EFFECTS OF HYPO- AND HYPERTHYROIDISM ON THE REPRODUCTIVE SYSTEM OF THE FEMALE RAT

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ABSTRACT

EFFECTS OF HYPO- AND HYPERTHYROIDISM ON THE REPRODUCTIVE SYSTEM OF THE FEMALE RAT

By

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The effects of hypothyroidism and hyperthyroidism on the female rat reproductive system were studied in three separate experiments.

1. The purpose of Experiment 1 was to determine the effects of early hypo- and hyperthyroidism induced by feeding propylthiouracil (PTU) or injecting thyroxine (T_4) on body growth, age of onset of puberty, regularity of the estrous cycle, and pituitary prolactin content. PTU treatment, begun prior to and continuing after birth, resulted in dwarfed, sexually immature animals with relatively unstimulated ovaries and uteri. Puberty was not reached in the majority of these rats. In comparison, control untreated rats reached puberty at 36.7 $\pm$ 0.6 days of age, and thyroxine-treated animals at 44.4 $\pm$ 1.2 days of age. Precocious puberty (31.7 $\pm$ 1.0 days) resulted when goitrogen treatment was withdrawn at 21 days of age. Anterior pituitary prolactin content was significantly decreased in the severely hypothyroid rats.

2. The purpose of Experiment 2 was to determine the effects of early hypo- and hyperthyroidism on anterior pituitary follicle stimulating hormone (FSH) and lutenizing hormone (LH) content and on the hypothalamic content of FSH-RF. Also, thyroxine treatment was withdrawn in a second group of rats after 21 days to determine the effects of early treatment on the onset of puberty. PTU treatment begun prior to and continuing after birth resulted in decreased pituitary content of FSH and LH, while the content of both hormones increased greatly in the hyperthyroid rats.

Hypothalamic FSH-RF content did not vary significantly between groups. Puberty was found to be significantly delayed after discontinuing thyroxine treatment.

3. The third experiment was designed to determine whether or not a critical period exists after which time thyroxine and goitrogen treatments have no effect on the onset of puberty. Rats treated with thyroxine from the 21st day of life until puberty showed no significant delay of vaginal opening. Hypothyroidism also had no effect on vaginal opening.

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INTRODUCTION

The thyroid gland of all vertebrates secretes thyroid hormones into the circulation. In man and in higher animals, these substances have a profound influence on the development of the body. Although much is known about the biochemistry and biological effects of the principle thyroid hormones, L-thyroxine (t_4) and 3,5,3'-L-triiodothyronine (t_3), little work has appeared on their effects on the gonadotropins of the pituitary and on reproduction in general.

In humans, it is known that decreased thyroid activity or hypothyroidism from infancy leads to sexual immaturity and infertility. Hypothyroidism in the adult woman is commonly associated with loss of libido, failure to ovulate and irregular menstrual patterns.

Hyperthyroidism, or increased thyroid hormone in the circulation, also causes abnormal sexual development. Female patients with this condition, (thyrotoxicosis), often have disturbed menstrual cycles which in severe cases cease altogether. When ovulation does occur, fertility is low and abortion is common. It has also been reported that puberty is delayed in some of these patients. It is a reasonable working hypothesis to assume that abnormalities in sexual development of human subjects with thyroid disorders are directly related to altered secretion of pituitary gonadotropins, although direct effects of the thyroid on the gonads are also possible. While some anomalies remain, the changes in sexual development observed in the rats in these studies generally support this contention.

Although there have been many investigations on the effects of abnormal thyroid secretion on the reproductive system, there is little in the literature to explain the mechanisms by which thyroid hormones influence the gonads. The studies described here were designed to further elucidate the mechanism of thyroid action at the pituitary and hypothalamic levels. The pituitary content of gonadotropins and of prolactin in the female rat were measured under normal, hyperthyroid and hypothyroid conditions. The hypothalamic content of follicle stimulating hormone releasing factor (FSH-RF) was also measured, and ovarian and uterine weights were recorded. The pattern of the estrous cycles for each rat and the age of onset of puberty were recorded.

REVIEW OF THE LITERATURE

General Effects of Thyroid Hormones

In warm-blooded animals thyroid hormones accelerate energy production and processes related to increased metabolism in most normal tissues (Lardy, 1955). Without thyroid hormones, protein synthesis is decreased and profound shifts in protein stores occur leaving such organs as the liver and kidneys small and protein deficient (Pitt-Rivers and Tata, 1959).

The effects of thyroid hormones on carbohydrate metabolism are modified by other hormones, such as insulin and epinephrine, and are dependent upon the levels of thyroid hormones present. Small doses of T_4 stimulate glycogen synthesis while large doses cause hepatic glycogen depletion (Wolff and Wolff, 1964). In general, thyroid hormones tend to increase the blood concentration of glucose by increasing intestinal absorption of glucose, by depleting liver glycogen and by increasing insulin degradation (see Hoch, 1962). This is somewhat offset by the increased energy and oxygen requirements that T_4 is known to invoke.

Thyroid hormones also influence lipid metabolism. T_4 stimulates lipid synthesis, mobilization and degradation although the predominant effect of high levels of T_4 is catabolic. In hypothyroidism an increase in blood cholesterol and phospholipids is generally found (Kritchevsky, 1964).

The metabolism and excretion rate of hormones are also influenced by the thyroid hormones, although this has not been thoroughly investigated. In hypothyroidism, androgen secretion has been found to decrease and

testosterone and dehydroepiandrosterone have been reported to be transformed into etiocholanolone rather than androsterone (Gallagher et al., 1960). Thyroid treatment also increases the excretion of androsterone in the male, and patients with untreated myxedema excrete decreased amounts of androsterone (Campbell et al., 1965). Fishman et al., (1965) found that hypothyroidism also alters the metabolism of estradiol, converting it into estriol more often than into 2-hydroxyestrone. The same authors reported that hyperthyroidism causes a decrease in the conversion of estradiol to estriol and an increase in the 2-methoxyestrone fraction (Fishman et al., 1962).

Thyroid Abnormalities.

The absence of thyroid hormones during early post-natal life causes a child to become a cretin. When left untreated, such a child developes into a short, dwarf-like person with definite and irreversible mental retardation. Thyroxine replacement therapy is effective in reversing this condition only when administered at or near birth (Ingbar and Woeber, 1968).

The severe shortage of thyroid hormones affects the development of nearly every system in the cretin: growth and maturation are retarded, the rate of metabolism is decreased, there is reduced activity in the alimentary and renal systems, blood flow and blood volume are decreased, muscle contraction and relaxation time is slowed and the reproductive system functions poorly (Ingbar and Woeber, 1968). Cretins rarely exhibit normal sexual development, and in the case of the female, rarely have normal menstrual cycles or successful pregnancies (Steinbeck, 1963).

Some of the symptoms of hypothyroidism may be caused by changes in other endocrine glands such as the ovaries or the pituitary. Lack of thyroid hormones is thought to depress secretion of adrenocorticotropic

hormone (ACTH), growth hormone (GH), prolactin (LTH), and the gonadotropins (LH and FSH) of the pituitary, while thyroxine is thought to stimulate secretion of these hormones (Eartly and Leblond, 1956; Nicoll and Meites, 1963; Rall et al., 1964). However, a lack of thyroid hormones does not decrease anterior pituitary metabolism (Reichlin, 1966).

When hypothyroidism occurs in humans after infancy, it is referred to as myxedema. Physical lethargy and mental dullness mark this condition, although in severe cases most of the symptoms of cretinism are present. Because the disease does not occur during the critical period of early development the symptoms are reversible with proper hormone replacement therapy (Ingbar and Woeber, 1968).

Hyperthyroidism produces rapid blood flow, increased metabolism and increased nervous responses. This condition does not lead to increased strength and enhanced ability to function, however, and weakness and fatigue are common symptoms (Ingbar and Woeber, 1968).

Hyperthyroidism is also known to affect the reproductive system. In early life hyperthyroidism may cause delayed puberty and in later life may cause abnormal menstrual cycles, decreased fertility and increased risk of abortion in women (see Steinbeck, 1963).

Mechanism of Action of Thyroid Hormones

Cretinism and thyrotoxicosis have been mimicked in laboratory animals and in tissue culture in an effort to elucidate the way in which thyroid hormones act. Such mechanisms are studied on two levels: 1) the organ or system level, and 2) the cellular or biochemical level.

Little is known about the true action of thyroid hormones at the cellular level. Following administration of moderate doses of T_4, changes in mitochondrial structures have been seen (Wolf and Wolf, 1964), and enhanced protein synthesis has been observed for T_4 stimulated mitochondria

(see Tata, 1964). Recently it was discovered that nuclear RNA turnover rate increases upon T_4 stimulation as does DNA dependent RNA polymerase. All classes of RNAs are apparently effected by this stimulation (Tata, 1970). During the last decade the hypothesis has been generated that the ubiquitous constituent of plasma membrane, adenyl cyclase, is directly involved in the action of hormones (Robison, 1968) and that cyclic 3' -5' AMP mimics the effects of hormones on their target cells. It has been shown that hormones can act on tissues to increase the levels of 3'5'-AMP. These hormones include the catecholamines, glucagon, ACTH, vasopression, LH, and TSH (thyroid stimulating hormone) (Sutherland, 1965). It is thought that hormones such as the steroids and T_4 may relay their messages by similar systems (Sutherland, 1965). The effects of thyroid hormone on the brain, the pituitary and the gonads will be reviewed.

Thyroid Influence on Brain Development

In the rat, a species whose brain is poorly developed at birth, there appears to be a critical period before 15 days postpartum when the presence of thyroid hormones is necessary for normal cerebral maturation (Campbell, 1965). If thyroid hormones are absent at this time, the capacity to learn is retarded (Eayrs, 1961), and the age at which innate responses can first be elicited is increased (Eayrs, 1964b). During the past twenty years the etiology of thyroid-related brain disturbances has been studied. It has been found that there is a decrease in cell processes and impairments in the axonal networks of the cortex, reducing the probability of axo-dendritic interaction in the hypothyroid rat (Uttley, 1955). Hypothyroidism has also been found to alter the shape of the endocranium and to cause abnormal brain vascular pattern, increasing the probability of brain damage (Eayrs, 1954).

An excess of thyroid hormone has been found to cause hyperplasia of

the cerebellar folia and a hastened regression of the fetal cortex in young rats (Tusques, 1956).

Thyroid hormones also act on various enzyme systems in brain tissue. Hamburgh and Flexner (1957), demonstrated that the development of cerebral cortical succinic dehydrogenase was irreversibly impaired in animals made hypothyroid before the 15th day of age. Others have reported decreased gamma-amino butyric acid dehydrogenase activity in the cerebrum of thyroidectomized rats (Argiz et al., 1967). According to Rall et al., 1964, thyroid hormones probably act mainly as chelating agents, binding metals that normally inhibit enzymes such as the dehydrogenases.

Control of the Reproductive System

The reproductive system in mammals has long been represented by the 'negative feed-back' or ' servo' hypothesis first applied to the pituitary-thyroid interrelationship (Hoskins, 1949). The gonads and the pituitary were thought to secrete hormones that effected each other, achieving a balance within which reproduction could occur. It is now understood that the hypothalamus, located in the brain above the pituitary, secretes small descrete polypeptides known as 'releasing' or 'inhibiting' factors which directly influence the pituitary, and hence reproduction.

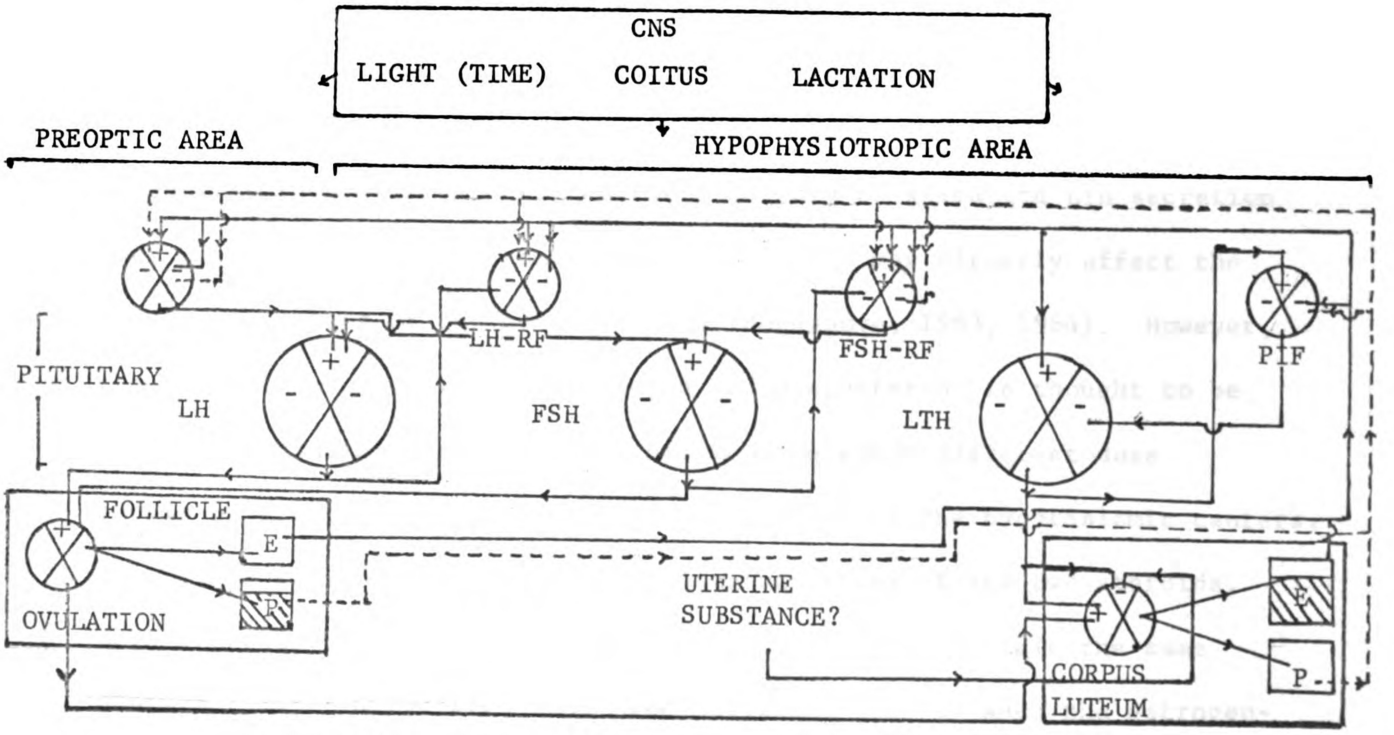
The hypothalamic factors related to reproduction are follicle stimulating hormone releasing factor (FSH-RF) and luteinizing hormone releasing factor (LH-RF). Prolactin inhibiting factor (PIF) may also be concerned with reproduction.

The pituitary hormones related to reproduction are known as FSH and LH in the female. Prolactin or lactogenic hormone (LTH) is concerned with the maintenance of corpora lutea in animals such as rats and mice and with milk production in all mammals.

It is known that hormones from the hypothalamus stimulate (in the

case of PIF, inhibit) the anterior pituitary which in turn produces hormones that stimulate the gonads (and the mammary glands). Until recently, the gonads were thought to produce steroids that selectively inhibited or stimulated pituitary production of one or both gonadotropins (FSH or LH) (see Greep, 1961). It is now known that the control of mammalian reproduction is much more complex.

The following hypothetical model depicts the control of reproduction in the adult female rat as it is now understood (see Schwartz, 1967; Schwartz and Hoffman, 1967; McCann et al., 1967; Motta et al., 1969; Flerko, 1966; and Davidson, 1969).



A MODEL OF THE CONTROL OF REPRODUCTION IN THE FEMALE RAT

E = estrogen
P = progestrone
LH = luteinizing hormone
FSH = follicle stimulating hormone
LTH = prolactin, lactogenic hormone
-RF = releasing factors
PIF = prolactin inhibiting factor

In the preceding model the hypothalamus is divided into two areas; an anterior area, the preoptic area, and the hypophysiotropic area including the median eminence (ME). The preoptic area controls the dynamic flow of pituitary LH and FSH, allowing a large discharge of these hormones to occur which precipitates ovulation in the mature female rat (see Flerko, 1966). In the ME region the production and release of LHRF and FSHRF ensure a static level of pituitary stimulation (Flerko, 1966). Ablation of this area (Szentagothai and Halasz, 1964) or removal of the pituitary from beneath the ME (Nikitovitch-Winer and Everett, 1957) depresses the production of LH and FSH, causing the ovaries to atrophy. Pituitary production of prolactin, however, is not inhibited when pituitary-hypothalamic connections are severed (Everett, 1956).

Prior to ovulation, LH and especially FSH stimulate the ovaries to produce estrogen and some progesterone (see Schwartz, 1967). Estrogen appears to feed back directly on the pituitary to stimulate LTH secretion (see Meites, 1959; Nicoll and Meites, 1962) and may directly affect the pituitary content of the gonadotropins (Bogdanove, 1963, 1964). However, the major site of action for estrogen and progesterone is thought to be at the hypothalamic level. It is not known whether different dose levels of estrogen and progesterone per se control the hypothalamic centers, or whether it is the effect of the changing ratios of the two steroids. Both steriods have been reported to stimulate and to inhibit the same centers (Sawyer & Everett, 1959; see Davidson, 1969). When the estrogen-progesterone ratio increases to a certain level the hypothalamic centers appear to release a surge of LHRF which triggers ovulation and causes the development of corpora lutea from the ovulation site (see Schwartz, 1967). If mating has occurred and plasma LTH is relatively high (Amenomori et al., 1970), the corpora lutea are maintained and systemic

progesterone levels increase (see Everett, 1961). It is thought that LH (see Rothchild, 1965) or in some species a uterine substance (Nalbandov, 1964) may limit the life of the corpora lutea.

Other central nervous system inputs influence the reproductive cycle. Schwartz and Bartosik (1962) have shown that there is an internal regulator governing the ovulatory surge of LH and it has been shown that the length of daylight sets this internal clock (Everett and Sawyer, 1950). Lactation and suckling also influence the release of LH, FSH and prolactin via the central nervous system (see Meites, 1966).

Recent experiments have further complicated the story. Corbin, (1966) discovered that there was short feedback effect of FSH on its own releasing factor. Later Fraschini et al., (1968) confirmed this. Corbin, 1966 showed that implantation of small amounts of LH into the ME region of the hypothalamus selectively decreased pituitary and plasma LH. MacLeod, (1966) showed, via disk electrophoresis,that prolactin secreting pituitary tumors suppressed endogenous pituitary prolactin and Chen et al., (1967) independently observed this using the pigeon crop method for LTH determination. The latter also reported an increase in hypothalamic PIF. These results were confirmed in the same laboratory by different techniques (Clemens and Meites, 1968; Welsch et al., 1968).

Superimposed upon this basic model are the other endocrine glands concerned with reproduction. The thyroid will be reviewed here.

Interactions of the Thyroid and the Reproductive System

The interactions between the thyroid and the reproductive system have been studied in various experimental animals. Soliman and Reineke, (1954), reported that the female rat thyroid gland fluctuated in its iodine uptake in accordance with the estrous cycle, collecting the maximum iodine during estrus and reaching a minimum at proestrus. The

same authors found that endogenous estrogen treatment had similar effects on the thyroid while progestrone antagonized the stimulatory effects of estrogen (Soliman and Reineke, 1955). Others have found similar effects of estrogen on radioactive iodine uptake in the rat (Feldman, 1956; Brown-Grant, 1962). Iodine release rates appear unaffected in the rat, however, (Brown-Grant, 1962). A review of the literature (see Feldman, 1956; Florsheim, 1958) reveals extensive work on this subject with many conflicting results, partly explained by the variety of dose levels of estrogen used and the degree of inanition resulting.

The effects of estrogen on the thyroid may be direct (Feldman, 1956; Florsheim, 1958) although Soliman and Reineke (1955) found no stimulatory effect of estrogen or progesterone on the thyroids of hypophysectomized rats. More recently, the pituitary and higher centers have been implicated. Brown-Grant (1963) has shown that the administration of nembutal on the day of proestrus blocked the ovulatory surge of LH and suppressed the increase in thyroid gland activity usually seen at estrus. It has been suggested by the same author that there may be a spread of impulses from the LHRF center to the TRF (thyroid stimulating hormone releasing factor) center and that this is blocked by nembutal. Yamada et al., (1966) has also reported that the pituitary is involved in the thyroidal response to estrogen. Thyroid weight gains usually seen with estrogen stimulation are not seen in similarly treated hypophysectomized rats and only slight iodine uptake is noted in these animals.

In humans, estrogen stimulation has been found to increase the thyroxine-binding proteins (Ingbar and Freinkel, 1960) and the level of PBI (Engbring and Engstrom, 1959) while leaving total thyroid function unaltered (Dowling et al., 1959).

It has long been known that the thyroid also influences the

reproductive system. In humans, thyroid therapy is one of the most effective means of correcting menstrual abnormalities (Foster and Thornton, 1939). In 1921, Evans and Long reported that thyroidectomy caused a pause in the estrus cycles of rats. Others have found that either thyroidectomy or goitrogen administration causes irregularities of the cycle in many experimental animals (see Reineke and Soliman, 1953). Thyroid feeding has been reported to delay impregnation in rats (Gudernatsch, 1915) and to suppress ovulation when fed in large quantities (Evans and Long, 1921).

In acute hypothyroidism, the ovarian picture in the rat is one of follicular growth (see Reineke and Soliman, 1953), and in the human with myxedema, ovarian cyst formation is often found (Leathem, 1959). In patients with myxedema due to destruction of the thyroid, precocious menstruation accompanied by breast development and galactorrhea have been reported (Nalbandov, 1963).

In acute hyperthyroidism the luteal cells predominate. Weichert and Boyd (1933) reported that rats fed thyroid powder appeared pseudo-pregnant. Long term treatment with thyroid powder, however, has been reported to inhibit the normal growth and maturation of the ovaries (Ershoff, 1948).

There has been some investigation on the mechanism of action of thyroid hormones on reproduction. Fluhmann (1934) found that thyrotropic hormone (TSH) contamination decreased the stimulatory effects of pituitary gonadotropin on ovarian weight in the rat. In 1936, Leonard reported that thyroidectomy augmented the ovarian and uterine response to FSH. Johnson and Meites (1950) showed that hyperthyroidism in rats decreased the ovarian response to pregnant mares' serum, while short term treatment with the goitrogen, thiouracil, augmented the response. Chronic administration of the goitrogen, however, reduced the ovarian

response was stimulated by hyperthyroidism in mice, suggesting that this species reacted differently from the rat and secreted less than an optimal amount of thyroid hormone for maximal response of the ovaries to gonadotropin administration.

Severinghaus (1937) reported that hypothyroidism caused a decrease in pituitary acidophil granulation, although there has been disagreement as to whether the growth hormone (GH) content of such pituitaries decreased. Schooley et al., (1966) pointed out that this discrepancy may be due to the synergistic stimulation of the assay animals by contaminating TSH. McQueen-Williams (1935) and Meites and Turner (1947) have shown that pituitary prolactin (also produced by acidophils) is decreased in hypothyroidism. Further, Nicoll and Meites (1962) showed that thyroid hormones accelerate pituitary prolactin release in vitro. Macleod (1965) found that thyroid hormones stimulate the growth of the pituitary tumor M_tTW_5, a predominantly acidophilic tissue which secretes both prolactin and growth hormone.

Contopoulos et al. (1958) did a series of experiments on gonadectomized and thyroidectomized male rats. It was found that thyroidectomy alone increases plasma TSH and decreases plasma GH. The anterior pituitaries from these animals were found to be deficient in FSH, ICSH (interstitial cell stimulating hormone), GH and TSH. Gonadectomy superimposed upon thyroidectomy reinstated the low levels of plasma and pituitary gonadotropins, showing that the pituitary of thyroidectomized animals is capable of responding to a decrease in steroid feedback. Measurements were not specific for ICSH and FSH and were accomplished by the method of ovarian and uterine weight response in hypophysectomized, immature female rats.

There is general agreement that experimental hyperthyroidism causes

an increase in the size and number of pituitary basophils (Kojima, 1917; Halmi, 1952 and Severinghaus, 1937). Several investigators have attempted to correlate a pituitary change in gonadotropin content with hyperthyroidism. In 1930, Evans and Simpson thyroidectomized female rats and after five weeks assayed the pituitaries for total gonadotropin. The pituitaries from the hypothyroid animals were compared with normal pituitaries and with pituitaries from a third group of animals fed thyroid powder for five weeks for their relative ability to advance sexual maturity in immature host rats. It was found that hypothyroidism decreased the potential of the pituitaries, and hyperthyroidism stimulated it. Smith and Engle (1930) however, reported no change in anterior pituitary gonadotropin content when female rats were thyroidectomized. In 1931, Van Horn observed that pituitaries taken from female rats fed large doses of thyroid powder and then implanted into immature female rats stimulated the gonads of the host more than did pituitary grafts taken from untreated animals. However, direct measurements of pituitary LH and FSH after thyroid hormone manipulation have not been recorded.

EXPERIMENT I

Effects of Early Hypo- and Hyperthyroidism on Reproductive Functions

The purpose of this experiment was to determine the effects of early hypothyroidism and hyperthyroidism on the reproductive system in the female rat. The hypothyroid animals were compared with euthyroid and hyperthyroid animals for age of puberty, regularity of the estrous cycle and body weight. Organ weights and pituitary prolactin content were measured in the hypothyroid and control rats.

Mature male and female rats of the Spraque-Dawley strain (Spartan Animal Farms, Haslett, Michigan) were housed in a constant temperature room (25 ±C) with automatically controlled lighting (14 hours light,10 hours dark), and were given food and water ad lib. One male and five females were placed in each of six cages and allowed to breed. The females were checked daily for vaginal plugs and for the appearance of sperm in the vaginal smears at the time of estrus, as an indication of insemination. Estrous cycles were followed until a definite diestrous pattern was noted, indicating pregnancy, at which time each female was placed in a separate cage with adequate nest-building material. These cages were not kept in temperature-controlled animal rooms and the room temperatures fluctuated greatly. On the fifteenth day of pregnancy the females were separated into three groups and treated as follows:

Group 1. control rats. 8 animals, 4 injected with saline, 4 not injected.

Group 2. hypothyroid rats. 15 animals, all fed 0.1% PTU (propylthiouracil) in feed.

Group 3. hyperthyroid rats. 7 animals, all injected with 10 μg L-T_4 (thyroxine) per 100 gm body weight.

At parturition all the male young were discarded. The young females of mothers in the control group were counted, marked, weighed when necessary and allowed to remain with their mothers until 21 days of age. Half of the young were injected with 0.1 ml saline daily.

All the young of the hypothyroid mothers received the goitrogen (PTU) from the milk of their mothers who were fed 0.1% PTU for 21 days after parturition. Fifteen young rats were injected with 5 mg PTU/day for 20 days. However, all of the hypothyroid young appeared similarly affected regardless of the route of PTU administration, and were combined at 20 days of age. On the 21st day of life, 10 of the hypothyroid young were placed in separate cages and fed a regular diet of Wayne Lab Blox. These animals were not treated with PTU further. The remaining animals were placed in new cages (approximately 10 rats per cage) and continued on a diet of 0.1% PTU in ground Wayne Lab Blox. Those young that were severely hypothyroid were allowed to remain with their mothers for several additional weeks. All hypothyroid young that continued to be treated were kept in cages with nesting materials to conserve body heat.

The nursing mothers in the hyperthyroid group were injected daily with 10 μg T_4 per 100 gm body weight until weaning. The pups of the hyperthyroid mothers were allowed to suckle until their 21st day of life, at which time they were placed in new cages. A dose of 10 μg T_4/100 gm body weight was injected subcutaneously in the pups from day 15 until sacrifice.

The young from all groups were weighed and checked for vaginal opening and the stage of the estrous cycle. At approximately 60 days of age, the animals were sacrificed and the pituitaries, thyroids, ovaries, and uteri of 17 hypothyroid and 11 euthyroid (control) rats were dissected out and weighed. The pituitaries were quickly weighed, frozen and stored

for prolactin assay. Body weight and length were also measured.

Prolactin assay

Prolactin content in the control and hypothyroid pituitaries was assayed in six to eight week old White King squabs by the intradermal pigeon crop sac method of Lyons, (1937). A direct comparison was made between samples by injecting the control sample over one side of the pigeon crop sac and the experimental sample over the opposite side. The prolactin responses in each bird were rated by the method of Reece and Turner (1937) and were converted into IU from a standard dose-response curve established with NIH-B1 prolactin in pigeons of similar age and breed. The results of the assays were analyzed by the 't'-test for paired experiments.

Results

The female rats that were bred became pregnant within two weeks of each other. In the control group, 6 females littered a total of 25 female pups. In the hypothyroid group, 12 mother rats gave birth to 57 females, 27 of which died during the course of the experiment. The severity of the hypothyroid condition varied greatly from animal to animal but was independent of the route of PTU administration. In the hyperthyroid group, only 3 female rats gave birth to live young. A total of 12 female pups was obtained from these animals.

Body weights were measured daily in all groups of young rats prior to, during and after puberty. These data are shown in Figure 1. Both T_4 and PTU treatments decreased body weight. The T_4-treated animals did not increase in weight as linearly as did the controls, but they did not differ from the control animals greatly in their overall weight. Continuous PTU treatment significantly retarded growth, and so severely dwarfed the animals that their body weights averaged only 55 grams at

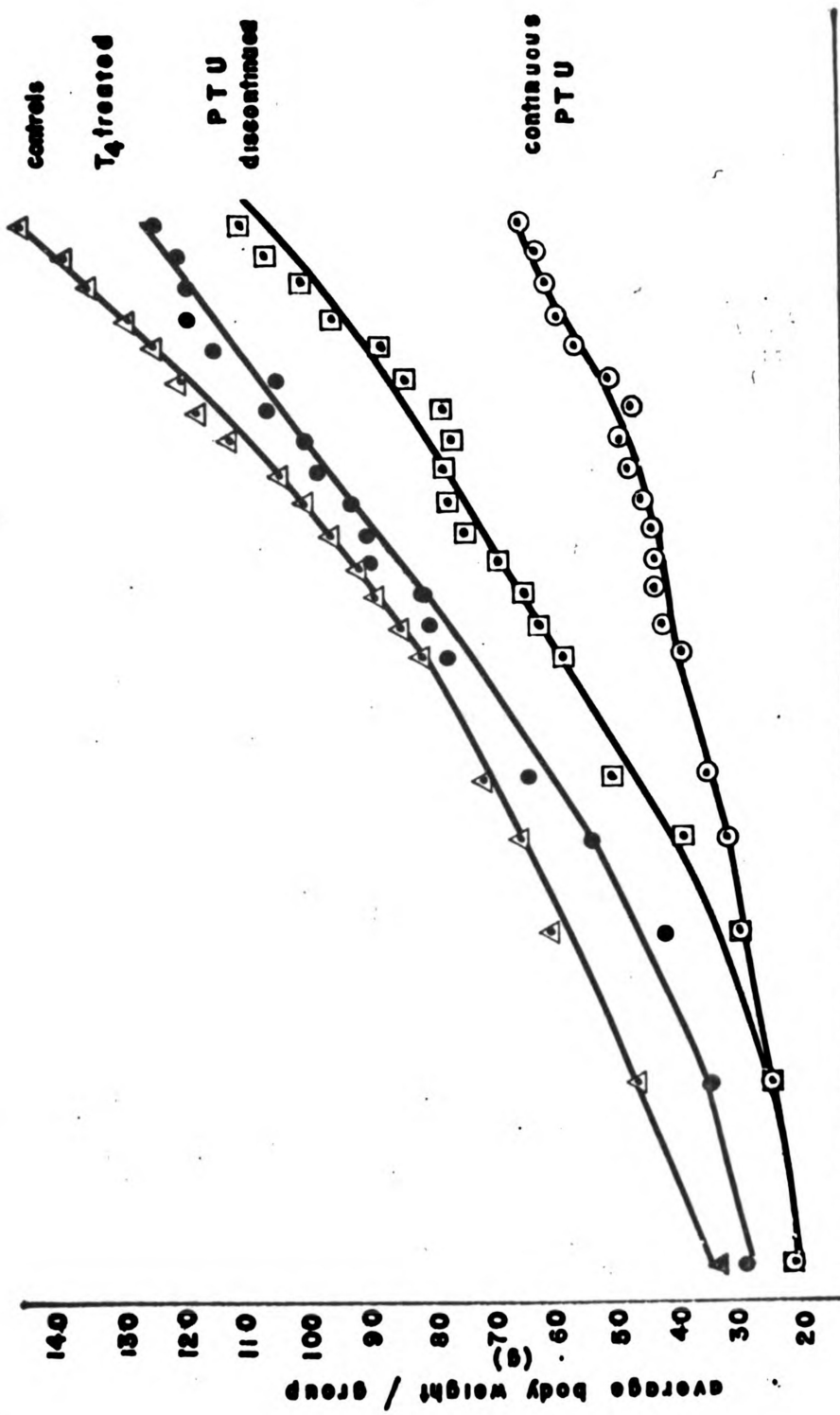


FIGURE I

BODY WEIGHTS OF CONTROL, HYPERTHYROID AND HYPOTHYROID RATS

forty days of age. The young animals whose PTU treatment was terminated after 21 days gained weight rapidly. However, they never became as heavy as either the control rats or the T_4-treated animals.

The age at which puberty was attained was recorded as the day of vaginal opening. A vaginal smear of each animal was taken at that time. All of the animals showed either cornified cells or a mixture of cornified cells and leukocytes, confirming the presence of estrogen. The average ages of vaginal opening for all groups are shown in Table 1. The average age of vaginal opening in the control group was 36.7 $\pm$ .6 days while the range was from 34 to 41 days. Puberty was first observed in the T_4-treated animals at 39 days and seen as late as 51 days with a mean of 44.4 $\pm$ 1.2 days of age. These animals were slightly heavier than the controls at puberty. Continuous PTU treatment retarded body growth and caused almost complete cessation of puberty. Several such animals showed no signs of vaginal opening after 100 days of age. The ten animals from the hypothyroid group taken off PTU feedings after 21 days of age showed precocious signs of puberty. The first animal to exhibit vaginal opening in this group was 27 days old; the average age of puberty for this group was 31.7 $\pm$ 1.0 days. The size and body weights of these animals were significantly below those of the control rats at puberty.

For one month beginning on the first day of vaginal opening, vaginal smears were taken and a record of the estrous cycle was kept. In Figure 2, three representative cycles from each group are shown. The peaks represent the time of estrus (E) when the smear showed only cornified cells, and the lowest levels represent diestrus (D) when the majority of the cells were leukocytes. In the control animals approximately 48% of the cycles are represented by estrus-proestrus, and neither the T_4-treated nor the animals with discontinued PTU-treatment differed from this

TABLE 1

AGE OF VAGINAL OPENING IN CONTROL,
HYPERTHYROID AND HYPOTHYROID RATS

Treatment	No. of rats per group	Body weight at vag. opening	Age at vag. opening
Controls	18	110.2	36.7 $\pm$ 0.61
Thyroxine treated *1	12	121.0	44.4 $\pm$ 1.20
Continuous PTU *2	20	-	>50 *3
PTU discontinued (at 21 days of age)	10	64.0	31.7 $\pm$ 1.0

*1. 10.0 μg T_4 injected/day after 15 days of age. Mothers of these rats treated with 10 μg/100gm body weight/day from 15th day of gestation until weaning at 21 days.

*2. 0.1% PTU placed in feed

*3. In several animals vaginas were not open at 100 days of age.

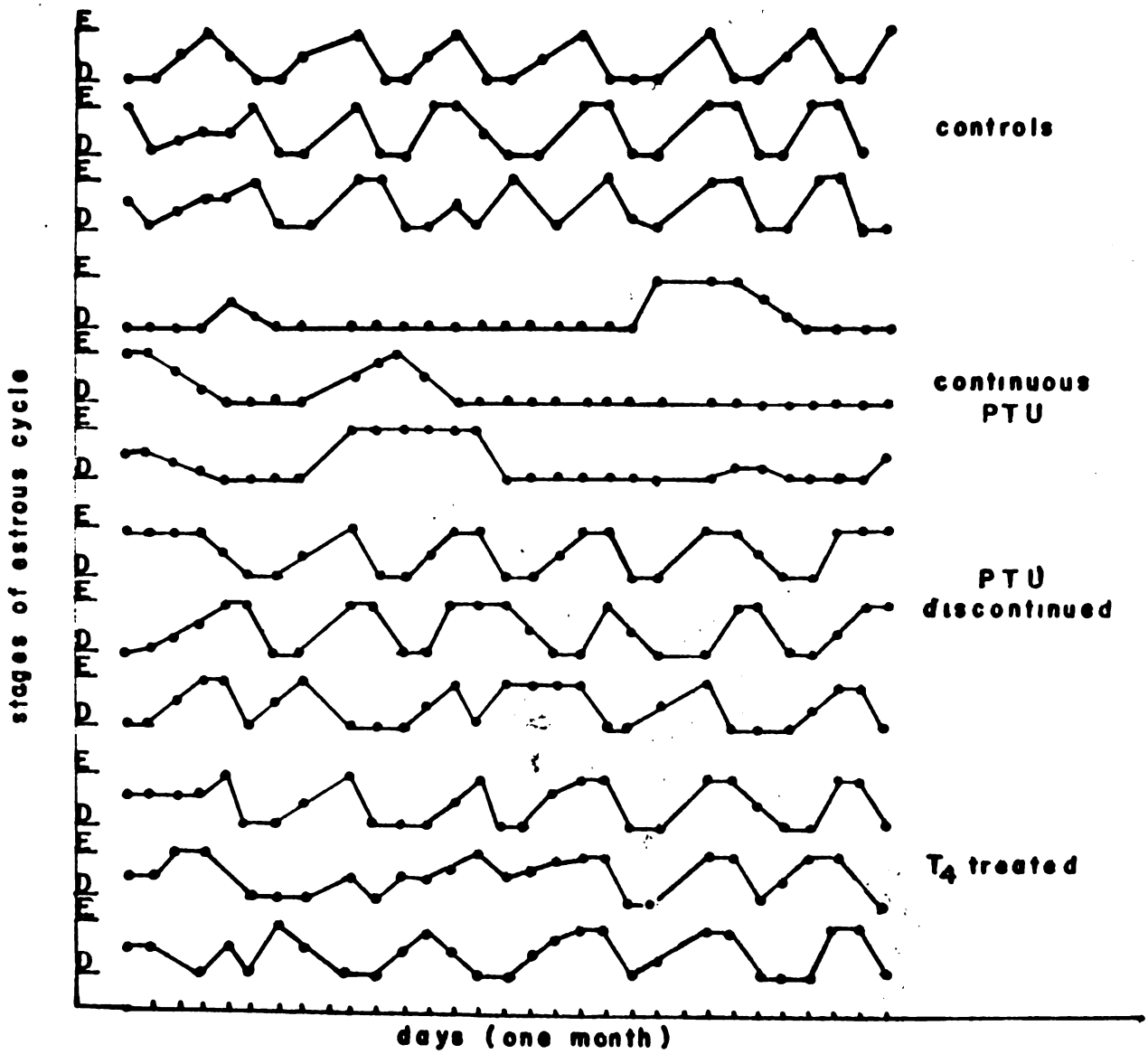


FIGURE 2

ESTROUS CYCLES OF CONTROL, HYPOTHYROID AND HYPERTHYROID RATS

significantly. However, in the group of rats treated continuously with PTU, those animals that did cycle were very irregular and exhibited estrus-proestrus only about 30% of the time.

The weights of the various organs of 11 animals from the control group and 17 animals from the hypothyroid group are shown in Table 2. The hypothyroid animals had dwarfed bodies with weights less than one-third that of the controls. The ovaries of these 'cretins' were greatly reduced in weight and the uteri were small and showed no sign of estrogen stimulation. The thyroids were approximately 5 times as large as the control thyroids, when expressed as mg/100g body weight and the pituitaries, when similarily expressed, weighed slightly more than did pituitaries from control rats.

Anterior pituitary prolactin concentration and content for the 11 control and 17 hypothyroid rats can be seen in Table 3. Prolactin levels were significantly higher in the cycling, control females than in the sexually immature, PTU-treated rats of the same age.

From the data in Experiment 1, it may be concluded that chronic hypothyroidism depresses body growth and retards sexual maturity. Pituitary prolactin concentration and content are also significantly decreased in such animals. In those animals that do reach puberty the estrous cycles are irregular and show a lack of normal follicular development. Puberty is also significantly delayed in female rats made continuously hyperthyroid by T_4 administration from an early age, although body growth and sexual development apparently are not seriously impaired. From this experiment it can also be seen that precocious puberty follows the removal of PTU in female rats under certain experimental conditions.

TABLE 2.

ORGAN WEIGHTS OF CONTROL AND PTU-TREATED RATS

ORGANS	CONTROL	PTU
Ovary (single) (mg)	31.4 ± 1.6	6.6 ± 1.1
Uterus (mg)	379.6 ± 26.8	83.1 ± 11.9
Thyroid (mg)	15.2 ± 0.7	21.8 ± 0.5
Thyroid (mg/100gm body wt.)	6.6 ± 0.3	31.4 ± 0.6
Pituitary (mg)	11.8 ± 0.8	4.8 ± 0.4
Body weight (g)	230.7 ± 2.8	71.8 ± 1.1
Body length (cm)	21.7 ± 0.2	13.5 ± 0.3

TABLE 3.

PROLACTIN CONCENTRATION AND CONTENT
IN PTU-TREATED AND CONTROL RATS

	CONTROL	PTU
No. of rats per group	11	17
Pituitary weight (mg)	11.8	4.8
Reece-Turner Units (RTU) LTH per mg pituitary	0.50	0.28
RTU LTH per pituitary	5.92	1.33
Significance level	1%	
No. of birds per group	10	10

EXPERIMENT 2

Effects of Hypo-and Hyperthyroidism on Pituitary FSH and LH and Hypothalamic FSH-RF

The purpose of Experiment 2 was to determine the effects of hypothyroidism and hyperthyroidism on pituitary FSH and LH content and on the hypothalamic content of FSH-RF. It is known that the continuous state of hyperthyroidism may change the metabolism of steroids and increase their breakdown in the body. The effect of such an event on estrogen would mimic pituitary gonadotropin inhibition. A group of rats in Experiment 2 was treated with T_4 from the 15th day of gestation until 21 days of age and allowed to reach puberty without further treatment. The purpose of this was to determine the effects of early treatment with T_4 on sexual maturation without the metabolic effects of excess T_4 in the circulation at the time of puberty.

Sixty adult Spraque-Dawley rats were housed, bred and fed as in Experiment 1. On the 15th day of pregnancy the females were placed in separate cages and treated as follows:

Group 1. control rats. 15 animals per group, no treatment.

Group 2. hypothyroid rats. 16 animals per group, all given 0.1% PTU in feed.

Group 3. hyperthyroid rats. 20 animals per group, all injected with 10 μg T_4/100 gm body weight.

At parturition all the male young were discarded and the female young were treated as in the first experiment, with one exception. Several days after giving birth, 5 mothers, with a total of 24 female pups, were set aside. These nursing females were injected subcutaneously daily with 10 μg T_4/100 gm body weight until the pups were weaned at 21 days. The pups were then allowed to mature without further treatment.

The time of puberty was measured in these young and in a corresponding control group by observing the day of vaginal opening by observing an estrous smear.

At 45 days of age 10 rats from the control group, 17 hypothyroid rats, and 12 rats treated continuously with T_4 were sacrificed. The pituitaries were quickly removed, weighed and frozen for future FSH and LH assay. The hypothalami were rapidly removed and stored in 0.1N HCL at -20°C. The ovaries, uteri and adrenals were removed and weighed and the ovaries were fixed, sectioned and stained for histological examination.

Preparation of Hypothalamic Extract

Prior to incubation, the hypothalami were thawed, homogenized in chilled 0.1N HCl and centrifuged at 12,000 xG for 40 minutes at 4°C. The supernatants were added to protein free medium 199 (Difco Labs., Detroit, Michigan) containing $NaHCO_3$, and the pH was adjusted to 7.4 by the addition of 0.1N NaOH. (Refer to Table 4 for exact amounts of all reagents used). After neutralization, the hypothalamic extract was immediately added to the incubating pituitaries.

Incubation of Pituitaries with Hypothalamic Extract

Male rats were killed by decapitation and each anterior pituitary (AP) was removed, separated from the posterior lobe and placed in a petri dish on filter paper moistened with cold medium 199. The incubation procedure for FSH-RF (Mittler and Meites, 1966) was as follows:

Amount HE/AP	No. AP's per flask	No. flasks per group	Pre-incub. time (min.)	Incub. time (hrs.)
0.25	4	4	30	6

In order to minimize the differences between donor pituitaries, each AP was bisected and the halves distributed so that each group had halves from a different pituitary. The equivalent of 16 pituitaries or 32 halves was placed in groups 1 and 2; one-half of each anterior pituitary

TABLE 4.

PROCEDURE FOR FSH-RF INCUBATION

	CONTROL	T_4-TREATED	PTU-TREATED
No. of hypothalmi in 3.0 ml of 0.1N HCL per group	10	12	17
Hypothalamic equivalents after centrifugation	7.3	8.8	13.0
Amount of Medium 199 added (ml)	0.81	0.98	1.40
Amount of 1.0N NAOH added (ml)	0.22	0.22	0.22
Amount of 10% $NaHCO_3$ added (ml)	0.10	0.12	0.17
Amount of H_2O added (ml)	4.78	6.28	10.39
Total volume after additions (ml)	8.1	9.8	14.5
Amount added to each incubation flask (ml)	1.0	1.0	1.0

was placed in a flask in group 1, the second half in a flask in group 2. Sixteen more pituitaries were halved and similarly placed in flasks of group 2 and group 3. A final 16 pituitaries were halved and placed in flasks of group 1 and group 3. The incubation was carried out in a Dubnoff metabolic shaker (60 cycles/minute) under constant gassing with 95% 02 - 5% CO2 at 37°C. The pituitaries were preincubated in medium 199 for 30 minutes and the medium was discarded. Fresh medium and hypothalamic extract were then added to each flask to initiate the 6 hour incubation. At the termination of the incubation, the pituitary halves were weighed and discarded, and the medium was stored at -20°C until assayed several days later.

FSH Assay

FSH activity in control, hyperthyroid, and hypothyroid animals was measured by the HCG augmentation method of Steelman and Pohley (1953) as modified by Parlow and Reichert (1963). The incubation media collected from each of the three groups was diluted with saline and HCG so that each assay animal received a total dose of 3 ml of medium containing 50 IU HCG. NIH-FSH-S4 reference standard was tested at 50 and 100 microgram levels. The mean and 95% confidence limits were calculated for each group and students' "t" test was used to determine the significance of differences in FSH activity between groups.

LH Assay

LH activity was assayed by the ovarian ascorbic acid depletion method of Parlow (1961). NIH-LH-S8 reference standard was assayed at doses of 0.4 and 1.78 mg, the equivalent of 0.0013 and 0.0003 NIH-LH-S1 units. The pituitary material from control, hyperthyroid and hypothyroid rats was homogenized in saline and injected at a dose of 1 mg/assay animal into 5 animals per group. The mean and 95% confidence limits were

calculated for each group and the results were analyzed by students' "t" test.

Results

In the control group, 4 of the 15 females did not become pregnant; the remaining 11 rats bore a total of 47 live female pups. In the hypothyroid groups, 13 of the 16 breeding females littered a total of 53 females. Twenty-one of these pups died before termination of the experiment. In the hyperthyroid group, 15 out of 20 animals gave birth to live young, bearing a total of 56 female pups. One of the young rats was eaten and 5 died shortly after birth.

Twenty of the 24 young rats that discontinued receiving T_4 at 21 days of age developed into healthy adult animals. Four rats succumbed to respiratory infections. All of the surviving rats that received T_4 until 21 days of age showed significant delays in sexual maturation. The age of vaginal opening for these rats and their controls is shown in Table 5.

At the time of sacrifice, at 45 days of age, all of the control rats were cycling normally. None of the 12 rats kept continuously on T_4 had reached puberty and only 2 of the 17 hypothyroid animals were cycling.

The body and organ weights from the PTU-treated, continuously T_4-treated, and control animals, at the time of sacrifice, are shown in Table 6. As in Experiment 1, the body and organ weights of the hypothyroid animals were smaller than in the controls. The ovaries of the non-cycling animals in this group were very small with few developed follicles and no corpora lutea, and the uteri showed no significant sign of estrogen stimulation. Although the T_4-treated animals did not appear dwarfed, they weighed significantly less than the control animals and had immature ovaries and uteri. The ratio of adrenal weight/body weight did not

TABLE 5.

THE EFFECT OF EARLY THYROXINE TREATMENT
ON THE ONSET OF PUBERTY

Treatment	No. of rats	Age at vaginal opening
Intact controls	24	38.4 ± 0.61
Hyperthyroid animals*	20	46.0 ± 1.10

* The rats in this group suckled females that were injected with 10 μg T_4 per 100gm body weight from the 15th day of gestation until weaning. When the pups were 21 days of age thyroxine treatment was discontinued.

TABLE 6.

ORGAN WEIGHTS OF ANIMALS TREATED WITH

THYROXINE AND PROPYLTHIOURACIL

	CONTROL	PTU-TREATED	CONTINUOUS T_4
Body weight (gm)	185.3 ± 5.2	56.7 ± 2.5	103.5 ± 3.0
Anterior pituitary (mg)	8.4 ± 0.5	3.6 ± 0.2	3.5 ± 0.4
Ovaries (mg)	46.2 ± 2.7	8.6 ± 0.8	14.9 ± 0.7
Uterus (mg)	331.4 ± 42.6	53.1 ± 11.5	30.3 ± 1.0
Adrenals (mg)	47.2 ± 2.5	16.5 ± 0.8	38.5 ± 1.5
State of sexual maturity	10/10 cycling	15/17 immature	12/12 not cycling

change significantly between the treated and control animals. Both hypothyroidism and hyperthyroidism decreased the gross weight of the pituitaries. However, when expressed as mg wet AP tissue/100gm body weight, hypothyroidism increased the average weight from 4.53 to 6.35 while hyperthyroidism decreased AP weight to 3.38.

The results of the assays for FSH and FSH-RF in the control, hypothyroid and hyperthyroid groups are presented in Table 7. In the PTU-treated group, pituitary FSH concentration was slightly decreased and, due to the small size of the pituitaries, total pituitary FSH content was significantly lower than in the control group. In the T_4-treated animals, pituitary FSH concentration was more than twice that of the controls. Pituitary FSH content was also significantly greater in the T_4-treated group. As can be seen from Table 7 there was no significant difference between the hypothalamic FSH-RF contents of the three groups.

Anterior pituitary LH concentration and content were measured in the same rats as above. The results are summarized in Table 8. Thyroxine treatment greatly increased the LH concentration and content while PTU depressed LH content significantly.

It may be concluded from Experiment 2 that early treatment with T_4 delays sexual maturation in the female rat and that this delay is not dependent upon the presence of excess T_4 at the time of puberty. Rats continuously treated with T_4 from the 15th day of gestation until sacrifice at 45 days of age have increased FSH and LH per mg AP tissue, as compared with control pituitaries, but show no signs of normal estrogen secretion. The high FSH and LH in the pituitaries may represent storage rather than release. Continuous PTU treatment decreases the AP content of LH and FSH. Because neither hyperthyroidism nor hypothyroidism had an appreciable effect on the hypothalamic content of FSH-RF, it is possible that T_4 and PTU may act directly on the pituitary gland.

Table 7.

EFFECTS OF PROPYLTHIOURACIL AND THYROXINE TREATMENT ON PITUITARY FSH CONTENT AND CONCENTRATION AND HYPOTHALAMIC FSH-RF CONTENT.

	CONTROLS	PTU-TREATED	T_4-TREATED
No. of rats per group	10	17	12
Pituitary weights (mg)	8.35	3.56	3.52
Pituitary FSH*1 concentration (μg/mg)	7.67±0.07	6.97±0.35	23.00±2.25
Pituitary FSH*1 content (μg/gland)	64.0	24.8	81.0
Hypothalamic FSH-RF (μg*2/mg pit./ml)	10.21	9.43	11.12

*1. NIH-FSH-S_4 standard. Relative potency = 1.37 U/mg.

*2. FSH-RF is expressed as μg of FSH released into incubation medium.

TABLE 8.

EFFECTS OF PROPYLTHIOURACIL AND THYROXINE TREATMENT

ON PITUITARY LH CONTENT AND CONCENTRATION.

	CONTROLS	PTU-TREATED	T_4-TREATED
No. of rats per group	10	17	12
Pituitary weights (mg)	8.35	3.56	3.52
Pituitary LH* concentration (μg/mg)	(.5-1.3) 0.80	(.43-.57) 0.50	(9.1-11.0) 10.00
Pituitary LH* content (μg/gland)	6.68	1.78	35.20

*. NIH-LH-S_8 standard. Relative potency = 0.73 U/mg.

EXPERIMENT 3

Effects of Treatment with PTU or T_4 beginning at 21 Days of Age on Onset of Puberty

The third experiment was designed to determine whether or not a critical period exists after which time the administration of T_4 and PTU would have no effect on the onset of puberty.

Fifty-three 21-day-old female Spraque-Dawley rats were placed in cages housing approximately 5 rats each, were separated into 4 groups, and were treated as follows:

Group 1. control rats. 12 rats per group, no treatment.

Group 2. T_4-treated rats. 20 rats per group, 10 μg T_4/100 gm body weight/day until puberty.

Group 3. continuous PTU-treated rats. 11 rats per group, 0.1% PTU placed in feed until puberty.

Group 4. discontinuous PTU-treated rats. 10 rats per group, 0.1% PTU placed in feed until 28 days of age.

The animals were given food and water ad lib. Daily checks for vaginal opening were made and at puberty, vaginal smears were taken.

Results

None of the animals in the four groups differed significantly from each other in age of sexual maturation. Table 9 shows the body weights of the rats at the onset of puberty. The rats that were continuously treated with PTU lost weight and showed a slight but insignificant delay in the onset of puberty.

It is concluded from this experiment that the changes in the onset of puberty seen in female rats where thyroid treatment was begun before the 21st day of life, are not observed in similar animals whose treatment began after the 20th day of life.

Table 9.

AGE OF VAGINAL OPENING IN ANIMALS TREATED WITH THYROXINE AND PROPYLTHIOURACIL FROM THE TWENTY-FIRST DAY OF LIFE.

TREATMENT	NO. OF RATS PER GROUP	BODY WEIGHT AT VAGINAL OPENING (gm)	AGE AT VAGINAL OPENING (Days)
CONTROLS	12	153.1	34.7
T_4-TREATED*[1]	20	156.1	35.5
CONTINUOUS PTU TREATMENT*[2]	11	103.7	37.2
PTU-TREATMENT DISCONTINUED*[3]	10	159.7	35.2

*1. 10.0μg T_4/100gm body weight injected daily until puberty.

*2. 0.1% PTU placed in feed until puberty.

*3. 0.1% PTU treatment discontinued at 28 days of age.

GENERAL DISCUSSION

The effects of early abnormal thyroid function on the reproductive system in the female rat were studied in Experiments 1 and 2. The number and viability of young born to pregnant rats treated with T_4 was reduced as compared with the control rats in experiment I. Continuous administration of T_4 to the surviving female pups did not significantly reduce their growth rate as compared to control rats but did decrease their total body weight somewhat. Although hypothyroidism did not affect the number of viable young, many of the young pups that suckled PTU-treated mothers became weak and died. The continuous administration of PTU severely retarded growth and reproductive development.

It can be concluded from these experiments that the age at which treatment is begun is important. Treatment with T_4 from the 15th day of gestation until 21 days of age delayed puberty to the same extent as similar treatment continued beyond 50 days. Thyroxine treatment given after 21 days of age had no effect on the onset of puberty. Continuous PTU given from the 15th day of gestation until 60 days of age caused severe growth retardation and a delay or complete cessation of puberty. The same doses of PTU given to weanling rats for a period of approximately 15 days had no effect on the onset of puberty. In Experiment I, rats made hypothyroid from gestation until day 21 responded to the withdrawal of PTU by exhibiting precocious puberty and this was followed by normal estrous cycles.

It has been shown that climate, stress and changes in temperature can advance puberty (Donovan and van der Werff ten Bosch, 1965), as can the presence of males, increased handling and crowded housing conditions. It is also known that nutrition influences puberty and it has been stated that the attainment of sexual maturity in most species corresponds

closely to the attainment of adult size (Donovan and van der Werff ten Bosch, 1965). More recently, puberty has been advanced experimentally by the administration of various median eminence extracts (Gellert et al., 1964; and Andjus and Kamberi, 1966), estrogen treatments (Rameriz and Sawyer, 1965) and anterior hypothalamic implants of estrogen (Smith and Davidson, 1967). In the rats in the experiments described herein puberty normally occurred between 34-41 days of age. The advancement of puberty to 31.7 $\pm$ 1.0 days by the withdrawal of long term PTU treatment cannot be explained by stress or nutrition, and puberty occurred long before the rats reached adult size. There are, however, several rational explanations. It is possible, although unlikely, that as the blood level of PTU decreased the ensuing mild hypothyroid condition stimulated pituitary gonadotropin release. Another explanation might be that during the hypothyroid period, when the pituitary gonadotropins were suppressed, an increase in gonadotropin-RF's was produced (due to the decrease in the 'short' negative feedback of LH and FSH on the hypothalamus). When the pituitary was no longer suppressed, following the removal of PTU, it over-responded to the increase of gonadotropin-RF's and the ovaries were prematurely stimulated. A third possibility is that a severe lack of T_4 may influence both the pituitary and the ovaries, making the latter more sensitive to gonadotropins while inhibiting the growth and function of the pituitary. After the removal of PTU, increased ovarian response to pituitary LH and FSH could result in increased estrogen secretion and the advancement of puberty.

The literature presents what appears to be contradictory evidence concerning the effective role of T_4 in murine reproduction. Study of ovarian cytology shows that short-term hypothyroidism stimulates follicular growth (implying either increased plasma FSH or increased

ovarian sensitivity to FSH), while mild hyperthyroidism augments luteal development (implying either increased plasma LH or increased ovarian sensitivity to LH and increased prolactin secretion). Johnson and Meites (1950) found that hyperthyroid rats did not respond to exogenous pregnant mares' serum (FSH) as much as did untreated control rats and that thyroidectomy evoked the opposite response. Their results suggest that the effect of thyroid hormone is primarily on the ovaries. Others have reported, however, that pituitary LH (ICSH in males) and FSH is decreased in hypothyroid male rats (Contopoulos et al., 1958). Attempts have been made to show that hyperthyroidism has the opposite effect on pituitary gonadotropins but changes in these hormones have not been measured directly.

It appears from the present data that severe hypothyroidism in the rat, as in the human cretin, stunts body growth and all of the processes related to growth and maturation. The onset of puberty is delayed or totally absent and there is little sign of ovarian maturation and steroid production. The pituitaries, on first examination, appear small with decreased LH and FSH content (Experiment 2) and prolactin (Experiment I) content. However, when the hormone content of these small pituitaries is expressed in terms of 100 gm of body weight, only prolactin content remains decreased. The content of LH and FSH per average body weight per group does not differ significantly between the controls and those treated with PTU. Since the pituitary weights of these rats decreased in direct proportion to the decrease in body weights, the most valid measurement appears to be hormone concentration rather than content. As seen from Tables 3, 7 and 8, only prolactin concentration decreased significantly. The delay of puberty and the sexual immaturity of the severely hypothyroid rats may, in fact, reflect the general retardation of cellular processes

and not specific inhibition of pituitary gonadotropin synthesis or release.

Hyperthyroidism appears to act differently. Although pituitaries from T_4-treated animals are as small as those from PTU treated rats, body growth and general appearance are approximately normal. The concentration of LH and FSH is greatly increased in the hyperthyroid rats although there is no sign of gonadotropin release.

Kragt and Ganong (1967) and Corbin and Daniels (1967) have shown that pituitary FSH concentration increases from about the 15th day of life until the 25th day, peaking around day 20, and then falls precipitously prior to puberty. The average pituitary content of FSH in the cycling rat returns to the levels seen around day 15 but the concentration of FSH remains low. A drop in the hypothalamic content of FSH-RF at the onset of puberty has also been reported (Rameriz and Sawyer, 1966). The highest levels of FSH-RF found prior to puberty are also found in the normal cycling adult in late proestrus (Rameriz and Sawyer, 1965). In Experiment 2 it was shown that chronic T_4-treatment delayed puberty significantly. The pituitaries from these animals showed a 3-fold increase in FSH concentration as compared to normal, cycling 45-day-old rats. The increase corresponded to the high levels of FSH previously reported for normal 15-25-day-old rats. Since the drop in FSH-RF at puberty (Rameriz and Sawyer, 1966) and during estrus (Rameriz and Sawyer, 1965) is thought to be due to the increased negative feedback by estrogen on pituitary LH, causing a decrease in the negative 'short' feedback loop between LH and the hypothalamus, one might expect to find an increase in FSH-RF in those rats that remained immature with little sign of pituitary LH release. However, the slight increase observed for FSH-RF in the T_4-treated group was not statistically significant for the number of animals used.

It has been reported by Rameriz and McCann (1963) that LH concentration is approximately 3-fold higher in immature female rats than in cycling adults. The same investigators found that ovariectomy of adult rats increased pituitary LH concentration approximately 2-fold. In the present experiments it was found that T_4 treatment increased the concentration of LH more than 10 times that of normal cycling controls.

Because the rats treated with T_4 from the 15th day of gestation to the 21st day of life, or until sacrifice, exhibited marked delay in puberty, indicating a lack of ovulation, and because pituitaries from T_4-treated rats contained abnormally large amounts of LH and FSH as in prepubertal rats, it is concluded that T_4 acts at least in part to prevent the release of LH and FSH from the anterior pituitary.*1 The possibility remains that T_4 also acts directly on the ovaries to render them less sensitive to the gonadotropins.

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*1. This author (unpublished data) has found that low doses of T_4 increase pituitary LH content in adult male rats. High doses of T_4 or treatment with PTU did not change the pituitary LH concentration significantly.

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