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ADRENAL INFLUENCE ON PUBERTY ONSET IN THE RAT

by

Method Anthony Duchon

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

February

1976

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BIOGRAPHY

Method Anthony Duchon was born in Jacksonville, Florida, on January 26, 1949. He graduated from St. Peter's Grammar School and Boylan Central Catholic High School in Rockford, Illinois. In August of 1969, he was married to Martha Louise Witkowski. He graduated from the University of Illinois, Urbana, in February, 1971, and joined the Physiology Department of Loyola University, Maywood, Illinois. In September of 1971, he also entered the Loyola University, Stritch School of Medicine, receiving his Doctor of Medicine in December, 1974.

ABSTRACT

Previous investigations in our own laboratory have demonstrated an adrenal - ovarian interaction period in the maturational steps culminating in puberty in the female albino rat. The question confronted was what role these adrenal secretions play in the onset of puberty in the male rat. Therefore, the role of the adrenal gland in the timing of puberty in the male rat was investigated. Further, the adrenal - ovarian interaction period was investigated in the female by an indirectly-induced advancement of puberty, and the latter's effect upon the adrenal - ovarian interaction period was studied.

Male rats were received at 18 days of age, from ARS/Sprague-Dawley (Madison, Wisconsin), and housed two per cage under controlled lighting of 14 hours light and 10 hours dark, at a constant temperature of $24 \pm 1^{\circ}$ C. Penile smears were taken via a saline lavage of the tip of the glans penis. Presence of spermatozoa in the smear was considered a positive indicator of puberty onset.

ABSTRACT (continued)

In the first of three studies, ten male animals were smeared daily from 50 to 71 days of age. The results agreed closely with previous reports of this technique in the rat and the male golden hamster.

The second study was confirmatory in nature. It assessed further the correlation between a positive smear and the onset of puberty in the male rats raised in our animal quarters. Groups of ten animals each were autopsied at 43, 45, 47, 49, 51, 53, and 55 days of age. Penile smears were obtained at the day of autopsy. Body, adrenal, testis, thymus, and seminal vesicle weights were recorded. Results revealed a close association between the first positive penile smear and full maturation of the reproductive apparatus. Simultaneous smears from the vas deferens revealed no false positive penile smears.

The third study consisted of controls and animals bilaterally adrenalectomized or sham operated at 19, 26, 33, 40, and 47 days of age. Penile smearing commenced at

ABSTRACT (continued)

45 days of age, and autopsy was performed on the day of the first positive penile smear. Adrenalectomized animals were given a 1% saline solution to drink. Body, adrenal, thymus, testis, and seminal vesicle weights were recorded. Sections of testicular tissue were preserved for histological study. Results of this study revealed that adrenalectomy had no effect on the time of puberty onset in the male rat. There were no significant differences in accessory sex organ weights in any of the animal groups. Thymic weights showed a good adrenal-related response. No histological differences were seen among control and treatment groups.

Studies four and five were essentially similar in nature. Female rats were received at 18 days of age and housed as described. However, they were exposed to constant illumination to achieve an advancement of puberty. Animals were autopsied on the day of vaginal opening. Constant illumination resulted in an advancement of

ABSTRACT (continued)

puberty when compared to light - dark controls. Further, the adrenalectomy-induced delay period was also shifted by a significant amount. Also, a period of sham-induced advancement was seen to be phase-shifted. Study five was performed to confirm these results, but were inconsistent throughout.

It is concluded that whatever factor(s) is(are) responsible for an adrenal-mediated response in the female is(are) not operative in the male in determining the time of puberty onset. It is also concluded that in the female constant light exposure advances puberty and is related to the adrenal - ovarian interaction period in a yet unexplained way.

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CHAPTER I

LITERATURE REVIEW

The maturation and functioning of the reproductive system in both the male and female remains as complex and perplexing as ever. The beginning of this functioning is puberty when maturation is theoretically complete, but this is not entirely the case. Puberty derives from the Latin word "pubes" indicating hair or the age at which certain body parts began to be covered with hair. However, even the ancients knew that this did not indicate complete maturity and had other terms for someone who was able to marry and bear children. Thus, even with more advanced techniques and penetrating observations of brilliant minds, we still seem to be little advanced from these simple observations. The purpose of this literature review is to explore the mechanism of puberty in both the male and female and the gonads' relationship with the adrenals.

A. Brain - Pituitary - Testicular Interactions

The events that lead to the production of mature

spermatozoa are complex and perhaps less understood than the reproductive cycle of the female. The question is: How does the process begin and what is involved in maintaining it?

1. Spermatogenesis

- a. Anatomical changes

The testicles are paired reproductive organs with a dual function - the elaboration of steroid hormones and the production of gametes. The synthesis of hormones was inferred quite early by Claude Bernard's use of dog testicular extract to rejuvenate his flagging sexual interest and by Berthold's classic experiment with the rooster (34). The production of gametes is the function that was probably recognized in antiquity (4).

The morphology of the testicles was studied in detail by Hagitt (47), and in a more quantitative fashion Clermont (14 - 19) has given a reasonably complete picture of the histology of spermatogenesis. What is in order is a brief summary of the steps of spermatogenesis.

At birth, in the rat, the testes show primitive germ cells, gonocytes, that are present until about four days of age. At four to six days of age some of the gonocytes appear to be degenerating or the gonocytes appear to be completely absent. Early authors (102), working with birds, favored the view that the gonocytes

gave rise to the spermatogonia, while supportive elements gave rise to the Sertolic cells. Other workers, studying the rat (29), supported the view that the supporting elements gave rise to both the Sertoli cells and germ cells. However, Clermont marshalled convincing evidence that the gonocytes give rise to the germ cells; he, along with others (14), in a series of papers elucidated the basic events of spermatogenesis and arranged these events in stages. His first conclusion is that cells in the tubules of the testes are grouped in well defined associations. Secondly, these associations advance consecutively, with the most primitive cells near the basement membrane and the more differentiated cell types close to the tubular lumen. The cell types may be summarized as follows.

Gonocytes, the primitive germ cells, give rise to dark staining type A_1 spermatogonia. These cells divide mitotically to renew the undifferentiated germ cell compartment and to yield pale staining type A_2 spermatogonia. The type A_2 cells divide mitotically to yield the more highly differentiated type B_1 cells. These type B_1 cells undergo two mitotic divisions and give rise to primary spermatocytes. The primary spermatocytes then undergo a meiotic division to produce haploid secondary spermatocytes. These then equationally

divide to yield spermatids. The spermatids then undergo transformation in the process of spermiogenesis (15).

This brief summary leads to two important considerations. The first of these is the time that is required for the entire process. Oakberg (67) concluded, using the irradiated mouse testis, that the process from type A₁ spermatogonia to mature sperm takes approximately 34.5 days. The human cycle (49) is about 74 days in duration. Finally, the Sprague-Dawley rat's spermatogenic cycle is about 51 days in duration (19). Interestingly, the various strains show some variation in length of cycle in the rat (19). The second consideration is at what point hormonal effects are operative in the spermatogenic cycle.

b. Hormonal changes

Smith (95, 96), in a series of classic experiments, demonstrated that the testicles were unequivocally controlled by secretions of the pituitary gland. Greep and co-workers (42, 43) performed other classic experiments that led to the concept that FSH had influence over the spermatogenic process and LH controlled Leydig cell function. What followed these historic experiments were about 30 years of experiments delineating the function of the gonadotrophins. A full review of this work is not deemed advisable at this point (see

review by Steinberger, 100), but a few works are deserving of comment.

Woods and Simpson (110) did an extensive re-evaluation of the pituitary's control over the testis of the hypophysectomized rat using the purest gonadotrophin preparations available at the time. They concluded that Interstitial Cell Stimulating Hormone (ICSH) or LH, at very low doses, maintained spermatogenesis but that the weight of the testes were reduced. However, even high doses of ICSH alone failed to return testicular weights to normal. FSH alone produced only a slight effect on the testes, but when these small doses of FSH were given along with low doses of ICSH, the most marked effects were produced. They concluded that this was evidence for a synergism between the two hormones, and that ICSH was the primary hormone responsible for the maintenance of the seminiferous epithelium. They also presented data that suggested prolactin and Growth Hormone (GH) augmented the effect of ICSH and FSH, while Adrenocorticotrophic Hormone (ACTH) and Thyroid Stimulating Hormone (TSH) had no effect. Lostroh (57) also concluded that GH augmented the effect of gonadotrophin on the testis. To further confound the picture, Selye (92) has demonstrated that testosterone can, in sufficient doses, maintain spermatogenesis in the rat, but

this evidence is only qualitative and not quantitative.

Where the various hormones are active in the previously outlined spermatogenic cycle (Section 1. a.) has also been of considerable interest. Steinberger (97) has shown that, in tissue culture, gonocytes from newborn rats could be transformed into type A₁ spermatogonia without the presence of hormones. However, this may not be valid because Resko (88) documented a high level of testosterone in the testis of one-day old rats, and thus the cells may have already been stimulated by hormone. Qualitatively the spermatogenic cycle appears to be hormonally independent to the stage of spermatocytes. At the spermatocyte stage, testosterone appears to be required only to complete the transformation to spermatids (98). Finally, FSH is apparently required (99) for the late spermatid to mature into a spermatozoon.

With the advent of more sensitive assays, the peripheral concentrations of hormones in the prepuberal rat has received considerable attention. Pfieffer (73) originally showed that the neonatal testis is capable of hormone secretion. Resko (88) amplified this work with the demonstration that levels of testosterone in the testis and blood decline after one to two days of age. They then remain stable until 30 days of age

when they begin to rise in both the plasma and testis. The most marked rise occurs between 40 and 60 days of age, with adult levels reached at this time. Testosterone is also the major androgen in the male rat, with androstenedione playing a minor role (88). Serum FSH (37, 69) rises neonatally and then declines by 10 - 15 days of age; a peak is then observed at 30 days of age, with a gradual decline to adult levels at 70 days of age. Plasma LH is markedly elevated (69) neonatally, with a decline to minimum levels at 12 - 15 days of age; LH concentration then begins a slow rise to maximal levels at 70 days of age (101). Prolactin has also received some attention for its participation in the puberal changes in the male rat. Hafiez (45) has suggested that prolactin participates in the growth of accessory organ structures. Others (62) have shown that prolactin rises from low levels at 15 - 20 days of age to a peak at 25 days of age. This level was maintained until 50 days of age when it rises again, plateauing at 100 days of age.

c. Neural influences

The hypothalamic-pituitary axis is also undergoing change during the prepuberal period, and this is probably where the control of puberty onset resides. Harris and Jacobsohn (48) demonstrated that pituitaries

of very young male and female rats were capable of supporting normal adult gonadal function. From this work it can be inferred that the control over puberty onset rests above the level of the pituitary.

Supportive evidence concerning the neural aspect of puberty onset is the use of brain lesions. This work has primarily been done in the female, but some studies have been carried out in the male. Donovan and Van der Werff ten Bosch found that electrolytic lesions in the anterior hypothalamus of female rats resulted in premature vaginal opening and ovulation (24). This is evidence that hypothalamic centers are inhibiting gonadotrophin release. Relkin (86) has shown that, in the male rat, puberty, as judged by testicular descent, can be advanced by pinealectomy. He also demonstrated (87) that bilateral amygdaloid lesions, which had delayed puberty in the female, had no effect in the male. Other studies in the female rat (10) have shown beyond doubt that lesioning of the anterior hypothalamus produces sexual precocity. The fact that this appears not to be true in the male is not disturbing. The rat is perhaps a poor test animal for, as already noted, his spermatogenic cycle is initiated at birth and is relatively hormone independent.

A second line of evidence for hypothalamic

involvement that has recently been obtained is the discovery of hypothalamic releasing factors. Ramirez and Sawyer noted a decrease in bioassayed hypothalamic LH-RF at about the time of puberty in the female rat (80). Others (107) have shown decreases in the assayable amount of hypothalamic FSH-RF around puberty, although it should be remembered that this releasing factor is still only theoretical. These sudden drops in hypothalamic content, however, do not necessarily reflect release but might reflect decreased synthesis as a result of other puberty-initiating events. The sensitivity of the pituitary to the releasing factors is also important, and Debeljuk and co-workers found slight changes, possibly unimportant, in the responsiveness of the pituitary to LH-RF (23). Bearing on this question is development of feedback sensitivity. Yaginuma demonstrated the hemicastration of males on the day of birth resulted in compensatory testicular hypertrophy at three days of age (112). Ojeda (69) has demonstrated increased FSH on day 12 when males were hemigonadectomized at ten days of age. Finally, Bloch and co-workers (11) have shown that prepuberal males require less androgen than adults to suppress LH levels back to normal. Thus, the prepuberal male has both feedback sensitivity and gonadotrophin-producing capabilities that remain quiescent until the

onset of puberty.

d. Assessment of puberty in the male rat

The attainment of puberty in the male rat represents a perplexing problem in terms of assessment. The female has a vaginal opening which is discrete, easily assessed, and reproducible. Such a parameter is desirable in the male. Testicular descent is taken as one measure of puberty (87), but it occurs at approximately 40 days of age when true puberty is perhaps not yet reached. Attainment of an adult coital pattern occurs at 75 days of age (54) and may be indicative of the completion of puberty. What is needed is something that is a reflection of events occurring in the 50- to 60-day old rat, which is the age at which accessory organ weights are changing the most (44), fertility is achieved (13), and many hormonal patterns are achieving adult levels (see Discussion, Section a., b., c.).

The use of the penile smear has appeared recently in the literature (26, 104) and perhaps represents a means of assessing puberty in the male. Beach and Eaton noted (6) that spontaneous emissions occur in male hamsters, and that this is partially dependent on the amount of genital grooming (70). Recovery of these spontaneous emissions would thus represent an innocuous manner to assess puberty in the male. To speak of

puberty in the male as a discrete event is a little misleading. Puberty in the male is an acceleration of developmental processes involving the secondary sex characteristics and a final maturing of the spermatogenic process. The human male exhibits a more discrete puberty because of the length of the quiescent period between birth and puberty. As already noted, the male rat is perhaps from birth initiated into the events that culminate in puberty. Thus, the penile smear represents one way to score animals on the adolescent-to-adult growth continuum, but does not necessarily indicate a discrete change in function as occurs in menarche.

B. Brain - Pituitary - Adrenal Interactions

Concomitant with the developing gonadal system, the brain - adrenal axis is also undergoing maturation and is important to the functioning of the organism.

1. Adrenal Gland

a. Anatomical description

The adrenal gland is a paired endocrine tissue with two distinct anatomical and physiological areas. The medulla is composed of preganglionic terminals and postganglionic neurons of the sympathetic nervous system, and will not be reviewed. The cortex is a tripartite structure with large lipid-filled cells arranged irregularly around vascular sinusoids.

Three zones of the cortex have been described: first, the zona reticularis bordering the medulla; second, the fasciculata, widest of the areas with tall columns of cells; and thirdly, the glomerulosa with tufts of cells under the capsule (7). The growth and maintenance of the adrenal cortex is dependent upon continuing secretion of a pituitary hormone. It was demonstrated in 1946 (22) that hypophysectomy causes a marked atrophy of the fasciculata and reticularis while the glomerulosa is less affected. The pituitary factor responsible for this action was called adrenocorticotrophic hormone or ACTH. It has also been demonstrated that adrenalectomy can alter the pituitary structure (94), presumably causing the ACTH-secreting cells to undergo hypertrophic and hyperplastic changes. These changes are prevented by the administration of cortisol. Another histological alteration can be seen after an animal has been subjected to a stress. Selye (91, 93) showed that a noxious stimulus or environment (i.e. "stress") could alter the morphological character of the adrenal gland. These changes were most marked in the zona fasciculata with increases in lipid content, volume, and distribution. This was viewed as evidence of increased secretory activity. The administration of ACTH has been shown to mimic (52) these effects.

b. Hormonal changes

No discussion of the adrenal gland is complete without mention of Thomas Addison, who recognized the life-maintaining properties of the gland and described the disease of adrenal insufficiency which now bears his name (1; cited in 20, 30, 68, 93, 113). In 1927, Zwemmer (116) showed that the cortex was the life-sustaining portion of the gland; this led to the eventual isolation and crystallization of the cortical hormones. These hormones are steroids and are divided into three groups primarily on the basis of activity. The glomerulosa produces mainly mineralocorticoids, the fasciculata mainly glucocorticoids, and the reticularis mainly sex steroids, although each layer probably produces some of each type of hormone. This gave rise to the concept of a functional as well as anatomical zonation of the gland. The effects of these hormones are legion and thousands of publications have documented the effects of these hormones on almost every body system. What I would like to focus on are the developmental changes that are occurring in adrenal gland activity in the prepuberal rat. Bear in mind that the major corticoid in the rat is corticosterone and not cortisol as in the human.

The rat fetus has been shown to contain corticosterone during the last five days of gestation (9).

Estrogen and progesterone secretion have not been demonstrated to occur in utero (8), but at the twentieth day of gestation androgens can be produced (35). Following birth, the development of a mature adrenal rhythm has received considerable attention. The two-day old rat can release measurable amounts of corticosterone in response to an ether stress (41). The rat adrenal is also capable of responding to ACTH at birth (2), but these same workers state that the response to stress is not seen until approximately 15 days of age. Ramaley (76, 77) has shown the developmental changes of corticosterone secretion in the prepuberal rat, with Males showing a less consistent pattern of secretion than females. Changes in plasma concentration were demonstrated in the male at 19 - 20 days of age, but the pattern was poorly defined; diurnal variations were also seen at 30 - 38 days of age, 43 - 44 days of age, and after 48 days of age. In the female rat an adult pattern of adrenal secretion was noted at 18 days of age; however, the levels were attenuated. The diurnal variations are then more consistent in the female, with a marked elevation in the evening level of corticosterone on the day of puberty. Progesterone and androgens also have been demonstrated (12, 27) to be produced in the adrenal gland. Additional comments on the secretion of

sex steroids are contained in Section C.

c. Control of secretion

Both male and female rats show a 24-hour rhythm in corticosterone secretion (21); however, the basal levels in the female are higher than in the male (52). Both sexes demonstrate low levels of corticosterone at approximately 3 A.M. with peak levels occurring at 4 - 8 P.M. It should be remembered that the rat is a nocturnal animal, and its secretion rhythm is consistent with this activity pattern. The output of hormones from the cortex is influenced by ACTH which, in turn, is partially controlled by corticotrophin releasing factor (CRF).

The adrenalectomized animal has provided some insight into the control of adrenal activity and of CRF activity. Following adrenalectomy, the circadian rhythm of CRF activity persists (103), and ACTH is released in response to stress (51). The injection of ACTH into hypophysectomized adrenalectomized animals leads to a reduced content of CRF in the hypothalamus (90). This is evidence that the release of CRF has some neural influence and that a short feedback loop exists to control ACTH release. The classical feedback control of adrenal secretion involves the effect of corticosterone on circulating ACTH levels. It has been shown that physiological levels of corticosterone can suppress the

afternoon peak of corticosterone and that these same levels can suppress the adrenal response to stress (114, 115). Many sites in the brain have been implicated as possible feedback receptor sites, but it is also probable that the hormones act in part by inhibiting ACTH secretion at the level of the pituitary (39).

C. Adrenal - Gonadal Interactions

The major thrust of this thesis is the possible connection between gonadal and adrenal function. This area has received particular interest in the past few years such that a review has appeared (79) devoted entirely to this aspect of endocrinology. As in most other areas of endocrine research, the male has been slighted in favor of the female.

As early as the beginning of the nineteenth century it was suggested that there was a relationship between the gonads and the adrenals (see Nagle in 20). The turn of this century also saw allusions in the literature to the putative connection, but Corey and Britton (20) were perhaps the first workers to demonstrate adrenal gland extracts' ability to advance puberty; they stated that this effect was seen in both males and females, although more pronounced in the females. At about the same time, Freed and Brownfield (35) reported that adrenalectomy had profound effects on the testes, but

admitted to the terminal condition of their animals and that this may have been a factor. In 1952, Aterman and Greenberg (5) induced precocious development in the male rat by the administration of cortisone, while others (8) showed that cortisone acetate in prepuberal females induced increased uterine weights and greater hypophyseal gonadotrophic potency. Prior to this, Wade and Hazelwood (106) had demonstrated that ovariectomy, but not adrenalectomy, could delay puberty in the female. In concert with this was the demonstration that irregular estrous cycles followed adrenalectomy in the female (61), but it was later demonstrated that an animal maintained on NaCl would have normal estrous cycles (63). Thus, early work demonstrated a possible connection between adrenal and ovarian function, but many questions remained.

More recent evidence points to a possible "fine-tuning" role of the adrenal gland in the regulation of the onset and maintenance of the estrous cycle. This evidence falls into two basic categories: the prepuberal and puberal female rat, and the adult animal, respectively. Each will be considered.

In the prepuberal female rat, work in our own laboratory (30, 31, 40) has demonstrated that adrenalectomy up to 26 days of age has the effect of delaying

puberty. If adrenalectomy is performed after this time, the effects are more subtle, resulting in a different pattern of ovulation and changes in ovarian weight, but not a change in time of vaginal opening. Results also emerged from this work which indicated that the sham procedure performed at 26 to 30 days of age advanced the time of vaginal opening. Mandl (59) was perhaps the first to demonstrate that adrenalectomy reduced the response of prepuberal rats to PMS gonadotrophin. Later, Ramaley (75) demonstrated that Pregnant Mare's Serum (PMS) would induce ovulation in weanling rats only if the adrenals were intact. This was not the case in 27-day old rats where PMS induced ovulation in adrenalectomized animals but ovarian and uterine weights were reduced. This evidence must be interpreted cautiously because it has been shown that high levels of ACTH can, alone, blunt the response to PMS in immature animals (46); this was demonstrated in the intact animal and adrenalectomized animals showed a normal response to PMS in spite of high doses of ACTH. Aside from ACTH, there are three hormones (estrogen, progesterone, and corticosterone) possibly of adrenal origin that may have effects on maturation.

Weisz and Gunsalus (109) have questioned the origin of high levels of estrogen in the immature rat and

postulated adrenal origin because adrenalectomy considerably diminished the level. It was previously demonstrated by Ramirez and Sawyer (81) that estrogen can advance puberty onset. These results have been duplicated in our own and many other laboratories. So, it is known that estrogen participates in vaginal opening but its mode of action remains to be investigated. Since corticosterone shows its temporal pattern in the prepuberal rat, as discussed previously, the third candidate is progesterone.

It has been shown (78) that in immature rats there is a pattern of progesterone secretion that is established by 28 - 29 days of age. Further work in progesterone secretion of adrenal origin is confined to the adult animal, and so the evidence for the adrenal's participation in gonadal function in the adult will be discussed.

It has recently been demonstrated that the total plasma progesterone concentration is in large part of adrenal origin (60). Further, this adrenal progesterone can be released by ACTH (28) in the ovariectomized animal. The rise in plasma progesterone, which begins at 1 P.M. on the afternoon of proestrus, is probably of adrenal origin (28, 60), with the more marked rise at 4 P.M. of ovarian origin. Lawton (55) has shown that the stress of sham ovariectomy will advance the time of the critical period of LH release with the response

abolished by adrenalectomy. This line of evidence leads one to the conclusion that in the normal estrous cycle the adrenals act merely to "fine tune" events because the animals can function without its participation. Also, puberal events are in some manner synchronized by the adrenals.

Two questions come to mind: what is the effect of adrenalectomy on the onset of puberty in the male rat, and is there a manipulation of the time of onset of puberty that might demonstrate if the adrenal ovarian interaction period is a phenomenon related to the time of puberty onset. For, if the time of puberty onset in the female can be shifted, and the adrenal-ovarian interaction period also shifts, then this might be evidence for a direct relationship.

D. Statement of Problem

Previous investigations in our laboratory and a growing body of evidence support the contention that the adrenal gland plays a role in puberty onset in the female rat. Other studies portray adrenal secretions as operative in the normal estrous cycle. Both of these systems appear to be maturing during similar developmental periods and interacting with each other. The purpose of this research is to explore any possible adrenal effects upon the reproductive system in the male rat.

Further studies will also be done to obtain more information concerning the adrenal - ovarian interaction period.

CHAPTER II

MATERIALS AND METHODS

A. Animals and Housing

ARS/Sprague-Dawley male rats (Madison, Wisconsin) were received at 18 days of age and housed two per metal cage. The animals, originally raised under conditions of constant light, were placed under controlled lighting of 14 hours light and 10 hours dark (lights on 5 A.M. to 7 P.M.). The temperature was maintained at $24 \pm 1^{\circ} \text{C}$ and the relative humidity was maintained above 40%, although not controlled. Animals were fed Rockland/Teklad's mouse breeder diet ad libitum to minimize variations in feed steroid content (31). Animals were given tap water except for adrenalectomized animals which received 1% NaCl.

ARS/Sprague-Dawley female rats (Madison, Wisconsin) were received at 18 days of age and placed two per metal cage (Acme Metal Products), under constant illumination of fluorescent origin with an average intensity of 120 foot candles at the cage front. Animal groups were

arranged vertically in cage racks to minimize variations in floor to ceiling illumination. Control animals were housed in an adjacent room under controlled fluorescent illumination of 14 hours light and 10 hours dark (lights on 5 A.M. to 7 P.M.). Temperature in both rooms was maintained at $24 \pm 1^{\circ}$ C. Relative humidity was maintained above 40%, although not controlled. As in the male study, Rockland/Teklad's mouse breeder diet was fed to all animals ad libitum, and tap water was given to all animals except those adrenalectomized, which received 1% NaCl. All times given in this report are Central Standard Time, which was maintained throughout the year regardless of local time.

B. Identification of Puberty via Penile Smears

The presence of spermatozoa in a penile smear has been reported (26, 104) as an index of puberty onset in the male rat. In this experiment all rats were examined daily in the morning (7 A.M. - 11 A.M.) commencing at the appropriate age for the experimental design. The smear consisted of a modification of the two reported techniques (26, 104). The animal was gently removed from his cage and placed on his back. The tail was gently retracted and the tip of the glans penis was lavaged with a small amount of saline in an eyedropper. This was then placed on a glass slide and examined microscopically.

A smear with mature sperm was considered positive evidence for puberty onset; only intact sperm were acceptable.

C. Identification of Puberty by Vaginal Opening

Full canalization of the vagina with opening of the vaginal membrane and first ovulation are closely linked in the rat and frequently taken as a sign of puberty in the female (56). In these experiments, all rats were examined daily from 7 - 10 A.M. to ascertain if vaginal opening had occurred. If visual inspection revealed that vaginal opening had occurred, puberty onset was said to have taken place on that day.

D. Treatments

Laparotomies (sham-operations) or adrenalectomies were all performed under ether anesthesia. Bilateral subcostal flank incisions of approximately 2 centimeters in length were made. The adrenals were removed by careful grasping in forceps and transection of all vessels to the gland. Blood loss was minimal and no ligation of vessels was required. The sham-operation involved administration of anesthesia and a search for the adrenals without removal. In all operated animals the muscle was closed with simple interrupted silk sutures. The skin was closed with two or three metal clips. Total blood loss was usually insignificant and after

recovery from anesthesia the animals were returned to their cages.

E. Autopsy Procedures

Animals were autopsied on the day of puberty for most experiments. The animals were weighed and sacrificed under ether anesthesia. In the case of the males, left adrenal, testicle, and seminal vesicle wet weights were recorded; the entire trimmed thymus gland was also weighed. At the same time fluid was expressed onto a glass slide from the right vas deferens and examined for the presence of mature sperm.

In the case of the females, thymic and uterine wet weights were recorded, as were left adrenal and ovarian wet weights.

The mean and the standard error of the mean was computed for the data. Comparisons were made using the Student's t-test utilizing a PDP-12 computer. P values of less than .05 were considered to be significant.

F. Histology

Representative testicles and ovaries were fixed in Bouin's solution. These were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. In experiment two, care was taken to preserve the complete excurrent duct system to assess the movement of sperm as maturation was completed.

CHAPTER III

RESULTS

A. Study I: Validation of the Penile Smear as an Index of Puberty in the Male Rat

1. Experimental Design

This experiment was a repeat of that reported in the literature (104) on the golden hamster. Ten rats were received at 18 days of age. At 50 days of age, the rats were smeared daily and sacrificed at 71 days of age. Day 59 and 66 were omitted to see the effect of skipping the daily smear on subsequent smears. Organ weights were recorded for control purposes.

2. Results

The animals tolerated the smears well without signs of aversive behavior. The results of serial penile smears are presented in Table IA and B. This pattern of results agree quite closely with those obtained in the golden hamster (104). It is seen that the penile smear gives a discrete endpoint that is necessary for subsequent studies. The mean day of first positive penile smear was $53.8 \pm$

TABLE IA

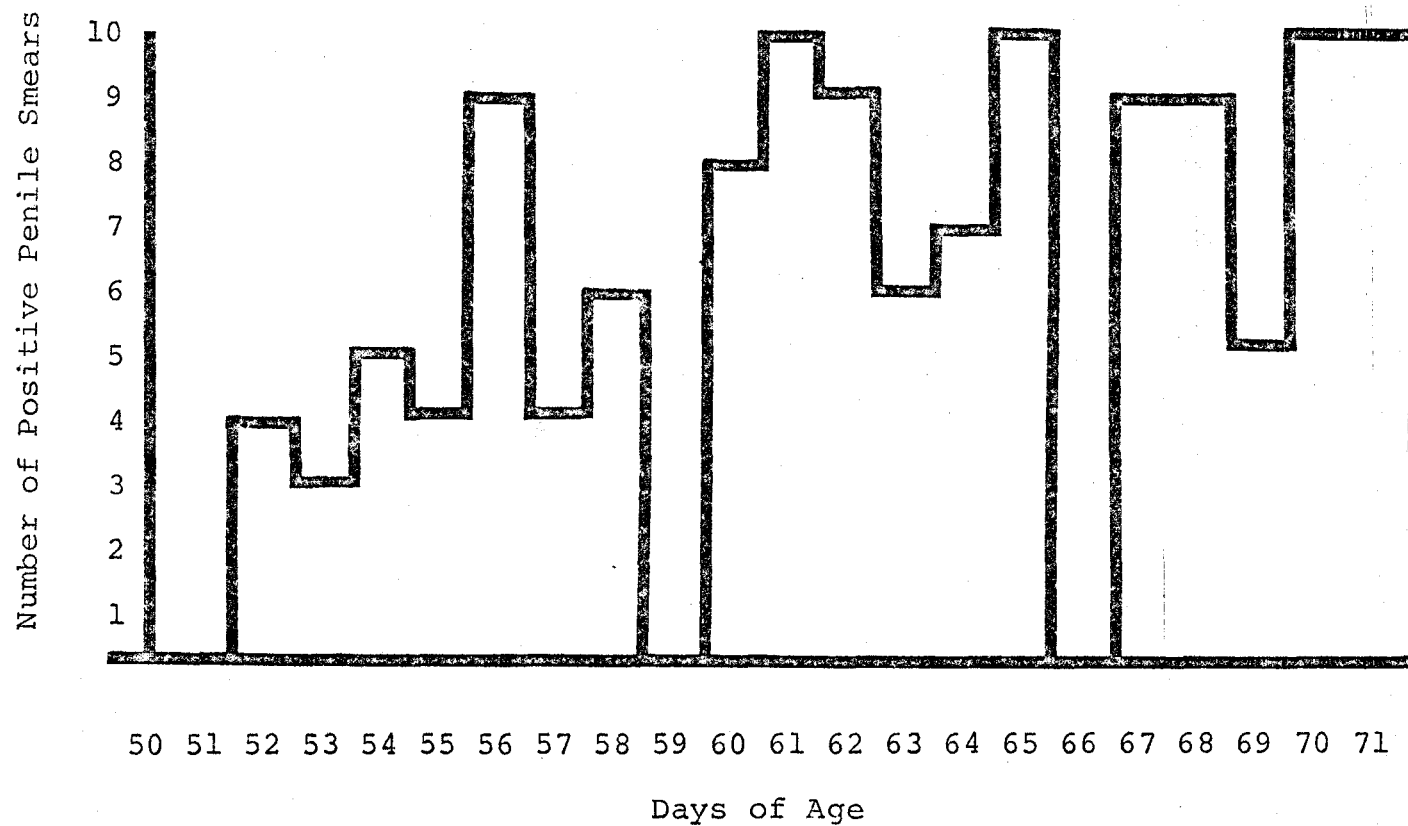
SERIAL PENILE SMEARS ON TEN ANIMALS FROM 50 TO 71 DAYS OF AGE

Animal	Age																					
	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71
1							+				+					+		+	+		+	+
2			+	+		+	+	+	+		+	+	+			+	+			+	+	+
3			+	+			+					+	+			+	+		+	+		+
4							+		+		+	+	+			+		+	+	+	+	+
5			+		+		+				+	+	+	+	+	+		+	+	+	+	+
6				+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+	+
7			+		+	+	+	+			+	+	+	+	+	+		+	+		+	+
8					+		+		+		+	+		+	+	+		+	+		+	+
9							+		+		+	+	+	+	+	+		+	+		+	+
10					+	+	+		+		+	+	+	+	+	+		+		+	+	+

+ = Sperm Present

TABLE IB

FREQUENCY HISTOGRAM OF POSITIVE PENILE SMEARS



.6733 S.E.M., which agrees quite closely with other reports of the time of puberty in the male rat (26). Any difference could easily be explained to strain variations and conditions of raising.

B. Study II: Histological Verification of the Penile Smear as an Index of Puberty

1. Experimental Design

The purpose of this experiment was to evaluate possible correlations between a positive penile smear, presence of sperm in the vas deferens and histological evidence that full spermatogenesis is occurring in the testes. This study consisted of groups of ten rats that were smeared a single time on the morning of sacrifice and autopsied. This was done at 43, 45, 47, 51, 53, and 55 days of age.

2. Results

Table II demonstrates that at 43 days of age there are no sperm present in the excurrent duct system of the male. It can be inferred from reports (13) that spermatogenesis has reached the mature stage and that approximately 2 weeks are required for migration of the sperm into the duct system. We see, also, that the penile smear does not become positive until after the animals have spermatozoa present in the vas deferens. Thus, the penile smear gives a discrete day which can be used to

TABLE II

PRESENCE OF SPERM IN VAS DEFERENS AND SMEAR IN RATS OF VARIOUS AGES

Age (Animal Number)	Number of Sperm Present	
	Vas Deferens	Penile Smear
43 D. (10)	0/10	0/10
45 D. (10)	0/10	0/10
47 D. (10)	5/10	0/10
49 D. (10)	10/10	2/10
51 D. (10)	10/10	5/10
53 D. (10)	10/10	9/10
55 D. (10)	10/10	9/10

assign the attainment of puberty.

Tables III and IV show that at this time the animals are rapidly gaining weight but that the most marked increases are occurring in the testicles and seminal vesicles. This is in accordance with the changes associated with puberty reported in the literature (44).

Histologically, the 43-day old animals showed the appearance of spermatozoa in a few isolated tubules. The 45-day old animals showed a much fuller complement of tubules in which spermatogenesis had gone to completion, and yet the head and tail of the epididymis remained without mature sperm. The 47-day old animals showed completion of spermatogenesis in most of the tubules and these testes were difficult to distinguish from adult. The 49-day old animals were similar and by 51 days of age, sperm had occupied the excurrent duct system. In the animals examined, those 55 days of age were seen to be very similar to the adult. These observations are general in nature and were intended to relate events in the testes to the appearance of sperm in the penile smear. It can be seen that sperm are recoverable from the vas deferens (Table II) at 47 days of age and yet specimens revealed very poor filling of the vas. This is thought to represent the continual nature of sperm production with small numbers of sperm reaching the vas that could

TABLE III

BODY AND ADRENAL WEIGHT OF ANIMALS AT VARIOUS AGES

Mean Weight \pm S.E.M.

Age (Animal Number)	Body Wt. (Gm.)	Adrenal Wt. (Mg.)
43 D. (10)	172 \pm 2.4	13.4 \pm .63
45 D. (10)	186 \pm 4.5	16.6 \pm .62
47 D. (10)	190 \pm 4.0	16.4 \pm .52
49 D. (10)	206 \pm 3.7	17.1 \pm .59
51 D. (10)	223 \pm 7.1	17.6 \pm .71
53 D. (10)	237 \pm 3.7	20.3 \pm 1.04
55 D. (10)	255 \pm 4.0	13.7 \pm .67

TABLE IV

TESTIS, THYMUS, AND SEMINAL VESICLE WEIGHTS OF ANIMALS AT VARIOUS AGES

Mean Weight \pm S.E.M.

Age (Animal Number)	Testis Wt. (Gm.)	Thymus Wt. (Gm.)	Seminal Vesicle (Gm.)
43 D. (10)	1.0388 \pm .0273	.5748 \pm .0231	.0765 \pm .0051
45 D. (10)	1.0843 \pm .0259	.6205 \pm .0182	.0939 \pm .0067
47 D. (10)	1.1818 \pm .0153	.6486 \pm .0150	.1130 \pm .0057
49 D. (10)	1.3013 \pm .0269	.6779 \pm .0255	.1343 \pm .0084
51 D. (10)	1.3015 \pm .0266	.6916 \pm .0305	.1701 \pm .0173
53 D. (10)	1.3774 \pm .0326	.6673 \pm .0266	.1902 \pm .0144

more easily be seen by examining the fluid in the vas than by histological study.

C. Study III: Effect of Adrenalectomy on Puberty Onset

1. Experimental Design

The animals in this study were divided into an untreated control group and ten treatment groups. The treatments consisted of laparotomy (sham control operation) or bilateral adrenalectomy. The animals were further subdivided according to what age the treatment took place. Treatments were performed at 19, 26, 33, 40, and 47 days of age. Penile smears were begun at 45 days of age and animals were autopsied on the day of the first positive penile smear.

2. Results

Table V shows the first day of positive penile smears, puberty, for all the animal groups. No significant differences existed for any of the groups for the mean day of puberty onset. In mean body weights shown in Table VI, we see a significant reduction in body weights in animals adrenalectomized at 19 days of age when compared with controls ($p < .001$). However, the fall in body weight was similar in both adrenalectomized and sham-operated groups. There is a less marked difference ($p < .025$) between the 19-day old sham-operated and control animals. In the group undergoing treatment

TABLE V

EFFECT OF ADRENALECTOMY AND LAPAROTOMY ON PUBERTY ONSET

Treatment Group	Puberty Onset ⁺		
Controls	53.1	\pm 1.02	(12)
Day 19 - Sham	51.0	\pm .28	(12)
Adrx.	51.5	\pm .56	(10)
Day 26 - Sham	51.0	\pm .61	(12)
Adrx.	51.3	\pm .39	(12)
Day 33 - Sham	52.0	\pm .44	(12)
Adrx.	52.0	\pm .63	(11)
Day 40 - Sham	52.6	\pm 1.05	(12)
Adrx.	52.0	\pm .44	(11)
Day 47 - Sham	52.7	\pm .64	(12)
Adrx.	52.3	\pm .55	(10)

⁺ = Mean Day of Age \pm S.E.M. (Animal Number)

TABLE VI

EFFECT OF TREATMENT ON BODY, TESTICULAR, SEMINAL VESICLE WEIGHTS

Treatment Group	Body Weight ⁺ (Gm.)		Left Testicle ⁺ Weight (Gm.)	Seminal Vesicle ⁺ Weight (Gm.)
Controls	227.8	± 5.4 (12)	1.3921	± .0217 .2366 ± .0179
Day 19 - Sham	209.5	± 4.5 (12)	1.3752	± .0292 .2130 ± .0142
Adrx.	192.0	± 8.8 (10)	1.2164	± .0392 .2119 ± .0263
Day 26 - Sham	225.4	± 6.5 (12)	1.3971	± .0257 .2275 ± .0158
Adrx.	191.9	± 6.6 (12)	1.2960	± .0260 .1968 ± .0107
Day 33 - Sham	218.1	± 2.4 (11)	1.4029	± .0295 .2158 ± .0172
Adrx.	209.2	± 9.8 (11)	1.3415	± .0183 .2012 ± .0111
Day 40 - Sham	232.5	± 8.8 (12)	1.4505	± .0350 .2478 ± .0213
Adrx.	215.3	± 9.5 (11)	1.3429	± .0345 .2365 ± .0186
Day 47 - Sham	227.4	± 2.9 (12)	1.3947	± .0285 .2273 ± .0120
Adrx.	213.2	± 4.9 (10)	1.3786	± .0325 .2136 ± .0388

+ = Mean ± S.E.M.

at 26 days of age, a significant difference ($p < .005$) in body weight does exist between the adrenalectomized animals and shams. There are no further significant differences in body weights among groups except in the group undergoing surgery at 46 days of age where shams differ from adrenalectomized animals ($p < .025$). The mean testicular weights for all the groups were similar when sham operations were compared to controls and adrenalectomies compared to shams. Seminal vesicle mean weights were similar except for the controls versus those adrenalectomized at 26 days of age ($p < .05$). However, no difference existed between the adrenalectomized animals and shams. Table VII shows the thymic weights; the increases in weight are generally regarded (30) to reflect a loss of adrenocortical hormones. The thymuses of animals adrenalectomized at 19, 33, 40, and 47 days of age were significantly heavier than their respective shams ($p < .05$, $p < .001$, $p < .005$, $p < .001$). Thymic weights in those adrenalectomized or sham-operated at 26 days of age were not significantly different. The animals laparotomized at 47 days of age were lighter than controls ($p < .01$) probably due to the stress of surgery close to the time of autopsy.

Histological examination of testicular tissue from animals of the various groups revealed no discernible

TABLE VII
EFFECT OF TREATMENT ON THYMIC WEIGHTS

Treatment Group	Thymus Weight (Gm.)
Controls	.7260 \pm .0387
Day 19 - Sham	.6860 \pm .0352
Adrx.	.7849 \pm .0450
Day 26 - Sham	.6810 \pm .0261
Adrx.	.7154 \pm .0251
Day 33 - Sham	.6538 \pm .0284
Adrx.	.8334 \pm .0289
Day 40 - Sham	.7028 \pm .0185
Adrx.	.8154 \pm .0281
Day 47 - Sham	.5850 \pm .0314
Adrx.	.7805 \pm .0390

+ = Mean \pm S.E.M.

differences. Greater than 90% of the tubules examined showed full spermatogenesis to be occurring, regardless of treatment.

D. Study IV: Adrenalectomy and Constant Light Exposure in Puberty Onset in the Female

1. Experimental Design

The animals in this study were divided into light/dark (LD)-exposed controls and constant light (LL)-exposed animals. The LL-exposed animals were further subdivided into control and treatment groups. The treatment groups varied according to what age the treatment took place. Animals in these groups were adrenalectomized or sham-operated at 18, 22, 23, 24, 25, and 26 days of age. Animals were inspected daily for the presence of the vaginal membrane and autopsied on the day of vaginal opening (puberty).

2. Results

Table VIII summarizes the results of the experiment. Exposure to LL advanced puberty significantly ($p < .002$) as expected. It can be seen that adrenalectomy at 18 and 22 days of age delays puberty, but that adrenalectomy on or after day 23 has no effect. Thus, the normal period when adrenalectomy should have delayed puberty has been advanced by about 3 days. The sham procedure can be seen to advance puberty when performed at 24 or 25 days of age.

TABLE VIII
EFFECT OF CONSTANT LIGHT (LL) AND TREATMENT
ON PUBERTY ONSET

		P Values		
Treatment Group (N.)		Day of Vaginal Opening	LD or Sham Controls Versus LL Controls	Adrx. Versus Shams
LD Controls	(10)	36.2 \pm .4	<.002	
LL Controls	(12)	33.4 \pm .4		
Day 18 - Sham	(10)	33.9 \pm .6	N.S.	<.0005
Adrx.	(9)	39.4 \pm 1.2	<.0001	
Day 22 - Sham	(9)	32.8 \pm .5	N.S.	<.00005
Adrx.	(9)	39.8 \pm 1.1	<.00005	
Day 23 - Sham	(10)	33.1 \pm .8	N.S.	N.S.
Adrx.	(7)	36.0 \pm 1.9	N.S.	N.S.
Day 24 - Sham	(9)	31.0 \pm .6	<.01	<.005
Adrx.	(9)	36.3 \pm 1.5	N.S.	<.005
Day 25 - Sham	(10)	30.8 \pm .5	<.005	N.S.
Adrx.	(10)	33.4 \pm 1.2	N.S.	
Day 26 - Sham	(10)	33.4 \pm .8	N.S.	N.S.
Adrx.	(9)	34.2 \pm .6	N.S.	N.S.

N.S. = Not significant; $p > 0.05$

Adrx. = Bilateral adrenalectomy

+ = Mean \pm S.E.M.

This is also advanced over the usual age range (days 26 - 30) at which the sham procedure will advance puberty.

E. Study V: Effect of Adrenalectomy and Constant Light Exposure in Puberty Onset in the Female Rat

1. Experimental Design

This experiment was a duplicate of Experiment IV with the animals under LL conditions and treatments performed at 18, 22, 23, 24, 25, and 26 days of age. Sham surgery was only performed at 26 days of age due to the lack of significant findings in the previous experiment. This experiment was thought to be necessary because of the small number of animals in some groups and also because the results were so striking in the first attempt.

2. Results

The results of Experiment V are presented in Table IX. The first thing that one sees is that puberty onset in the LD control animals is occurring later than usual; this is significant ($p < .05$) when compared to the LD control animals in the previous experiment. This was the first time in 3 - 4 years that a shift in the normal time of puberty onset was seen. When this group of animals was compared to 30 LD-control animals in two previous experiments, significant differences ($p < .005$) were noted. Thus, the experimental comparisons in Experiment V are with reference to control animals exhibiting some

TABLE IX

EFFECT OF CONSTANT LIGHT AND TREATMENT ON PUBERTY ONSET

		P Values		
Treatment Group (N.)		Day of Vaginal Opening	LD or Sham Controls Versus LL Controls	Adrx. Versus Shams
LD Controls	(12)	40.8 \pm .6	<.05	
LL Controls	(13)	38.0 \pm .9		
Day 18 - Sham	(6)	38.8 \pm .9	N.S.	N.S.
Adrx.	(6)	41.6 \pm 1.7	<.05	N.S.
Day 22 - Sham	(6)	41.6 \pm .5	<.02	N.S.
Adrx.	(8)	43.8 \pm .9	<.005	N.S.
Day 23 - Sham	(6)	41.6 \pm 1.3	<.05	N.S.
Adrx.	(8)	43.3 \pm .8	<.005	N.S.
Day 24 - Sham	(8)	38.8 \pm 1.2	N.S.	<.0005
Adrx.	(8)	44.6 \pm .2	<.0005	<.0005
Day 25 - Sham	(6)	36.1 \pm .9	N.S.	<.005
Adrx.	(5)	42.6 \pm 1.2	<.01	<.005
Day 26 - Sham	(6)	38.0 \pm 1.2	N.S.	

N.S. = Not significant; $p > 0.05$

Adrx. = Bilateral adrenalectomy

+ = Mean \pm S.E.M.

abnormal phenomenon. The LL-exposed animals showed an advancement as in the previous experiment. The adrenalectomy-induced delay period was seen when surgery was performed at 18 - 25 days of age.

CHAPTER IV

DISCUSSION

The results of Studies A and B indicate that there is excellent concordance between the attainment of puberty and a positive penile smear. Prior to 45 days of age, the testes show some spermatogenesis at completion, and this is not disturbing because it is now agreed (68) that Clermont's (18) projection of 51 days of age for the Sprague-Dawley cat's spermatogenic cycle is based upon the choice of too immature a stem cell for the beginning of spermatogenesis. Thus, 41 days is probably closer to the real value and explains why in the present study completion of spermatogenesis was seen in the 43-day old animal. The vas deferens fluid was also devoid of sperm until 47 days of age, with all animals having sperm in the vas by 49 days of age. The penile smear did not become positive until all animals demonstrated sperm in the vas deferens and did so for at least a week. It is concluded that the penile smear is an adequate test for the attainment of puberty in the male and that false

positive smears are not a factor to alter the results.

In Study C it is seen that adrenalectomy in no way alters the time course of puberty in the male rat. As was pointed out in the Literature Review, the rat has initiated spermatogenesis at or slightly after birth. Further, the spermatogenic cycle is relatively hormone-independent. Therefore, the absence of a delay is not surprising. What was not analyzed in this study, and perhaps is deserving of further scrutiny, are the subsequent events. The first wave of spermatogenesis appears not to be affected by adrenalectomy, but the question of long-term effects remains. A second point not considered was the relative cell populations. If quantitative cell counts had been performed, a difference might have been detected between the adrenalectomized and control animals. Militating against this were the testicular weights which showed no differences among groups. Seminal vesicle weights also showed no significant differences, which might indicate a change in stimulatory factors, be they androgens, prolactin, growth hormone, or other entities.

A third possibility is that a period where adrenalectomy might have been effective was not studied. In the female, the effect of adrenalectomy is seen to occur over about a seven-day period. In this study,

adrenalectomies were performed every seven days and an "effective" period may have been missed. This is, however, unlikely because the total time that sham or adrenalectomy is effective in the female is longer than a week and it would seem unlikely that the longer period of development seen in the male is coupled to a shorter adrenal - gonadal interaction period than seen in the female. Also, it has been shown (52) that adrenal hormone secretion is less consistent in the male prepuberally and that the levels are lower in the male than in the prepuberal female. This is perhaps further inferential evidence that the adrenal secretions are less important in the male than in the female. It is concluded that adrenalectomy does not affect the onset of puberty in the male as it does in the female.

If we look at the previous studies in this laboratory (30, 31), we can see both an adrenalectomy-induced delay period and a sham-induced advancement period in the female rat. This may be diagrammed as follows (Firlit, personal communication):

	Day	
	18	
	19	
First Period:	20	
Adrx.-induced delay	21	
in puberty onset and	22	
decrease in ova	23	
	24	
	25	
	26	
Adrx. decrease in ova	27	Second Period:
	28	Sham-induced advance
	29	of puberty onset
	30	
	31	
	32	
	33	
	34	
	35	Advanced puberty
	36	
	37	
	38	Normal puberty onset
	39	
	40	
	41	
	42	
	43	Delayed puberty onset
	44	

It is seen that adrenalectomy at or before 18 days of age and through 25 days of age delays puberty. Adrenalectomy after this period does not delay puberty onset, but a decrease in ova are seen. A second period has been defined in which the sham operation will advance puberty; this period extends from 26 to 30 days of age.

It is seen in Study D that these periods have been altered by the exposure to LL. The adrenalectomy-induced delay period is seen to be absent after 23 days of age. Also, the sham-induced advancement period is present at

24 and 25 days of age. It can be inferred from this experiment that these two periods are in some manner coupled to puberty. They are shifted in time by LL exposure and maintain their temporal association. It can also be inferred that the action of LL in advancing puberty is occurring at a site other than the adrenal.

In the repeat of this experiment, the control animals demonstrated a shift in the normal time of puberty onset. This was the first time that this had occurred in the course of a few years of experiments in our laboratory. A careful search was then initiated to secure the cause of this shift, because a host of factors have been shown to alter puberty onset in the female (58). The animal breeder was contacted (personal communication) and questioned as to any possible changes in procedure; this was without significant finding. The change of seasons was a factor reported by Ramaley (74) to influence puberty onset, but this was not seen in our laboratory (30). Food was constant in both experiments, as were cages, rooms, delivery times, time of surgery, and methods of handling. One factor that was not controlled was the room itself because it had remained idle for a number of months between experiments. It is concluded that an unknown factor was operative in shifting the time of puberty onset in the control animals, and its source

has not been discovered.

It was also seen that the results of the other portions of Study E were inconsistent with Study D. Adrenalectomy delayed the onset of puberty in LL-exposed rats when performed between day 18 and 25. The sham-induced advancement period was not seen, rather the sham procedure performed at 22 days of age delayed puberty onset ($p < .02$). Taken in toto, these results indicate that the rats were not achieving puberty as in the previous experiment. Ramaley (personal communication) has recently duplicated the results of Study D in her laboratory and this indicates that Study E represents an abnormal, and as yet unexplained, situation. It is concluded that some factor was operative in Study E that invalidates any firm statement concerning results.

It was noted that LL exposure shifts the adrenal - ovarian interaction period and that this indicates a mechanism of the action of LL other than the adrenal gland. Fiske (33) observed that exposure to LL for long periods resulted in abnormally small pineal glands, suggesting a role of the pineal in the onset of puberty. Wurtman (111) has shown that the ovaries of LL-maintained animals were equivalent to those of constant dark, pinealectomized animals. In the male, enucleation of the eyes leads to a retardation of testicular and secondary sex organ

growth, the effects being more pronounced in the case of the secondary sex organs (82, 83). Removal of the pineal or section of the superior cervical ganglion negates the effect of darkness (84). Therefore, the pineal gland seems to mediate the response of the reproductive system to light and is separate from the adrenal - ovarian interaction.

CHAPTER V

SUMMARY

Substantial evidence has accumulated to indicate a functional relationship between the adrenal glands and gonads. Recent evidence in our own laboratory has shown an adrenal - ovarian interaction period in the female rat. The purpose of the present studies was two-fold: to see if such a period existed in the male rat, and to ascertain if the interaction period of the female rat was subject to manipulation.

Two studies were conducted to verify the use of the penile smear as an index of puberty in the male rat. In a third study, male rats were prepuberally adrenalectomized and then autopsied on the day of the first positive penile smear, i.e. puberty. Results indicated no effect on the time of puberty onset among control, sham-operated or adrenalectomized animals. Accessory sex organ weights were not significantly different among animal groups and histological examination of gonadal tissue revealed no discernible differences.

Constant light exposure has been shown to advance the time of puberty onset in the female rat. This (constant light) was used to advance the time of puberty onset in the female rat and study its effect on the adrenal - ovarian interaction period. Results in the first study demonstrated an advancement in the adrenal - ovarian interaction period with constant light exposure. The sham operation also was shown to effect puberty onset in the female rat.

From these studies it is concluded:

1. The penile smear is a fast, non-invasive procedure to assess puberty onset in the male rat.
2. Prepuberal adrenalectomy does not alter the time of puberty onset in the male rat.
3. Constant light exposure advances puberty onset in the female rat.
4. Prepuberal adrenalectomy and constant light exposure alter the adrenal - ovarian interaction period in the female rat.

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

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