. An external sign of ovulation in the adult female hamster is the characteristic stringy, mucous estrous discharge expelled from the hamster vagina upon applied pressure to this area. This estrous discharge occurs the morning after

ovulation and designates the first day of the hamster 4-day estrous cycle. Day 1 is the day of estrus; day 2, metestrus; day 3, diestrus; and day 4, proestrus (Orsini, 1961). It has been postulated in this thesis that this characteristic estrous discharge should be indicative of puberty onset in young female hamsters. In initial experiments, an animal "positive" for this characteristic estrous discharge was said to have reached puberty on that same day.

Adrenal-Gonadal Interactions

To understand what role the adrenal glands play in the onset of puberty, it important to understand adrenal-gonadal relationships. The gonads affect the hypothalamic-pituitary-adrenal axis at a number of sites. Gonadal effects on the morphology of the adrenal cortex exist in that sex steroids secreted by the gonads affect adrenal weight (Kitay, 1968). In the intact hamster, in which the female adrenal gland is larger than the male, prepubertal gonadectomy results in a decrease in adrenal weight in both females and males. Estradiol (or testosterone) replacement therapy restores adrenal weights to control values (Gaskin and Kitay, 1970). Gonadal hormones can also affect the secretion of corticosteroids. Circulating levels of corticosterone are decreased *in vivo* and *in vitro* after ovariectomy in rats (Kitay, 1968). However, treatment of ovariectomized animals with estradiol restores the *in vivo*

and in vitro secretion of corticosterone (Kitay, 1968). In female hamsters, prepubertal gonadectomy results in an increased amount of ACTH in the pituitaries of adult castrates (Gaskin and Kitay, 1971). Estradiol replacement does not restore the ACTH content of the pituitary to normal levels.

The adrenal glands have an effect on reproductive function. The adrenal cortex has been implicated as being the indirect "timer" in the ovulatory cycle in the rat, especially during puberty (Ramaley, 1974). The timing of adrenal output of steroids can enhance or depress fertility, depending upon whether the peak output of corticoids coincides with the critical period of the estrous cycle. The critical period refers to that interval of time on the day of proestrus during which ovulation can be blocked by pharmacological agents that interfere with the hypothalamic activation of the pituitary. In adrenalectomized rats, the "predictability" of the cycles was reduced, and the timing of the critical period was delayed (Feder et al., 1971). The hormones of the adrenal cortex have the capacity to serve as the signal that integrates adrenal and gonadal function. Ramaley (1974) has postulated that either corticosterone or progesterone could serve as this phase signal. Adrenalectomy removes both adrenocortical steroids and progesterone. In the female rat, adrenalectomy performed before 26 days of age delays vaginal opening and

ovulation, ie. puberty (Gorski and Lawton, 1974; Ramaley and Bunn, 1972). Puberty was significantly delayed by adrenalectomy performed on days 18 and 25 of age, but not affected when adrenalectomy was performed on day 35 of age. Gorski and Lawton (1973) proposed that the periodicity (rhythmicity) of the adrenocortical secretions had not been established by day 25, and thus, whatever adrenal factor was responsible for the timing of the onset of puberty was removed by adrenalectomy. By day 35, one can infer that periodicity within the hypothalamic-pituitary-gonadal system was matured and whatever contribution the adrenal makes to this process had already occurred. Gorski and Lawton (1973) also examined some other effects of adrenalectomy in the female rat. They found that adrenalectomy did not have a significant effect on ovarian or uterine weights or on cycle length (when animals eventually reached puberty). Laparotomy, or sham adrenalectomy, did not have any effect on puberty onset in rats. It is the purpose of this thesis to determine the effects of adrenalectomy on the onset of puberty in the female golden hamster.

CHAPTER III

MATERIALS AND METHODS

Animals: Golden hamsters obtained from Charles River (Lakeview, MI) were bred to produce females which were divided into experimental groups. The day of birth was designated as day 0. Animals were weaned on day 21. All animals were maintained on a light:dark cycle of 14:10 and given Purina Rat Chow and tap water ad libitum. All adrenalectomized animals were given 0.2% saccharine/1.0% NaCl drinking solution ad libitum instead of tap water after surgery to compensate for mineralocorticoid loss (Nickerson and Molteni, 1971; Salber and Zucker, 1974).

Surgical Procedures: Surgery was performed at 0900-1000h. Adrenalectomy and sham adrenalectomy were performed via bilateral dorsal incisions under Metofane anaesthesia. Sham adrenalectomy is surgical manipulation without removal of the adrenal glands.

External indicators of Puberty: In Experiment 1, stringy vaginal discharge, characteristic of estrus in the adult hamster (Orsini, 1961), was used as indicator of puberty onset, since it seemed to be an easy and convenient external

way of detecting estrus without performing surgery or autopsy. In the second, third, and fourth experiments, new criteria were added to provide more conclusive evidence of puberty onset. Daily vaginal lavages were taken using distilled or deionized water. These lavages were used for two purposes. The first was to test for the presence of crystalline concretions of calcium carbonate. These concretions are found dispersed in the vaginal lavages and could be seen with a light microscope (100X). They occur on days 3 and 4 of the estrous cycle, and therefore indicate that ovulation has already occurred 2 and/or 3 days earlier (Alleva et al., 1976). They also serve to predict the occurrence of the next estrus. Secondly, bromothymol blue was added to the vaginal lavage to test the pH of the vaginal secretions. At the onset of ovulation, estradiol levels peak, causing the pH of the vagina to fall, resulting in a color change in bromothymol blue. The test is positive when bromothymol blue turns yellow and indicates that ovulation has occurred; when bromothymol blue remains blue, the test is considered negative and indicates that an animal has not ovulated. In the third and fourth experiments, internal indicators of puberty were added to classify an animal as "postpubertal". These included 1) the presence of corpora lutea in the ovaries; 2) the presence of hemorrhagic follicles; 3) the presence of ova in the oviducts; and, 4) a high uterine weight.

Autopsy Procedures: Animals were sacrificed by decapitation. Trunk blood was collected for the possible future measurement of estrogen and progesterone levels by radioimmunoassay. Blood was centrifuged and the serum stored at -20 C. All adrenalectomized animals were checked for the presence of adrenal regeneration. The reproductive tract of each animal was removed and cleaned of adipose tissue. Uteri were weighed to the nearest 0.1 mg. The two ovaries were weighed together, along with one intact oviduct. These were then fixed for possible future histological studies. The other oviduct was dissected out under a dissecting microscope, and the number of ova (if present) was counted. Dissection was carried out using 28 G, 1/2" Stylex needles, the tips of which were blunted by sanding so as not to puncture the oviduct. Mesotubal tissue was broken apart using the needles. Slides of the unraveled oviducts were made using one drop of saline and a coverslip. Ova were counted under a light microscope with a magnification of 100X.

Protocol Experiment 1:

At weaning, female golden hamsters were divided into the following experimental groups (n=6-10/group): sham

adrenalectomy at 21, 25, or 29 days of age, adrenalectomy at 21, 25, or 29 days of age, and an intact group.

Starting on the day of weaning, animals were weighed and checked daily for the characteristic estrous discharge by applying pressure to both sides of the vagina (Orsini, 1961). The first discharge from the vagina was thought to be indicative of the onset of puberty. On the day an animal showed this discharge, it was sacrificed between 1100-1200h.

Protocol Experiment 2:

At weaning, the females were divided into the following treatment groups (n=5-7/group): an intact group, sham adrenalectomy performed on days 23 or 26, adrenalectomy performed on days 23 or 26, and adrenalectomy performed on days 23 or 26 followed by cortisone acetate administration. The days of surgery were changed from the first experiment to days 23 and 26 of age, since animals did not tolerate surgery well when performed on day 21. Also, day 29 had no effect on puberty, and therefore the day of surgery was lowered to day 26 of age. Day 26 was chosen because it is the age at which follicular maturation is almost completed. The addition of cortisone acetate was administered subcutaneously in a group of animals in order to compensate for glucocorticoid loss and to detect whether it is the lack of these which causes puberty to be delayed.

Those animals in the adrenalectomy plus cortisone acetate group were given 0.1 ml of 3.0 mg/ml cortisone acetate subcutaneously once weekly (starting on the day of surgery) to compensate for glucocorticoid loss. Starting on the day of weaning, animals were weighed and checked daily for the external indicators of puberty onset. On the morning of first estrous discharge and/or when bromothymol blue turned yellow, animals were sacrificed between 0930-1030h.

Protocol Experiment 3:

Animals were weaned on day 21, and on day 26 they were either left intact, sham adrenalectomized, or adrenalectomized (n=12-19/group). Animals were weighed and checked daily for the characteristic estrous discharge and were given vaginal lavages, starting on the day of surgery (day 26). Animals in each group were either sacrificed on day 30 or on day 34. Animals were sacrificed between 1000-1200h.

Protocol Experiment 4:

Animals were weaned on day 21, and on day 26 they were either left intact, sham adrenalectomized, or adrenalectomized. Starting on day 26, all animals were weighed and checked daily for external signs of ovulation. Some animals in all three groups were injected

subcutaneously with 20 IU Pregnant Mares Serum Gonadotropin (PMS) starting at 0800h on either day 26 or day 30. An equivalent of 20 IU human chorionic gonadotropin (HCG) was injected subcutaneously into these animals 54 hours later (Moore and Greenwald, 1980). Those animals given PMS on day 26 were autopsied on day 30, while those animals given PMS on day 30 were autopsied on day 34. Animals were sacrificed between 1100-1200h. Other animals in these three groups (intact, sham, and adrenalectomy) served as controls in that no PMS or HCG was given to these animals. Again, some animals were autopsied on day 30, while others were autopsied on day 34. There were 8 animals in the intact/day 30 autopsy groups, 12 in the intact/day 34 autopsy groups, 8 in the sham adx/day 30 autopsy groups, 4 in the sham adx/day 34 autopsy groups, 6 in the adx/day 30 autopsy groups, and 6 in the adx/day 34 autopsy groups. An outline of this protocol is shown in Table 1.

Statistics:

The statistical tests that were used to analyze the data from these experiments were analysis of variance, Student's t test, Chi-square analysis, and linear regression analysis. The tests were applied wherever appropriate.

Table 1. Protocol Experiment 4.

Day 26	Day 30	Day 34
Intact-----		
1) PMS/HCG	autopsy	
2) control	autopsy	
Intact-----		
	1) PMS/HCG	autopsy
	2) control	autopsy
Sham adx---		
1) PMS/HCG	autopsy	
2) control	autopsy	
Sham adx---		
	1) PMS/HCG	autopsy
	2) control	autopsy
Adx--------		
1) PMS/HCG	autopsy	
2) control	autopsy	
Adx--------		
	1) PMS/HCG	autopsy
	2) control	autopsy

CHAPTER IV

RESULTS

EXPERIMENT 1

In the intact animals, the range of ovulation was found to be from day 30-39, with an average age of puberty onset at 34.6±1.0 days of age (Table 2). Sham adrenalectomized animals in all three groups (ie. at 21, 25 and 29 days of age) exhibited normal puberty onset, as can be seen by comparing the average ages of first estrus for the three sham adrenalectomized groups to that of the intact group. Thus, sham adrenalectomy performed on days 21, 25, and 29 seemed to have no effect on the onset of puberty. The postpubertal ratio, or the number of animals that were found to indeed have ovulated per total number of animals autopsied in that group, was calculated for each group. The postpubertal ratios of the sham adrenalectomized groups compare very closely with that of the intact group, indicating that for these groups the external indicator of ovulation was accurate.

Adrenalectomy performed at 21 days of age seemed to delay puberty, which did not occur in any (0 out of 3) of the animals that were autopsied in this group up to 41 days of age. Since 5 other animals in this group died 7-19 days following removal of the adrenals (an average of 32.4±2.4 days of age), however, the effect of adrenalectomy at 21 days of age on puberty onset could not be determined.

Table 2. Average age in days at autopsy and first estrus and number of postpubertal animals per total number autopsied.

Group	Average Age at Autopsy	Postpubertal Ratio*	Average Age First Estrus	PSR**
intact	34.6±1.0	10/10	34.6±1.0	---
sham adx- d21	33.3±1.7	7/7	33.3±1.7	7/7
adx- d21	38.7±1.4	0/3	-----	3/8
sham adx- d25	32.7±0.8	5/6	33.0±1.7	6/7
adx- d25	31.3±2.3	2/3	31.0±1.4	3/7
sham adx- d29	34.2±0.8	6/6	34.2±0.8	6/6
adx- d29	35.9±1.3	5/8	36.6±2.0	8/9

* postpubertal ratio: # postpubertal/# autopsied
** PSR=postsurgical survival rate: # survivors/ # receiving surgery

Two of the three animals which had been adrenalectomized on day 25 were postpubertal at the time of autopsy. These animals reached puberty at 30 and 32 days of age, or an average of 31 days of age. There were 4 other animals in this group which died from 8-15 days post-operative, ie. at 33-40 days of age. These animals failed to show the characteristic estrous discharge, or any other signs of puberty, upon examination. Thus, a total of 5 out of 7 animals in this group never reached puberty.

Adrenalectomy performed at 29 days of age seemed to have no effect on puberty onset. Five out of 8 animals in this group were found to be positive for estrus at autopsy. Out of the remaining three animals, two were found to have high uterine weights at autopsy; one of these latter two animals was found to contain two ova in its oviduct. This indicates that ovulation has already occurred in these two animals, yet they failed to show the characteristic estrous discharge. Upon examination, waxy plugs and powdery secretions were inconsistently present.

The average number of ova ovulated per one oviduct was calculated for each group (Table 3). Ten out of 10 animals in the intact group were found to contain ova in their oviducts. These animals ovulated an average of 4.7 ± 0.6 ova. In the 21 day old sham adrenalectomized animals, 5 out 7 animals had ova in their oviducts. For the other 2 animals in the group, oviduct dissection was unsuccessful. The

Table 3. Number of animals with ova present per total number of animals and average number of ova per one oviduct.

Group	Number of animals with ova/ total number present	Average number of ova/one oviduct
Intact	10/10	4.7±0.6
Sham adx d21	5/7	5.2±2.3
Adx d21	0/3	---
Sham adx d25	3/5	2.1±1.0
Adx d25	1/3	1.0
Sham adx d29	6/6	3.8±0.3
Adx d29	6/8	5.0±1.7

average ova count in this group was 5.2±2.3 eggs (n=5). Three out of five 25 day old and 6 out of six 29 day old sham adrenalectomized animals had an average of 2.1±1.0 and 3.8±0.3 ova, respectively (n=3, n=6). These values were not significantly different (ANOVA: $F=1.92$; $d.f.=3,20$; $p>0.05$).

Upon dissection of one oviduct from the animals in the 21 day old adrenalectomized group, no ova were found (n=3). However, in the 25 day old adrenalectomized animals, one animal showed a single ovum in the oviduct; the other two animals in the group showed no ova. Adrenalectomy performed on day 29 seemed to have no effect on ovulation rate, since the average number of eggs for the animals that were found to have ova was 5.0±1.7 eggs per oviduct (n=6) (Student's t test: 29 day old sham vs 29 day old adx, $p>0.05$).

The mean ovarian and uterine weights were calculated for each group and analyzed by analysis of variance (Table 4). The ovarian weights of all animals were found to be similar, regardless of the group ($F=1.53$; $d.f.=6,35$; $p>0.05$). Thus, adrenalectomy seemed to have no significant effect on ovarian weight.

Analysis of variance of the postpubertal uterine weights indicated a significant difference between the groups ($F=2.78$; $d.f.=5,30$; $p<0.05$). However, further analysis of these data indicated that there were no significant differences between intact and sham-operated animals ($p>0.05$) or between sham-operated and

Table 4. Mean body weights, ovarian, and uterine weights for animals in each group.

Group	n	BW (g)	Ovarian Wt (mg)	Uterine Wt (mg) pre	Uterine Wt (mg) post
intact	10	71.8±2.7	26.6±0.8	--	117.0±12.5
sham-21	7	63.6±3.7	21.5±2.2	--	93.9±17.8
adx-21	3	66.3±8.8	22.9±4.4	40.9±13.6	--
sham-25	5	58.6±5.5	24.6±4.7	59.2 (1)	114.2±24.4 (4)
adx-25	3	65.3±0.8	20.5±2.1	28.0 (1)	87.6±4.0 (2)
sham-29	6	73.2±2.3	26.5±0.8	--	152.6±15.8
adx-29	8	73.3±2.1	23.7±1.0	53.6 (1)	124.9±13.0

adrenalectomized animals ($p>0.05$) in postpubertal uterine weight.

RESULTS

EXPERIMENT 2

The average age of first estrus in the intact group was 36.7±1.6 days of age (Table 5). All animals in the group were found to be in estrus at autopsy. The small number of animals in this group was due to the fact that those animals which had abnormally low body weights at weaning were deleted from the group. There were 2 such animals whose body weights were 18 and 20 g at weaning. The average body weight at weaning for the intact animals was 30.3 g.

Sham adrenalectomy performed on days 23 and 26 seemed to have no effect on puberty onset, since the mean ages at first estrus were not significantly different from that of the intact group.

Only one out of five animals in the 23 day old adrenalectomized group was positive for estrus, and this animal reached puberty at 37 days of age. The four other animals in this group were found not to be in estrus upon examination of internal structures at autopsy (ie. they were false positives). One out of two 26 day old adrenalectomized animals was positive for estrus, and this occurred on day 36. The other animal in this group failed to reach puberty up to 44 days of age. There were 5 other animals in the 26 day old adrenalectomized group. However,

Table 5. Mean age in days at autopsy and first estrus and number of postpubertal animals per total number autopsied.

GROUP	MEAN AGE AT AUTOPSY (DAYS)	POSTPUBERTAL RATIO*	MEAN AGE 1ST ESTRUS	PSR**
Intact	36.7 ± 1.6	3/3	36.7± 1.6	--
Sham adx d23	34.5 ± 0.8	6/7	34.5± 0.8	7/7
Adx d23	33.0 ± 1.7	1/5	37.0	5/6
Adx + CA d23	35.8 ± 1.8	6/6	35.8± 1.8	6/6
Sham adx d26	36.4 ± 3.4	5/5	36.4± 3.4	5/5
Adx d26	40.0 ± 5.7	1/2	36.0	2/7
Adx + CA d26	41.4 ± 3.2	5/5	41.4± 3.2	5/5

*postpubertal ratio: #postpubertal/ #autopsied
**PSR=postsurgical survival rate: # survivors/ # receiving surgery

they died at an average of 12.2±5.0 days post operative before autopsy procedures could be performed.

Animals in the 23 day old adrenalectomy plus cortisone acetate group showed normal puberty onset. Thus, cortisone acetate restores the ability of the animal to ovulate. However, animals in the 26 day old adrenalectomy plus cortisone acetate group showed a somewhat higher, but not significantly different (Student's t test: $p>0.05$) puberty onset, compared to either intact or day 26 sham adrenalectomized animals. Post hoc examination of the daily records of these animals indicated that previous ovulations could have occurred prior to sacrifice in four of the five animals in this group.

In the intact group, the animals had an average of 5.3±0.4 ova in their oviducts upon dissection. This average was very similar to that found in the first experiment (4.7±0.6 ova for 10 out of 10 animals). In the 23 day old sham adrenalectomized group six out of seven animals had ova in their oviducts, and of those animals, the average number of ova counted was 3.0± 0.8. Five out of five 26 day old sham adrenalectomized animals contained ova in their oviducts, and the average number for this group was 4.2±1.0. The values of average ova count for the sham adrenalectomized animals did not differ significantly from that of the intact group (ANOVA: $F=1.28$; $d.f.=2,11$; $p>0.05$), showing that sham adrenalectomy had no significant effect on

ovulation rate. Three ova were found in one out of five of the 23 day old adrenalectomized animals. This animal reached puberty at 37 days of age; while the other four animals in this group were found neither to be in estrus nor to contain any ova in their oviducts upon dissection. One out of two animals in the 26 day old group of adrenalectomized animals contained three ova in the oviduct on day 36 of life; no ova were found in the other animal in this group. More information was needed concerning these groups to make a conclusion on the effect of adrenalectomy on the number of ova ovulated. In the adrenalectomy plus cortisone acetate groups, five out of six animals in the 23 day old adrenalectomized group, and five out of five animals in the 26 day old group, had average ova counts of 3.8 ± 0.7 and 2.6 ± 0.5, respectively. These values were not significantly different ($p>0.05$) compared to the respective sham values.

The mean ovarian weight at autopsy did not differ significantly among the groups (ANOVA: $F=0.36$; d.f.$=6,24$; $p>0.05$) (Table 6). Therefore, ovarian weight was not affected by either sham adrenalectomy, adrenalectomy, or by treatment with cortisone acetate.

Mean uterine weights of prepubertal and postpubertal animals in each group were calculated (Table 6). All animals (n=3) in the intact group were found to be postpubertal at autopsy, and thus, the mean uterine weights

Table 6. Mean body weights, ovarian, and uterine weights for each group.

Group	n	BW (g)	Ovarian Wt (mg)	Uterine Wt (mg) pre	Uterine Wt (mg) post
intact	3	65.3±6.4	21.6±1.4	--	97.6±20.7
sham d23	6	68.7±2.5	24.8±2.9	--	104.3±15.0
adx d23	4	59.8±5.5	24.7±3.4	29.9±5.7 (3)	80.0 (1)
adx d23 +CA	6	59.8±1.8	23.2±2.2	--	77.0±8.8
sham d26	5	60.4±2.7	24.0±2.3	--	95.8±17.4
adx d26	2	70.5±3.5	27.2±1.7	30.6 (1)	89.4 (1)
adx d26 +CA	5	64.4±3.6	25.9±3.1	--	85.0±7.8

were high, as expected. The postpubertal uterine weights for 23 and 26 day old sham adrenalectomized animals were not significantly different from the intact group. (ANOVA: $F=0.10$; $d.f.=2,11$; $p>0.05$). One animal in each adrenalectomized group was postpubertal (both were autopsied at late ages of 36 and 37 days). The 23 and 26 day old adrenalectomized plus cortisone acetate groups were found to be postpubertal upon autopsy. The average uterine weights of both adrenalectomized plus cortisone acetate groups were somewhat lower, but not significantly different (ANOVA: $F=0.88$; $d.f.=4,20$; $p>0.05$), from those of the postpubertal intact and sham adrenalectomized groups.

RESULTS

EXPERIMENT 3

In Experiment 3, the use of internal and external indicators of puberty onset, such as 1) estrous discharge, 2) bromothymol blue tests, 3) vaginal concretions, 4) the presence of hemorrhagic follicles, 5) the presence of corpora lutea, 6) the presence of ova in the oviducts, and 7) uterine weight, allowed for a confident assessment of puberty onset. Where external indicators failed to demonstrate puberty onset, internal indicators provided substantial proof of puberty onset. Animals were classified as prepubertal or postpubertal on the basis of whether or not they showed more than one of the indicators of puberty. By day 30, two out of five, or 40%, of the intact animals ovulated, ie., were postpubertal (Table 7). By day 30, three out of seven, or 43%, of the sham adrenalectomized animals were postpubertal. None of 12 animals adrenalectomized at 26 days of age were postpubertal when autopsied at 30 days of age. There were no signs of estrous discharge, corpora lutea or hemorrhagic follicles, and no ova in the oviducts. Chi-square analysis revealed a significant difference ($p<0.05$) among the groups autopsied at 30 days of age. All of the animals in the intact group had ovulated by day 34. For animals sacrificed on day 34, the number of sham adrenalectomized animals that were

Table 7. Number of postpubertal animals per total number autopsied on day 30 or day 34.

Group	Day 30	Day 34
intact	2/5 or 40%	7/7 or 100%
sham adx (day 26)	3/7 or 43%	6/6 or 100%
adx (day 26)	0/12 or 0%	1/7 or 14%

postpubertal was the same as the number of intact animals that were postpubertal. Adrenalectomy performed on day 26 delayed puberty. Chi-square analysis showed a significant difference ($p<0.01$) between the groups sacrificed at 34 days of age. Only 1 out of 7 or 14% of the adrenalectomized animals sacrificed on day 34 reached puberty. This animal showed all the signs of puberty onset including a mucous, stringy estrous discharge, a positive bromothymol blue test, hemorrhagic follicles, corpora lutea, ova in the oviducts, and a high uterine weight (103.6 mg).

The weight of the uterus increases at ovulation as a result of increased estrogen levels. Figure 1 plots uterine weight for each group on the two days of sacrifice. Both the intact and sham postpubertal uterine weights were high compared with prepubertal intact and sham uterine weights on day 30. Postpubertal uterine weight in sham adrenalectomized animals appeared somewhat lower, but was not significantly different ($p>0.05$) than that of corresponding intact controls. By day 34, all intact and sham adrenalectomized animals were postpubertal, and the uterine weights of these animals were very high and not significantly different (Student's t test; $p>0.05$).(Table 8). The mean uterine weight for 26 day old adrenalectomized animals, all of which remained prepubertal by day 30, was low. By day 34, only 1 adrenalectomized animal out of 7 was postpubertal and its uterus weighed 103.6 mg.

Figure 1. Prepubertal and postpubertal uterine weights for intact, sham adrenalectomized, and adrenalectomized animals on days 30 and 34 of sacrifice. The number of individuals is shown at the bottom of each bar.

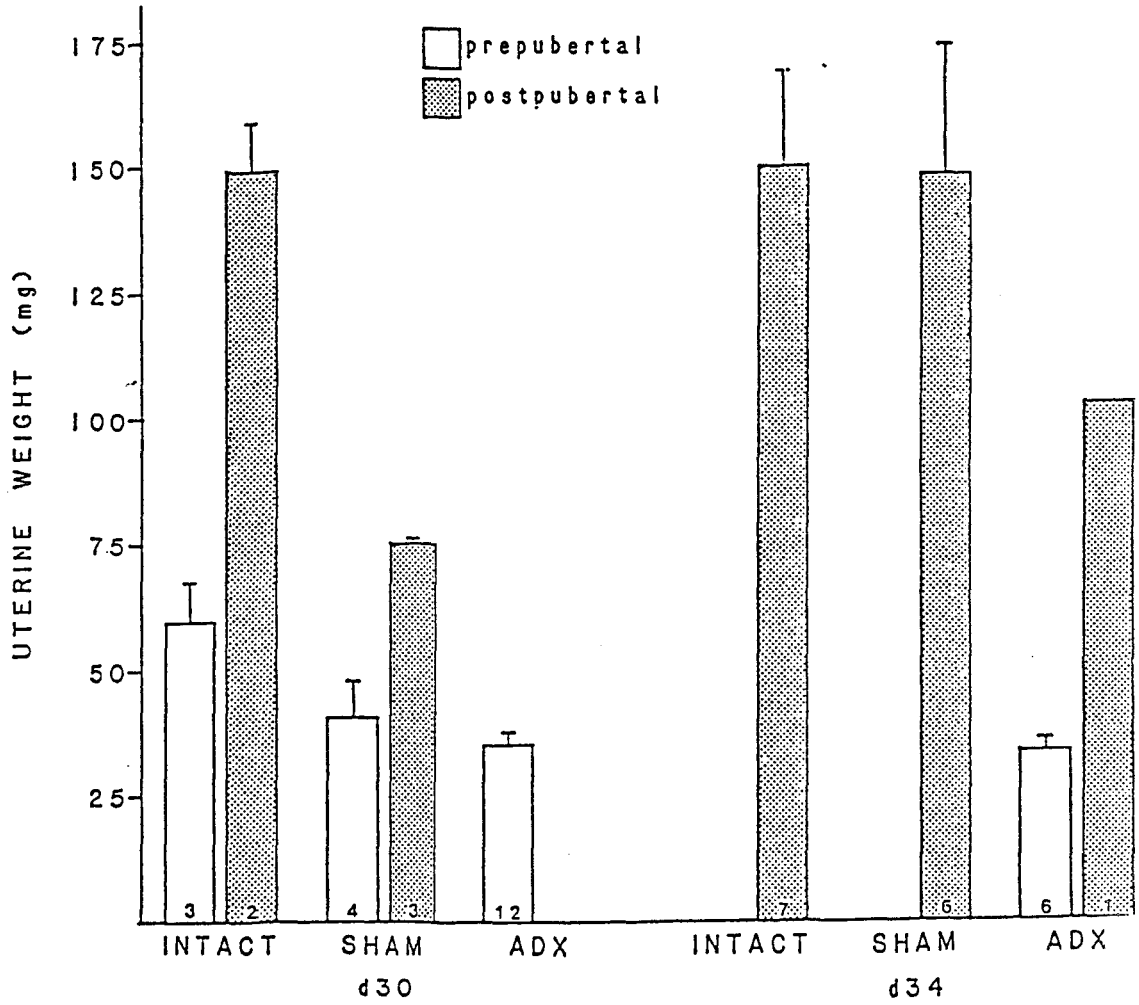

prepubertal
postpubertal
UTERINE WEIGHT (mg)
175
150
125
100
75
50
25
3
2
4
3
12
7
6
6
1
INTACT
SHAM
ADX
INTACT
SHAM
ADX
d30
d34

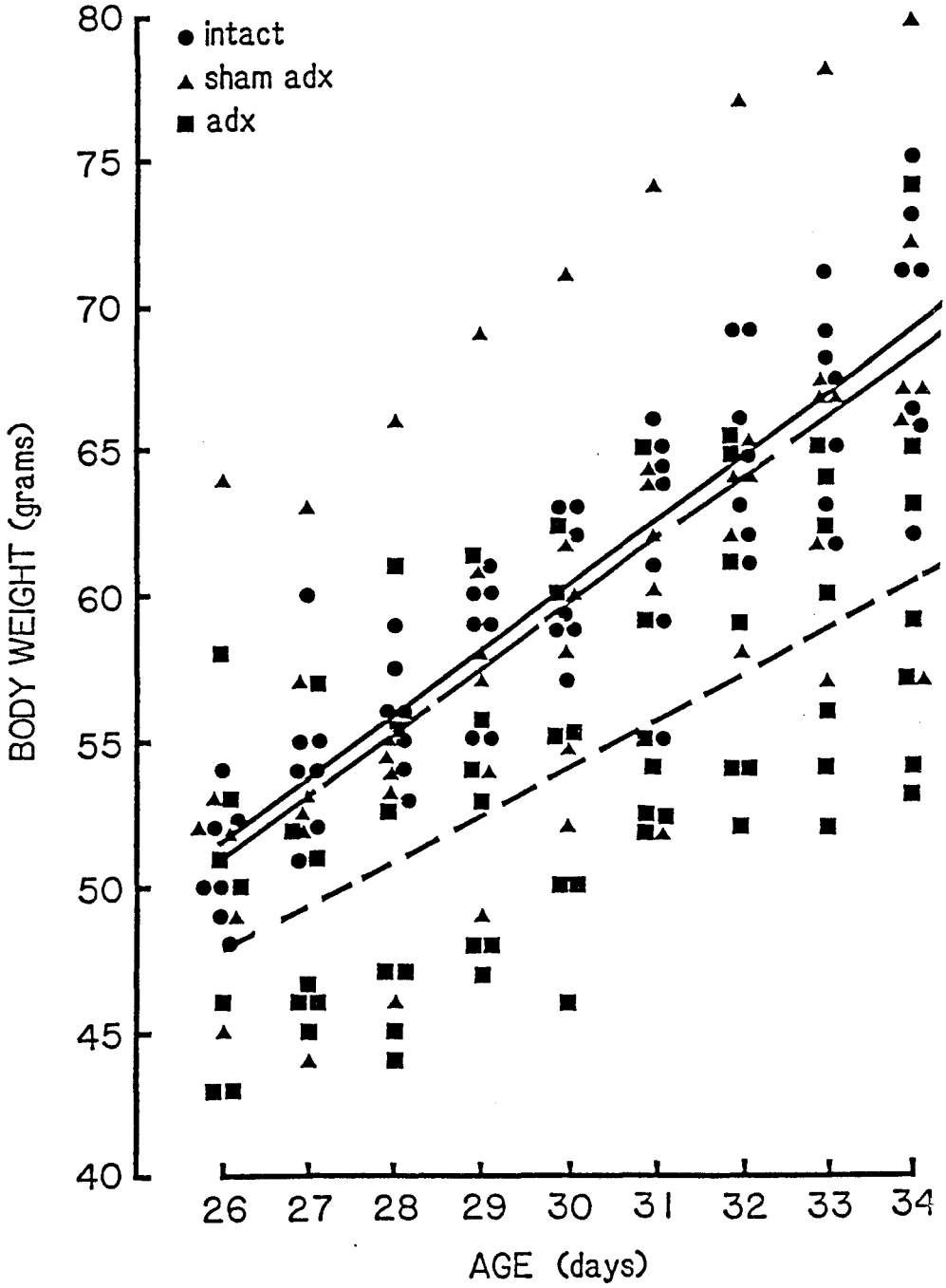
Table 8. Mean body weights, ovarian and uterine weights for each group autopsied on day 30 or day 34.

Group		n	BW (g)	Ovarian Wt (mg)	Uterine Wt (mg) pre	Uterine Wt (mg) post
intact	d30	5	57.8±5.2	22.2±0.9	59.0±10.8 (3)	149.1±14.5 (2)
	d34	7	69.1±3.8	24.5±1.6	--	150.0±20.0 (7)
shamadx	d30	7	58.0±4.0	22.0±1.2	40.5±11.4 (4)	75.0±1.3 (3)
	d34	6	68.5±5.7	24.2±1.5	--	148.5±27.4 (6)
adx	d30	12	54.3±3.6	18.9±0.5	35.0±2.8 (12)	--
	d34	7	60.7±5.7	22.5±0.7	33.6±3.0 (6)	103.6 (1)

The mean ovarian weight was calculated for each group on the two days of sacrifice (Table 8). In contrast to uterine weights, ovarian weight did not change significantly between prepubertal and postpubertal animals (ANOVA: $F=0.01$; $d.f.=1,42$; $p>0.05$). The mean ovarian weights for the sham adrenalectomized groups on days 30 and 34 were virtually the same values as the corresponding controls. The mean ovarian weights of adrenalectomized animals autopsied at 30 days of age were significantly different (ANOVA: $F=6.63$; $d.f.=2,21$; $p<0.01$) from those of the corresponding intact or sham adrenalectomized groups. However, there was no significant difference in ovarian weights between the groups autopsied at 34 days of age (ANOVA: $F=0.77$; $d.f.=2,17$; $p>0.05$).

In Figure 2, body weights for all animals in each group that was autopsied at 34 days of age were plotted against age (from day 26 through day 34). The lines represent linear regression analysis of all the data points for each group. These three lines did not differ in y-intercept (intact vs sham: $t=0.16$, $p>0.05$; sham vs adx: $t=0.82$, $p>0.05$; intact vs adx: $t=1.37$, $p>0.05$). There were no significant differences in the slopes, or rate of growth, between intact (slope=2.4) and sham adrenalectomized (slope=2.1) groups ($t=0.19$, $p>0.05$), or between sham adrenalectomized and adrenalectomized animals ($t=1.275$, $p>0.05$). However, there was a significant difference in the

Figure 2. Mean body weights for intact, sham adrenalectomized, and adrenalectomized animals against time (from day 26 through day 34).


intact
sham adx
adx
80
75
70
65
60
55
50
45
40
BODY WEIGHT (grams)
26
27
28
29
30
31
32
33
34
AGE (days)

slopes of the regression lines for the intact and the adrenalectomized groups ($t=2.09$, $p<0.05$).

RESULTS

EXPERIMENT 4

The response to PMS and HCG in intact, sham adrenalectomized, and adrenalectomized animals autopsied on day 30 or day 34 is shown in Tables 9 and 10, respectively. Treatment of intact, sham, and adrenalectomized animals with PMS and HCG induced ovulation 2-3 days later. The response to this treatment was one of increased ovarian weight, uterine weight, and number of ova ovulated.

Ovarian weights increased by 57.8% and 57.4% for the intact groups treated on days 26 and 30, respectively. Thus, the response to PMS/HCG treatment on ovarian weight was very similar for both days of treatment in intact animals. The same was true for the response in sham adrenalectomized animals. Ovarian weights increased with PMS/HCG treatment in sham adrenalectomized animals. The percent increases for treatments on days 26 and 30 were 42.5 and 42.7%, respectively. For adrenalectomized animals, ovarian weights increased by 12.3 and 29.7%. Thus, the increase in ovarian weight induced by PMS/HCG was highest in the intact groups, lower in the sham groups, and the least in the adrenalectomized groups. Analysis of variance showed a significant difference in ovarian weights among the groups which received PMS/HCG at 26 days of age and were autopsied

Table 9. Per cent animals that ovulated, mean number of ova, and mean ovarian and uterine weights for animals treated on day 26.

Treatment on Day 26		% Ovulated	# Ova	Ovarian Wt (mg)	Uterine Wt (mg)
Intact	no PMS/HCG	0/2	0 (2)*	26.6±4.6	65.5±16.1 (n=2 all pre)
	w/PMS/HCG	6/6	11 (3)	65.5±12.8	181.9±14.4 (n=6 all post)
Sham	no PMS/HCG	0/2	0 (2)	26.1±2.9	36.6±1.4 (n=2 all pre)
	w/PMS/HCG	6/6	16 (3)	49.1±16.2	156.9±20.6 (n=6 all post)
Adx	no PMS/HCG	0/1	0 (1)	23.6 (1)	29.4 (n=1 all pre)
	w/PMS/HCG	5/5	19 (5)	32.9±3.5	159.0±23.0 (n=5 all post)

* (n)=number of animals whose ova were successfully counted.

Table 10. Per cent animals that ovulated, mean number of ova, and mean ovarian and uterine weights for animals treated on day 30.

Treatment on Day 30		% Ovulated	# Ova	Ovarian Wt (mg)	Uterine Wt (mg)
Intact	no PMS/HCG	3/3	0 (3)*	23.9±2.4	102.3±21.8 (n=3 all post)
	w/PMS/HCG	9/9	17 (3)	66.6±9.2	197.9±33.5 (n=9 all post)
Sham	no PMS/HCG	1/1	1 (1)	26.2 (1)	145.2 (n=1 all post)
	w/PMS/HCG	3/3	19 (3)	53.1±4.8	178.3±31.8 (n=3 all post)
Adx	no PMS/HCG	0/1	0 (1)	26.2 (1)	42.4 (n=1 all pre)
	w/PMS/HCG	5/5	25 (5)	41.4±3.6	152.6±20.9 (n=5 all post)

* (n)=number of animals whose ova were successfully counted.

at 30 days of age ($F=5.90$; d.f.=2,14; $p<0.025$). There was also a significant difference among the groups which received PMS/HCG at 30 days of age and were autopsied at 34 days of age ($F=10.79$; d.f.=2,4; $p<0.005$). However, there were no significant differences between the intact and sham-operated group ($p>0.05$) or the sham-operated and adrenalectomized group ($p>0.05$) at either autopsy time, when compared by Student's t test.

Uterine weights in all three groups were increased upon administration of PMS/HCG, as compared with the control groups. Uterine weights increased 62.8 and 37.6% for the intact groups, 75.1 and 4.4% for the sham groups, and 77.2 and 69.1% for the adrenalectomized groups on the two days of treatment, respectively. This analysis shows that PMS/HCG treatment did not affect uterine weight similarly between groups. Uterine weights may be inconsistent due to the normal increase in uterine weight upon reaching puberty. It is important, however, to note that uterine weight did increase in PMS/HCG treated adrenalectomized animals. Furthermore, these uterine weights were very similar to those of intact and sham adrenalectomized animals treated with PMS/HCG on both days. There was no significant difference in uterine weight among intact, sham adrenalectomized, and adrenalectomized animals given PMS/HCG and autopsied at 30 (ANOVA: $F=1.72$; d.f.=2,14; $p>0.05$) or 34 days of age (ANOVA: $F=2.23$; d.f.=2,14; $p>0.05$).

The number of ova ovulated also increased in all groups upon treatment with PMS/HCG. The most ova found in either the intact or sham adrenalectomized untreated animals was one in this experiment (seven were found in intact animals from the previous experiment), whereas in PMS/HCG treated animals, the number of ova ranged from 8-25 for these two groups. Adrenalectomized animals ovulated a large number of ova; an average of 19.4 ova were found in the oviducts of animals autopsied on day 30, while an average of 21.2 ova were shed in those animals autopsied on day 34.

The non-PMS/HCG treated animals in all three groups on both treatment days confirmed the results of experiment 3 in that 100% of intact, 100% of sham, and 0% of adrenalectomized animals reached puberty by day 34. Thus, it can be concluded that puberty was delayed by adrenalectomy performed on day 26 of life.

CHAPTER V

DISCUSSION

The experiments in this study were designed to determine whether or not the adrenal glands play a role in the onset of puberty in the female golden hamster and the mechanism by which adrenalectomy has its effect on puberty. In order to do this, some reliable indicator of first ovulation had to be found so that this hypothesis could be tested. The characteristic estrous discharge was used initially (Orsini, 1961). Using this as an indicator, the range of "first ovulation" in the intact animals of the first experiment was found to be from day 30-39. However, it is very likely that previous ovulations were occurring before day 39, since animals showed higher uterine weights and ova counts, both being indicative of prior ovulation. Animals had also shown waxy plugs and powdery secretions upon examination prior to autopsy. The characteristic stringy, mucous estrous discharge was not always evident in these animals. Thus, estrous discharge proved to be an unreliable indicator of first ovulation. This finding is in agreement with that of Diamond and Yanagimachi (1970) who found that the estrous discharge is not consistently correlated with first ovulation, although it is the earliest indicator of impending behavioral estrus. Since estrous

discharge was not efficient in indicating ovulation, the initial experiment could not determine whether or not adrenalectomy delayed puberty. Therefore, the second experiment was performed with new procedures that would help to identify first ovulation more accurately.

However, criteria for the day of first estrus did not provide conclusive information. The characteristic estrous discharge was not present in all animals. Bromothymol blue tests were also inconsistent. For some animals, a "positive" result for estrus was yellow, for others it was green, and still for others it was yellow-green or blue-green, using the same bromothymol blue. In addition, vaginal concretions were not always detected in the smears of those animals known to have ovulated.

In the third experiment, internal as well as external criteria were used to detect whether or not ovulation had occurred. The internal criteria were, by far, more reliable indicators of ovulation than the external. Thus, if an animal had corpora lutea, hemorrhagic follicles, ova in the oviducts, and/or a high uterine weight, then it was certain that the animal had ovulated.

In summary, it can be concluded that the first ovulation in the female hamster may be a "silent" one, since external indicators of puberty were not always present at first ovulation. Diamond and Yanagamachi (1970) found similar results in some of their females in that first

ovulation was not always preceded by vaginal indications of estrus (anaestrous ovulation= "silent heat"); however, they also found that starting at age 32 days, all females did ovulate after vaginal estrus.

Using just the external indicators of puberty, the estimated age of first estrus was slightly higher than expected in the intact animals, as well as the sham adrenalectomized animals (36.7±1.6 days of age for the intact group and 34.5±0.8 and 36.4±3.4 days of age for the 23 and 26 day old sham groups, respectively). Greenwald and Peppler identified first ovulation by examining ovaries histologically to identify corpora lutea and tubal ova and reported that the earliest spontaneous ovulation ranges from day 29-33 in intact hamsters (Greenwald and Peppler, 1968). The data in Experiment 3 clearly show that two out of five, or 40%, of the intact animals ovulated by day 30 (Table 7). These data agree with those of Greenwald and Peppler (1968) who found that 30% of their intact animals ovulated by day 29. In the present investigation, 100% of the animals in the intact group ovulated by day 34. Greenwald and Peppler (1968) found similiar results in that seven out of ten animals ovulated by day 35.

In Experiment 3, sham adrenalectomy had no effect on puberty onset, since there was no significant difference between the percentage postpubertal animals sacrificed on either day 30 or day 34, as compared to the intact group.

At this point, it can be affirmed that adrenalectomy performed on day 26 did delay puberty up to at least 34 days of age. In adrenalectomized animals, there was no sign of estrous discharge, bromothymol blue tests were negative, and there were no vaginal concretions in the lavages. Furthermore, no ova were found in the oviducts, and no signs of hemorrhagic follicles nor corpora lutea were seen except in the case of one animal. Earlier, it had been concluded that adrenalectomy performed at 29 days of age does not delay puberty. This is most likely due to the fact that follicular maturation was not completed until about 28 days of age, and by age 29, it can culminate in spontaneous ovulation (Greenwald and Peppler, 1968). A similar situation was found in the rat in that adrenalectomy performed on days 18 and 25 significantly delayed puberty, whereas when performed on day 26, it had no effect on puberty onset (Gorski and Lawton, 1974). Gorski and Lawton (1973) proposed that the periodicity of the adrenocortical secretions had not been established by day 25. Thus, whatever factor the adrenals contributed to the timing of the onset of puberty was removed by adrenalectomy. By day 35 in the rat, adrenalectomy no longer affected the onset of puberty, thereby indicating that the periodicity within the hypothalamic-pituitary-gonadal system was matured and whatever contribution the adrenal makes to this process had already occurred.

The uterine weights of adrenalectomized animals were small both by day 30 and by day 34, and were mostly prepubertal with the exception of the one animal mentioned above. This animals showed all the signs of puberty onset and had a comparatively higher body weight than the other animals at autopsy. It has previously been demonstrated that puberty onset is correlated with body weight in rats (Kennedy and Mitra, 1963; Glass et al., 1976, 1979; Frisch et al., 1977; Wilson et al., 1983) and mice (Hansen et al., 1983). Thus, it was of interest to determine if this was the case in hamsters. No significant difference was found in the slopes, or rate of growth, between intact, sham adrenalectomized, or adrenalectomized animals. Linear regression analysis did not support a correlation between puberty onset and body weight.

In the final experiment, the purpose was to determine at what level adrenalectomy has its effect. Could the effect of adrenalectomy be due to some influence of adrenalectomy on the pituitary secretion of other hormones such as the gonadotropins? Does it have its effect at the follicular level? Is follicular development halted, or does follicular maturation occur, yet the absence of the signal for ovulation causes puberty to be delayed? In the present investigation, the last hypothesis was tested, because it was one of the easiest to test.

It has been shown that in prepubertal intact hamsters, the ability to induce ovulation coincides with the onset of spontaneous ovulation. Furthermore, spontaneous ovulation coincides with the appearance of antral follicles, therefore, indicating that follicular development is complete (Greenwald and Peppler, 1968). Thus, if one can induce ovulation by extraordinary stimuli, follicular development must be completed. This can be confirmed by histological analysis of the ovaries. Upon administration of exogenous gonadotropins--PMS and HCG--to 26 day old adrenalectomized female hamsters, ovulation was induced. A large number of ova were found in the oviducts of these animals. Treatment with PMS/HCG in adrenalectomized, as well as intact and sham, animals also increased ovarian and uterine weights significantly, as compared with untreated animals. Treatment with PMS/HCG seemed to induce the animals to ovulate in the normal range of time for ovulation (ie., before day 34). It has been shown by Ramaley and Bartosik (1975) that PMS given to 22 day old intact and adrenalectomized rats induced ovulation 3 days later at 0400h in the intact animals and (3 days later) at 1600h in the adrenalectomized animals. Animals injected with PMS on day 26 in the latter experiment ovulated at 2400 on day 28 when left intact, and ovulated on day 29 by 1600 h when adrenalectomized. Thus, adrenalectomized PMS-treated animals showed a delay in the timing of ovulation.

Adrenalectomy plus PMS in rats also delayed vaginal opening and uterine ballooning, and raised progesterone levels. Ramaley and Bartosik (1975) postulated that PMS causes the adrenal to stimulate the ovary to release progesterone, thus facilitating ovulation. They also postulated that the adrenal normally acts as a primer to facilitate gonadotropin release in rats. They then suggested a possible correlation between the pattern of progesterone secretion elicited by PMS and the priming effect of endogenous progesterone on gonadotropin secretion. Thus, they proposed that the pattern of progesterone release induced by PMS could be related to when ovulation takes place. In the present experiment, adrenalectomy and PMS treatment on days 26 and 30 induced ovulation in all animals in both groups. The occurrence of ovulation in these animals was indicated by both external cues (characteristic estrous discharge, vaginal concretions, positive bromothymol blue test) and internal cues (high uterine and ovarian weights, hemorrhagic follicles, etc.). The effects of adrenalectomy and PMS treatment in Ramaley and Bartosik's rats showed similarities in comparison with the effects seen in the hamsters in the present experiment. One similarity is that in both adrenalectomized rats and hamsters, PMS induced ovulation approximately 2-3 days later. Also in agreement was the uterine weight response to PMS in adrenalectomized rats and hamsters, as compared with intact PMS-treated animals.

Uterine weights in adrenalectomized PMS-treated rats were not consistently different from those of intact PMS-treated rats, just as uterine weights of adrenalectomized PMS-treated hamsters were very similar to those of intact PMS-treated hamsters. The effects of adrenalectomy plus PMS treatment were also similar in the two studies in regard to ovarian weights (Tables 9,10). Shaha and Greenwald (1983) have shown that the ovaries of prepubertal PMS-treated hamsters increased in weight probably due to an increased proliferation of the interstitium. Ramaley and Bartosik (1975) showed that adrenalectomy resulted in decreased ovarian weights in response to PMS treatment, as compared with ovarian weights of intact animals. A similar result was seen in the adrenalectomized hamsters of the present experiment. Ovarian weights increased with PMS/HCG treatment, but remained consistently lower than ovarian weights in the intact group (Tables 9,10).

Ovulation can be induced by the administration of PMS/HCG in adrenalectomized hamsters. As stated earlier, the ability to induce ovulation coincides with the onset of spontaneous ovulation which, in turn, coincides with the appearance of antral follicles (thereby indicating that follicular development is complete). Therefore, since ovulation was induced with PMS/HCG in adrenalectomized hamsters, follicular development must have occurred. Moreover, the normal events which lead to follicular

development were restored after adrenalectomy by PMS/HCG treatment. In a future experiment, it would be interesting to look at the pattern of progesterone secretion in intact and adrenalectomized hamsters given PMS and HCG, and to determine if a correlation exists between this pattern and the timing of the onset of ovulation.

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Approval Sheet

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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

12/5/86
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