THE INFLUENCE OF VARIATIONS IN THE THYROID STATE ON REPRODUCTION AND LACTATION IN FEMALE ALBINO MICE

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THE INFLUENCE OF VARIATIONS IN THE THYROID STATE ON REPRODUCTION AND LACTATION IN FEMALE ALBINO MICE

By

Dorothy Hupp Ward

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DEDICATIONS

This thesis is affectionately dedicated to my husband whose constant understanding and helpful encouragement have made completion of this work possible and to my parents, Mr. and Mrs. Charles D. Hupp, for their understanding guidance and inspiration.

Dorothy Hupp Ward

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INTRODUCTION

Although considerable research has been reported in this field there is still much disagreement regarding the role of the thyroid secretion in reproduction in the female. Clinically it has been shown that many women with basal metabolic rates in the normal range, and with no outward signs of myxedema, have reproductional disturbances. These disturbances include menstrual irregularities, low fertility approaching sterility, and termination of pregnancy with abortion, miscarriage, or stillbirth. Correction or prevention of these disturbances is often accomplished through constant medication with prophylactic or therapeutic doses of thyroxine or desiccated thyroid.

It has been reported by various workers that induced mild hyperthyroidism increases both milk and fat yield of lactating cows and goats. Many studies have been carried out on rats, rabbits, and guinea pigs regarding the influence of daily thyroxine treatment and the influence of hypothyroidism resulting from surgical ablation or pharmacological suppression of the thyroid gland on estrous cycle, length of gestation, resorption of fetuses, abortion, litter size, and lactation.

The results reported, especially concerning the influence of variations in the thyroid state on gestation, littering, and lactation often have been conflicting. Little experimentation on this subject has been reported in which mice were the experimental subjects. Results of the existing work indicate that a definite interrelationship exists between the thyroid state and these processes.

This study was initiated in an attempt to determine the influence hypothyroidism, produced by thiouracil administration, and varying degrees of hyperthyroidism, resulting from the administration of varied doses of d,l-thyroxine, exert on the physiological processes of reproduction and lactation in female albino mice.

REVIEW OF LITERATURE

The Influence of Variations in the Thyroid State on Growth and on Food and Water Intake

An excellent review on the effect of experimental changes of thyroid secretion status on various aspects of animal production has been made by Blaxter et al. (1949). These aspects of production include growth and fattening, fertility, reproduction, mammary growth, and lactation. This report discusses the use of various thyroactive iodinated proteins, such as 'Protamone' and iodinated casein, and of various synthetic goitrogens, such as thiourea and thiouracil, to change the thyroid secretion status experimentally in ruminants, swine, and poultry. Barker (1949) presented a good review of the influence of thiouracil on reproduction and growth in the rat.

The influence of variation in environmental temperature and thyroid status on growth, feed consumption, and sexual development in the growing male mouse has been studied by Maqsood and Reineke (1950a, 1950b). They reported that thiouracil feeding depressed both feed and water consumption in mice. Mice receiving graded amounts of thyroprotein in their feed showed an increase in food and water intake proportionate to the thyroprotein dosage. Thyroprotein given as both 0.025 and 0.05 per cent of the ration caused highly significant weight gains. They found also that thiouracil administration caused a decrease in the weights of the testes and of the seminal vesicles; there were some atrophic and degenerative changes, with limited spermatogenesis. Mild hyperthyroidism stimulated spermatogenic activity in the testes and stimulated sexual development.

Soliman (1950) found that mild doses of thyroprotein given to growing female mice produces in them a rate of growth exceeding that of the

non-treated controls. This indicates that the rate of thyroid hormone secretion in mice apparently is less than the optimal. The rate of growth and maturation are further decreased by thiouracil. Thiouracil decreased food and water consumption; thyroprotein increased the food and water consumption.

The Influence of Variations in the Thyroid State on the Estrous Cycle

Smear Techniques and Normal Cycle Lengths

As early as 1917 Stockard and Papanicolaou made use of a small nasal speculum for vaginal examination of guinea pigs. The speculum permitted an examination of the entire surface of the vaginal canal and smears were made daily from the substances present in the lumen. These investigators studied the smears and observed the existence of a typical estrous cycle.

Allen (1921, 1922) used the same technique, slightly modified for use on the mouse. He examined some animals three times daily to get the exact time relations of the various phases of cell changes occurring in their vaginal linings. One daily examination was found to be adequate for accumulation of data as to cycle length. He designated the various stages in the cycle as diestrus, a period of relative quiescence averaging from one to three days; proestrus, a period of augmented growth and congestion often lasting less than a day; estrus, the period of sexual excitement or heat usually lasting one or two days; and metestrus, divided into two stages each usually lasting a day and consisting of a return to the diestrus stage. The smears associated with the various stages of this cycle were described. He found the average deviation of the entire

cycle to be from four to six days. Observations were made that ovulation in some mice is spontaneous at every estrus, and the ovaries are chiefly masses of corpora lutea. He found, however, that not all mice ovulate spontaneously during estrus. Some ovulate only sporadically and corpora lutea may be entirely absent. Normal estrous cycles were experienced in both types of mice.

Long and Evans (1922) made a very complete study of sexual maturity in the female rat. They concluded that first estrus occurred at an average age of 92.7 days and that vaginal introitus occurred at an average age of 76.5 days with first ovulation in the majority of the females delayed approximately five days after the occurrence of vaginal introitus. The first estrus cycle was found to be significantly longer than any succeeding one. Blandau et al. (1939) studied the normal reproductive cycles of female rats by use of the copulatory response and found the average duration of heat periods to be 13.7 hours with the onset of three-quarters of the heat periods studied occurring between 5 P. M. and 11 P. M. They found four-day cycles in 58 per cent of the cases studied and five-day cycles in 24 per cent of the cases. Blandau and Money (1943) found that the average age for onset of heat in 200 albino female rats was 49.4 days. The length of the first heat averaged 9.07 hours. They also observed that the first heat period was significantly shorter than succeeding ones and the first and second reproductive cycles were significantly longer than the remaining ones. The majority of the first heat periods began between 7 P. M. and 5 A. M.

Blandau et al. (1941) reported that tests of estrous condition of the female rat can be made at frequent intervals over a considerable period of time without disturbing the normality of the cycle. This was

accomplished by ascertaining the degree of copulatory response to manual manipulation in the vulvar region. Indivudual animals tended to show a consistency in character of the cyclic behavior as measured by the length of heat, the length of the cycle, and the time of day at which heat began. Lee (1927, 1928) reported an increased basal heat production in female rats during the last 10 hours of diestrus and in the first six hours of proestrus.

Boughton and Stoland (1943) found that all the mature albino rats they used as experimental subjects exhibited estrous cycles at less than 25 weeks of age. Of these, 84 per cent were four-day cycles, and the sequence of four-day cycles was quite regular up to about 50 weeks of age, becoming increasingly irregular beyond this time. An increasing proportion of the animals over 25 weeks of age failed to show evidences of heat on vaginal smears, and of those that did an ever larger proportion deviated from the four-day cycle.

Hypothyroidism

Using thyroidectomized female rats Lee (1925) found that the first cycle following the operation and sometimes the second one was often much lengthened, however this also occurred after a sham operation only, apparently due to trauma or slight infection. The succeeding cycles showed an average lengthening of about one day above those of normal controls due to a lengthened diestrus. Freedman et al. (1935) confirmed this prolongation of the cycle in diestrus, with the lengthening of diestrus ranging from one to five days. Ross (1938) also found that the duration of the estrous cycles increased from the normal four or five days to seven or eight days in thyroidectomized female rats due to an extension of the diestrual phase.

Nelson and Tobin (1937) followed the vaginal smears of non-pregnant thyroidectomized rats and noted that the estrous cycles definitely were disturbed with a resulting tendency toward extended diestrus. Evans and Long (1921b) did not find permanent alteration in the length of the estrous cycles following thyroidectomy. They reported that the operation usually was followed by a pause in the estrous cycles of six to 27 days; but this, in turn, was succeeded by normal estrous cycles. Dragstedt et al. (1934) reported that complete absence of the thyroid and the parathyroid gland in dogs did not prevent regular appearance of estrus in the bitches.

Krohn and White (1950) studied the effects of hypothyroidism on the reproductive cycle of female albino rats, using both surgical ablation and pharmacological suppression with thiouracil. They found that estrous cycles tended to be longer, especially in the thyroidectomized animals, and more variable in length. Richter (1933) observed marked disturbances in the formerly very regular four to five-day reproductive activity cycles after thyroidectomy. The intervals between peaks of activity were found to vary from three to 25 days. During the period between activity peaks the smears showed a consistently diestrous picture. When the animals were active, the estrous cornified cell stage had a tendency to be prolonged beyond the normal interval.

Several workers observed the effect of administration of thiouracil on estrous cycles in rats. Nelson (1948) found that after either thyroidectomy or administration of thiouracil female rats show very irregular periods of estrus and they frequently may be absent for several weeks. Mann (1945) found disrupted estrous cycles in all rats given thiouracil. There were greatly lengthened periods between estrous stages,

exemplified by 14-day periods between periods of cornification in some cases. He stated that the inhibitory action of thiouracil apparently is very rapid since some animals had only one estrous cycle after the addition of thiouracil to the diet.

Hyperthyroidism

Evans and Long (1921a) observed that estrous cycles usually were not greatly disturbed when rats normally showing four-day cycles were fed 0.25 to 1.50 gm. of fresh beef thyroid daily. However, when they were fed one-fourth to one-half of a gland daily the cycle was greatly lengthened or totally inhibited. Pronounced loss in body weight occurred and some animals died showing a degree of toxicity with this large dosage. Drill et al. (1943) found that female rats maintained continuous diestrus after 10 to 17 days of dosage with 100 mg. of thyroid gland in the daily diet. The administration of a yeast supplement during 79 days of thyroid feeding prevented this abnormality.

Reiss and Pereny (1928) found that thyroid hormone inhibited heat in normal female rats and also suppressed the action of ovarian hormone applied to castrate animals. This result was confirmed by Van Horn (1933) who found that approximately three times as much theelin was required to produce an estrous condition in a castrate rat made hyperthyroid by oral feeding of desiccated thyroid as was required in an ordinary castrate animal. He believed that the increased metabolism induced by the hyperthyroidism was instrumental in the elimination of theelin, thus keeping the amount of theelin in the body below that required for the estrous threshold. He also observed a prolonged diestrus in hyperthyroid rats and an irregularity of estrous cycles when sexually mature female rats with normal cycles were thyroidectomized. Hill (1948) studied the effect

of both age and thyroid state on the response of ovariectomized rats to estrone. It was shown that young rats given tolerable doses of thyroxine, 7 ug. per 100 gm. body weight, for a 10-day period, show a decreased response to estrone. In old rats, 13 to 14 months of age, this same treatment increases the estrus response. Excessive doses of thyroxine, 70 ug. per 100 gm. body weight, administered daily for 10 days to old rats decrease both estrus response and body weight. She stated that the sensitivity to estrogen decreased and the sensitivity to thyroxine increased in rats sometime between 11½ and 14 months of age.

Halpern and Hendryson (1935) raised the metabolic rate of female rats with dinitrophenol and found that heightened metabolism per se did not lead to an increased rate of estrin elimination and had scarcely any effect on the estrous cycles, ovaries, and pituitaries. On the other hand there were marked changes in the estrous cycles, ovaries, and pituitaries of thyroid-fed rats indicating that the thyroid has a specific action on them.

Weichert and Boyd (1933) did considerable work on induced hyperthyroidism in female rats and the ensuing diestrus. They showed that animals fed doses of 0.25 or 0.5 gm. of desiccated thyroid daily had none, one, two, or three regular estrous cycles after the beginning of thyroid feeding followed by a prolonged diestrous period of 10 to 22 days in duration. As long as thyroid feeding was continued, a series of such lengthened diestrous intervals occurred separated from each other by one or two normal estrous periods. Normal and regular estrous cycles followed again if thyroid feeding was discontinued during a prolonged diestrous period. These unusually long diestrous periods after thyroid treatment proved to be pseudopregnancy since placentomata could be produced experimentally

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during these periods. They also stated that it was possible for pseudo-pregnancy to occur after mechanical stimulation of the vagina, even from taking smears.

Mechanical Stimulation

Several groups of workers found that mechanical stimulation of the vagina, as in swabbing or taking smears, often results in epithelial proliferation and cornification. Wade and Doisy (1935) used a small amount of cotton wrapped about the end of a toothpick and moistened with water to insert into the vaginal canal of spayed rats and rotate gently a few times to obtain smears. They found that the spayed animals which were neither smeared nor injected presented a thin, smooth vaginal epithelium, usually two cell layers thick when the vaginae were examined after the animals were killed. A typical section from the vagina showed a progressive thickening of the epithelium up to twelve or more layers with the usual desquamation of the surface cells when the animals had been smeared once, twice, or three times daily. Likewise the vaginal smear underwent a progressive change. About one quarter of the spayed animals showed fully positive estrus smears on the third or fourth day of treatment when the smears were made three times daily.

Emery and Schwabe (1936) agreed that frequent examination, four times a day, by this cotton swab method brought about positive estrus smears in the castrated rat instead of the usual negative diestrus smear. Lactating mothers also were brought into an estrous-like condition by frequent examinations with the cotton swab. In normal virgin rats the estrous cycles became irregular and estrus occurred more frequently than normal when examinations were made four times a day with the cotton swab. They found, on the other hand, that the pipette and lavage method of

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taking vaginal smears only rarely changed the diestrous smear of the castrated rat to a positive estrous-like smear. The cycles of normal virgin rats also were little affected by the lavage method. They considered it necessary that extreme care be observed when taking daily smears with the pipette to insert only the tip into the vagina and not to move it back and forth while taking the smear. They warned that otherwise there may be sufficient pressure and irritation to give a positive smear. Rogers and Allen (1937) observed this same stimulation of the vaginal epithelium by rotation of a cotton swab rotated several times in the vagina. They used a moist toothpick for taking smears and failed to observe these changes.

The Influence of Variations in the Thyroid State on Fertility

Hypothyroidism

Hypothyroidism decreases fertility. Nelson and Tobin (1937) observed that non-fertile copulations were more frequent in thyroidectomized rats than in control rats. Litzenberg (1926) reported that myxedema is certainly a cause of sterility. He stated that lesser degrees of hypothyroidism also cause or are an index of a cause of sterility. He found clinically that 39 per cent of 68 married women with low basal metabolic rates but without signs of myxedema were sterile. There were functional disturbances of menstruation in 40 per cent of 114 women, both married and unmarried, with low basal metabolic rates. Of 44 sterile women who came for treatment half had basal metabolic rates of -10 or below. Thyroid medication was given to 18 of those who did have lower rates. Within two months six of them became pregnant.

Litzenberg and Carey (1929) observed that in a large percentage of cases even a moderate degree of lowered metabolism interferes with the reproductive function of women. Abnormal menstruation was found in one-third of all the women with low metabolic rates who were studied and in nearly two-thirds of the sterile women with low rates. Nearly half of all the women with decreased metabolism whom they observed were sterile and more than half of the sterile women had below normal basal metabolic rates. These workers found that in many cases restoring the basal metabolic rate to normal by thyroid medication improved menstruation and permitted conception.

Foster and Thornton (1939) reported on the results they observed after treatment with desiccated thyroid on healthy women patients with normal pelvic findings who were seen because of menstrual irregularities and who had basal metabolic rates ranging from -1 to -33 with an average of -15. Of 25 cases who complained of dysmenorrhea or difficult and painful menstruation 17 had complete relief and 5 had partial relief. Of 17 women with a scanty or infrequent menstruation or oligomenorrhea all experienced complete relief. Among 13 patients with metrorrhagia or hemorrhage from the uterus 12 experienced complete relief. Six out of seven with excessively profuse menstruation or menorrhagia had complete relief. They believed that a therapeutic trial of thyroid is indicated if the basal metabolic rate is zero or below in those individuals complaining of menstrual irregularities who have no demonstrable pathological symptoms.

Similarly, Winkelstein (1940) studied a group of sterile patients who had been married at least one year with normal congress and who had used no contraceptives. He found that a few of these women had slight

clinical hypothyroidism and some had subclinical hypothyroidism. Some were overweight and were made to lose. All of them were free of pathological symptoms. The normal range for basal metabolic rate was considered by him to be -10 per cent to +10 per cent. He gave doses of desiccated thyroid starting with 1 grain per day and increasing the dose 0.5 to 1 grain per day at semiweekly intervals until a maintenance tolerance was reached and then continued indefinitely at this optimal dosage. Pregnancy occurred in most of these patients.

Hyperthyroidism

Gudernatsch (1915a, 1915b) found that female rats made hyperthyroid by the feeding of fresh thyroid gland would not breed and pregnancy did not occur until a period of time had elapsed after the thyroid feeding was discontinued. Pregnancy did not occur in normal female rats when they were mated to hyperthyroid males. Clute and Daniels (1930) reported that a diminution of menstrual function and a lack of fertility often accompanies severe hyperthyroidism; in fact, the more severe the hyperthyroidism the less active is the ovarian function.

The Influence of Variations in the Thyroid State on Gestation

Hypothyroidism

Long and Evans (1922) found that 90 per cent of the gestation periods in normal rats fall between 21.5 and 22 days in length. Ukita (1920) reported that rabbits thyroidectomized from the seventh to the tenth day of pregnancy had their gestation period increased to 60 to 70 days in length. Krichesky (1935) disagreed with these results. He performed thyroidectomies on rabbit does from two hours to 12 days after

their mating and found no apparent differences between the length of the gestation period in operated and unoperated does. Normal rabbits have an average 31-day gestation period ranging from 29 to 32 days; the thyroidectomized does ranged from 30 to 33 days in length of gestation period. Krichesky (1939) again reported that thyroid removal during the first 12 days of gestation in rabbits did not alter gestation length. Furthermore, these animals successfully terminated a second pregnancy within the limits of a normal gestation period.

Litzenberg (1926) found that a normal basal metabolic rate apparently is necessary to a normal continuance of pregnancy. Clinically, he gave thyroid medication to 18 of 22 sterile women with basal metabolic rates of -10 per cent or below and six or one-third of them became pregnant and had normal full term pregnancies. Two of these women had had one or more previous pregnancies terminating in abortion, miscarriage, or stillbirth. One of these six women also had two normal pregnancies later while under thyroid medication. He suggested that women who habitually abort should have their basal metabolic rate measured. Litzenberg and Carey (1929) reported that 52 sterile women with a metabolic rate below normal were treated with desiccated thyroid to restore the rate to normal. Of these women 30 per cent became pregnant following treatment and had normal full term pregnancies except two who discontinued treatment after conception and aborted. Fifteen of these women who had normal full term pregnancies during treatment had aborted one or more times before treatment. Thus this work indicates that a normal basal metabolic rate should be maintained during pregnancy. These findings are not difficult to understand if it is true that reproductive tissue cells are more subject to deleterious influences than any other cells in the body.

The Japanese worker, Nojima (1933), thyroidectomized female rats and observed the effect on reproduction. He found that the mating rate decreased to 22.7 per cent of normal on the hundredth to the one hundred and fiftieth day after thyroidectomy, and the conception rate decreased to 33.3 per cent of normal on the eighty-eighth to the hundredth day after operation. Abortion occurred in a few of these thyroidectomized rats when pregnancy occurred and in those that did not abort there was a very slight tendency toward prolongation of the gestation period. If thyroidectomy were performed after the beginning of pregnancy it caused an increase in intra-uterine death rate, a decrease in fetal weight and size, and a hypertrophy of the fetal thyroid.

Dragstedt et al. (1934) reported that complete absence of the thyroid and parathyroid glands in dogs did not prevent pregnancy. Nelson and Tobin (1937) found that thyroidectomy apparently has no harmful effects on pregnancy and parturition in rats and guinea pigs. Fifteen rats thyroidectomized during the last half of their gestation continued to term and delivered normal litters. Ten rats thyroidectomized prior to the incidence of pregnancy delivered one or more litters successfully, 17 in all. Parturition occurred in a normal fashion in six guinea pigs thyroidectomized during the last 10 days of pregnancy. Ross (1938) found that pregnancy with normal numbers of embryos occurred in three-quarters of the thyroidectomized adult female rats studied. There was, however, a decided increase in number of resorptions with only one third of the animals producing normal litters.

King and Herring (1939) found that a low basal metabolic rate of -10 to -30 per cent in women often was followed by abortion. They even encountered abortion in cases where the husband had rather marked

hypothyroidism. It was found advisable to give prophylactic doses of desiccated thyroid to pregnant women with a basal metabolic rate of -6 to -9 per cent, especially if there were a record of previous abortions.

Sachs (1939) found that the basal metabolism of thyroidectomized rats falls severely within one month after the operation and, although fertilization occurred when the animals were mated, the advanced embryos died. Two parenteral injections of 1 mg. of pure thyroxine before mating raised the basal metabolic rate to normal, and another such injection after mating led to normal pregnancy and normal birth. They found that additional injections of thyroxine on the thirteenth to the eighteenth day of pregnancy had no effect, indicating that thyroxine was no longer necessary after the thyroid gland of the embryo began to function. Folley et al. (1942) reported that pregnancy often was prolonged when thyroidectomized rats were mated. They delivered their young but seldom began to rear their litters.

Jones et al. (1946) studied the effect of thiouracil-induced hypothyroidism upon the reproductive system of the adult rat. Males did not lose their ability to sire litters. In female rats prolonged thiouracil administration interfered with continuation of gestation by causing resorption of embryos in all of the cases. If, however, the drug was given over a period of less than 100 days, some rats did deliver litters which were normal in growth and development and reproduced normally. They judged the occurrence of pregnancy and resorption by finding sperm in the smear, followed by diestrus smears and finally bleeding. To be positive that this sequence of events was not given by pseudopregnancy they examined the animals by palpation for fetuses at the sixteenth day of gestation and performed laparotomies on seven animals.

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They found normally palpable fetuses in only one rat; the other rats had pregnancies which resorbed before the palpable stage. Of the laparotomized animals four had recognizable resorbing implantation sites, and the others had metrial glands (inflammatory areas). They concluded that treatment with thiouracil for at least 100 days may be necessary to produce severe hypothyroidism in rats.

Krohn and White (1950) reported that hypothyroid rats conceived but due to resorption of fetuses at varying stages of gestation, they brought fewer young to term than they had in a previous normal pregnancy, whether they were made hypothyroid by surgical ablation of the thyroid gland or by pharmacological suppression with thiouracil. They also observed a slight prolongation of gestation. To be sure rats were hypothyroid at the time of mating, these workers waited at least 54 days after starting thiouracil injections and at least 28 days after thyroidectomy before mating. Preheim (1940) observed that gestation periods of thyroidectomized rats were prolonged approximately 24 hours.

Hyperthyroidism

Gudernatsch (1915a, 1915b) found that feeding fresh thyroid tissue delayed pregnancy. If pregnancy did occur, it resulted in abortion or the young died soon after birth. In rabbits cretinized by thyroidectomy at three weeks of age, and then after several months made severely hyperthyroid by feeding desiccated thyroid, Kunde et al. (1929) found, in most instances, that although fertilization and implantation occurred the young were never born. Instead, resorption of all or many of the fetuses occurred during the latter two-thirds of pregnancy. Finding of sperm on the vaginal smears of isolated does placed in mating cages no longer than one hour and palpation of fetuses about eight days later

was taken as proof of pregnancy. In some of the rabbits laparotomy incisions were made and fetuses were counted on the seventh to fifteenth day of gestation. It was reported by Rose (1947) that muscular maturation of the fetal rat may be delayed if the mother is made hyperthyroid by feeding desiccated thyroid during the early portion of the pregnancy.

Weichert (1930) noticed a prolonged gestation period in pregnant rats fed 0.25 gm. desiccated thyroid daily. He stated that in normal rats 90 per cent of parturition occurs at 21.5 and 22 days but one of these hyperthyroid animals delivered on the twenty-third day, three on the twenty-fourth, three on the twenty-fifth, and only one on the twenty-first day of gestation. He believed this prolongation to be due to inability of the rats to deliver rather than to their failure to come to full term at the normal time, possibly because of inability of the estrus-producing hormone to act on uterine muscles to produce the normal contractions of parturition. Possibly the increased metabolism is instrumental in eliminating this hormone. Three guinea pigs given 300 mg. thyroid daily from the tenth, the twentieth, and the thirtieth days of pregnancy were found by Carloni (1930) to have gestation periods of 61, 60, and 57 days, respectively. Young were born alive, and the litters of the first two animals showed no effects of the thyroid administration. The guinea pigs in the third litter were hyperactive. The length of pregnancy was 55 days, 51 days, and 53 days when 400, 500, and 600 mg., respectively, of thyroid were administered daily from the thirtieth day of gestation. The guinea pigs from mothers fed thyroid were longer and thinner than normal, and litters were all small.

Danforth and Loumos (1936) found that pregnant rats are more tolerant to desiccated thyroid than are non-pregnant rats. Danforth et al. (1937) administered large doses of estrone, estriol, emmenin, progesterone, and the anterior pituitary-like factor of pregnancy urine to female albino rats for a week and then fed 100 mg. of desiccated thyroid to each rat daily for two weeks. There was significantly less increase in the oxygen consumption rate in all these doubly-treated groups than in control rats receiving only thyroid. This inhibition of the metabolic effect of orally administered thyroid indicates the reason for the ability of pregnant rats to tolerate doses of desiccated thyroid which are toxic when fed to non-pregnant animals. Bodansky and Duff (1936) reported the remarkable tolerance to thyroxine of pregnant rats. The number of young per litter was approximately the same in treated and untreated animals. They found, however, that a greater incidence of still-births occurred in the thyroxine-treated rats than in the normal controls. They observed a normal gestation period in a considerable proportion of those rats treated with thyroxine. Normally 21.5 to 22 days is the gestation period in rats. They found, however, that two of their six untreated controls delivered on the twenty-third day and the rest by the twenty-fourth day. Of the thyroxine-treated rats, three delivered on the twenty-third day, two on the twenty-fourth day, and one on the twenty-sixth day. Since they had made no attempt to fix the actual insemination time, these results are questionable.

Weichert and Boyd (1934) began giving a daily dose of 0.5 gm. desiccated thyroid mixed to a thin paste with water and fed directly to the rat by a medicine dropper as soon as sperm were found in the vaginal smears indicating fertile copulation. Of 21 thyroid-fed animals used 10

had to be discarded due to resorption of embryos during the first half of pregnancy. They did not observe any prolonging of gestation, probably due to their general practice of discontinuing thyroid feeding some time before parturition.

The Influence of Variations in the Thyroid State on Parturition and Litter Performance

Normal Littering Responses

Rat litters normally are born in the late afternoon of the twentieth day of gestation or, more often, on the morning of the twenty-first day according to Hall and Kaan (1942). Blandau and Money (1943) reported that the average litter size for first litters in normal female rats was six with a range of three to 10.

Blandau and Soderwall (1941) made direct observations on parturition in normal rats. They found that the interval between delivery of the first and last members of a litter may vary considerably, even in animals bearing the same number of young. The largest number of pregnant mothers littered between 12 noon and 6 P. M. and delivery was complete in less than 70 minutes in the majority of cases. Sturman-Hulbe and Stone (1929) reported that the parturient rat normally manifests a strong nest building tendency closely associated with the birth and care of her young. This maternal nesting response lasts until the young begin to leave the nest at 17 to 20 days after parturition.

Crozier and Enzmann (1935) reported that mice giving birth to more young than they could adequately suckle killed a proportion of the litter. During pregnancy there was a permanent increase of the weight of the mother depending upon the size of the litter carried. After birth

of the litter, the mother killed off part of her litter on reaching the limits of her milk-producing capacity. Falconer (1947) also observed that the practice of infanticide seems to be rather widespread among mice. He found that individual weights in litters of normal mice at 12 days of age were greatest in litters of two, three, or perhaps four, gradually decreasing with increasing litter size, the reduction being approximately 0.075 gm. for each additional mouse in the litter.

Hypothyroidism

Nelson and Tobin (1937) found that 15 rats thyroidectomized during the last half of pregnancy delivered normal litters. This was also true of 10 rats thyroidectomized prior to the onset of pregnancy. Nojima (1933) reported an unusual result of thyroidectomy. He observed in rats that conception on the twenty-fourth to the thirty-fifth day after thyroidectomy produced 80 per cent of females in the litter, instead of the normal 48.5 per cent. He did not attempt an explanation. Folley et al. (1942) found that although thyroidectomized rats mated and delivered their young, they seldom began to rear their litters.

Hyperthyroidism

Weichert and Boyd (1934) observed that feeding of 0.5 gm. of desiccated thyroid daily to pregnant rats for as long as 18 days after fertilization resulted in failure of the animals to care for their young. Weichert (1930) also observed that hyperthyroid rats not only did not care for their young but also ate many of them. Kraatz (1939) reported a larger litter size in rats made slightly hyperthyroid for a short time. An average litter size of 13 resulted when adult female rats were treated with daily administration of 0.25 to 0.3 gm. of thyroid substance for

three to five days and then mated within four days to normal males as compared to an average litter size of nine in untreated littermates. An increase in the amount of thyroid given proved deleterious to reproduction.

The Influence of Variations in the Thyroid State on Mammary Gland Development and on Lactation

Transplacental and Transmammary Transmission of Thiouracil and of Thyroactive Substances

Several workers reported transplacental transmission of thiouracil. Williams (1944) found significant quantities of thiouracil in the fetuses of rats given 0.1 per cent thiouracil in their drinking water during gestation. Kauffman et al. (1948) presented evidence indicating that thyroid follicle and colloid formation began in the mouse on the sixteenth day of fetal life. They observed depressed colloid formation and inhibited follicle formation in the fetal thyroids when the mother had received feed containing 0.2 per cent thiouracil. These workers believed this effect to be due to placental transfer of thiouracil. Goldsmith et al. (1945) reported an enlargement of thyroids in fetuses from rats fed thiouracil attributed either to the result of passing of thiouracil, of the thyrotropic hormone, or of a combination of both across the placenta.

Tobin (1941) concluded that thyrotropin did not pass across the placenta since it is a very large protein molecule. He determined the number of implantation sites in pregnant rats by laparotomies on the tenth day of pregnancy and intraperitoneal injections of thyrotropic hormone were begun the next day. On the sixteenth day the animals were sacrificed and the number of living and dead embryos were counted. He found

that the percentage of living embryos was inversely proportional to the duration of the thyrotropin administration rather than to the dosage. Although the maternal thyroids showed histological signs of stimulation when the animals had been treated for two days or more, the thyroids of the embryos failed to show such signs. He attributed the embryonic deaths to secretion from the stimulated maternal thyroid.

Hughes (1944) observed both transplacental and transmammary effects of thiouracil. Thiouracil was administered in the drinking water of pregnant female rats beginning four to eight days before parturition and continued after the birth of the young. The young of thiouracil-treated mothers appeared to be normal but showed thyroid hyperplasia as early as one day of age and definitely retarded development at 10 days. Freiesleben and Kjerulf-Jensen (1946, 1947) also observed both transplacental and transmammary transmission of thiouracil derivatives. Fetuses of pregnant rats which were given a diet containing 4-n-propyl-2-thiouracil were mixed in the food of normal rats. This food-fetus mixture brought about thyroid hyperplasia in rats eating it. When 6-methyl-2-thiouracil was administered to suckling rats immediately after parturition, thyroid hyperplasia was found in their young within one week and a maximal hyperplasia at 12 to 14 days. Goldsmith et al. (1944) found that young rats, suckled by mothers maintained on a laboratory stock diet containing 0.5 per cent thiourea, had hyperemic and enlarged thyroids.

Williams et al. (1944b) reported that infant rats, whose only possible source of thiouracil was the mother's milk, showed a thiouracil concentration of 5.6 mg. per cent in the blood while that of the mother's blood was 12 mg. per cent. Williams et al. (1944a) reported that human

patients receiving from 0.2 to 1.2 gm. of thiouracil daily showed a thiouracil concentration in the blood varying from 0.8 to 6.4 mg. per cent and about three times that concentration in their milk.

Reineke and Turner (1944) conducted experiments to determine whether cows fed 'Protamone' for stimulating increased lactation would transmit any thyroidal substance in their milk. They fed guinea pigs milk from 'Protamone-fed' cows and found no changes in basal metabolic rates. In a similar experiment on goats thyroidectomized when young there was no improvement of the cretinism and no metabolic stimulation. However these animals responded markedly to 0.25 gm. 'Protamone' fed directly. Similarly, two human beings showed no elevation of basal metabolic rate as the result of daily consumption of one quart of milk from 'Protamone-fed' cows but did have significant elevation after receiving 0.5 to 1.0 grain of 'Protamone' per day orally. They concluded that the mammary gland does not permit passage of biologically detectable amounts of thyroidal hormone when 'Protamone' is fed to lactating cows. Lukacs (1930) found that six litters (51 rats) suckled by mothers made hyperthyroid by thyroid feeding gained only 46 to 69 per cent as much as did the young in four control litters (31 rats) suckled by normal mothers. Lukacs regarded this as evidence that the thyroid hormone produced in the maternal organism was secreted in the milk. This seems to be fallacious reasoning. The cause of the smaller weight gain observed in the young rats suckled by the hyperthyroid mothers probably was decreased lactation.

Evaluation of Normal Lactation

Falconer (1947) found that milk production of mice could be evaluated satisfactorily from measurements of litter growth. He used the 12-day

total weight of the litter suckled as a convenient measure of milk production. The weight was taken at this age since young mice begin to take solid food when their eyes open at about 13 to 15 days of age and thus 12 days after birth was considered to be the latest time at which a measurement would represent growth due only to milk consumed. To allow for difference of litter size he used an index of performance independent of the size of the litter suckled to express the milk production of a mouse. This index of performance was obtained by expressing the litter weight produced by a mouse during a particular lactation as a percentage of the average weight of litters of that number. Of course, the index measures only the response of a mouse to a standard stimulus and gives no indication of the maximum capacity of the mouse. Even with the indexes of performance it is uncertain whether the comparisons of the lactation of mice having different numbers of young are completely valid and whether real differences of performance will be detected.

Hypothyroidism

Several studies have been made concerning lactation in thyroidectomized animals. Grimmer (1918) reported a temporarily diminished milk secretion believed due to operative trauma following thyroidectomy in the goat. Graham (1934b) found that thyroidectomy of cows caused a marked lowering of milk fat secretion as well as a diminution in milk secretion but a similar decrease accompanied control operations. Dragstedt et al. (1934) reported that complete thyro-parathyroidectomy in dogs did not prevent the hypertrophy of the mammary glands during pregnancy or the secretion of milk after delivery provided tetany was controlled. Nelson and Tobin (1937) found that rats thyroidectomized during the last half of pregnancy suckled one or more litters successfully.

After thyroid removal during the last 10 days of pregnancy, guinea pigs lactated in a normal fashion.

Preheim (1940) found that thyroidectomy in rats reduced the growth of their offspring 12 per cent, indicating that lactation was affected. Davenport and Swingle (1927) found that thyrocauterized mothers lactated poorly. Karnofsky (1942) observed that the thyroid gland was not essential for lactation in the rat. However, thyroidectomy before conception, during gestation, or immediately after delivery was found to diminish lactation. Folley (1938) likewise found that removal of all the thyroparathyroid tissue from lactating rats caused an immediate marked decline in lactation as judged by growth rates of sucklings. When these operated rats were subsequently remated, parturition was normal but lactation was definitely subnormal. Folley et al. (1942) confirmed this work. They were, however, able to maintain lactation partially in thyroidectomized rats by injecting them daily with parathyroid extract. They concluded that the observed failure of lactation after thyroidectomy is due, at least in part, to parathyroid deficiency.

Smithcors and Leonard (1942) thyroidectomized normal immature male rats and observed an inhibition of mammary duct growth and a stimulation of alveolar development as compared with their normal controls. In young female rats Leonard and Reece (1941) observed a thickening of the mammary gland ducts and an increase of the number of lateral end buds after thyroidectomy. Nelson and Hickman (1937) also found that mammary development may occur in the absence of the thyroid. Mammary glands of thyroidectomized rats receiving estrone showed marked development as did normal controls receiving estrone.

Oddly enough Smithcors (1945) reported that thiouracil administered to normal or castrated male and female rats failed to produce the lobule-alveolar growth in the mammary glands similar to that obtained following thyroidectomy. Since the period of thiouracil treatment was for only 35 days he believed that possibly the period of treatment was not sufficiently long for the changes to occur. Meites and Turner (1947) administered 0.1 per cent thiouracil in the feed of young female rats for 24 days. They observed that this treatment reduced the lactogenic hormone content of the pituitary below that in the case of normal rats. When estrogen and thiouracil together were administered to rats for 10 days subsequent to a two-week preliminary treatment with thiouracil alone, the estrogen and thiouracil failed to maintain even the normal level of lactogenic hormone.

Trentin et al. (1948) reviewed the effects of thiouracil on reproduction and growth in mice and rats with special attention to mammary growth. They concluded that experimentally induced mild hyperthyroidism is conducive to enhanced mammary responsiveness in the mouse, whereas the opposite is true of hypothyroidism. In the rat, on the contrary, experimentally induced hypothyroidism is conducive to mammary gland growth and responsiveness. Work which these workers did confirmed these facts. They administered diethylstilbestrol to one group of castrate male rats and thiouracil and diethylstilbestrol to another group of male castrates and then compared these groups with a group of control castrate male rats. Those receiving both thiouracil and stilbestrol showed extensive alveolar development with good duct extension and a striking advancement of mammary development as compared to the controls and a less marked improvement over those receiving only stilbestrol. They repeated the

experiment on intact male albino mice and detected no difference in the response to estrogen in the stilbestrol and thiouracil treated mice from those treated only with estrogen.

Hyperthyroidism

The influence of the thyroid gland on lactation has been clearly established by administration of thyroid hormone to pregnant and lactating animals. Weichert and Boyd (1934) observed that experimental hyperthyroidism in pregnant rats resulting from the feeding of 0.5 gm. desiccated thyroid daily starting on the first day of pregnancy caused earlier mammary development than occurred in controls. They also reported that the thyroid-fed rats had an earlier appearance of secretion in the alveoli than did the controls. While working with the lactating cow in declining lactation, Graham (1934a, 1934b) found that thyroxine injections or thyroid feeding quickly caused a marked temporary increase in milk fat production and a lesser increase in milk secretion to a higher level than previously after which the gradual fall, which is normal during this portion in the lactation curve, continued. During the early stages of lactation following parturition such treatment had no apparent effect on the amount of milk secreted. He also observed that feeding excessive quantities of thyroid to a thyroidectomized animal caused a diminution in milk secretion and an even greater diminution in fat secretion.

In the goat de Fremery (1936) found that low thyroxine dosage produced no demonstrable change in the milk output. On the other hand, high dosage of thyroxine, 15 mg. daily, caused severe hyperthyroidism with reduced milk output. Contrary results were reported by Reineke and Turner (1942). They fed thyrolactin, an artificially formed

thyroprotein, to goats in the declining stages of lactation at the rate of 5 to 10 gm. daily. There was an increase in milk production ranging from 0.8 to 40.8 per cent and averaging 10.51 per cent. An average increase in milk yield of 8.59 per cent occurred in cows fed 50 to 100 gm. of thyrolactin daily. There also was an average increase of 6.77 per cent in milk fat percentage and an increase of 13.9 per cent in fat yield.

Gardner (1942) fed small amounts of desiccated thyroid to intact male mice and reported that their mammary glands showed duct proliferation and hyperplastic end buds; no mammary growth showed in castrate male mice similarly treated. Mixner and Turner (1942) reported that an optimal dose of thyroxine increased by about 25 per cent the efficiency of minimal doses of progesterone and estrone in stimulating mammary lobule-alveolar growth in castrate female mice. On the other hand, thyroidectomy inhibited the ability of these mice to respond to progesterone and estrone.

Weichert et al. (1934) reported the initiation of mammary secretion in a normal virgin rat fed desiccated thyroid and occurring only during the pseudopregnant period induced by the experimental hyperthyroidism. Gudernatsch (1915a, 1915b) reported a decrease in milk output from feeding thyroid to rats. When rats were extremely hyperthyroid the young in their litters showed a diminished ability to grow and stayed behind the normally-fed rats of litters of corresponding age in relative size.

The subject of lactation was adequately reviewed by Folley (1940). He summarized the influence of the thyroid gland on lactation by stating that the thyroid gland is essential for normal lactation and that thyroxine stimulates milk secretion in the cow. In many species lactation can proceed in the absence of the thyroid, but only to a slight degree.

The Use of Thiouracil as a Goitrogen

History

Until recently it was necessary to perform thyroidectomy to produce experimental hypothyroidism. Kennedy (1942) reported that an attempt to isolate the goitrogenic substance in rape seed suggested that it might be a derivative of thiourea. Consequently he gave daily doses of 20 mg. allyl thiourea to rats for eight weeks. Upon examination these rats had thyroids three to four times the normal weight with the glands showing extreme hypertrophy and hyperplasia and almost complete lack of colloid. His experiments with thiourea produced similar results. Several different groups of workers reported experiments testing the effect of thiourea on the thyroid gland during the next year. The Mackenzies (1943) observed the production of a hyperemia and an enlargement of the thyroid with a reduction in colloid and an increase in the height of the thyroid epithelium accompanied by severely lowered basal metabolic rates in rats, mice, and dogs administered sulfonamides and thiourea. The reaction did not occur in chicks or guinea pigs. The effect of the drugs was not prevented by a number of dietary factors including liver, p-aminobenzoic acid, vitamin C, and iodine. However, thyroxine at proper dosage level prevented the effect entirely. Astwood *et al.* (1943) confirmed these results and reported that the goitrogenic activity of thiourea was higher than that of any of the sulfonamides tested. Williams and Bissell (1943) used thiouracil, a thiourea derivative, to treat thyrotoxicosis effectively.

Mode of Action

Thiouracil apparently lowers the basal metabolic rate by a decrease in the production of thyroid hormone in the same manner as do thiourea and sulfonamides. Glock (1949) fed 0.1 per cent thiouracil in the diet to a litter of puppies and to adult dogs. Although there were no outward manifestations of hypothyroidism in any of the dogs or puppies, their thyroids were considerably enlarged with marked hyperplasia and the loss of colloid generally was relatively small.

Many investigators have attempted to determine the mechanism of thiouracil action. Malkiel (1946) found that thiouracil does not inactivate preformed and circulating thyroxine and is not antagonistic to it. Rawson et al. (1944) found that thiouracil acts to block the usual easy and ready uptake of iodine by the thyroid, that is, thiouracil acts to interfere with the iodination of the thyroglobulin molecule preventing the elaboration of any physiologically active thyroid hormone. Astwood and Bissell (1944) found that the administration of thiouracil to young rats was followed by a nearly complete disappearance of iodine from the thyroid gland as well as by a threefold increase in the size of the gland. Franklin et al. (1944) showed that thiouracil fed to rats interfered with the incorporation of injected radioactive iodine into diiodotyrosine and thyroxine by their thyroid glands.

Jandorf and Williams (1944) found that subcutaneous injection of thyrotropic hormone into rats caused an increase in the rate of oxygen consumption of all organs studied and that especially liver and muscle showed increased metabolism. This effect was abolished by simultaneous treatment with thiouracil. The hyperplasia of the thyroid was greater when the two were given simultaneously than when either was given alone.

They concluded that this effect was due to the inhibition of thyroid hormone formation by the thiouracil. Himsworth (1943) also concluded that thiourea acts by interfering with the synthesis of the thyroid hormone. Keston et al. (1944) reported that analyses showed no appreciable amounts of radioactive iodine in any form in the thyroid glands of thiourea-treated rats.

Its Use in a Method for Assay of Thyroid Secretion Rate

Several workers have reported the use of thiouracil together with thyroxine as a method for assaying thyroid hormone secretion rate. Dempsey and Astwood (1943) reported using the method as a basis for an assay procedure for thyroid hormone. They found that maintenance or restoration of normal thyroid weight by simultaneous administration of 0.1 per cent thiouracil in the drinking water and of graded doses of thyroxine by injections to rats gave a measure of the amount of thyroid hormone secreted by the thyroid gland. At room temperatures averaging 25° C. a quantity of thyroid hormone equivalent to 5.2 ug. of thyroxine was required daily to maintain a thyroid of normal weight in young male rats. Reineke et al. (1945) used this method and found that, when young male albino rats were given 0.1 per cent thiouracil in their drinking water for two weeks, daily injections of 4.75 ug. of d,l-thyroxine returned their metabolic rate to normal and daily injections of approximately 4.8 ug. were required to return the thyroid weight to normal. Hurst and Turner (1947) determined the thyroid secretion rate in mice using the simultaneous thiouracil-thyroxine administration method of assay. They found that mature female mice kept at an average temperature of 80° F. produced the equivalent of 5.5 ug. of d,l-thyroxine per 100 gm. body weight daily.

Reineke and Turner (1945) determined the comparative thyroidal activity of l- and of d,l-thyroxine by their relative ability to reduce the thyroid weight of thiouracil-treated chicks and rats to normal. They found that l-thyroxine produced twice the effect of the racemic mixture in every species tested.

Advantages and Disadvantages

The effects of thiouracil and thyroidectomy were compared by Gordon et al. (1946). Both methods reduced food and water consumption and inhibited body growth of rats. In the thyroidectomized group these effects occurred more rapidly and were more pronounced. Oxygen consumption was depressed to approximately similar levels by both methods of thyroid ablation. Astwood (1943) tested the relative effectiveness of 106 chemical compounds in inhibiting the function of the thyroid. He found 2-thiouracil the most effective compound tested and stated that 3 to 4 mg. daily of thiouracil induced maximal responses in young rats.

There are several advantages in the use of thiouracil over thyroidectomy for producing hypothyroidism experimentally. Meyer et al. (1944) reported that thiouracil is relatively non-toxic. Krohn and White (1950) found that pharmacological suppression of the thyroid was more effective in causing a consistent depression of metabolic rate than was surgical ablation. Meyer and Ransom (1945) reported that thiouracil is more reliable than thyroidectomy in producing reduction in metabolic rate. Thiouracil acts on any thyroid tissue in the organism, irrespective of location, and thus is effective in animals which show no reduced basal metabolic rate after thyroidectomy due to the presence of accessory thyroid tissue. Chapman (1941) found that small amounts of thyroxine

apparently can be formed even in the complete absence of the thyroid gland. From his experimental data on thyroidectomized animals receiving an iodine supplement, he concluded that iodine may play a role in body metabolism in the absence of thyroid tissue, possibly by producing a thyroxine-like substance in the tissues.

Ely et al. (1948) studied the absorption and elimination of thiouracil in calves and goats receiving oral and subcutaneous doses of the drug. Maximum blood plasma thiouracil concentrations were reached four to eight hours after oral administration. Thiouracil was practically eliminated from the blood stream within 24 hours after cessation of administration. These results give both an advantage and a disadvantage of thiouracil suppression of the thyroid. It is advantageous that the blood plasma so quickly after oral administration has a maximum concentration of thiouracil acting to suppress further formation of thyroxine by the thyroid and it often is an advantage to be able to restore the experimental animals to normal. This is impossible with thyroidectomized animals except by constant thyroxine administration. One disadvantage of thiouracil is that it must be continuously administered to be effective. Barker (1949) observed in rats that even after 16 months of depressed thyroid function, withdrawal of thiouracil resulted in temporary renewal of growth, and reproductive activity was restored.

Hughes (1944) listed disadvantages of thiouracil administration by daily subcutaneous injection as the frequent handling necessary, the trauma incident to the daily injection, and the necessity for constant and continuous treatment. He noted that if growing animals stunted by prolonged treatment with the drug were accidentally without it for a day they would show a subsequent gain. Jones et al. (1946) concluded

as a result of their experimental observations that 100 days of treatment with thiouracil may be necessary to produce severe hypothyroidism in rats and that there may be only a borderline hypothyroidism if treatment with thiouracil has been for fewer than 100 days.

PROCEDURE

Female albino mice were used in this series of experiments. The 30 animals in the first experiment were from a group of mature mice averaging 33 gm. body weight and all of which previously had borne one or two litters. These mice originally were obtained from Rockland Farms, New City, N. Y., and had been maintained in this laboratory since reaching sexual maturity.

The 60 animals used in the second experiment were obtained especially for these experiments from Rockland Farms and averaged 20 gm. body weight at the time of their arrival. Since female mice are considered to approach sexual maturity at around 25 gm. body weight, this was the average body weight of the animals at the time the experiment was started.

The mice were fed a balanced stock diet* ad libitum, and drinking water was available at all times. They were kept in wire cages measuring $16\frac{1}{4}$ in. by $9\frac{3}{4}$ in. by 7 in. high with five mice per cage. The laboratory was maintained at a constant temperature of 75 degrees Fahrenheit throughout all of the experiments.

In the first experiment 30 mice were divided into six groups of five animals each and labeled as Groups A through F. In the second experiment 60 mice were divided into six groups of 10 animals each labeled as Groups G through L; each group of 10 animals was subdivided into two cages of five mice each. Each individual mouse in a group was identified by means of a system of marking with picramic acid.

In both experiments each mouse was weighed to the nearest tenth gm. once a week. A record of the food and water consumption for each

* - The stock diet consisted of ground or whole Purina Laboratory Chow.

group was maintained to the nearest gm. during the period previous to breeding. During the time records of intake were being made, the food was ground chow and was placed in special food-saving dishes to prevent wastage. These dishes were of metal with a sliding inverted-V-shape top in which there were eight small holes, four on each side, of a size such that the mice could get their heads in to get food but could not get their front paws in to scratch out the food. It was found that the mice wasted some food even from these dishes if they were filled completely. Consequently, at no time were they kept more than half full.

Experiment I was started on April 16, 1948, at which time the records of the 30 mice and of their food and water consumption were started. Beginning June 20, 1948, vaginal smears were taken on all 30 mice twice daily commencing at 8:30 A.M. and at 4:30 P.M. each day through July 22, 1948, 29 days comprising two weeks both before and after injections were started.

A glass medicine dropper pipette with its tip pulled out fine and fire-polished was used for taking the smears. A small amount of distilled water was drawn into the medicine dropper, washed in and out of the vagina, and transferred to a slide to dry. The slides were flamed slightly to fix the smear. Staining was accomplished by means of immersion in 0.1 per cent methylene blue solution for two minutes, after which the slides were washed with distilled water, dried, and examined at a magnification of 100 times.

Subcutaneous injections were started for each of the 30 mice on July 8, 1948, and were administered once daily throughout the remainder of the experiment. Each mouse received 0.1 ml. of solution in each injection. Group A was the control group; Group B was the thiouracil

group deprived of thyroid activity by virtue of a 0.1 per cent thiouracil solution received in place of their drinking water starting simultaneously with the injections and continuing for the remainder of the experiment. These two groups received daily injections of physiological saline (0.85 per cent).

The other four groups received varying daily doses of d,l-thyroxine solution as shown in Table I. A concentrated stock solution was made up using 70 mg. of d,l-thyroxine powder per 100 ml. of solution. The average body weight of the mice considered in making up each of the thyroxine solutions was 33 gm.

TABLE I Thyroxine Doses Used in Experiment I

Group	Rate	d,l-thyroxine used in the injections	
		Per 100 gm. body weight (in ug.)	Per each mouse (in ug.)
C	x	5.5	1.8
D	2x	11.0	3.6
E	4x	22.0	7.2
F	8x	44.0	14.4

Under rate in Table I let x stand for the rate of thyroxine secretion found by Hurst and Turner (1947) to be normal for albino mice; that is, 5.5 ug. of d,l-thyroxine per 100 gm. body weight.

After 14 days of injection males were placed with each of these six groups. One week later these males were replaced with different males. The second male was removed after a second seven-day period*. In both experiments each male placed with a thiouracil group was given a daily injection of thyroxine, x dose, to prevent his becoming hypothyroid from the thiouracil drinking water. Records of food and water consumption

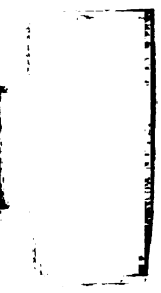
* - All the males used in both experiments were proved fertile.

were discontinued upon the placing of males with the groups. The special food-saving dishes were removed, and the mice received whole chow cubes. When the breeding period was over and the males were removed, each mouse was placed in an individual littering cage. These wire cages measured $4\frac{1}{4}$ in. by 7 in. by 5 in. high and were fitted with metal trays in the bottom to facilitate the preparation of nests. Shavings were furnished.

As soon as each litter was observed, each member of the litter was weighed to the nearest mg. The young were weighed again in this same manner at one week of age. At two weeks of age the young were weighed to the nearest tenth gm. The dates on which the young mice had complete hair coats and on which they had their eyes open were recorded. The litters were sexed as soon as a partial hair coat was present so that the teats of the females were perceptible and the females were marked with picramic acid. At three weeks of age the litters were weaned, and the mothers were sacrificed.

Females that did not litter and that obviously were not pregnant were sacrificed with ether and autopsied three weeks and one day after the second male had been removed from the groups.

Practically the same procedure was followed in Experiment II with the 60 younger mice. Vaginal smears were taken once daily during the four-week period from July 13, 1948, through August 11, 1948, commencing at 4:15 P.M. each day and comprising 30 days with two weeks before and two during injections. Food and water consumption were recorded during this time. The first males were with these groups for six days starting August 12, 1948. Males were rotated after six and 12 days, respectively. The last males were removed August 26, 1948, and the 60 mice were placed in individual cages for littering.



Daily subcutaneous injections were started July 29, 1948, two weeks after the experiment started, and were continued throughout the remainder of the experiment. The groups and the injections were as follows. As in Experiment I daily injections were 0.1 ml. in size. Group G was the control group and received exactly the same treatment as that of Group A. Group H was the hypothyroid thiouracil group and was treated in the same manner as Group B. The remaining four groups were treated with d,l-thyroxine as shown in Table II. A concentrated stock solution was prepared in the same manner as in the first experiment. The average weight of these mice used in preparing the thyroxine solutions for each of the groups was 25gm.

TABLE II Thyroxine Doses Used in Experiment II

Group	Rate	d,l-thyroxine used in the injections	
		Per 100 gm. body weight (in ug.)	Per each mouse (in ug.)
I	$\frac{1}{2}x$	2.75	0.7
J	x	5.5	1.4
K	$2x$	11.0	2.8
L	$4x$	22.0	5.6

As before, let x be the normal thyroid secretion rate as found by Hurst and Turner (1947).

Statistical methods used in all calculations are from Mode (1946) and Snedecor (1946). The values of t were compared for significance with the values in Table 3.8 (Snedecor, 1946).

RESULTS

General Appearance

The 30 mice in Experiment I showed more definite changes in physical appearance due to the experimental variations in the thyroid state than did the 60 younger mice in Experiment II. About 10 days after injections were started the animals in Group B, the thiouracil group, began to appear and feel greasy. Their hair coats became matted and dirty. The entire cage surface was greasy and dirty. Within five days these symptoms disappeared and the mice were clean and appeared no different from the controls.

The animals in Group C, x thyroxine mice, had the general appearance of good health. Their hair coat was very sleek, glossy, and clean. They were alert and active. Their general appearance seemed even better than that of the controls.

As the dose of thyroxine increased, its effects became more apparent. The animals receiving 2x thyroxine, Group D, became more excitable than usual. They were somewhat nervous and scratched in the litter more often than did the controls. They were more busy exploring their cage as well as more curious than usual and they often started immediately to crawl out of the cage when it was opened.

The Group E mice, 4x thyroxine, showed definite symptoms of hyperthyroidism. They breathed rapidly, almost panting, and were nervous and easily excited. Their eyes seemed quite bright, and their movements were very jerky. They were irritable and very apt to bite when being injected or when vaginal smears were being taken. They did not bite when being handled before injections were started.

The 8x thyroxine animals, Group F, evidently were suffering from thyrotoxicosis. Most of the time they made little excited chattering squeaks. Their movements were very nervous and jerky and they always were in motion while they were awake. They scratched nervously and often stuck their noses through the holes of the wire sides of their cages. Their eyes were bright and glassy. Their breathing was extremely rapid and shallow even while they were sleeping or resting. Their attitude while resting or sleeping was not that normally taken by mice. They stretched out completely with their paws extended instead of being hunched up with their paws under them as is usually the case.

These thyrotoxic symptoms increased throughout the period of thyroxine treatment. The thyrotoxicosis was fatal to all except one of the mice. One mouse in the group died nine days after treatment started. Another died after 15 days of thyroxine treatment. After 20 and 29 days of injections, respectively, two others died. The one remaining animal was sacrificed after 51 days of injections, this being 21 days after the breeding period ended. Before this mouse was sacrificed she had a very shabby, scraggly hair coat, especially around the face. She had protruding exophthalmic eyes. She lay resting in the cage looking very dejected with her ears laid back, her back hunched up a little, and her eyes open, panting and making no unnecessary movements. She was gradually losing weight. An autopsy showed that the animal had not been pregnant.

The thiouracil mice of Experiment II, Group H, exhibited a greasy appearance for several days approximately two weeks after the treatment was started. The thyroxine-treated animals in this second experiment did not show any definite changes in appearance.

Food and Water Intake

Records of food and water intake before and during injections are presented in detail for Experiments I and II in Appendix Tables I and II, respectively. Analysis of the records of food and water intake before the beginning of the injections shows some puzzling results. In both experiments there were initial differences between each of the groups and their control group. Many of these differences in Experiment I were significant at the one or five per cent levels of probability. The differences found in this regard in Experiment II were not significant.

A clue to these puzzling differences is found in the group mean weights at the beginning of the experiments. Although the mice in each experiment were chosen from animals of the same age and approximately the same weight, they were not grouped to give exactly the same initial group mean weight. These mean weight differences were taken into account in the food and water intake data by determining the ratios between the mean food weights and mean water weights for each group and the group mean body weights of all animals in the group for the period under consideration. These ratios and the percentages of change in mean food and water intakes during injection from these intakes previous to injection are presented in Table III.

The Group C mice, x thyroxine, showed little change in food and water intake as a result of the injections. The animals in the thyrotoxic Group F, 8x thyroxine, showed both a decreased food and water intake.

The two intermediate thyroxine doses, 2x and 4x, given to Groups D and E, respectively, increased both food and water intake, with the

TABLE III Daily Food and Water Intake Calculated on Body Weight Basis

Group	Treatment	Food			Water		
		Before Injections (gm./100 gm.)	During Injections (gm./100 gm.)	Change (%)	Before Injections (gm./100 gm.)	During Injections (gm./100 gm.)	Change (%)
			Experiment I				
A	Control	11.6	13.8	+19	22.3	19.1	-14
B	Thiouracil	11.2	12.4	+11	25.5	17.6	-31
C	Thyroxine, x	13.3	13.0	-2	25.5	26.2	+3
D	Thyroxine, 2x	12.8	13.5	+5	21.2	23.0	+8
E	Thyroxine, 4x	12.9	14.8	+15	22.7	25.7	+13
F	Thyroxine, 8x	12.5	10.7	-14	23.8	23.0	-3
			Experiment II				
G	Control	13.6	15.2	+12	23.0	28.0	+22
H	Thiouracil	15.3	14.5	-5	22.5	18.4	-18
I	Thyroxine, ½x	14.1	14.9	+6	21.8	23.4	+7
J	Thyroxine, x	13.7	13.6	-1	22.8	20.7	-9
K	Thyroxine, 2x	13.2	15.3	+16	26.0	27.1	+4
L	Thyroxine, 4x	13.8	16.3	+18	20.8	25.8	+24

larger dose causing the greater increase. The animals in Experiment II receiving these same thyroxine doses, Groups K and L, showed corresponding results.

The smaller thyroxine doses in Experiment II, $\frac{1}{2}x$ and x, injected daily into Groups I and J, respectively, did not produce much change in food and water intake. The daily food and water intakes were slightly increased for Group I and slightly decreased for Group J.

Both control groups, A and G, showed increased food intake. Their water intakes during treatment differed. The Experiment I animals showed decreased water intake, while the mice in the second experiment had increased water intake.

The Experiment II thiouracil group, H, showed both decreased food and water intakes. Group B in Experiment I had decreased water intake but a very slightly increased food intake.

Body Weight

Only the weights of mice that littered were considered in determining the group mean weights for each week of both experiments, except that in Group F which had no litters, the weights of all animals in the group were averaged each week. Appendix Tables III, IV, V, VI, VII, and VIII, part A, summarize the mean weights of the animals in Experiment I and Appendix Tables IX, X, XI, XII, XIII, and XIV, part A, give the corresponding results for the Experiment II animals. Littering dates and the dates of last weighing are presented in part B of the same tables. Record of infanticide, death, or sacrifice is also found in these tables. All other mothers raised some or all of their young to two weeks of age and at that time their injections and weekly weighing were discontinued.

The relative weight changes of each of the groups in Experiments I and II are shown graphically in Figures I and II, respectively. The means for Groups F and L represent lessening numbers of animals as is indicated on the figures. Infanticide was the cause for this decrease in number of animals considered in the Group L means and death from thyrotoxicosis was the cause for it in Group F.

Figure I clearly shows the effect of the thyrotoxicosis of the 8x thyroxine dosage on the weight of the animals in Group F. After only two days of thyroxine treatment the mean weight of the group had started to drop and at no time afterward did the weight reach that of the beginning. The control group, A, showed an initial decrease in weight, possibly due to handling. As the animals grew accustomed to daily handling their weight began to rise. They showed a general increase in weight parallel with their gestation periods as did all the other groups which had litters, and a gradual slight decrease in weight following birth of their young, to a weight about five per cent above their starting weight. Groups B, C, D, and E, showed a permanent increase in weight after pregnancy which was greater than that of the control group. The x thyroxine group, C, gained weight even after parturition to levels above those reached during gestation as compared to decreases in weight during lactation evidenced by Groups A, B, and D, and a slight increase at a lower level by Group E.

Examination of Figure II shows that all groups in the second experiment had terminal weights 40 to 55 per cent higher than their original weights. Groups H and K, thiouracil and 2x thyroxine, respectively, showed slight increases in weight during lactation but their weights were below those recorded during gestation. All other groups showed slight decreases in weight during lactation.

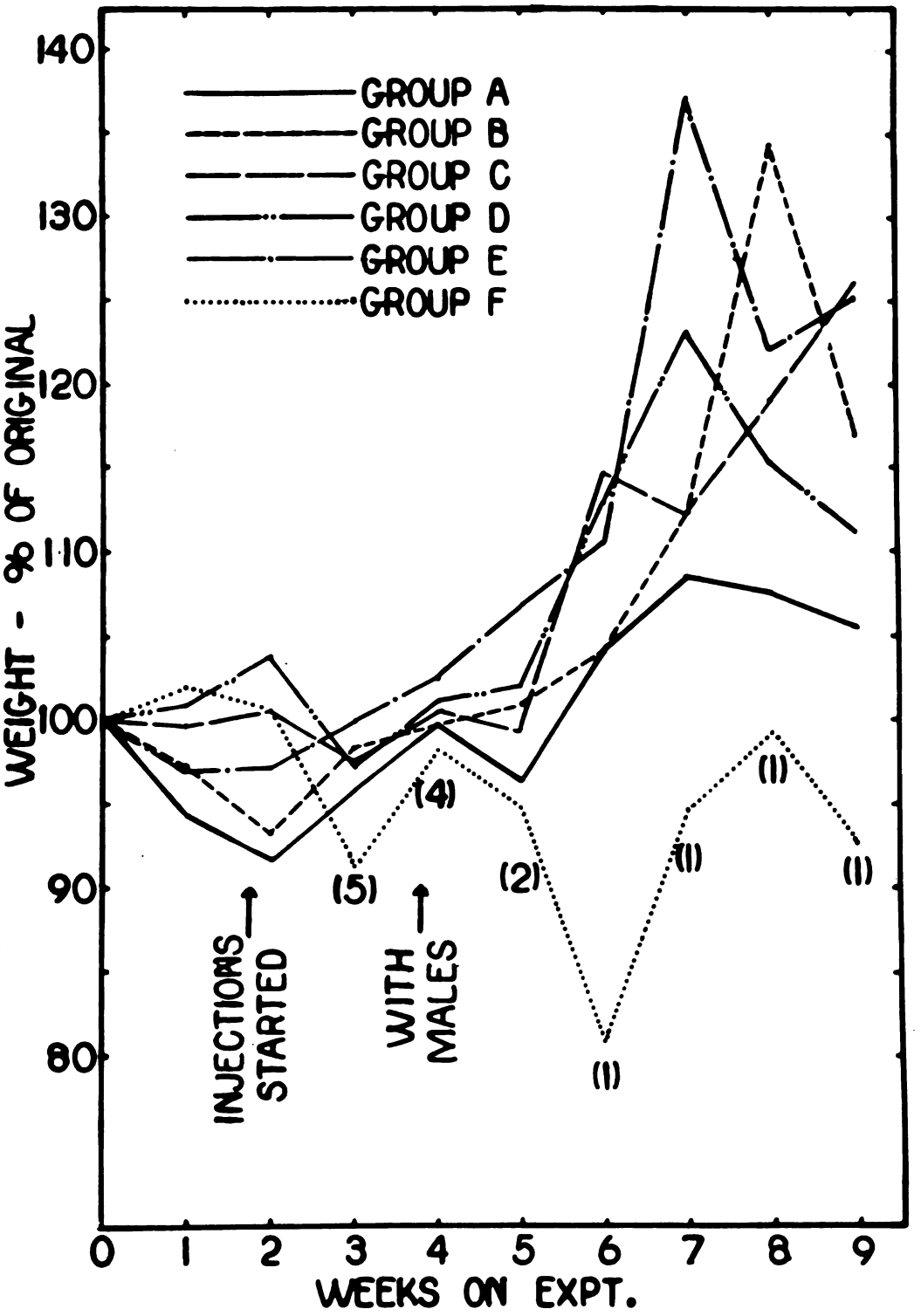


FIGURE I Percentage Weight Comparisons, Experiment I

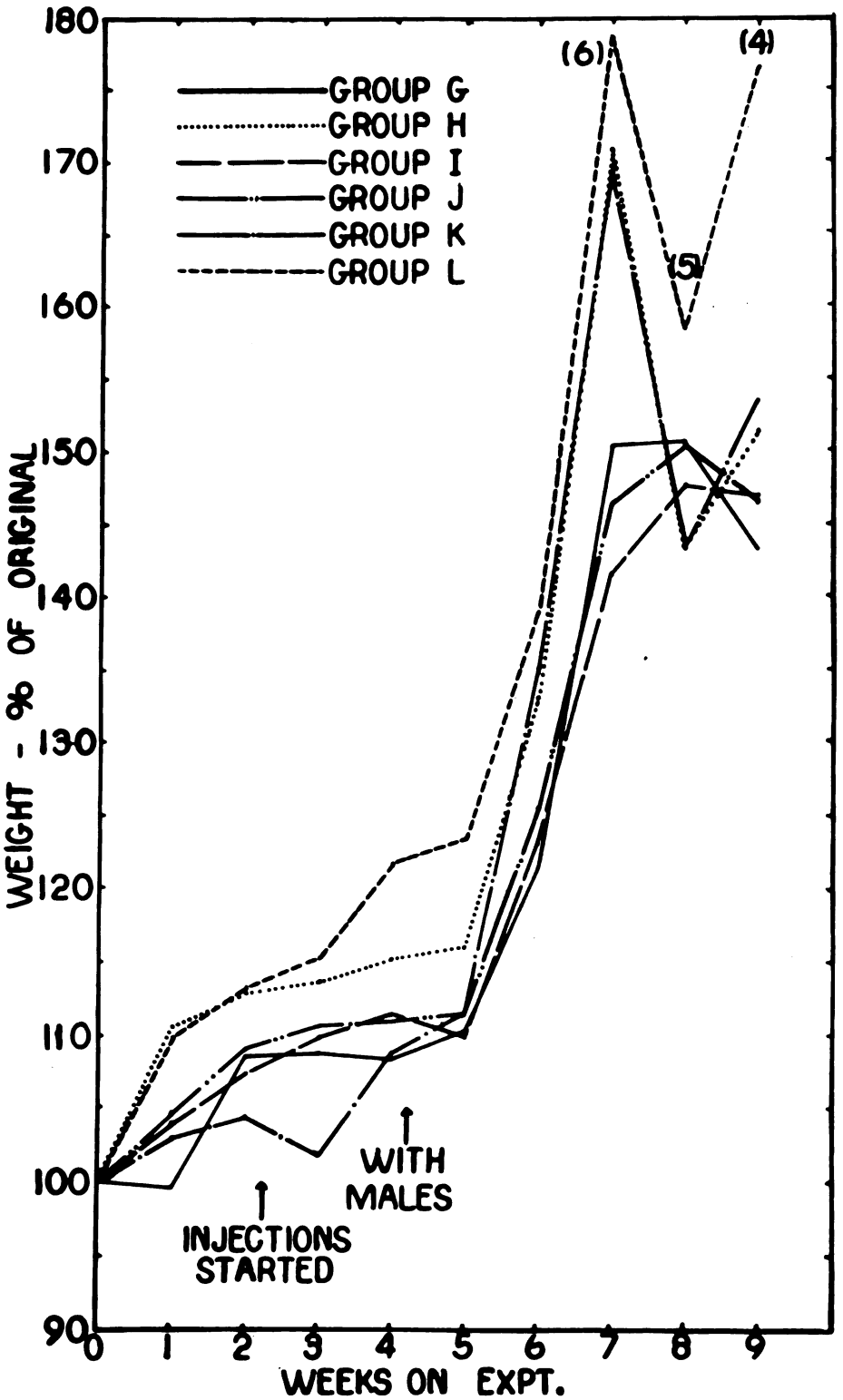


FIGURE II Percentage Weight Comparisons, Experiment II

Estrous Cycles

The vaginal smears were rated carefully as to the stage in the estrous cycle evident in each. The number of days between the peaks of the estrous stages both before and during injections and the mean cycle length for each mouse are presented in Appendix Tables XV and XVI for Experiments I and II, respectively. The average lengths of estrous cycles for all groups before and during injection are summarized in Table IV.

The analyses of estrous cycle lengths for Experiment I suggest that hypothyroidism in mice may lengthen estrous cycle and hyperthyroidism may decrease the length of estrous cycles. The control group and the x thyroxine group showed no change in length of cycle. The 2x, 4x, and 8x thyroxine groups had a progressive decrease in cycle length. The thiouracil group exhibited a greatly lengthened estrous cycle.

Gestation

It is almost impossible from these experiments to state whether or not the length of gestation in albino mice is affected by changes in the thyroid state. Since no definite copulation plugs were observed and vaginal smears were discontinued as soon as males were with the females for mating, it is impossible to state with any certainty when fertilization occurred. Appendix Tables XVII and XVIII summarize detailed data concerning birth of litters for Experiments I and II, respectively.

To attempt to estimate the length of gestation in each mouse, the average estrous cycle length for mice in her group, or a multiple of this length, was subtracted from the number of days elapsing between the last recorded estrus and the date of littering. These estimated

TABLE IV Estrous Cycle Length

Group	Treatment	Mean Length of Estrous Cycles Before Injections (days)	During Injections (days)	Change (%)
		Experiment I		
A	Control	5.7	5.8	+2
B	Thiouracil	5.0	8.8	+76
C	Thyroxine, x	6.3	6.5	+3
D	Thyroxine, 2x	6.0	5.5	-8
E	Thyroxine, 4x	7.7	6.0	-22
F	Thyroxine, 8x	7.0	6.0	-14
		Experiment II		
G	Control	6.2	6.8	+10
H	Thiouracil	6.2	6.0	-3
I	Thyroxine, ½x	6.6	5.8	-12
J	Thyroxine, x	6.1	6.2	+2
K	Thyroxine, 2x	5.4	6.1	+13
L	Thyroxine, 4x	5.6	5.3	-5

gestation periods also are shown in Appendix Tables XVII and XVIII. Mean lengths by groups of the estimated gestation periods are included. All of these estimated periods range from 17 to 22 days in length with the majority 18 to 20 days in length. Group mean lengths of estimated gestation range from 18.5 to 20.4 days.

Animals which did not litter were autopsied in an attempt to determine whether fertilization and implantation had occurred with resorption of fetuses interrupting gestation. None of the autopsied animals in Experiment I showed any evidences that fertilization had occurred. The one 8x thyroxine animal, Group F, which survived apparently had not mated.

Since the mice in Experiment II never had been mated previous to their use in this experiment, uteri normally were small if there had been no gestation. If the uterus of any autopsied animal of this experiment was larger, it was considered evidence of possible interrupted gestation. Animals J3 and J4, mice that received x thyroxine doses, K'2, a mouse that received 2x thyroxine, and two animals that received 4x thyroxine, L1 and L'1, had large uteri and may have had interrupted pregnancies. A control animal, G1, and a thiouracil animal, H'1, also had rather large uteri. No uterus which was examined was inflamed. It is impossible to state with any certainty that pregnancy had occurred in any of these animals.

Litter Weight and Size

Data concerning litter weight and size are presented in Appendix Tables XIX and XX for Experiments I and II, respectively. These data are summarized in Tables V and VI. In Experiment I litters from the control group averaged seven young at birth; the thiouracil group, eight

TABLE V

Summary of Litter Data--Experiment I

Group	Treatment	No. of Litters	Litter Size			Individual Weight			Total Litter Weight		
			Mean	S. E.	t	Mean (gm.)	S. E. (gm.)	t	Mean	S. E.	t
					At Birth						
A	Control	4	7.0	1.3		1.538	0.037		10.763	2.298	
B	Thiouracil	4	8.5	1.2	0.847	1.565	0.030	0.567	13.300	1.458	0.932
C	Thyroxine, x	5	10.4	1.4	1.780	1.534	0.028	0.086	15.950	1.547	1.873
D	Thyroxine, 2x	3	11.0	1.5	2.010	1.453	0.027	1.852	15.980	3.030	1.373
E	Thyroxine, 4x	2	6.5	1.5	0.251	1.656	0.039	2.199	10.764	2.044	0.000
F	Thyroxine, 8x	0	---	---	---	---	---	---	---	---	---
					At 7 Days of Age						
A	Control	3	4.7	1.9		3.820	0.156		17.829	7.715	
B	Thiouracil	3**	6.5	0.5	0.918	3.428	0.242	1.364	22.280	6.953	0.428
C	Thyroxine, x	5	9.4	1.2	2.088	3.407	0.113	2.145	32.028	3.652	1.663
D	Thyroxine, 2x	3**	6.5	4.5	0.369	3.500	0.082	1.822	22.747	16.537	0.269
E	Thyroxine, 4x	2	6.5	1.5	0.744	4.618	0.085	4.488*	30.014	5.804	1.263
					At 14 Days of Age						
A	Control	3	4.7	1.9		6.48	0.22		30.2	13.1	
B	Thiouracil	3	6.0	0.6	0.653	5.89	0.30	1.595	35.3	2.2	0.383
C	Thyroxine, x	4	9.2	1.2	2.000	5.72	0.15	2.825*	52.9	4.4	1.645
D	Thyroxine, 2x	3	6.3	2.6	0.498	5.43	0.28	2.964*	34.4	11.0	0.246
E	Thyroxine, 4x	2	6.5	1.5	0.744	6.72	0.19	0.814	43.7	6.5	0.925

* - Significant at the 5% level of probability

** - One litter not weighed

TABLE VI

Summary of Litter Data--Experiment II

Group	Treatment	No. of Litters	Litter Size			Individual Weight			Litter Weight		
			Mean	S. E.	t	Mean (gm.)	S. E. (gm.)	t	Mean (gm.)	S. E. (gm.)	t
					At Birth						
G	Control	5	8.0	0.5		1.411	0.027		11.290	0.288	
H	Thiouracil	5	7.8	1.1	0.165	1.478	0.033	1.566	11.527	1.263	0.184
I	Thyroxine, $\frac{1}{2}x$	7	7.4	0.5	0.845	1.507	0.019	2.909*	11.197	0.543	0.151
J	Thyroxine, x	6	6.7	1.0	1.161	1.548	0.029	3.452**	10.320	1.302	0.729
K	Thyroxine, $2x$	5	6.6	1.3	1.007	1.409	0.019	0.064	9.300	1.747	1.124
L	Thyroxine, $4x$	6	9.8	1.6	1.071	1.434	0.021	0.681	14.106	2.115	1.322
					At 7 Days of Age						
G	Control	5	6.4	0.7		3.618	0.126		23.152	2.469	
H	Thiouracil	5	7.8	1.1	1.077	3.187	0.141	2.281	24.859	1.221	0.621
I	Thyroxine, $\frac{1}{2}x$	7	7.1	0.4	0.864	4.137	0.067	5.633**	29.549	1.435	2.245*
J	Thyroxine, x	6	6.3	0.8	0.094	4.050	0.151	2.195	25.651	2.271	0.746
K	Thyroxine, $2x$	5	6.6	1.3	0.135	3.879	0.092	1.669	25.600	4.552	0.473
L	Thyroxine, $4x$	4***	10.0	0.6	3.913**	3.636	0.049	0.131	36.361	1.895	4.247**
					At 14 Days of Age						
G	Control	4	6.2	0.8		5.70	0.20		35.6	3.4	
H	Thiouracil	5	6.4	1.0	0.156	4.55	0.22	3.859**	29.1	3.9	2.407*
I	Thyroxine, $\frac{1}{2}x$	7	7.3	0.4	1.236	6.13	0.14	1.743	44.9	2.6	3.796**
J	Thyroxine, x	6	6.2	0.9	0.000	6.15	0.20	1.603	37.0	3.8	0.522
K	Thyroxine, $2x$	5	6.0	1.3	0.131	5.45	0.25	0.779	32.7	6.0	0.948
L	Thyroxine, $4x$	4	10.0	0.4	4.270**	4.86	0.15	3.318*	48.6	6.2	4.194**

* - Significant at the 5% level of probability
** - Significant at the 1% level of probability
*** - One litter not weighed

and a half; x thyroxine, 10.4; 2x thyroxine, 11; and 4x thyroxine, six and a half. In no case was the number significantly different from that of the control group. When comparisons were made between groups of the number of survivors at seven and at 14 days of age, there likewise were found to be no significant differences.

The mean litter size for the various groups in Experiment II at birth were eight for controls, 7.8 for thiouracil, 7.4 for $\frac{1}{2}$x thyroxine, 6.7 for x thyroxine, 6.6 for 2x thyroxine, and 9.8 for 4x thyroxine. There was no significant difference in litter size at birth between the control and any other group, but the 4x thyroxine group showed a highly significantly greater number of survivors at seven and at 14 days of age.

In both experiments the decreases in litter size at one and two weeks of age were due to infanticide. In many of the litters some of the young which were killed were partially or completely eaten by the mothers.

There were no significant differences in individual birth weights in Experiment I. At seven days of age the mean individual weight of young in Group E, 4x thyroxine, was significantly greater than that of the control group, A. At 14 days of age the mean individual weights of young in the x and 2x thyroxine groups, C and D, were significantly less than that of Group A. In experiment II the mean individual birth weight of the $\frac{1}{2}$x thyroxine group, I, was significantly greater and of the x thyroxine group, J, was highly significantly greater than that of the control group, G. At seven days of age the mean individual weight of young in the $\frac{1}{2}$x thyroxine group, I, was highly significantly greater

than that of the control group. At 14 days of age the mean individual weight of the thiouracil group litters was highly significantly lower and that of the 4x thyroxine group litters was significantly lower than that of the control group.

Since total litter weight reflects litter size and mean individual weight, variations are easily of large magnitude. In Experiment I the x thyroxine litters led the list in this measurement throughout the lactation period. The 4x thyroxine group also had large total litter weight values at seven and at 14 days of age. In no case were the differences significant. In the second experiment at seven days the $\frac{1}{2}$x thyroxine group had significantly greater and the 4x thyroxine group had highly significantly greater total litter weights than those of the control group. At 14 days the thiouracil group had significantly smaller total litter weights and the $\frac{1}{2}$x and 4x thyroxine groups had highly significantly greater total litter weights than those of the controls.

Nesting and Care of Litters

In Experiment I the mice in the x and 2x thyroxine groups were excellent mothers and the 4x thyroxine mice were good mothers. The mothers in these three groups manifested very strong nesting tendencies and formed large deep nests. The x thyroxine animals kept their nests quite clean by pushing feces and waste food out of the cage with a rooting procedure. The thiouracil-treated animals piled up fairly good nests. The control mice did not exhibit very strong nesting tendencies. Animals C3, C4, and E5 carried their young back to the nests in their mouths if any were out. Group C, x thyroxine, seemed to enjoy nursing and seemed quite relaxed. The animals in other groups did not stretch out completely relaxed while nursing.

In Experiment II very little difference in nesting tendency was noted. All groups seemed to contain equally good mothers. G2, a control animal, was nervous and restless and was a very poor mother. She ate six of her ten young. G'5 was a good mother. One of the x thyroxine animals, I'2 was an excellent mother. She kept her nest very clean and carried her young back if they were out of the nest. She also attempted to bite when her young were removed from the cage for weighing. Animal L3, a 4x thyroxine mouse, killed all of her young and ate some of them. One thiouracil mouse, H'4 had a very well-made neat nest.

Hair Coat and Opening of Eyes

In Experiment I the numbers of animals in each group having litters that were raised to weaning age were only two or three. Therefore, hair coat and eye opening were not recorded. Appendix Table XXI shows a record of the number of days of age at which hair coat became complete and at which all young in the litter had their eyes open for Experiment II. There were no significant differences apparent between each of the treated groups and the control group. The average age at which hair coat was complete was nine and a half days with a range of nine to 9.8 days. The average age for opening of eyes was 14.5 days with a range of 14.2 to 14.6 days.

Sex of Litters

The litters were sexed as soon as the teats of the females were perceptible. At three weeks of age the litters were separated from their mothers. The sexes of the young of each litter at weaning are given in Appendix Table XXII. The sexes of the young at weaning are presented by groups in Table VII. The mean sex ratio of all groups in

TABLE VII Sex Ratio at Weaning

Group	Treatment	Sex Distribution at Weaning		Sex Ratio	
		Males	Females	Males (%)	Females (%)
A	Control	9	5	64	36
B	Thiouracil	8	10	44	56
C	Thyroxine, x	13	24	35	65
D	Thyroxine, 2x	10	9	53	47
E	Thyroxine, 4x	7	6	54	46
Totals	Experiment I	47	54	46.5	53.5
G	Control	6	19	24	76
H	Thiouracil	19	18	51	49
I	Thyroxine, ½x	29	22	57	43
J	Thyroxine, x	18	20	47	53
K	Thyroxine, 2x	15	15	50	50
L	Thyroxine, 4x	19	20	49	51
Totals	Experiment II	106	114	48.2	51.8

Experiment I was 46.5 males to 53.5 females and of all groups in Experiment II was 48.2 males to 51.8 females weaned. The control group in the first experiment weaned 64.0 per cent males. In Experiment II the controls weaned 24.0 per cent males. This was the lowest ratio of males in both experiments. The $\frac{1}{2}$x thyroxine group had a higher ratio of males, 57.0 per cent, than any other group in the second experiment. The sex ratios indicate no definite trend or relationship between thyroid state and sex of litters.

Lactation

The data on lactation are given by individuals in Appendix Tables XXIII and XXIV. These data for Experiment II are summarized by groups in Table VIII. The total weight of mice in the litter at 14 days of age was used to measure the growth due to milk consumed. The total litter weight at 14 days of age was expressed as a percentage of the average total weight of all litters of that number at 14 days. This ratio is the index of performance.

Indexes of performance were not considered valid for litters in which the number of young at 14 days of age differed from the number at birth since some of the milk produced went to young which died or were killed and were not considered in the total litter weight at 14 days. The indexes of performance of the Experiment I animals are not summarized by groups because there were only seven animals which had the same numbers in their litters both at birth and at 14 days of age.

In the second experiment the index of performance for the thiouracil group was less than that of the controls. All four groups which received thyroxine had higher indexes of performance than did the controls. In no case was this difference significant.

TABLE VIII

Lactation

Group	Treatment	Number of Indexes	Index of Performance		t
			Mean (%)	S.E. (%)	
G	Control	4	93.7	3.8	
H	Thiouracil	5	83.6	8.6	1.069
I	Thyroxine, $\frac{1}{2}x$	7	106.6	5.1	2.028
J	Thyroxine, x	5	102.6	6.0	1.250
K	Thyroxine, $2x$	3	103.3	5.9	1.366
L	Thyroxine, $4x$	3	102.0	10.4	0.746

DISCUSSION

The more clear-cut changes in physical appearance due to experimental variations in the thyroid state exhibited by the 30 older mice used in the first experiment suggest declining thyroid secretion in mice with increasing age. Hill (1948) found that the response to thyroid in rats normally increases with increasing age.

The excellent general appearance of the Group C, x thyroxine, mice indicate that this dosage is an optimal one for older mice. These mice showed better performances by most measures used than did the control animals. Larger doses of thyroxine gave increasing symptoms of hyperthyroidism, including thyrotoxicosis at the 8x dose of thyroxine. The sizes of the thyroxine doses used in the second experiment consequently were halved for the four thyroxine groups.

The results on food and water intake during injection shown in Table III in general parallel those of Soliman (1950) and those of Maqsood and Reineke (1950a). The x thyroxine group in Experiment I and the $\frac{1}{2}$x and x groups in Experiment II showed little change in food and water intake as a result of the injections. These facts indicate that the estimated thyroid secretion rate of 5.5 ug. per 100 gm. of body weight per day for mature female albino mice found by Hurst and Turner (1947) and used as the x thyroxine dose is near the normal thyroid secretion rate of the mice used in both experiments.

The increased food intakes exhibited by both control groups possibly may be explained by the increased handling after injections were begun. The apparent partial loss of appetite of the Group F, 8x thyroxine, mice shown by the reduced food and water intake is an

indication of the severity of the thyrotoxicosis. Thompson (1942) lists loss of appetite and loss of weight among the symptoms of crisis in toxic goiter.

Maqsood and Reineke (1950a) reported depression of both food and water consumption by thiouracil feeding and reported that food and water intake increased proportionately with graded increase in thyroprotein dosage. Gordon et al. (1946) reported reduced food and water consumption after thiouracil feeding. Soliman (1950) also found that food and water consumption were increased by thyroprotein administration while both were decreased by thiouracil administration. These results are confirmed by these experiments.

The rise in mean weight of all groups after gestation to a weight above their original weight agrees with the work of Crozier and Enzmann (1935) showing that pregnancy in mice results in a permanent increase in the weight of the mother. The very decided increases in weight of the Experiment II groups after gestation reflect the fact that these were the first pregnancies and the first litters for these mice.

The increase in weight during lactation exhibited by Group C, x thyroxine, Group E, 4x thyroxine, and Group K, 2x thyroxine, may be a characteristic associated with the increase in milk secretion resulting from the thyroxine injections although this is a quite variable response. This increase in milk secretion has been reported by Graham (1934a) to occur in cattle when the thyroxine is injected during the period of declining lactation but not if the treatment was given during the early stages of lactation following parturition.

The variations of mean estrous cycle length before injections, from 5.0 to 7.7 in the first experiment and from 5.4 to 6.6 in Experiment II

possibly may be explained by the mechanical stimulation of the vagina occurring when the smears were taken. Emery and Schwabe (1936) found irregular estrous cycles occurring in normal virgin rats as a result of frequent vaginal examination by the cotton swab method. They considered extreme care necessary in taking daily smears by the pipette and lavage method to avoid false smears from mechanical stimulation. The smaller vaginae of the mice made it extremely difficult to avoid some mechanical stimulation. Wade and Doisy (1935) found a progressive change in the vaginal smears of spayed rats smeared once, twice, or three times daily. The larger variation in mean cycle lengths in Experiment I may be explained by the fact that smears were taken twice daily in that experiment and only once daily in the second experiment. Allen (1921, 1922) observed that one daily examination was adequate for accumulation of data as to cycle length. He found the average deviation of the entire cycle in mice to be from four to six days. The groups in Experiment I averaged about six and a half days for cycle length before treatment started and the average for Experiment II was six days.

Another possible explanation of the larger variation in cycle lengths for the older mice used in the first experiment is their advanced age. Boughton and Stoland (1943) found that an increasing proportion of mature albino rats over 25 weeks of age failed to show evidences of heat on vaginal smears, and an ever larger proportion of those which did deviated from the four-day cycle observed in the majority of the rats below 25 weeks of age. Beyond 50 weeks of age the sequence of four-day cycles became increasingly irregular.

Since smears were taken for only two weeks before and two weeks after the beginning of experimental treatment the results on the estrous

cycle length are not conclusive. Lee (1925), Freedman et al. (1935), and Ross (1938) found an average lengthening of estrous cycles in thyroidectomized female rats due to a lengthened diestrus with the prolongation ranging from one to five days or with the duration increased from the normal four or five days to seven or eight days. Nelson (1948) and Mann (1945) both observed very irregular or greatly lengthened periods of estrus in rats given thiouracil. Krohn (1947) found that daily subcutaneous injections of propylthiouracil disturbed the normal estrous rhythm of mature albino mice, causing lengthening, irregularity, or complete disappearance of the cycles. The lengthening of mean cycles in the Group B thiouracil animals during treatment confirm these results.

The progressive decrease in estrous cycle length with increasing doses of thyroxine observed in Experiment I is contrary to the results reported by most investigators. Evans and Long (1921a) observed little disturbance of the normal four-day cycles of rats fed small amounts of fresh beef thyroid daily. With the administration of large amounts of thyroid they found the cycle was greatly lengthened or totally inhibited. Continuous diestrus in rats was maintained with 100 mg. of thyroid gland daily (Drill et al., 1943). Reiss and Pereny (1928) and Van Horn (1933) observed that the thyroid hormone inhibited heat and was antagonistic to the action of ovarian hormone.

In analyzing the effects of the varying doses of thyroxine used in these experiments on gestation it must be remembered that the thyroxine doses used throughout the two experiments were based on average weights of 33 gm. and 25 gm., respectively, and weights in both experiments increased permanently during gestation.

The duration of gestation in mice normally is 19 days, varying from 18 to 20 days (Parkes, 1926-1927, and Asdell, 1946). MacDowell et al. (1927) stated that most litters are born before the end of the nineteenth day and Enzmann et al. (1932) reported that the period of gestation in non-lactating mice is 20 days.

In these experiments the exact time of fertilization was not observed, but the estimated gestation periods ranged from 17 to 22 days in length with the majority between 18 to 20 days suggesting that there were no effects of variation in the thyroid state on gestation length in these experiments. There are many conflicting results in previous work. Ukita (1920) reported greatly lengthened gestation periods in thyroidectomized rabbits, but Krichesky (1935, 1939) found no alteration of gestation length in rabbits after thyroid removal. Nelson and Tobin (1937) found no harmful effects on pregnancy and parturition in rats and guinea pigs from thyroidectomy and Nojima (1933) found a slight tendency toward prolongation of the gestation period in thyroidectomized female rats which did not abort when pregnancy occurred.

Weichert (1930) observed a prolonged gestation period in pregnant rats fed desiccated thyroid daily. On the other hand, Weichert and Boyd (1934) observed no prolongation of gestation, possibly because they discontinued thyroid feeding some time before parturition. The normal duration of gestation in guinea pigs is about 67 to 68 days (Asdell, 1946) but Carloni (1930) observed gestation periods in guinea pigs of 51, 53, 55, 57, 60, and 61 days under different conditions of thyroid administration during pregnancy.

Many workers have reported resorption of embryos in rats or rabbits both from thiouracil feeding or thyroidectomy and from thyroxine

administration. Jones et al. (1946) reported resorption in rats after prolonged thiouracil administration of at least 100 days. If treatment were for less than 100 days previous to mating some rats did deliver normally. Krohn and White (1950) also found resorption of fetuses when rats were mated at least 54 days after starting thiouracil injections or at least 28 days after thyroidectomy. Chu (1945) found that thyroidectomy in the rabbit at an early stage of pregnancy caused resorption and abortion of the embryos, while still-born young were delivered if the operation was performed at a late stage of pregnancy. Possibly the fact that the Group B and Group H mice had received thiouracil in their drinking water for only two weeks previous to mating explains the fact that there were no definite evidences of resorption for any of these mice.

Kunde et al. (1929) found resorption of fetuses in severely hyperthyroid rabbits and Bodansky and Duff (1936) found a greater incidence of still-births in thyroxine-treated rats than in normal controls.

The average number of young for first litters in normal rats is six with a range of three to 10 (Blandau and Money, 1943). Kraatz (1939) found an average litter size of 13 in rats made slightly hyperthyroid for a short time before mating as compared with an average size of nine in untreated littermates. In mice Asdell (1946) reported that mean litter sizes vary from four and a half to seven and a half with the second litter the largest and a steady decrease afterwards.

For both experiments the mean litter sizes at birth for each group showed no significant differences from the control groups. These experiments do not confirm the result of Kraatz (1939) that hyperthyroidism in rats increased average litter size at birth, possibly because thyroxine

dosage was continued throughout gestation in these experiments while it was discontinued after mating in his experiments.

The fact that the $\frac{1}{2}x$ and the x thyroxine dosages in the second experiment resulted in increased individual weights of young at birth shows that these administrations were beneficial to gestation. This effect did not carry through in the 14-day lactation. It is interesting to note that thiouracil and $4x$ thyroxine administration exerted parallel effects on lactation as observed from the lower mean individual weights of young at 14 days of age in both these groups.

Parkes (1926) found that the rate of growth of young albino mice during the suckling period was inversely proportional to the size of the litter. Falconer (1947) also observed that individual mouse weights at 12 days of age were greatest in small litters falling off gradually with increasing litter size with the reduction being about 0.075 grams for each additional mouse in the litter.

The average age for opening of the eyes of the young was 14.5 days and no differences appeared from the experimental variations in the thyroid state of the mothers. This age was within the normal age range for mice. Falconer (1947) stated that the eyes of young mice open from 13 to 15 days of age. The average age at which hair coat was observed to be complete was nine and a half days with no appreciable differences between groups. Many workers have reported transplacental and transmammary transmission of thiouracil. Transplacental transmission was observed in rats by Williams (1944) and Kauffman et al. (1948). Williams et al. (1944a, 1944b) found transmammary transmission of thiouracil in human patients and in rats, respectively. Hughes (1944)

and Freiesleben and Kjerulf-Jensen (1946, 1947) observed both transplacental and transmammary transmission of thiouracil. Under the conditions of these experiments transplacental and transmammary transmission of thiouracil are not demonstrated by these measures of physical development. The varying doses of thyroxine given the mothers are not reflected in the rate of hair coat growth or the age of opening of the eyes of their young. This is in agreement with the observations of Reineke and Turner (1944) that the mammary gland does not permit passage of biologically detectable amounts of thyroid hormone when thyroprotein is fed to lactating cows.

In rats conceiving 24 to 35 days after thyroidectomy Nojima (1933) observed 80 per cent of females in the litters instead of 48.5 per cent females as in litters of normal rats. Parkes (1926-1927) agrees with this ratio as normal for mice. He reported about 52 per cent males normally in mice.

It must be remembered that the sex ratios given in these experiments are for the young weaned. No records were made of the sex ratio at the time of sexing, and some of the young were killed by their mothers between this time and the weaning time. Some young likewise were killed at birth or before sexing was possible. The fact that the controls in the first experiment were 36 per cent females at weaning and the controls in the second experiment were 76 per cent females indicates that any conclusions drawn from these data as to the effect of variations in the thyroid state on the sex of mice litters would be invalid.

The practice of infanticide in mice at birth of the litter or during the suckling period was observed by both Crozier and Enzmann (1935) and Falconer (1947). This practice occurred whenever the mother

reached the limits of her lactating capacity. No attempts were made in these experiments to record infanticide at birth. In Experiment I both of the Group E, 4x thyroxine, animals which littered had the same number of young in their litter at 14 days as at birth. In each of the other groups some animals had the same number of young at 14 days as at birth and some did not. In the second experiment each group contained both animals without infanticide during the suckling period and animals with infanticide during suckling. These results do not indicate that infanticide among mice is made any more or any less prevalent as a result of changes in the thyroid state. The numbers of young in the two Group E litters were five and eight. Neither of these litters was large in comparison with the mean litter sizes in mice of four and a half to seven and a half reported by Asdell (1946), so the fact that there was no infanticide during suckling in these two litters does not necessarily signify that these mothers had a larger capacity for milk production.

There are many conflicting reports concerning the effects of hypo- and of hyperthyroidism on milk secretion. Nelson and Tobin (1937) found that thyroidectomized rats and guinea pigs lactated in a normal fashion. Contrary to this was the work of Preheim (1940), Davenport and Swingle (1927), and Folley (1938) who found poor lactation in thyroidectomized rats. Graham (1934a, 1934b) found temporary increases of milk fat production and milk secretion in lactating cows injected with thyroxine or fed thyroid during declining lactation but no such effect during the early stages of lactation following parturition. Excessive quantities of thyroid caused a diminution in both milk and fat secretion. Reineke and Turner (1942) observed the same general results

with goats as the experimental animals. On goats de Fremery (1936) observed contrary results. Low thyroxine dosage produced no change and high dosage reduced milk output.

The validity of the indexes of performance for the evaluation of lactation in these experiments is questionable since an insufficient number of animals were used. Means of 14-day litter weights were calculated from litters in all groups, regardless of treatment, having the same number of young. Consequently the group indexes of performance actually are compared to the mean for all groups rather than to the mean of the control group alone.

These indexes of performance do, however, indicate possible trends of decreased lactation due to thiouracil hypothyroidism and of increased lactation due to thyroxine administration provided the dosage is not sufficiently large to be thyrotoxic to the animal. The group mean litter weights also represent a measure of lactation performance. These values for Experiment II show trends similar to those indicated by the indexes of performance.

SUMMARY AND CONCLUSIONS

The effects of thiouracil-induced hypothyroidism and of varying degrees of hyperthyroidism produced by graded doses of thyroxine on the food and water consumption, body weight, estrous cycles, gestation, littering, and lactation in female albino mice were observed.

1. Food and water consumption were depressed by thiouracil feeding and increased proportionately with graded increase in thyroxine dosage, with the exception of dosage at the estimated level of eight times the normal thyroid secretion.
2. Dosage at the estimated level of eight times the normal thyroid secretion was thyrotoxic.
3. All mean group body weights were permanently increased after gestation.
4. Estrous cycles were not significantly altered by thiouracil or by the thyroxine dosages used in this experiment.
5. Dosage at the estimated level of the normal thyroid secretion and at half that level was beneficial to gestation in young mice having their first litter.
6. Thiouracil and thyroxine dose levels used in this experiment did not significantly alter the number of young in litters at birth.
7. Mice receiving thyroxine at the estimated level of four times the normal thyroid secretion showed a highly significantly greater number of survivors in their litters at seven and 14 days of age than did the controls.
8. The thiouracil administration used in this experiment did not significantly alter mean individual weights of the young at birth.

9. Thyroxine administration at the estimated level of the normal thyroid secretion and at half that level, respectively, produced highly significantly greater and significantly greater mean individual weights of the young at birth in young mice having their first litter.

10. Tendencies for decreased lactation with thiouracil administration and for increased lactation with thyroxine administration were observed.

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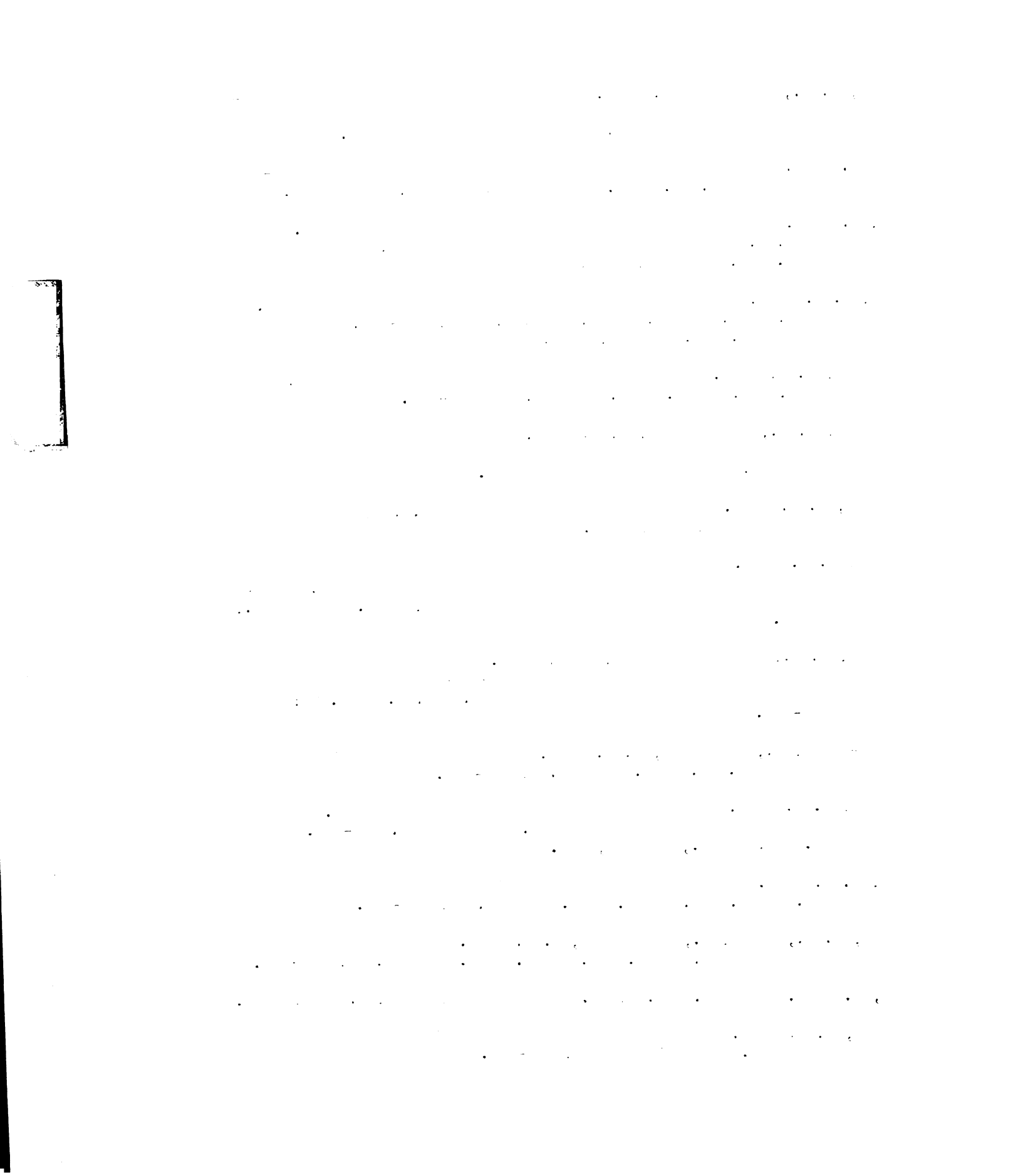

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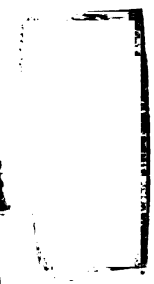
APPENDIX

APPENDIX

TABLE I Food and Water Intake--Experiment I

Group	Treatment	Previous to Injections: Days Averaged (no.)	Daily Consumption: Mean (gm.)	Std. Error (gm.)	t	During Injections: Days Averaged (no.)	Daily Consumption: Mean (gm.)	Std. Error (gm.)	t	Change (%)
					Food					
A	Control	8	17.12	0.48	--	9	19.33	0.76	--	+12.91
B	Thiouracil	8	18.38	1.05	1.091	14	18.86	1.24	0.324	+ 2.61
C	Thyroxine, x	8	23.38	0.63	7.944**	14	23.00	1.12	2.700*	- 1.62
D	Thyroxine, 2x	8	22.00	0.87	4.934**	14	23.57	1.15	3.077**	+ 7.14
E	Thyroxine, 4x	8	21.50	0.46	6.586**	14	24.79	1.40	3.432**	+15.30
F	Thyroxine, 8x	8	23.12	0.74	6.787**	10	19.50	1.27	0.115	-15.66
					Water					
A	Control	19	32.89	1.42	--	9	26.78	1.61	--	-18.58
B	Thiouracil	19	41.79	1.55	4.236**	9	26.67	1.38	0.052	-36.18
C	Thyroxine, x	19	44.95	0.98	6.991**	14	46.43	2.08	7.466**	+ 3.29
D	Thyroxine, 2x	19	36.63	1.72	1.679	14	40.07	1.80	5.494**	+ 9.39
E	Thyroxine, 4x	19	37.84	1.28	2.590*	14	42.93	1.89	6.496**	+13.45
F	Thyroxine, 8x	19	44.00	1.62	5.163**	10	41.90	3.40	4.014**	- 4.77

* - Significant at 5% level of probability
** - Significant at 1% level of probability



APPENDIX

TABLE II — Food and Water Intake--Experiment II

Group	Treatment	Previous to Injections: Days Averaged (no.)	Previous to Injections: Daily Consumption Mean (gm.)	Previous to Injections: Daily Consumption Std. Error (gm.)	t	During Injections: Days Averaged (no.)	During Injections: Daily Consumption Mean (gm.)	During Injections: Daily Consumption Std. Error (gm.)	t	Change (%)
					Food					
G	Control	8	32.88	1.91	--	14	37.14	0.59	--	+12.96
H	Thiouracil	8	39.50	2.77	1.966	14	37.50	0.68	0.400	- 5.06
I	Thyroxine, $\frac{1}{2}x$	8	34.75	1.87	0.699	14	40.64	0.80	3.528**	+16.95
J	Thyroxine, x	8	35.50	1.12	1.182	14	35.86	0.74	1.350	+ 1.01
K	Thyroxine, $2x$	8	31.00	1.65	0.745	14	37.93	0.54	0.988	+22.35
L	Thyroxine, $4x$	8	34.12	1.71	0.484	14	44.79	1.25	5.516**	+31.27
					Water					
G	Control	14	55.57	3.49	--	14	68.50	2.83	--	+23.27
H	Thiouracil	14	58.14	1.41	0.683	14	47.36	1.63	6.477**	-18.54
I	Thyroxine, $\frac{1}{2}x$	10	53.60	3.12	0.421	14	63.79	2.53	1.240	+19.01
J	Thyroxine, x	14	59.00	3.35	0.709	14	54.14	2.44	3.842**	- 8.24
K	Thyroxine, $2x$	14	60.79	3.25	1.095	14	67.00	3.01	0.363	+10.22
L	Thyroxine, $4x$	14	51.43	1.77	1.058	14	70.79	3.19	0.537	+37.64

* - Significant at 5% level of probability
** - Significant at 1% level of probability

APPENDIX

TABLE III Weight and Littering Data--Group A, Control

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	Change (%)
June 26	0	4			31.42	1.59	
July 3	1	4			29.60	1.42	-5.79
July 10	2	4			28.78	1.10	-8.40
July 17	3	4			30.10	1.24	-4.20
July 24	4	4			31.35	1.03	-0.22
July 31	5	4	3		30.28	0.30	-3.63
Aug. 7	6	4	4		32.75	1.62	+4.23
Aug. 14	7	4	2	2	34.10	4.19	+8.53
Aug. 21	8	4	1	3	33.80	5.20	+7.57
Aug. 28	9	4		4	33.15	4.38	+5.51
Sept. 4	10	1		1	43.20	--	--
Sept. 11	11	1		1	41.40	--	--

B

Mouse	Date of Littering	Last Date Weighed
A1*	Aug. 15	Aug. 28
A2*	Aug. 22	Sept. 11
A3*	Aug. 14	Aug. 28
A4*	Aug. 14	Aug. 28 (Ate her young)
A5	--	July 17 (Died)

* - Only those that littered were used in determining mean weights.

APPENDIX

TABLE IV Weight and Littering Data
Group B, Thiouracil

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
June 26	0	4			33.60	1.83	1.187	
July 3	1	4			32.62	0.66	1.929	-2.92
July 10	2	4			31.30	0.73	1.909	-6.84
July 17	3	4			33.02	1.04	1.804	-1.73
July 24	4	4			33.50	1.06	1.455	-0.30
July 31	5	4			33.92	1.36	2.614*	+0.95
Aug. 7	6	4	4		34.95	1.61	0.963	+4.02
Aug. 14	7	4	4		37.78	2.20	0.778	+12.44
Aug. 21	8	4	3	1	45.22	3.94	1.750	+34.58
Aug. 28	9	3		3	39.37	1.39	1.354	+17.17
Sept. 4	10	3		3	37.47	0.29	--	--
Sept. 11	11	2		2	37.05	3.95	--	--

* - Significant at the 5% level of probability

B

Mouse	Date of Littering	Last Date Weighed
B1*	Aug. 22	Sept. 11
B2*	Aug. 24	Aug. 21 (Died)
B3*	Aug. 23	Sept. 11
B4	--	Aug. 21 (Sacrificed)
B5*	Aug. 21	Sept. 4

* - Only those that littered were used in determining mean weights.

APPENDIX

TABLE V — Weight and Littering Data
Group C, Thyroxine, x

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
June 26	0	5			35.26	1.06	2.010	
July 3	1	5			35.20	1.17	3.044*	-0.17
July 10	2	5			35.40	1.51	3.544**	+0.40
July 17	3	5			34.40	1.31	2.384*	-2.44
July 24	4	5			35.38	0.94	2.890*	+0.34
July 31	5	5	4		35.04	1.60	2.924*	-0.62
Aug. 7	6	5	5		40.46	1.82	3.164*	+14.75
Aug. 14	7	5	2	3	39.64	1.73	1.222	+12.42
Aug. 21	8	5		5	42.02	0.51	1.573	+19.17
Aug. 28	9	5		5	44.44	1.47	2.444*	+26.04
Sept. 4	10	1		1	52.40	--	--	--

* - Significant at the 5% level of probability
**- Significant at the 1% level of probability

B

Mouse	Date of Littering	Last Date Weighed
C1	Aug. 21	Sept. 4
C2	Aug. 18	Aug. 28 (Killed her young)
C3	Aug. 14	Aug. 28
C4	Aug. 12	Aug. 28
C5	Aug. 14	Aug. 28

All animals were used in determining mean weights.

APPENDIX

TABLE VI — Weight and Littering Data
Group D, Thyroxine, 2x

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
June 26	0	3			35.40	0.68	2.302	
July 3	1	3			35.70	1.25	3.224*	+0.85
July 10	2	3			36.77	0.78	5.925**	+3.87
July 17	3	3			34.40	0.76	2.956*	-2.82
July 24	4	3	1		35.77	0.74	3.485*	+1.04
July 31	5	3	2		36.13	0.70	7.681**	+2.06
Aug. 7	6	3	3		40.03	3.15	2.055	+13.08
Aug. 14	7	3	1	2	43.67	2.11	2.040	+23.36
Aug. 21	8	3		3	40.77	2.64	1.195	+15.17
Aug. 28	9	3		3	39.50	3.48	1.135	+11.58
Sept. 4	10	1		1	52.40	--	--	--

* - Significant at the 5% level of probability
** - Significant at the 1% level of probability

B

Mouse	Date of Littering	Last Date Weighed
D1	--	Aug. 28 (Sacrificed)
D2*	Aug. 11	Aug. 28
D3*	Aug. 21	Sept. 4
D4*	Aug. 14	Aug. 28
D5	--	July 17 (Died)

* - Only those that littered were used in determining mean weights.

APPENDIX

TABLE VII — Weight and Littering Data
Group E, Thyroxine, 4x

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
June 26	0	2			33.55	0.25	1.323	
July 3	1	2			32.60	0.40	2.034	-2.83
July 10	2	2			32.60	1.40	2.146	-2.83
July 17	3	2			33.55	1.25	1.959	0.00
July 24	4	2	1		34.45	0.45	2.758	+2.68
July 31	5	2	2		35.80	1.80	3.025*	+6.71
Aug. 7	6	2	2		37.10	6.00	0.700	+10.58
Aug. 14	7	2	1	1	46.05	6.05	1.624	+37.26
Aug. 21	8	2		2	41.00	0.00	1.385	+22.21
Aug. 28	9	2		2	42.00	0.18	2.016	+25.19

* - Significant at the 5% level of probability

B

Mouse	Date of Littering	Last Date Weighed
E1	--	Aug. 28 (Sacrificed)
E2	--	Aug. 28 (Sacrificed)
E3	--	July 23 (Died)
E4*	Aug. 15	Aug. 28
E5*	Aug. 11	Aug. 28

* - Only those that littered were used in determining mean weights

APPENDIX

TABLE VIII Weight and Littering Data
Group F, Thyroxine, 8x

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
June 26	0	5			36.26	2.10	1.838	
July 3	1	5			36.98	1.33	3.793**	+1.99
July 10	2	5			36.46	1.47	4.183**	+0.55
July 17	3	5			33.06	2.29	1.137	-8.82
July 24	4	4			35.55	2.18	1.742	-1.96
July 31	5	2			34.40	2.60	1.574	-5.13
Aug. 7	6	1			29.30	--	2.130	-19.19
Aug. 14	7	1			34.30	--	0.048	-5.40
Aug. 21	8	1			36.00	--	0.423	-0.72
Aug. 28	9	1			33.60	--	0.103	-7.34

** - Significant at the 1% level of probability

B

Mouse	Date of Littering	Last Date Weighed
F1	--	July 31 (Died)
F2	--	July 17 (Died)
F3	--	July 23 (Died)
F4	--	Aug. 28 (Sacrificed)
F5	--	July 23 (Died)

All animals living were used in determining mean weights

APPENDIX

TABLE IX Weight and Littering Data
Group G, Control

A

Date	Weeks on Expt.	Animals	Preg.	Littered	Weight		Change
					Mean	Std. Error	
		(no.)	(no.)	(no.)	(gm.)	(gm.)	(%)
July 13	0	5			22.52	1.42	
July 20	1	5			22.44	1.94	-0.36
July 27	2	5			24.44	1.10	+8.53
Aug. 3	3	5			24.46	1.10	+8.61
Aug. 10	4	5			24.42	1.29	+8.44
Aug. 17	5	5	3		24.84	1.10	+10.30
Aug. 24	6	5	5		27.34	0.92	+21.40
Aug. 31	7	5	5		33.84	1.85	+50.27
Sept. 7	8	5	2	3	33.92	2.62	+50.62
Sept. 14	9	5		5	32.30	0.43	+43.43
Sept. 21	10	1		1	34.50	--	--

B

Mouse	Date of Littering	Last Date Weighed
G1	--	Sept. 14 (Sacrificed)
G2*	Sept. 3	Sept. 14 (Died)
G3	--	Sept. 14 (Sacrificed)
G4	--	Sept. 14 (Sacrificed)
G5*	Sept. 10	Sept. 21
G'1	--	Sept. 14 (Sacrificed)
G'2*	Sept. 3	Sept. 14
G'3*	Sept. 9	Sept. 14 (killed her young and died)
G'4	--	Sept. 14 (Sacrificed)
G'5*	Sept. 5	Sept. 14

* - Only those animals that littered were used in determing mean weight

APPENDIX

TABLE X — Weight and Littering Data
Group H, Thiouracil

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
July 13	0	5			23.10	0.78	0.358	
July 20	1	5			25.54	0.74	1.493	+10.56
July 27	2	5			26.08	0.72	1.247	+12.90
Aug. 3	3	5			26.24	0.89	1.258	+13.59
Aug. 10	4	5			26.62	0.56	1.564	+15.24
Aug. 17	5	5	5		26.78	0.72	1.476	+15.93
Aug. 24	6	5	5		30.74	1.10	2.371*	+33.07
Aug. 31	7	5	5		39.46	2.33	1.889	+70.82
Sept. 7	8	5		5	33.14	0.51	0.292	+43.46
Sept. 14	9	5		5	34.96	0.92	2.619*	+51.34

* - Significant at the 5% level of probability

B

Mouse	Date of Littering	Last Date Weighed
H1	--	Sept. 14 (Sacrificed)
H2*	Sept. 5	Sept. 14
H3	--	Sept. 14 (Sacrificed)
H4	--	Sept. 7 (Died)
H5*	Sept. 1	Sept. 14
H'1	--	Sept. 14 (Sacrificed)
H'2*	Sept. 4	Sept. 14
H'3*	Sept. 3	Sept. 14 (Died)
H'4*	Sept. 3	Sept. 14
H'5	--	Sept. 14 (Sacrificed)

* - Only those animals that littered were used in determining mean weights

APPENDIX

TABLE XI Weight and Littering Data
Group I, Thyroxine, $\frac{1}{2}x$

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
July 13	0	7			25.31	0.74	1.742	
July 20	1	7			26.29	0.77	1.845	+3.87
July 27	2	7			27.17	0.71	2.085	+7.35
Aug. 3	3	7			27.77	0.60	2.642*	+9.72
Aug. 10	4	7			28.19	0.73	2.544*	+11.38
Aug. 17	5	7	3		27.80	0.67	2.298*	+9.84
Aug. 24	6	7	7		31.14	0.74	3.218**	+23.03
Aug. 31	7	7	6	1	35.84	1.58	0.822	+41.60
Sept. 7	8	7	2	5	37.36	1.50	1.139	+47.61
Sept. 14	9	7		7	37.16	0.93	4.743**	+46.82
Sept. 21	10	3		3	35.30	2.48	0.323	--

* - Significant at the 5% level of probability
** - Significant at the 1% level of probability

B

Mouse	Date of Littering	Last Date Weighed
I1	--	Sept. 14 (Sacrificed)
I2*	Sept. 9	Sept. 21
I3	--	Sept. 14 (Sacrificed)
I4*	Aug. 31	Sept. 14
I5*	Sept. 3	Sept. 14
I'1	--	Sept. 14 (Sacrificed)
I'2*	Sept. 6	Sept. 14
I'3*	Sept. 1	Sept. 14
I'4*	Sept. 10	Sept. 21
I'5*	Sept. 7	Sept. 21

* - Only those animals that littered were used in determining mean weights

APPENDIX

TABLE XII Weight and Littering Data
Group J, Thyroxine, x

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
July 13	0	6			24.93	0.99	1.392	
July 20	1	6			26.03	0.80	1.711	+4.41
July 27	2	6			26.90	0.74	1.856	+7.90
Aug. 3	3	6			27.55	0.58	2.485*	+10.51
Aug. 10	4	6			27.67	0.97	2.014	+10.99
Aug. 17	5	6	3		27.80	0.60	2.362*	+11.51
Aug. 24	6	6	6		31.28	0.71	3.390**	+25.47
Aug. 31	7	6	6		36.48	1.36	1.150	+46.33
Sept. 7	8	6	2	4	37.48	2.74	0.939	+50.34
Sept. 14	9	6		6	36.55	0.94	4.111**	+46.61
Sept. 21	10	3		3	40.37	0.64	--	--

* - Significant at the 5% level of probability
** - Significant at the 1% level of probability

B

Mouse	Date of Littering	Last Date Weighed
J1	--	Sept. 7 (Died)
J2*	Sept. 8	Sept. 21
J3	--	Sept. 14 (Sacrificed)
J4	--	Sept. 14 (Sacrificed)
J5*	Sept. 11	Sept. 21
J'1*	Sept. 7	Sept. 21
J'2*	Sept. 3	Sept. 14
J'3*	Sept. 3	Sept. 14
J'4*	Sept. 4	Sept. 14
J'5	--	Sept. 14 (Sacrificed)

* - Only those animals that littered were used in determining mean weights

APPENDIX

TABLE XIII Weight and Littering Data
Group K, Thyroxine, 2x

A

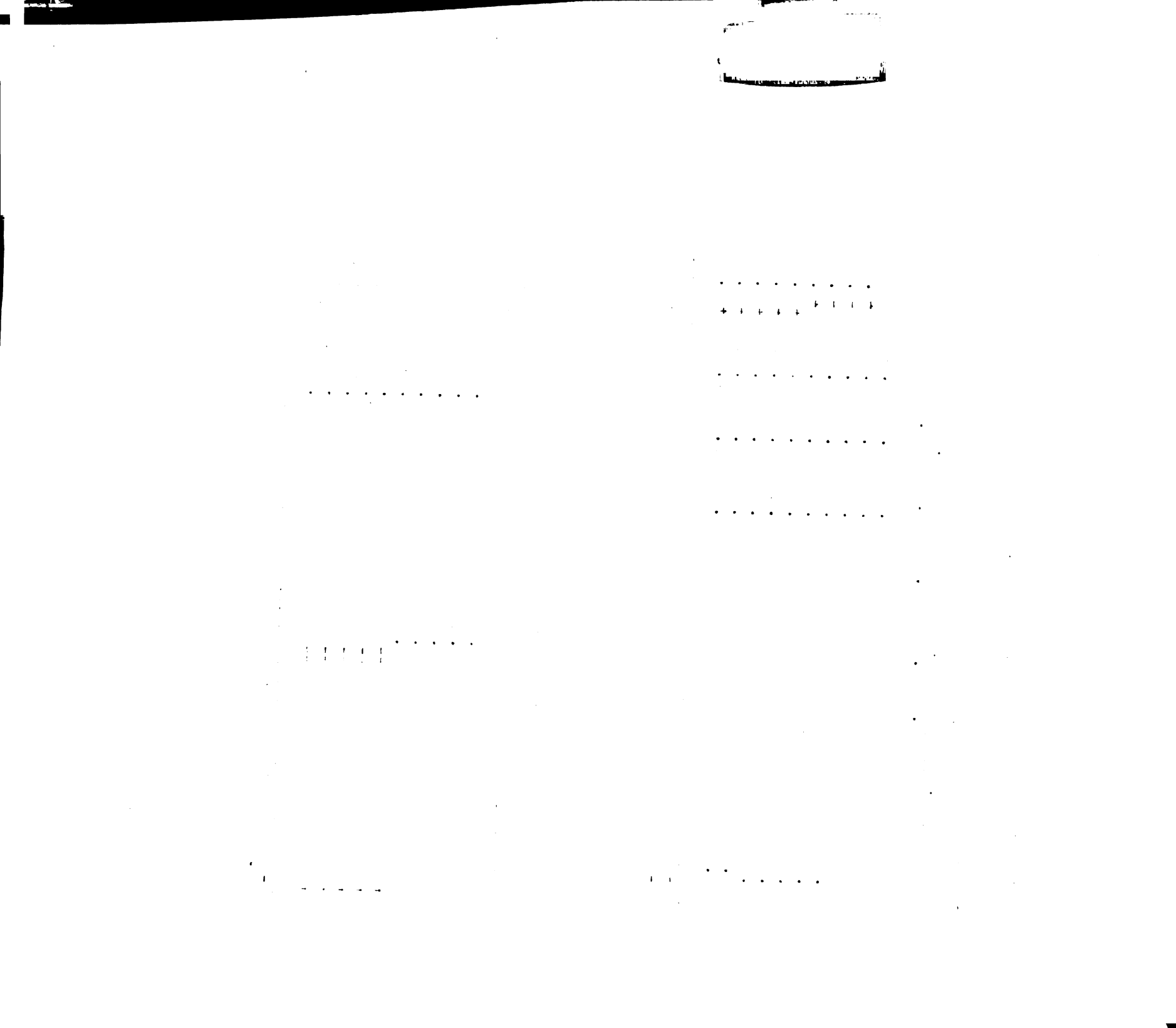
Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
July 13	0	5			23.62	0.43	0.741	
July 20	1	5			24.32	0.51	0.937	+2.96
July 27	2	5			24.62	0.57	0.145	+4.23
Aug. 3	3	5			24.04	0.50	0.348	+1.78
Aug. 10	4	5			25.72	0.58	0.919	+8.89
Aug. 17	5	5	5		26.34	0.63	1.183	+11.52
Aug. 24	6	5	5		31.92	0.54	4.293**	+35.14
Aug. 31	7	5	5		40.00	1.22	2.780*	+69.35
Sept. 7	8	5		5	33.84	0.76	0.029	+43.27
Sept. 14	9	5		5	36.32	1.14	3.299*	+53.77

* - Significant at the 5% level of probability
** - Significant at the 1% level of probability

B

Mouse	Date of Littering	Last Date Weighed
K1*	Sept. 4	Sept. 14
K2*	Sept. 1	Sept. 14
K3*	Sept. 3	Sept. 14
K4*	Sept. 3	Sept. 14
K5*	Sept. 1	Sept. 14
K'1	--	Sept. 7 (Died)
K'2	--	Sept. 14 (Sacrificed)
K'3	--	Sept. 14 (Sacrificed)
K'4	--	Sept. 14 (Sacrificed)
K'5	--	Sept. 14 (Sacrificed)

* - Only those animals that littered were used in determining mean weights



APPENDIX

TABLE XIV — Weight and Littering Data
Group L, Thyroxine, 4x

A

Date	Weeks on Expt.	Animals (no.)	Preg. (no.)	Littered (no.)	Weight Mean (gm.)	Weight Std. Error (gm.)	t	Change (%)
July 13	0	6			23.53	1.31	0.523	
July 20	1	6			25.87	1.13	1.528	+9.94
July 27	2	6			26.63	1.09	1.414	+13.17
Aug. 3	3	6			27.15	1.15	1.690	+15.38
Aug. 10	4	6			28.63	1.43	2.186	+21.67
Aug. 17	5	6	4		29.02	1.47	2.277*	+23.33
Aug. 24	6	6	6		32.68	2.12	2.311*	+38.89
Aug. 31	7	6	6		42.02	4.23	1.772	+78.58
Sept. 7	8	5	1	4	37.26	2.47	0.928	+58.35
Sept. 14	9	4		4	41.52	3.14	2.909*	+76.46

* - Significant at 5% level of probability

B

Mouse	Date of Littering	Last Date Weighed
L1	--	Sept. 14 (Sacrificed)
L2*	Sept. 3	Sept. 14
L3*	Aug. 31	Sept. 7 (Killed her young)
L4	--	Sept. 14 (Sacrificed)
L5	--	Sept. 14 (Sacrificed)
L'1*	Sept. 12	Sept. 14 (Killed her young)
L'2*	Sept. 6	Sept. 14
L'3*	Sept. 3	Sept. 14
L'4*	Sept. 4	Sept. 14
L'5	--	Sept. 14 (Sacrificed)

* - Only those animals that littered were used in determining mean weights

APPENDIX

TABLE XV Estrous Cycle Length as Measured Between Peaks of Estrus---Experiment I

Treatment and Animal No.	Time between Peaks		Mean Cycle Length	
	Before Injections (days)	During Injections (days)	Before Injections (days)	During Injections (days)
Control				
A1	3, 7	10, 4	5.0	7.0
A2	5, 5	8, 5	5.0	6.5
A3	3, 5, 4	5, 4, 6	4.0	5.0
A4	4, 5	6, 4, 4	4.5	4.7
A5*	10	8, 4	10.0	6.0
Thiouracil				
B1	2, 5, 5	7	4.0	7.0
B2	5, 5	6, 5, 5	5.0	5.3
B3	**	18	--	18.0
B4*	5	10, 6, 5	5.0	7.0
B5	6, 6	7, 6	6.0	6.5
Thyroxine, x				
C1	6	11, 5	6.0	8.0
C2	8	7, 6	8.0	6.5
C3	7	5, 5, 6	7.0	5.3
C4	8, 3	8, 6	5.5	7.0
C5	4, 6	6, 5	5.0	5.5
Thyroxine, 2x				
D1*	6	6, 5, 5	6.0	5.3
D2	4, 9	4, 4, 5	6.5	4.3
D3	5	6, 5	5.0	5.5
D4	4, 9	7, 5	6.5	6.0
D5*	6	8, 5	6.0	6.5
Thyroxine, 4x				
E1*	7, 6	7, 5	6.5	6.0
E2*	10	7, 5, 5	10.0	5.7
E3*	9	7, 5	9.0	6.0
E4	6	6, 5, 5	6.0	5.3
E5	7	5, 6, 7	7.0	6.0
Thyroxine, 8x				
F1*	**	7, 7	--	7.0
F2*	4, 6	5, 7	5.0	6.0
F3*	9	8, 3, 4, 2	9.0	4.2
F4*	8	10, 4, 6	8.0	6.7
F5*	6	6, 6, 7	6.0	6.3

* - Animal had no litter

** - Only one estrus during period of examination

APPENDIX

TABLE XVI Estrous Cycle Length as Measured Between Peaks of Estrus--Experiment II

Treatment and Animal No.	Time between Peaks		Mean Cycle Length	
	Before Injections (days)	During Injections (days)	Before Injections (days)	During Injections (days)
Control				
G1*	6	8	6.0	8.0
G2	5	11, 6	5.0	8.5
G3*	6, 5	5, 7, 4	5.5	5.3
G4*	7	6, 5, 7	7.0	6.0
G5	7	7, 5	7.0	6.0
G'1*	6, 5	4, 6	5.5	5.0
G'2	6	6, 6	6.0	6.0
G'3	7	9, 6	7.0	7.5
G'4*	7	8, 5, 5	7.0	6.0
G'5	6	9	6.0	9.0
Thiouracil				
H1*	6	3, 9	6.0	6.0
H2	5, 5	5, 6, 5	5.0	5.3
H3*	8	6, 7, 4	8.0	5.7
H4*	6	6, 6	6.0	6.0
H5	6	5, 7, 6	6.0	6.0
H'1*	5, 5	5, 5, 6	5.0	5.3
H'2	6, 5	5, 5, 6	5.5	5.3
H'3	4, 7	7, 6, 4	5.5	5.7
H'4	7	6, 10	7.0	8.0
H'5*	8	7, 7	8.0	7.0
Thyroxine, ½x				
I1*	5, 5	6, 5	5.0	5.5
I2	10	10, 4	10.0	7.0
I3*	7, 7	6, 5	7.0	5.5
I4	7	5, 4, 5	7.0	4.7
I5	10	5, 6	10.0	5.5
I'1*	5, 6, 4	6, 7	5.0	6.5
I'2	5, 8	7, 6	6.5	6.5
I'3	5	5, 6	5.0	5.5
I'4	5, 5	6, 5	5.0	5.5
I'5	5, 5	5, 6	5.0	5.5

APPENDIX

TABLE XVI (Continued)

Treatment and Animal No.	Time between Peaks		Mean Cycle Length	
	Before Injections (days)	During Injections (days)	Before Injections (days)	During Injections (days)
Thyroxine, x				
J1*	5, 5, 5	6, 7	5.0	6.5
J2	6, 7	6, 7	6.5	6.5
J3*	6, 5	5, 5	5.5	5.0
J4*	7	5, 5, 5	7.0	5.0
J5	6	9, 7	6.0	8.0
J'1	6, 5	5, 5, 5	5.5	5.0
J'2	7	11, 6	7.0	8.5
J'3	6, 7	6, 5	6.5	5.5
J'4	7	4, 5, 6	7.0	5.0
J'5*	5, 5	8, 7	5.0	7.5
Thyroxine, 2x				
K1	5, 6	5, 5, 5	5.5	5.0
K2	3, 5, 5	5, 7	4.3	6.0
K3	5, 5	8, 6	5.0	7.0
K4	5, 5	9	5.0	9.0
K5	6	7, 5, 4	6.0	5.3
K'1*	5, 6	7, 5	5.5	6.0
K'2*	4, 4, 5	6, 5	4.3	5.5
K'3*	7	8, 8	7.0	8.0
K'4*	6, 5	5, 7	5.5	6.0
K'5*	6	6, 3, 3, 2, 3	6.0	3.4
Thyroxine, 4x				
L1*	5	6, 7	5.0	6.5
L2	6	5, 5, 4	6.0	4.7
L3	5, 6	8, 5	5.5	6.5
L4*	4, 5	6, 6	4.5	6.0
L5*	4, 5	5, 4, 5	4.5	4.7
L'1	5, 5	6, 6	5.0	6.0
L'2	5, 5	5, 6, 4	5.0	5.0
L'3	7, 6	6, 5	6.5	5.5
L'4	7	9, 6	7.0	7.5
L'5*	7, 7	6, 6	7.0	6.0

* - Animal had no litter.

APPENDIX

TABLE XVII — Estimate of Length of Gestation--Experiment I

Treatment and Animal No.	Last Smear Stage	Mean Cycle Length (days)	Last Recorded Estrus	Date of Parturition	Elapsed Time between Last Recorded Estrus and Parturition (days)	Elapsed Time between First Opportunity to Mate and Parturition (days)	Estimated Length of Gestation** (days)	Mean Length (days)
Control								
A1	P or P+	5.8	July 20	Aug. 15	26	23	20	
A2	D?		July 19	Aug. 22	34	30	17	
A3	E+		July 22	Aug. 14	23	22*	22	20.0
A4	P?		July 18	Aug. 14	27	22*	21	
Thiouracil								
B1	P	8.8	July 16	Aug. 22	37	30	20	
B2	E+		July 22	Aug. 24	33	32	17	
B3	P		July 17	Aug. 23	37	31	20	19.8
B5	M		July 21	Aug. 21	31	29*	22	
Thyroxine, x								
C1	P	6.5	July 18	Aug. 21	34	29	21	
C2	P		July 19	Aug. 18	30	26	17	
C3	P+ or E-		July 22	Aug. 14	23	22	22	20.4
C4	E-		July 22	Aug. 12	21	20*	20	
C5	P		July 16	Aug. 14	29	22	22	
Thyroxine, 2x								
D2	M	5.5	July 22	Aug. 11	20	19*	19	
D3	D		July 20	Aug. 21	32	29	18	19.0
D4	D		July 19	Aug. 14	26	22	20	
Thyroxine, 4x								
E4	E	6.0	July 22	Aug. 15	24	23	18	
E5	E		July 22	Aug. 11	20	19*	19	18.5

* - First in the group to litter

** - Calculated by subtracting the average length of estrous cycle or multiplies thereof from elapsed time between last recorded estrus and parturition.

APPENDIX

TABLE XVIII — Estimate of Length of Gestation--Experiment II

Treatment and Animal No.	Last Smear Stage	Mean Cycle Length (days)	Last Recorded Estrus	Date of Parturition	Elapsed Time between Last Recorded Estrus and Parturition (days)	Elapsed Time between First Opportunity to Mate and Parturition (days)	Estimated Length of Gestation** (days)	Mean Length (days)
Control								
G2	E	6.8	Aug. 11	Sept. 3	23	22*	22	
G5	P		Aug. 7	Sept. 10	34	29	20	
G'2	D-		Aug. 8	Sept. 3	26	22*	18	19.6
G'3	D		Aug. 9	Sept. 9	31	28	18	
G'5	?		Aug. 4	Sept. 5	32	24	19	
Thiouracil								
H2	E+	6.0	Aug. 11	Sept. 5	25	24	19	
H5	E+		Aug. 11	Sept. 1	21	20*	20	
H'2	M-		Aug. 10	Sept. 4	25	23	19	20.4
H'3	E		Aug. 11	Sept. 3	23	22	22	
H'4	E+ or M-		Aug. 11	Sept. 3	23	22	22	
Thyroxine, $\frac{1}{2}$x								
I2	P?	5.8	Aug. 7	Sept. 9	33	28	21	
I4	M?		Aug. 10	Aug. 31	21	19*	19	
I5	D?		Aug. 9	Sept. 3	24	22	18	
I'2	M+?		Aug. 8	Sept. 6	29	25	17	19.6
I'3	P		Aug. 6	Sept. 1	26	20	20	
I'4	P		Aug. 7	Sept. 10	34	29	22	
I'5	P?		Aug. 6	Sept. 7	32	26	20	

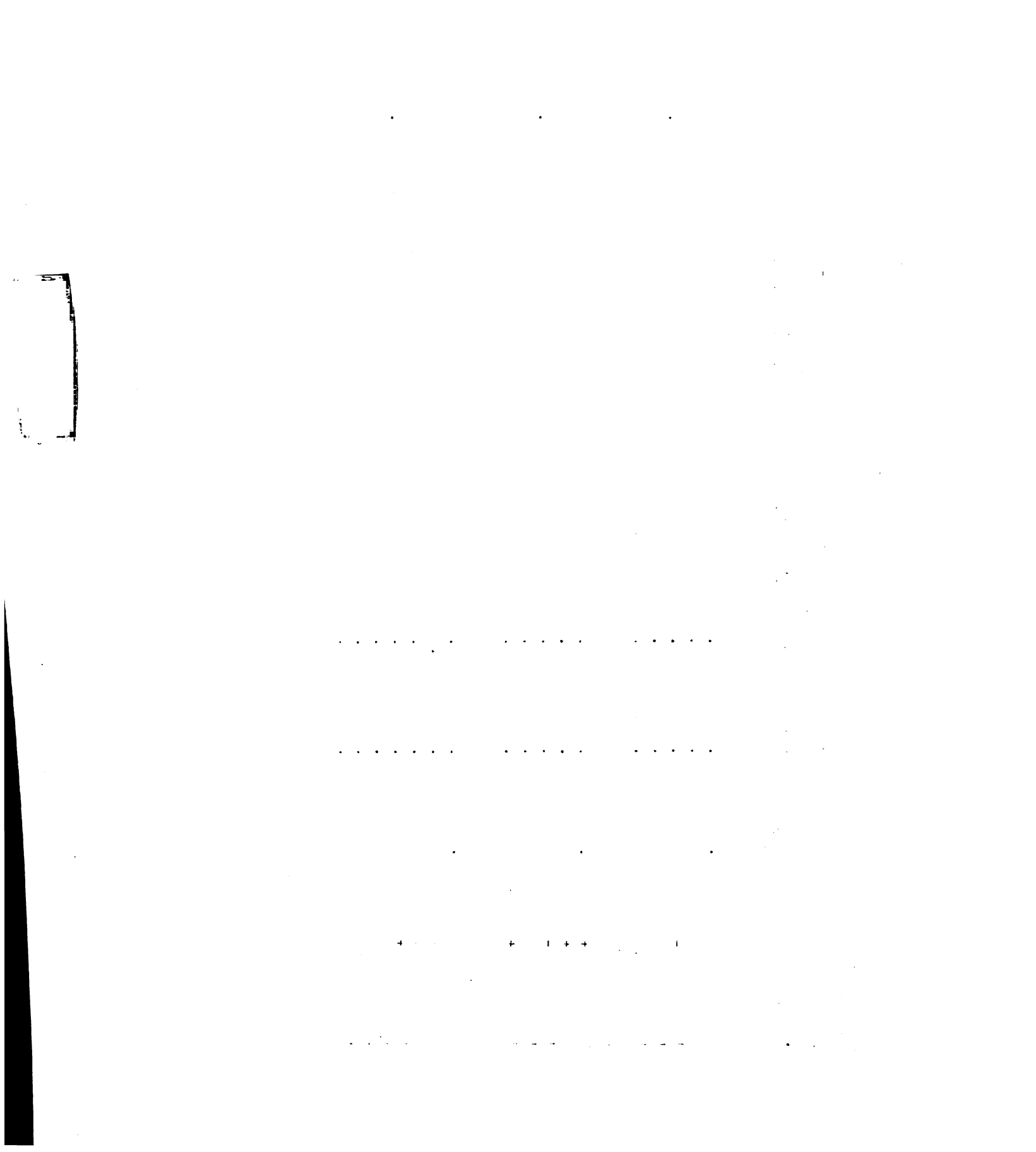


TABLE XVIII (Continued)

Treatment and Animal No.	Last Smear Stage	Mean Cycle Length (days)	Last Recorded Estrus	Date of Parturition	Elapsed Time between Last Recorded Estrus and Parturition (days)	Elapsed Time between First Opportunity to Mate and Parturition (days)	Estimated Length of Gestation** (days)	Mean Length (days)
Thyroxine, x								
J2	?	6.2	Aug. 9	Sept. 8	30	27	18	
J5	?		Aug. 5	Sept. 11	37	30	18	
J'1	E+ or M-		Aug. 11	Sept. 7	27	26	21	
J'2	?		Aug. 10	Sept. 3	24	22*	18	19.3
J'3	P		Aug. 7	Sept. 3	27	22*	21	
J'4	D		Aug. 9	Sept. 4	26	23	20	
Thyroxine, 2x								
K1	M?	6.1	Aug. 10	Sept. 4	25	23	19	
K2	?		Aug. 10	Sept. 1	22	20*	20	
K3	D?		Aug. 9	Sept. 3	25	22	19	19.2
K4	?		Aug. 4	Sept. 3	30	22	18	
K5	E		Aug. 11	Sept. 1	21	20*	20	
Thyroxine, 4x								
L2	P	5.3	Aug. 7	Sept. 3	27	22	22	
L3	P+		Aug. 7	Aug. 31	24	19*	19	
L'1	D		Aug. 9	Sept. 12	34	31	18	
L'2	E?		Aug. 11	Sept. 6	26	25	21	20.0
L'3	D+?		Aug. 8	Sept. 3	26	22	21	
L'4	E		Aug. 11	Sept. 4	24	23	19	

* - First in the group to litter

** - Calculated by subtracting the average length of estrous cycle or multiples thereof from elapsed time between last recorded estrus and parturition.

APPENDIX

TABLE XIX Individual-Litter Weight Data--Experiment I

Treatment and Animal No.	At Birth			At 7 Days of Age			At 14 Days of Age		
	No. in Litter	Weight		No. in Litter	Weight		No. in Litter	Weight	
		Mean (gm)	S. E. (gm.)		Mean (gm.)	S. E. (gm.)		Mean (gm.)	S. E. (gm.)
Control									
A1	8	1.674	0.040	7	3.699	0.012	7	6.51	1.84
A2	6	1.608	0.082	6	4.198	0.171	6	6.82	0.23
A3	4	1.202	0.038	1	2.405	--	1	4.20	--
A4	10	1.521	0.032	0	--	--	--	--	--
Thiouracil									
B1	7	1.793	0.044	7	4.176	0.118	7	5.07	0.09
B2	11	1.434	0.045	0	--	--	--	--	--
B3	6	1.582	0.033	6	2.554	0.054	6	5.23	0.12
B5	10	1.538	0.031	*	*	*	5	7.82	0.09
Thyroxine, x									
C1	12	1.527	0.043	12	3.535	0.097	12	5.35	0.20
C2	9	1.687	0.034	8	2.578	0.106	0	--	--
C3	14	1.441	0.028	12	2.773	0.075	10	4.96	0.10
C4	6	1.864	0.056	6	4.704	0.132	6	7.20	0.19
C5	11	1.354	0.045	9	3.956	0.126	9	6.08	0.15
Thyroxine, 2x									
D2	13	1.413	0.029	11	3.571	0.060	11	4.46	0.09
D3	8	1.587	0.049	*	*	*	6	6.87	0.03
D4	12	1.407	0.049	2	3.104	0.372	2	6.40	0.60
Thyroxine, 4x									
E4	8	1.601	0.046	8	4.477	0.096	8	6.28	0.13
E5	5	1.744	0.052	5	4.842	0.099	5	7.42	0.20

* - Litter not weighed

APPENDIX

TABLE XX Individual-Litter Weight Data--Experiment II

Treatment and Animal No.	At Birth			At 7 Days of Age			At 14 Days of Age		
	No. in Litter	Weight Mean (gm.)	Weight S. E. (gm.)	No. in Litter	Weight Mean (gm.)	Weight S. E. (gm.)	No. in Litter	Weight Mean (gm.)	Weight S. E. (gm.)
Control									
G2	10	1.156	0.029	4	3.850	0.176	4	6.58	0.45
G5	7	1.641	0.026	6	4.385	0.069	6	6.93	0.13
G'2	7	1.449	0.035	7	4.307	0.047	7	4.96	0.10
G'3	8	1.461	0.109	7	3.121	0.070	0	--	--
G'5	8	1.446	0.025	8	2.761	0.051	8	4.98	0.11
Thiouracil									
H2	8	1.681	0.032	8	3.303	0.051	8	4.30	0.09
H5	10	1.335	0.045	10	2.445	0.081	4	4.55	0.18
H'2	7	1.575	0.044	7	3.922	0.054	7	5.84	0.10
H'3	10	1.302	0.042	10	2.558	0.079	9	3.00	0.11
H'4	4	1.698	0.038	4	5.098	0.060	4	6.28	0.05
Thyroxine, $\frac{1}{2}$x									
I2	10	1.365	0.023	9	3.583	0.043	9	5.64	0.08
I4	7	1.542	0.042	7	3.633	0.069	7	4.63	0.07
I5	8	1.534	0.017	8	4.235	0.040	8	5.38	0.08
I'2	6	1.654	0.060	6	4.429	0.068	6	6.72	0.07
I'3	7	1.407	0.042	7*	4.183	0.082	7	6.50	0.15
I'4	6	1.682	0.029	6	5.048	0.037	6	8.07	0.04
I'5	8	1.474	0.014	8	4.166	0.045	8	6.52	0.09

TABLE XX (Continued)

Treatment and Animal No.	At Birth			At 7 Days of Age			At 14 Days of Age		
	No. in Litter	Weight Mean (gm.)	Weight S. E. (gm.)	No. in Litter	Weight Mean (gm.)	Weight S. E. (gm.)	No. in Litter	Weight Mean (gm.)	Weight S. E. (gm.)
Thyroxine, x									
J2	10	1.305	0.021	8	2.766	0.061	8	4.85	0.12
J5	8	1.646	0.031	8	4.089	0.063	8	6.39	0.07
J'1	8	1.495	0.031	8	3.584	0.053	8	5.50	0.12
J'2	6	1.743	0.035	6	4.799	0.132	6	6.40	0.46
J'3	3	1.658	0.026	3	5.721	0.081	3	8.67	0.07
J'4	5	1.661	0.070	5	4.890	0.191	4	7.35	0.22
Thyroxine, 2x									
K1	7	1.405	0.028	7	3.471	0.088	7	5.44	0.12
K2	6	1.430	0.024	6	4.274	0.074	6	6.70	0.11
K3	8	1.416	0.029	8	3.965	0.072	5	4.52	0.52
K4	2	1.566	0.226	2	4.846	0.929	2	7.50	3.00
K5	10	1.363	0.048	10	3.665	0.147	10	4.77	0.19
Thyroxine, 4x									
L2	15	1.329	0.036	11	3.501	0.104	10	5.00	0.11
L3	12	1.382	0.035	0	--	--	--	--	--
L'1	3	1.491	0.098	0	--	--	--	--	--
L'2	9	1.589	0.032	9	3.620	0.043	9	4.96	0.11
L'3	10	1.520	0.060	**	**	**	10	3.50	0.17
L'4	10	1.413	0.033	10	3.799	0.055	11***	5.90	0.12

* - One animal not weighed
** - Litter not weighed
*** - One extra weight recorded

APPENDIX

TABLE XXI — Opening of Eyes and Appearance of Complete Hair Coat

Group	Treatment	Age at Which Hair Coat Appeared Complete: Individual Litters (days)	Age at Which Hair Coat Appeared Complete: Mean (days)	Age at Which Litter Had Eyes Open: Individual Litters (days)	Age at Which Litter Had Eyes Open: Mean (days)
G	Control	10, 10, 9, 10	9.8	14, 14, 14, 15, 16	14.6
H	Thiouracil	9, 11, 8, 11, 10	9.8	15, 14, 14	14.2
I	Thyroxine, $\frac{1}{2}x$	11, 9, 8, 8, 9, 10, 10	9.3	15, 15, 14, 14, 15, 14, 14	14.4
J	Thyroxine, x	13, 10, 11, 8, 8, 8	9.7	16, 14, 15, 14, 14	14.6
K	Thyroxine, $2x$	9, 8, 10, 9, 9	9.0	15, 14, 15, 14, 15	14.6
L	Thyroxine, $4x$	10, 8, 10, 9	9.2	15, 14, 15, 14	14.5

APPENDIX

TABLE XXII Sex of Litters

Treatment and Animal No.	Sex at Weaning Males	Females	Treatment and Animal No.	Sex at Weaning Males	Females
Experiment I			**Experiment II**		
Control			Control		
A1	4	3	G2	2	2
A2	4	2	G5	0	6
A3	1	0	G'2	2	5
			G'5	2	6
Thiouracil			Thiouracil		
B1	4	3	H2	4	4
B3	2	4	H5	4	5
B5	2	3	H'2	4	3
			H'3	3	6
Thyroxine, x			H'4	4	0
C1	3	9			
C3	4	6			
C4	2	4	Thyroxine, $\frac{1}{2}$x		
C5	4	5	I2	5	4
			I4	2	5
Thyroxine, 2x			I5	6	2
D2	6	5	I'2	4	2
D3	3	3	I'3	3	4
D4	1	1	I'4	5	1
			I'5	4	4
Thyroxine, 4x					
E4	3	5	Thyroxine, x		
E5	4	1	J2	3	5
			J5	4	4
			J'1	4	4
			J'2	4	2
			J'3	0	3
			J'4	3	2
			Thyroxine, 2x		
			K1	2	5
			K2	4	2
			K3	1	4
			K4	1	1
			K5	7	3
			Thyroxine, 4x		
			L2	6	4
			L'2	4	5
			L'3	6	4
			L'4	3	7

APPENDIX

TABLE XXIII — Lactation

Mouse	Treatment	No. in Litter: Birth	No. in Litter: 14 Days	Litter Weight: Individ. (gm.)	Litter Weight: Mean* (gm.)	Index of Performance (%)
		Experiment I				
A3	Control	4	1	(4.2)	--	--
D4	Thyroxine, 2x	12	2	(12.8)	--	--
B5	Thiouracil	10	5	(39.1)		105
E5	Thyroxine, 4x	5	5	37.1	37.1	100
A2	Control	6	6	40.9		106
B3	Thiouracil	6	6	31.4		82
C4	Thyroxine, x	6	6	43.2		112
D3	Thyroxine, 2x	8	6	(41.2)	38.5	107
A1	Control	8	7	(45.6)		128
B1	Thiouracil	7	7	35.5	35.5	100
E4	Thyroxine, 4x	8	8	50.2	50.2	100
C5	Thyroxine, x	11	9	(54.7)	--	--
C3	Thyroxine, x	14	10	(49.6)	--	--
D2	Thyroxine, 2x	13	11	(49.1)	--	--
C1	Thyroxine, x	12	12	64.2	64.2	100
		Experiment II				
K4	Thyroxine, 2x	2	2	15.0	15.0	100
J'3	Thyroxine, x	3	3	26.0	26.0	100
G2	Control	10	4	(26.3)		105
H5	Thiouracil	10	4	(18.2)		72
H'4	Thiouracil	4	4	25.1		100
J'4	Thyroxine, x	5	4	(29.4)	25.1	117
K3	Thyroxine, 2x	8	5	(22.6)	--	--
G5	Control	7	6	(41.6)		99
I'2	Thyroxine, $\frac{1}{2}$x	6	6	40.3		96
I'4	Thyroxine, $\frac{1}{2}$x	6	6	48.4		116
J'2	Thyroxine, x	6	6	38.4		92
K2	Thyroxine, 2x	6	6	40.2	41.8	96

APPENDIX

TABLE XXIII (Continued)

Mouse	Treatment	No. in Litter		Litter Weight		Index of Performance
		Birth	14 Days	Individ. (gm.)	Mean* (gm.)	(%)
G'2	Control	7	7	34.7		91
H'2	Thiouracil	7	7	40.9		107
I4	Thyroxine, $\frac{1}{2}x$	7	7	32.4		85
I'3	Thyroxine, $\frac{1}{2}x$	7	7	45.5		119
K1	Thyroxine, $2x$	7	7	38.1	38.3	99
G'5	Control	8	8	39.8		90
H2	Thiouracil	8	8	34.4		78
I5	Thyroxine, $\frac{1}{2}x$	8	8	43.0		98
I'5	Thyroxine, $\frac{1}{2}x$	8	8	52.2		118
J2	Thyroxine, x	10	8	(38.8)		88
J5	Thyroxine, x	8	8	51.1		116
J'1	Thyroxine, x	8	8	44.0	44.1	100
H'3	Thiouracil	10	9	(27.0)		61
I2	Thyroxine, $\frac{1}{2}x$	10	9	(50.8)		114
L'2	Thyroxine, $4x$	9	9	44.6	44.6	100
K5	Thyroxine, $2x$	10	10	47.7		115
L2	Thyroxine, $4x$	15	10	(50.0)		121
L'3	Thyroxine, $4x$	10	10	35.0	41.4	85
L'4	Thyroxine, $4x$	10	11	(64.9)	--	--

* - Only those litters having same number of young at 14 days as at birth were used in this average. Litter weights in parentheses were not averaged.

APPENDIX

TABLE XXIV Index of Lactation Performance

Treatment	Mouse	No. in Litter		Index of Performance (%)
		Birth	14 Days	
		Experiment II		
Control	G2	10	4	105
	G5	7	6	99
	G'2	7	7	91
	G'5	8	8	90
Thiouracil	H2	8	8	78
	H5	10	4	72
	H'2	7	7	107
	H'3	10	9	61
	H'4	4	4	100
Thyroxine, $\frac{1}{2}x$	I2	10	9	114
	I4	7	7	85
	I5	8	8	98
	I'2	6	6	96
	I'3	7	7	119
	I'4	6	6	116
	I'5	8	8	118
Thyroxine, x	J2	10	8	88
	J5	8	8	116
	J'1	8	8	100
	J'2	6	6	92
	J'4	5	4	117
Thyroxine, $2x$	K1	7	7	99
	K2	6	6	96
	K5	10	10	115
Thyroxine, $4x$	L2	15	10	121
	L'2	9	9	100
	L'3	10	10	85