REPRODUCTIVE PERFORMANCE IN THE RAT IN RELATION TO SELECTION FOR TIME OF PUBERTY

by

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DEDICATION

TO MY WIFE, EVELYN

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REPRODUCTIVE PERFORMANCE IN THE RAT IN RELATION TO SELECTION FOR TIME OF PUBERTY

Chapter I

INTRODUCTION

The problem of attaining and perpetuating maximum reproductive efficiency in farm animals is one of utmost importance to the economy of the livestock industry. Much effort on the part of physiologists, geneticists, nutritionists and others has been directed toward the realization of this goal, particularly during recent years. The problem is very complex in nature, representing the combined effects of numerous factors of both genetic and environmental origin. The solution to this problem can be obtained only through detailed study of each of the many forces involved.

Relatively little effort has been directed toward the study of sexual development, other than in poultry, particularly with reference to the overall effect that the age of pubescence may bring to bear on fertility. Perhaps one of the principle reasons for the neglect of this approach to the problem stems from the idea that pregnancy and subsequent lactation occurring early in the life of the individual was thought to result in a reduction in mature size, fertility and quality of the offspring produced. It has been shown in recent years (78, p.66; 22, pp.368-370), however, that early-bred and lactating swine and rats reached the same adult size as those bred at normal age and further that early and regular reproduction served as

a stimulus to reproduction. A suggested explanation for this phenomenon (8, p.21) is that reproduction results in a greater unity between the gonads and the other endocrine glands concerned with regulating the reproductive processes. This line of reasoning leads one to the hypothesis that at least some of the factors affecting rate of sexual development may be associated with reproductive performance. Asdell (7, p.11) has stated that such is the case in several species and that accordingly early puberty and high initial fecundity may serve as good bases for economic selection.

If a criterion for selection, such as age of pubescence, is to be of practical value it is necessary that information regarding the effectiveness of selection for that character (heritability) as well as the genetic limits of variability be known. Knowledge of the physiology of sexual development would also be valuable. In view of this need it seemed advisable to carry out a more detailed study of the genetic and endocrine factors affecting sexual development and of the relationship of rate of sexual development (age of pubescence) to some productive characters in the female.

The present study was undertaken with the objectives of gaining information regarding the effects of some genetic and endocrine factors on sexual development and the relationship of age of pubescence to some productive characters in the female. The rat was used as the experimental animal since large numbers can be reared in a comparatively short time and with a minimum of expense due to large numbers of young per litter and short intervals between

generations. Further, genetic studies utilizing rats as the experimental animal permit experimental designs which are more adaptable to statistical analyses.

CHAPTER II

REVIEW OF LITERATURE

PUBERTY

"Puberty may be defined as the time at which reproduction first becomes possible, i.e., when germ cells are released," (7, p.10). Vaginal opening, changes in the vaginal smear, sexual receptitity and ovulation are all integral factors in the attainment of puberty in the female rat (17, p.198). The time of establishment of all of these factors is not necessarily identical; thus Long and Evans (70, p.244) found that in the majority of their females vaginal introitus preceded ovulation by approximately five days. Blandau and Money (17, p.215) have reported that vaginal opening generally occurred between 48 hours before and 12 hours after the onset of heat. These same investigators observed first ovulation in the majority of females between five and ten hours after the onset of first heat. They also reported that 30 per cent of the females mated during the first heat period failed to conceive as did 19 per cent of those inseminated at first heat. A small percentage of females did not become sexually receptive even though vaginal opening and ovulation had occurred. It is apparent from these data that no single one of these phenomena is a wholly reliable criterion of time of puberty. However, since on the average all of these phenomena occur within a period of about one day (7, p.10) no serious error is involved when one relies on any one of these.

The age of puberty of rats as reported in the literature is quite varied. Thus Long and Evans (70, p.244) found that their strain of rats reached puberty at an average age of 76.5 days, the range being

from 53 to 142 days. Fruedenberger (47, p.349) states that the Wistar strain attained puberty at 46.9 days after birth with a range of 36 to 66 days. In his laboratory the Long-Evans rats reached puberty at 52.7 days with a range of 39 to 101 days. Slonaker (88, pp.312-314) gives the average age of pubescence in his strain as 80.6 days. The average age of vaginal opening in intact normal rats at Cornell was observed to be between 58.6 and 61.1 days (22, p.358). The reports of other investigators (49,p.107; 62, pp.99-105; 42, pp.429-432; 19, pp.205-207; 94, pp.48-50) give further evidence of widespread differences in age of puberty.

FACTORS AFFECTING SEXUAL DEVELOPMENT

An unknown, but certainly relatively small portion of this variation in age of pubescence is undoubtedly due to the different criteria used by the various investigators in measuring time of puberty. The different environmental conditions existing in the several laboratories where such studies were carried on is another source of variation. The fact that a considerable part of the variability is due to genetic causes is borne out by the different values obtained for different strains in the same laboratory (47, pp.346-348; 19, pp.203-208. Further, Stone and Barker (94, pp.48-50) were able to establish by selection within a strain two divergent lines with regard to age of pubescence.

A further point of interest is the observation that the reported age of puberty has shifted downward by as much as 30 days over the last 50-year period. Stone and Barker (94, p.48) attributed this change in part to improved environment (diet and management), to use

of different criteria of measurement and to unrecognized causes, one of which may be selective breeding in which the genetically late pubescent animals have been unknowingly and unintentionally selected against.

AGE AND WEIGHT. The work of Asdell and Crowell (9, pp.14, 23) and the extensive studies of Bogart et al. (22, p.359), involving observations on 300 rats indicate that the time of vaginal opening is more closely related to age than weight. The coefficient of variability for age at vaginal opening, as reported by the latter group of workers, was 4.89 per cent while that for weight was 10.55 per cent. The coefficient of correlation for age and weight at the time of vaginal opening (after removal of variance due to treatment, location of animals and interaction) was +0.57. Blunn (19, p.212) also found that time of vaginal opening was to a considerable extent under the influence of age. Cole and Casady (33, p.122) reported that their earlier maturing strain of rats was heavier than the late maturing strain at 85 to 90 days of age.

RATE OF GROWTH. The work of Bogart et al. (22, pp.361-366) indicates a marked correlation of growth rate with time of vaginal opening. These workers found that the time of vaginal opening in a group of 50 castrate females occurred at the same point of change in the growth curve as it did in intact rats. These authors interpret this as an indication that certain of the factors affecting sexual development also govern growth rate. Asdell and Crowell (9, pp.21-23) studied the effect of retarded growth on the age of vaginal opening in the rat. They subjected 40-gram rats to severe energy inanition. The

ages of vaginal opening in three such rats were 377, 101 and 106 days, whereas their weights at the time of vaginal opening were 42, 43 and 54 grams, respectively. The average age at vaginal opening for the control group was 53.5 days. The suggested interpretation was that growth and sexual development were correlated by way of the hypophysis, secretion of hormones stimulating sexual development increasing as the production of growth stimulating hormone decreased. Evans and Bishop (45, p.335) have carried out similar experiments with like results. Blunn (19, pp.204-205) computed growth rate from weaning to the time of vaginal opening. He found a significant correlation between the growth rate and age of vaginal opening and concluded that the time of vaginal opening was a function of growth rate as well as of age.

SIZE OF LITTER AT WEANING. Engle, Crafts and Zeithaml (42, pp.427-432) found a definite relationship between size of litter raised to weaning and age of vaginal opening of the females in the litter. Females reared in the smaller litters reached puberty earlier than those reared in large litters. If their findings are correct one would expect selection for early puberty to result in a decrease in litter size. Blunn (19, pp.208-210), however, was unable to detect any significant effect of litter size at weaning on age of vaginal opening of the females in the litter.

NUMBER OF YOUNG BORN. Little information is available in the literature with regard to this factor. If number raised to weaning is a significant factor in determing time of puberty it would be expected that number of young born would exert a similar but lesser

effect since the former is a direct function of the latter. An effect of this factor might be exhibited through other secondary correlations such as a correlation of number born with suckling ability which in turn would be reflected in growth rate. Such an effect would not be expected to be great enough to be of any consequence. A direct genetic correlation between number born and time of vaginal opening is at least theoretically possible.

No studies with rats or other mammals have been reported in which an attempt was made to determine the heritability of age of pubescence. It is possible that such an estimate might be calculated from the original records of Blandau and Money (17, pp.197-215).

RELATION OF SEXUAL DEVELOPMENT TO PRODUCTIVE CHARACTERS

Very little evidence is presented in the literature relative to the effects of selection for early puberty on growth, mature size, fertility, length of reproductive life, suckling ability and other productive characters. Some definite evidence of a correlation between age of puberty and growth has been previously cited (22, p.358; 19, p.212). Cole and Casady (33, p.122) also found that their earlier "maturing" strain of rats was significantly heavier, at 85 to 90 days of age, than their later "maturing" strain.

Bogart et al. (22, p.360) found no evidence of a relationship between age of puberty and mature weight in rats. Phillips and Zeller (84, pp.398-399) on the other hand found that age of puberty was significantly different in the large and small types of Poland China swine.

One might logically postulate a correlation between sexual development and fertility (litter size) since both are, at least to some extent subject to the same endocrine regulation even though the former is admittedly qualitative in nature whereas the latter is quantitative. In this connection, Cole and Casady (33, pp.120-121) found highly significant differences in both age of puberty and prolificacy between two highly inbred strains of rats derived from the same parental stock. Of perhaps equal importance was the significantly greater ovarian weight of the early "maturing" more prolific strain. Further, Hetzer and Brier (52, pp.135-138) found that the larger, earlier "maturing" type of Poland China swine was also more prolific than the small, late "maturing" type. MacArthur (75, pp.229-231) selected mice for large and small size for several generations, and although he did not determine the effects of such selection on age of puberty, he did find that the larger strain produced significantly more young per litter. This was determined to be due primarily to a larger number of ova shed. He concluded that the correlation between body size and litter size depends on endocrinal links, such as the growth and gonadotropic hormones of the anterior pituitary rather than on differences in "litter size genes" or "fertility factors" as such in the two strains. While one may not hold exclusively to MacArthur's explanation, endocrine factors are unquestionably of considerable importance in such relationships. Accordingly, one can readily conceive of similar "links" between such characters as age of puberty and length of reproductive life and suckling ability.

RESPONSE OF TISSUES TO ENDOCRINE STIMULI

HYPERPLASIA AS AN INDEX OF ENDOCRINE STIMULATION. Stimulation of tissue may be manifested in either hypertrophy (increase in cell size) or hyperplasia (increase in cell numbers resulting from increased mitotic rate). By use of the colchicine technique for arresting mitoses in the metaphase, the normal and stimulated or depressed rates of mitoses have been studied in many different tissues (5, pp.321-329; 25, pp.933-936; 26, pp.135-165; 34, pp.195-210; 46, pp.266-268; 54, pp.263-275; 55, pp.133-138; 56, pp.127-137; 69, pp.811-901; 87, pp.181-189; 97, pp.265-273; 102, pp.137-153).

COLCHICINE TECHNIQUE FOR ESTIMATING MITOTIC RATE. Much of the credit for developing the colchicine technique of studying mitotic rate is due Dustin (39, pp.561-573; 40, pp.7-36) and Ludford (71, pp.411-441). Dustin (40, pp.30-35) first studied the effects of this alkaloid on malignant tumors and observed that cellular aberrations were produced. Ludford (71, pp.413, 430-440) found that the drug was effective on normal as well as malignant tissue and reported that when colchicine was injected into an animal, mitotic divisions were arrested in metaphase. He stated that "cells which divide during the period while colchicine is active are accumulated in easily recognizable condition." He attributed this action of colchicine to a "failure of spindle to form and function in the normal manner." Apparently he was the first to suggest the technique as a method of measuring growth. Evidence has been presented (69, pp.872-900) which indicates that colchicine also has some stimulating effect on mitosis. According to Worthington and Allen (102, pp.146-148) this stimulating effect of

colchicine does not render the method less useful as a relative measure of growth activity since the magnitude of stimulation is apparently proportional to the actual rate of mitosis. Dustin (40, pp.7-36) found the maximum number of accumulated mitoses in mice tissues nine and one-half hours after treatment, while Levine (68, p.179) reports that in mammalian tissue metaphases begin to accumulate within two to three hours and attain a maximum between ten and 15 hours after colchicine treatment.

The dosage generally recommended for rats is 0.1 mg per 100 grams of body weight.

In many of the studies reported in the literature the mitotic index has been based on the number of arrested mitoses per mm^2 of tissue element concerned (54, p.275; 55, p.138; 56; p.136; 102, pp.139-141) while in others mitotic stimulation has been expressed merely in terms of total number of arrested mitoses (4, p.180; 5, pp.325-326; 25, pp.933-936; 26, pp.138-158; 97, pp.271-272). In view of the possibility of extreme variation in number of cells per section or per unit area, resulting from different degrees of hypertrophy and/or hyperplasia induced by treatments, it would seem that these methods would be subject to considerable error. It would appear that a ratio of mitotic cells to total cells would be a more accurate measure for comparison of growth rates in tissues that had been subjected to different treatments.

HORMONE STIMULATION OF THE UTERUS.

Estrogen. Allen and Doisy (3, pp.819-821) first demonstrated the presence of the ovarian hormone theelin in 1923. Since

that time action of this and several other estrogenic substances (estrone, estriol, diethylstilbestrol) on female reproductive tissues has been intensively studied by these and other investigators (2, pp.191-195; 3, pp.819-821; 4, pp.133-181; 10, pp.25-31; 5, pp.321-329; 14, pp.401-411; 25, pp.933-936; 34, pp.195-210; 63, p.1048; 99, pp.247-294; 102, pp.137-153). It has been shown that these estrogenic substances are capable of causing increases in length, diameter and weight of the uteri or either normal, immature or ovariectomized females. These changes have been shown to be, primarily, the result of hypertrophy and hyperplasia of the uterine musculature (14, pp.405-410) and of the uterine endometrium (5, p.327; 63, p.1048; 102, pp.143-150). An increase in vascularity, contributing to the increase in weight also occurs. This is considered by Wothington and Allen (102, p.152) to be a secondary effect necessitated by the increased cellular activity. They conclude that "the epithelium may be considered as the specific end organ through which estrogen manifests itself."

Progesterone. The effects of progesterone, a second ovarian principle, on the uterus of normal, immature and castrate females have also been intensively studied (25, pp.933-936; 34, pp.195-210; 48, p.342; 63, p.1048; 99, pp.290-291). Crandall (34, pp.208-210) found that in rabbits marked hyperplasia of the uterine muscle occurred during the first four or five days of pregnancy, when the reproductive tract is normally subject to the action of progesterone. Gillman (48, p.342), working with castrate rabbits observed that progesterone, in proper combination with estradiol benzoate, caused marked vascular

changes in both the endometrium and myometrium. He reported also that progestational changes, due primarily to the action of progesterone, would take place or be inhibited depending upon the amounts of estradiol and progesterone administered. Williams (99, p.290) stated that progesterone alone was ineffective in promoting growth but that it exhibits a synergistic action with estrogen. The work of Bureau (25, pp.934-936) substantiates William's viewpoint. He reported that progesterone injections following pre-treatment with estradiol benzoate gave maximum mitotic response in the uterine epithelium of ovariectomized rabbits. On the other hand, some investigators are of the opinion that progesterone is antagonistic to estrogenic action. Thus, Korenchevsky and Hall (63, p.1048) reported that progesterone can prevent or decrease the metaplasia commonly induced in the uterine epithelium of ovariectomized or normal rats by injections of estrogenic hormones.

Thyroid Level and Uterine Response to Estrogens and Progesterone. Until recent years, studies concerning the thyroid gland secretion generally necessitated surgical ablation of the gland. It is now possible to maintain complete control over thyroid activity by feeding synthetic substances. Thus varying degrees of hyper- or hypothyroidism can be produced by feeding iodinated caseine or one of several goitrogens such as thiouracil, thiourea or thiobarbital (18, p.307). The effectiveness of these goitrogenic substances is attributed to an interference with thyroxine production (12, p.224; 76, pp.206-207). The usual range of dosage of thiouracil for rats is from 0.05 to 0.2 per cent of the diet.

According to Langham and Gustavson (66, p.760), Reiss and Pereny were the first to suggest that the hormone of the thyroid gland affected the response of vaginal mucosa to estrogen. In addition, subcutaneous injections of thyroid autocoid prevented heat in normal female rats and mice and also inhibited the action of the ovarian hormone in castrate animals. Other investigators (79, pp.847-849; 66, p.766) noted a decrease in the estrogen response of ovariectomized rats treated with d,l-thyroxine or dessicated thyroid. Langham and Gustavson (66, pp.763-766) observed the opposite effect when thiourea was administered or when the thyroid gland was removed surgically. They found further that the high estrone sensitivity accompanying hypothyroidism could be overcome, at least partially, by the administration of thyroxine. Van Horn (98, pp.152-162) has reported that approximately three times as much estrogen was required to produce estrous smears in hyperthyroid ovariectomized rats as in ovariectomized controls. Conversely, Richter (86, p.85) Krohn and White (65, pp.383-384), Barker (13, p.138) and Laqueur and Emge (67, pp.108-110) observed that hypothyroidism, resulting either from thyroidectomy or administration of goitrogens, resulted in absence of or marked irregularities in the normal estrous cycle of rats.

The available evidence with regard to this relationship in other species and also the effect of the thyroid hormone on mitosis in other tissues is equally controversal. Thus Brody and Frankenback (24) and Spielman et al. (90, p.337) reported complete absence of normal estrual behavior in the thyroidectomized cow. According to Engle (41, p.66) thyroidectomy interfers with the menstrual cycle of Rhesus

monkeys and prevents estrogenic response in ovariectomized monkeys. Still other workers (36, p.453) have reported that the uteri of hypothyroid mice were smaller than those of control animals.

Mixner (80, p.578) observed that goitrogens inhibited the normal stimulatory effect of estrogen on the growth of the mammary lobule-alveolar system of mice but a similar effect was not manifested in rats. Stein, Quimby and Moeler (91, p.263) state that thyroxine retards mitotic proliferation of the germinal epithelium of the mouse ovary, whereas Fleishmann and Breekler (46, pp.266-268) found that the mitotic activity of the corneal epithelium of rats was reduced as much as 50 per cent by thyroidectomy or feeding thiouracil. Stimulation of growth and development of the epiphyseal cartilage of castrate female mice by thyroxine has also been reported (87, p.189).

It is apparent that considerably more work is necessary before the full implications of thyroid functions are realized. In each of the studies cited above that dealt with thyroid-estrogen relationship, estrogen response was determined by the vaginal smear technique. No attempts have been made to study the effects of the thyroid gland secretion on the mitotic response of the uterine epithelium to estrogen or progesterone. Such information should be of value in assessing the role of the thyroid in sexual development.

CHAPTER III

MATERIAL AND METHODS

SELECTION STUDIES

Forty-two female and nine male albino rats of the Sprague-Dawley strain were obtained from the Holzman Rat Company, Madison, Wisconsin on March 21, 1950. The females, when received, were 43 days of age and all prepuberal, whereas the males were approximately three and one-half months of age and ready for service as breeding animals. The data presented are the records of performance of these individuals and three generations of their selected descendants reared during the period of March 21, 1950 to May 1, 1951.

Selection for both early and late pubescence was used to establish two divergent lines. They shall be referred to as the "early" line and "late" line. Ten females and four males were thus selected from first litters in each line as parents of the next succeeding generation of their respective line. Selection of breeding females was based solely on age of attaining pubescence as determined by date of vaginal opening. In the event of a choice between females the one chosen was from the best performing litter. Selection of breeding males was made on the basis of the performance of female littermates, one individual being selected at random from each of the four best performing litters. In the foundation stock, selection of males was entirely at random. The females were allowed to reproduce at regular intervals until death or sterility ensued or until termination of the experiment. No attempt was made to replace breeding

animals that died or were infertile regardless of possible cause.

Every effort was made to maintain a uniform environment throughout the experiment. The experimental animals were housed in an unlighted, well insulated room which was maintained at a temperature of 24 to 27 degrees Centigrade. Heat was supplied by use of an electrically operated, thermostatically controlled forced air heater.

Dornfeld's rat mix (Table 36) and water were fed ad libitum, the feed cups and water bottles being checked daily. Fresh pork liver was provided at the rate of 12 grams weekly for mature, nonlactating females and mature males. Lactating females received a minimum of 15 grams and immature rats received proportionate amounts per week depending upon age.

Mating was accomplished by placing the female in the cage with the male for a period of five days. She was then removed for a period of three days. This procedure was repeated, using a different male at each exposure period, until it was evident by palpation that the female had conceived. By the use of this method, it was possible to identify the sire of each litter without the expenditure of the time required to determine time of estrus in each female by daily vaginal smears. The method has the objection that some estrual periods occurred during the non-exposure period of three days and conception was delayed a few days beyond the earliest possible date. This, however, is of no particular consequence in the present study. All females were first exposed to the male at 100 days of age and at 31 days after delivery of each litter, regardless of whether or

not the litter survived. This procedure provided for a six-day-rest period after each litter was weaned. No attempt was made to mate particular females to certain males, excepting that brother-sister matings were not made for production of first litters.

Records taken were: date of birth of each litter; number born in the litter; number weaned and sex at 25 days of age; and individual weaning weights. After weaning, daily observations were made on the females for vaginal opening. The date and weight at the time of vaginal opening were recorded for each rat. A few of the females reared were not observed for vaginal opening as they were directed to other experiments at or shortly after weaning. Weights were taken on all first-litter females at 75 days of age.

STUDIES OF MITOTIC RESPONSE OF TISSUE TO ENDOCRINE STIMULI

These studies involved two separate but closely related experiments. In each experiment, 48 female rats were divided at random into eight equal groups. These were subjected to different treatments as indicated in the table below.

Table 1

Endocrine Treatment Received by Eight Groups of Rats in Each of Two Experiments

Experiment I and II			
Group	Thyroid Condition	Estrogen	Progesterone
1	Normal	None	None
2	"	None	Progesterone
3	"	Estrogen	None
4	"	Estrogen	Progesterone
5	Hypothyroid*	None	None
6	"	None	Progesterone
7	"	Estrogen	None
8	"	Estrogen	Progesterone

*Hypothyroidism was induced by feeding thiouracil at the rate of 0.2 per cent of the ration.

The hormone preparations were administered to the rats in the indicated groups by subcutaneous injections. The dosage of estrogenic substance at each injection was three Rat Units per rat; that for progesterone was 0.05 mg per rat. It was deemed advisable to avoid massive hormone doses in order to prevent abnormal effects. The progesterone preparation used was the same in both experiments, 1 mg per ml in corn oil. The animals in Experiment I were intact females 21 days of age at the start of the experiment. The ones given thiouracil received this during the eight days prior to the hormone treatment. No primer hormone injections were given. The estrogen used was a preparation of diethylstilbestrol in corn oil (60 Rat Units/ml). The rats used in Experiment II were mature females that had been ovariectomized ten days prior to the start of the experiment. Four of the groups, indicated above, received thiouracil for a period of 24 days. They received two identical hormone treatments five days apart, the first being given on the 18th day of thiouracil feeding. The estrogenic substance used was an aqueous solution of crystalline estradiol benzoate (60 Rat Units/ml).

All animals were sacrificed to obtain uterine specimens for study of relative mitotic rate. Each animal was given a subcutaneous injection of an aqueous solution of colchicine approximately 12 hours after the final hormone injections and nine hours prior to sacrifice. The concentration of the colchicine solution was 0.2 mg per ml and was administered at the rate of 0.1 mg colchicine per 100 grams of body weight.

The tissues were fixed in Bouin's solution to which a small amount of urea was added just prior to introduction of the tissues. The specimens were dehydrated by the Dioxan method (51, pp.64-66) and imbedded in paraffin. The uteri were sectioned at six micra, random sections were stained with iron-alum hematoxylin, destained with picric acid and washed thoroughly in a running water bath. The slides were cleared in xylol-creosote.

The relative mitotic activity, expressed as number of mitoses per 1000 cells, was determined for the endometrium of each rat. This mitotic ratio was calculated from the number of mitotic cells and an estimate of total cells in several (usually ten) random sections. The estimate of total cells was based on actual counts of total cells in two of the observed sections. Cell counts, both mitotic and total, were made using oil immersion at a magnification of 1,710 times.

STATISTICAL ANALYSES

The differences between the two divergent lines in the selection studies were analysed by use of analysis of variance (89, pp.214-252). The relation of puberty to productive characteristics was determined by multiple regressions after variance and covariance due to line and to litter sequence were removed (89,pp.340-373). Heritability estimates for age of vaginal opening were determined directly from the line differences (64, pp.3-15).

The effects of hormones on mitotic rate in the endometrium were studied by analysis of variance using the method of individual degrees of freedom following a log $(x + 1)$ transformation

(89, pp.400-430). Significance in all cases was determined at the 5 per cent level.

CHAPTER IV

RESULTS

SELECTION OF DIVERGENT LINES FOR AGE OF PUBERTY

HERITABILITY OF AGE AT TIME OF VAGINAL OPENING. The mean age of vaginal opening for first litter female progeny of each line in each generation is presented in table 2. The difference between the means of the two lines for each generation, also given in table 2, was 0.58, 0.95 and 2.25 days for the first, second and third generations, respectively. These values are estimates of the genetic difference which has accumulated as a direct result of selection in the preceding generations for opposite extremes of age at vaginal opening.

Table 2

Mean Ages (in days) at Time of Vaginal Opening for all First-litter Female Progeny by Lines and Generations

	Generation							
	0		1		2		3	
Line	No.	Mean Age	No.	Mean Age	No.	Mean Age	No.	Mean Age
Late	43	55.14	43	43.74	27	44.08	32	41.88
Early			38	43.16	23	43.13	30	39.63
L. - E.	-	-	-	0.58	-	0.95		2.25

The amount of selection practised for early and late vaginal opening, in each generation and line is represented by the average superiority or inferiority of the dams when compared with the means

of the population from which they were selected. The total amount of selection for each generation is represented by the sum of the amount in the two lines. These values are presented in table 3.

Table 3

Mean Superiority (+) or Inferiority (-) of Selected Dams in Age at Vaginal Opening (Days)*

	Dams of Generation					
	1		2		3	
	No.	Mean days	No.	Mean days	No.	Mean days
Late	10	(-)4.44	8	(-)3.41	7	(-)3.48
Early	9	(+)2.93	6	(+)3.77	7	(+)2.60
L. - E.	-	7.37	-	7.18	-	6.08

*In calculating the mean of the dams of each generation, each dam was weighted according to its number of female progeny. The mean for the whole population from which dams were selected was then subtracted from the mean for selected dams to obtain the average superiority or inferiority of dams.

Thus in the first generation the mean inferiority of the late-line dams of that generation was 4.44 days and the mean superiority of the early-line dams was 2.93 days. In other words the average age at the time of vaginal opening of the selected late-line dams was 59.58 (table 4) whereas the average age at the time of vaginal opening of the population from which they were selected was 55.14 (table 2) or a difference of (-) 4.44 days. Similarly, the average age at the time of vaginal opening of the selected early-line dams was 52.21 days (table 4) while that of the population from which they were selected

was 55.14 days (table 2) or a difference of (+) 2.93 days. Total selection in the first generation thus amounted to 7.37 days (4.44 + 2.93). Corresponding values for the second and third generations were calculated in the same manner. In calculating the mean age at the time of vaginal opening of the selected dams in each line of each generation it is necessary to adjust the value for each dam according to the number of female progeny raised. The adjusted means of the selected dams of each line and generation are presented in table 4.

Table 4

Mean Ages (in Days) at Time of Vaginal Opening of Selected Dams by Line and Generation

	Dams of Generation					
	1		2		3	
Line	No.	Mean Age	No.	Mean Age	No.	Mean Age
Late	10	59.58	8	47.15	7	47.56
Early	9	52.21	6	39.39	7	40.53

Estimates of heritability (h^2) are based on the association of the selected dams with their offspring. Estimates of heritability of age at the time of vaginal opening in the present study were calculated from the values given in tables 2 and 3. Thus h^2 for a given generation equals the difference between the means of the progeny of the two lines for that generation divided by the total accumulated selection. It is necessary to multiply the value obtained by two since selection was made on one parent (dams) only. Thus for the second generation:

$$h^2 = \frac{0.95 \text{ (table 2)} \times 2}{7.37 + 7.18 \text{ (table 3)}} = 0.1307 \text{ or } 13.07\%$$

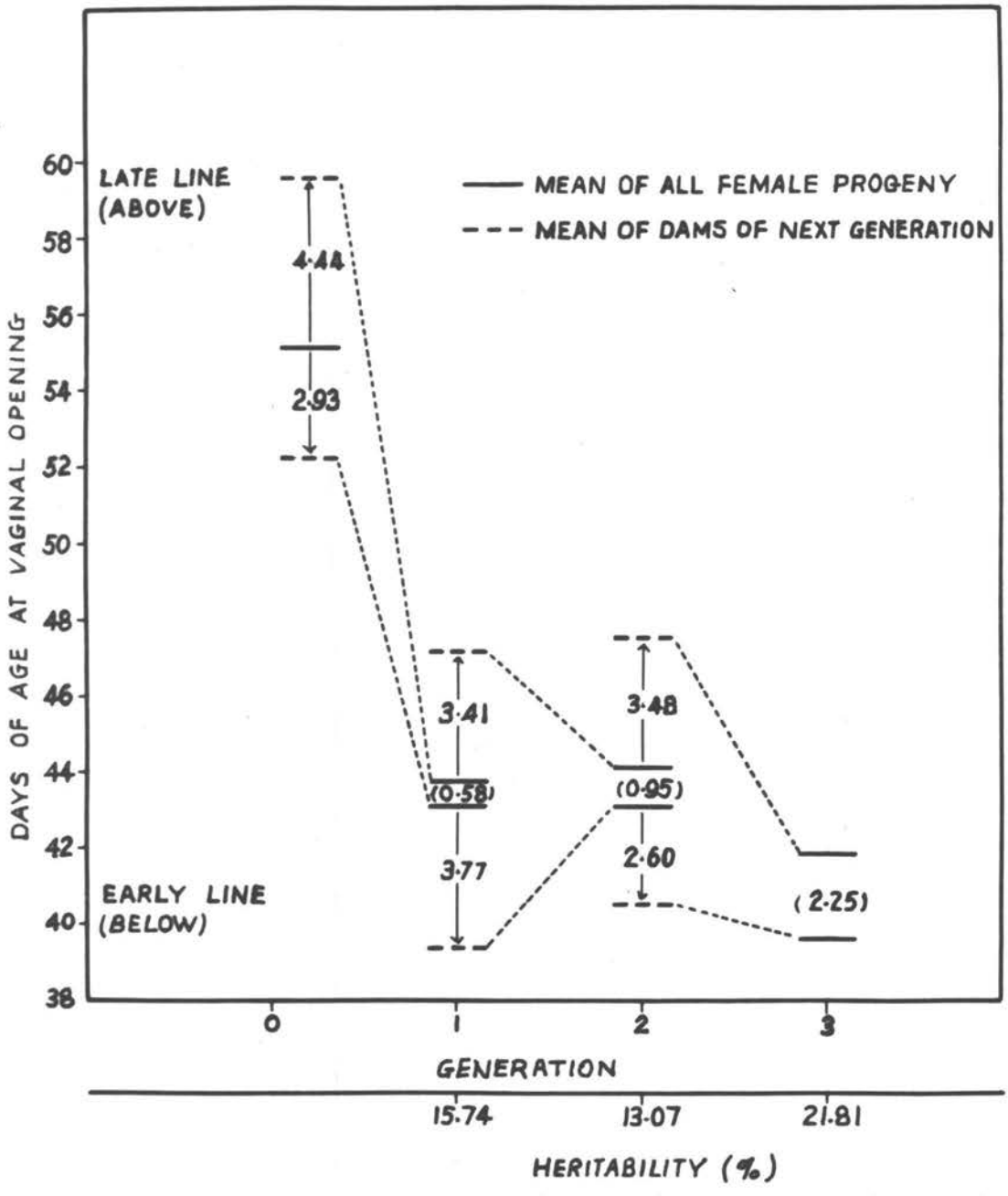
Heritability estimates for age at the time of vaginal opening were 15.74, 13.07 and 21.81 per cent for the first, second and third generations, respectively, (table 5). The average heritability for the three generations was 16.87 per cent (table 5). A diagram showing the amount of selection in each line for each generation and the accumulated genetic difference between lines during the three generations is presented in figure 1.

Table 5

Summary of Heritability Estimates of Age at Time of Vaginal Opening

Generation	Difference Between Lines	Accumulated Selection	$\frac{1}{2} h^2$	h^2
1	0.58 days	7.37 days	.0787	.1574
2	0.95	14.55	.0653	.1307
3	2.25	20.63	.1091	.2181
Average				.1687

FIGURE 1. MEAN AGE IN DAYS AT TIME OF VAGINAL OPENING FOR ALL FIRST-LITTER PROGENY AND FOR DAMS OF THE NEXT GENERATION, IN EACH GENERATION OF THE EARLY AND LATE LINES, SHOWING THE EFFECTS OF SELECTION. AVERAGE DIFFERENCES BETWEEN THE PROGENY OF THE TWO LINES IN EACH GENERATION ARE SHOWN IN PARENTHESES.



DIFFERENCES BETWEEN EARLY AND LATE LINES. Selection for the extremes in age at time of vaginal opening resulted in differences between the early and late lines with regard to age at the time of vaginal opening and certain productive characteristics.

Age at Time of Vaginal Opening. The mean age at which vaginal opening occurred was 41.86 days for early-line progeny and 44.40 days for late-line progeny. Thus vaginal opening occurred 2.54 days earlier in the early line (table 6).

Table 6

Average Age at Time of Vaginal Opening

Line	No. Females	Average Age V. O. (Days)
Early	190	41.86
Late	200	44.40
Difference		2.54

The difference in average age at which vaginal opening occurred in females of the two lines was significantly different from zero (table 7).

Table 7

Analysis of Variance for Age at Time of Vaginal Opening

Source of Variation	Degrees of Freedom	Mean Square	F
Total	389		
Between Lines	1	629.530	52.96*
Within Lines	388	11.888	

*Significant

Number of Young Born per Litter. The average number of young born per litter to females of the early-line was 9.66 whereas the average number per litter for females of the late-line was 8.69 (table 8). These means are based on the combined production of the foundation females and first and second generation females of each line. The mean litter size for early and late-line females for each of the three generations (0, 1 and 2) as well as the difference between means is also presented in table 8. The average litter size was 9.27 for early-line foundation females and 8.53 for late-line foundation females, or 0.74 more young were born per litter in the early-line. For first generation females the mean litter size was 9.84 for the early-line and 8.69 for the late-line; a difference of 1.15 young per litter. For second generation females the difference between lines was 1.50, the mean litter sizes being 10.90 for the early line as compared to 9.40 for the late line.

Table 8

Average Number of Young Born Per Litter

	Foundation ♀♀		1st Generation ♀♀		2d Generation ♀♀		Total	
Line	No. of Litters	Young per Litter	No. of Litters	Young per Litter	No. of Litters	Young per Litter	No. of Litters	Young per Litter
Early	44	9.27	25	9.84	10	10.90	79	9.66
Late	45	8.53	29	8.69	10	9.40	84	8.69
Difference		0.74		1.15		1.50		0.97

The difference between mean litter sizes of the early and late lines was significantly different from zero when the production of the three generations, involving a total of 163 litters, was combined (table 9).

Table 9

Analysis of Variance for Number of Young Born Per Litter

Source of Variation	Degrees of Freedom	Mean Square	F
Total	162		
Between Lines	1	38.1282	5.74*
Within Lines	161	6.6443	

*Significant

Number of Young Weaned per Litter. The average number of young weaned per litter, omitting litters in which none of the young survived, was 8.26 for the early line and 8.13 for the late line (table 10). These means are based on the production of the foundation (0 generation) females and first and second generation of the two lines.

Table 10

Average Number of Young Weaned per Litter and Per Cent of All Litters in Which No Young Survived

Line	No. Litters	Mean No. Weaned	Total No. Litters	Per Cent Not Raised
Early	53	8.23	73	27.4
Late	57	8.16	78	26.9
Difference		0.07		0.5

The percentage of total litters in which none of the young were raised was similar for the two lines. Early-line females failed to suckle 27.4 per cent of their litters and late-line females failed to suckle 26.9 per cent of their litters (table 10).

The difference between lines with regard to the mean number of offspring per litter raised to weaning (25 days) was not significantly different from zero (table 11).

Table 11

Analysis of Variance for Number of Young Weaned per Litter

Source of Variation	Degrees of Freedom	Mean Square	F
Total	109		
Between Lines	1	0.1289	0.019
Within Lines	108	6.9339	

Average Weaning Weight. The average weaning weight (25 day) of 190 early-line female offspring was 46.9 grams while that of 200 late-line female offspring was 51.3 grams, thus the late-line offspring were 4.4 grams heavier (table 12).

Table 12

Average Weaning Weights

Line	Number of Females	Weight (grams)
Early	190	46.9
Late	200	51.3
Difference		4.4

The average weaning weights of the female offspring of the two lines were significantly different (table 13).

Table 13

Analysis of Variance of Average Weaning Weights

Source of Variation	Degrees of Freedom	Mean Square	F
Total	389		
Between Lines	1	1,888.842	34.3*
Within Lines	388	55.040	

*Significant

Weight at Time of Vaginal Opening. The late-line female progeny (first, second and third generations) were, on the average, 14.82 grams heavier at the time of vaginal opening than the early-line female progeny. The average weight at the time of vaginal opening was 126.09 grams for late-line progeny and 111.27 grams for early-line progeny (table 14).

Table 14

Average Weight at Time of Vaginal Opening

Line	No. Females	Weight (grams)
Early	190	111.27
Late	200	126.09
Difference		14.82

The average weight of female progeny at the time of vaginal opening was significantly different between the two lines (table 15).

Table 15

Analysis of Variance of Weight at Time of Vaginal Opening

Source of Variation	Degrees of Freedom	Mean Square	F
Total	389		
Between Lines	1	21,389.441	98.36*
Within Lines	388	217.464	

*Significant

Weight at 75 Days of Age. The average weight of female progeny at 75 days of age was 175.88 grams for the early line and 179.82 grams for the late line. The late-line females were thus 3.94 grams heavier (table 16). These values are the averages of females raised in first litters of the first, second and third generations of each line.

Table 16

Average Weight at 75 Days of Age

Line	Number Females	Weight (grams)
Early	91	175.88
Late	101	179.82
Difference		3.94

The difference between lines lacked significance (table 17).

Table 17

Analysis of Variance of Weight at 75 Days of Age

Source of Variation	Degrees of Freedom	Mean Square	F
Total	191		
Between Lines	1	744.107	3.86
Within Lines	190	192.645	

Growth Rate. The difference between female progeny of the two lines with respect to average gain per day from weaning to time of vaginal opening was 0.04 grams. The average rate of gain during this period was 3.87 grams per day for early-line females and 3.83 grams per day for late-line females (table 18).

Table 18

Average Daily Gains from Weaning to Time of Vaginal Opening

Line	Number Females	Gain (grams/day)
Early	190	3.87
Late	200	3.83
Difference		0.04

The average daily gains of the two lines did not differ significantly (table 19).

Table 19

Analysis of Variance of Growth Rate

Source of Variation	Degrees of Freedom	Mean Square	F
Total	389		
Between Lines	1	0.1465	0.63
Within Lines	388	0.2333	

INTERRELATION OF FACTORS INFLUENCING AGE AT THE TIME OF VAGINAL OPENING. The multiple correlation coefficient of age at the time of vaginal opening on five variates (number of young born, number of young weaned, weaning weight, weight at the time of vaginal opening and gain per day from weaning to the time of vaginal opening) after line effects had been eliminated was found to be significantly different from zero (R = 0.89) by the analysis of variance (table 20). The corresponding value obtained for R^2 (0.79) indicates that 79 per cent of the variability in age at the time of vaginal opening is accounted for by the variation in the five other variates studied.

Table 20

Test of Significance of the Multiple Correlation Coefficient of Age at Time of Vaginal Opening on the Five Other Variates.

Source of Variation	Degrees of Freedom	Mean Square	F
Total	388		
Regression	5	727.6172	285.96*
Residual	383	2.5445	

*Significant

Partial regression coefficients for age at the time of vaginal opening on each of the five variates were obtained while keeping the remaining four variates constant. The five partial regression coefficients and their respective confidence intervals with a confidence coefficient of 0.95 are presented in table 21. Thus, for each additional rat born in a litter (within the range of 2 to 15)

Table 21

Partial Regression Coefficients and Their Respective Confidence Intervals of Age at Time of Vaginal Opening on Each of the Five Other Variates

Variate	Partial Regression Coefficient	Confidence Interval
Number Born	0.0848	-0.0415 to 0.2146 (days per no. of young)
Number Weaned	-0.1012	-0.2180 to 0.0156 (days per no. of young)
Weaning Weight	-0.2305	-0.2586 to-0.2024 (days per gram)
Weight at Time of V. O.	0.2020	0.1896 to 0.2143 (days per gram)
Gain per Day	-4.3046	-4.6673 to-3.9420 (days per gram)

the mean age at the time of vaginal opening of the females in the litter was increased by 0.08 days; for each additional rat weaned in a litter (within the range of 2 to 13) the mean age at the time of vaginal opening of the females in the litter was decreased by 0.10 days; for each increase of one gram in weaning weight (within the range of 31 to 79 grams) the mean age at the time of vaginal opening was reduced by 0.23 days; for each gram increase in weight at the time of vaginal opening (within the range of 83 to 201 grams) the mean age at the time of vaginal opening was increased by 0.20 days and for each gram per day increase in average rate of gain from weaning to time of vaginal opening (within the range of 2.27 to 5.11 grams per day) age at the time of vaginal opening was reduced 4.30 days.

The partial regression coefficients were tested for significance by use of the "t" test. The t-values for age at the time of vaginal opening on weaning weight, weight at the time of vaginal opening and gain per day from weaning to the time of vaginal opening showed that these three partial regression coefficients were significantly different from zero (table 22).

Table 22

Test of Significance of Each of the Five Partial Regression Coefficients

Variate	Degrees of Freedom	t
Number Born	383	1.28
Number Weaned	383	-1.70
Weaning Weight	383	-16.07*
Weight at Time of V. O.	383	31.96*
Gain per Day	383	-23.26*

*Significant

INTERRELATION OF FACTORS INFLUENCING RATE OF GAIN OF FEMALES FROM WEANING TO TIME OF VAGINAL OPENING. The multiple correlation coefficient (R) of rate of gain on five other variates (number of young born, number of young weaned, weaning weight, weight at the time of vaginal opening and age at the time of vaginal opening) after line effects had been eliminated was 0.81. This coefficient was found to be significantly different from zero by the analysis of variance (table 23). The corresponding value obtained for R^2 (0.66) indicates that 66 per cent of the variability in average

daily gain from weaning to the time of vaginal opening is accounted for by the variation in the five other variates studied.

Table 23

Test of Significance of the Multiple Correlation Coefficient of Rate of Gain on the Five Other Variates

Source of Variation	Degrees of Freedom	Mean Square	F
Total	388		
Regression	5	11.9455	148.54*
Residual	383	0.0804	

*Significant

Partial regression coefficients for gain per day on each of the five other variates were obtained while keeping the four remaining variates constant. The five partial regression coefficients and their respective confidence intervals with a confidence coefficient of 0.95 are presented in table 24.

Table 24

Partial Regression Coefficients of Gain Per Day on Each of the Five Other Variates

Variate	Partial Regression Coefficient	Confidence Interval
Number Born	0.0254	0.0024 to 0.0483 (gm per day/young)
Number Weaned	-0.0117	-0.0325 to 0.0091 (gm per day/young)
Weaning Weight	-0.0250	-0.0310 to -0.0190 (gm per day/gm)
Weight at Time of V. O.	0.0318	0.0290 to 0.0346 (gm per day/gm)
Age at Time of V. O.	-0.1360	-0.1475 to -0.1246 (gm per day/day)

Thus for each additional rat born per litter (between the range of 2 to 15) the mean gain per day of females in the litter increased 0.03 grams; for each additional rat weaned per litter (within the range of 2 to 13) the mean gain per day of females in the litter decreased 0.01 grams; for each gram increase in weaning weight (within the range of 31 to 79 grams) the mean gain per day decreased 0.02 grams; for each gram increase in weight at the time of vaginal opening (within the range of 83 to 201 grams) the mean gain per day increased 0.03 grams and for each additional day of age at the time of vaginal opening (within the range of 32 to 52 days) the mean gain per day was reduced 0.14 grams.

The partial regression coefficients were tested for significance by use of the "t" test. The t-values for number of young born per litter, weaning weight, weight at time of vaginal opening and age at time of vaginal opening showed that these four partial regression coefficients were significantly different from zero (table 25).

Table 25

Test of Significance of Each of the Five Partial Regression Coefficients

Variate	Degrees of Freedom	t
Number Born	383	2.16*
Number Weaned	383	-1.10
Weaning Weight	383	-8.22*
Weight at Time of V. O.	383	22.57*
Age at Time of V. O.	383	-23.26*

*Significant

Table 26

Analysis of Variance of Effects of Hormone Factors on Mitotic Activity in the Endometrium.

Experiment I

Source of Variance	Degrees of Freedom	Mean Square	F
Total	47		
Treatment	7	0.688	2.55*
Thiouracil	1	0.945	3.50
Progesterone	1	0.786	2.91
Estrogen	1	1.069	3.96
Interactions			
Thiouracil x Progesterone	1	0.021	0.08
Thiouracil x Estrogen	1	0.721	2.67
Progesterone x Estrogen	1	1.252	4.64*
Thiouracil x Progest. x Estrogen	1	0.023	0.09
Within Treatment	40		

*Significant

Table 27

Analysis of Variance of Effects of Hormone Factors on Mitotic Activity in the Endometrium.

Experiment II

Source of Variance	Degrees of Freedom	Mean Square	F
Total	47		
Treatment	7	0.816	3.90*
Thiouracil	1	0.876	4.18*
Progesterone	1	0.815	3.89
Estrogen	1	0.001	0.01
Interactions			
Thiouracil x Progesterone	1	0.009	0.05
Thiouracil x Estrogen	1	0.044	0.21
Progesterone x Estrogen	1	3.166	15.12*
Thiouracil x Progest. x Estrogen	1	0.800	3.82
Within Treatment	40		

*Significant

HORMONE FACTORS INFLUENCING MITOTIC ACTIVITY OF THE ENDOMETRIUM

Both of these experiments were 2 x 2 x 2 completely randomized factorials with six observations in each of the eight treatment combinations. The analyses of variance are presented in tables 26 and 27.

EFFECT OF THE THYROID. The average number of mitoses per 1000 endometrial cells was 5.41 for the hypothyroid (thiouracil fed) rats and 10.25 for the normal rats of Experiment II (castrates). In Experiment I (immature, intact females) the average number of mitoses for the hypothyroid group was 8.15 as compared to 15.26 for the normal group (table 28). Thus, in each of the experiments the mean mitotic activity of the hypothyroid group was approximately one-half of that of the corresponding normal group.

Table 28

Mean Number of Mitoses per 1000 Cells in the Endometrium of Normal and Hypothyroid Rats

Experiment	Normal	0.2% Thiouracil
I (intact females)	15.26	8.15
II (castrate females)	10.25	5.41

The difference between means of the hypothyroid and normal groups in Experiment II (castrate females was significantly different from zero (table 27) whereas the difference between means of the two groups in Experiment I (immature, intact females) was not significant (table 26).

EFFECT OF PROGESTERONE. The mean mitotic activity in the endometrium of rats receiving progesterone in Experiment II was 3.55 as compared to 12.11 for comparable controls (no progesterone). In Experiment I, corresponding means were 10.16 for rats receiving progesterone and 13.25 for the controls (table 29).

Table 29

Mean Number of Mitoses per 1000 Cells in the Endometrium of Progesterone Treated and Untreated Rats

Experiment	Untreated	0.05 mg Progesterone
I (intact females)	13.25	10.16
II (castrate females)	12.11	3.55

Although the differences between mean numbers of mitoses of the treated and untreated groups were considerable, especially in Experiment II (castrate females), they were not significantly different from zero (tables 26 and 27).

EFFECT OF ESTROGEN. The mean mitotic activity for the estrogen treated rats (estradiol benzoate) in Experiment II (castrate females) was 10.80 while that of the control rats (no estrogen) was 4.86. In Experiment I (intact females) the corresponding means were 11.95 for the treated rats (diethylstilbestrol) and 11.46 for the controls (no estrogen). See table 30.

The difference between mean numbers of mitoses of the treated and untreated groups was not significantly different from zero in either experiment (tables 26 and 27). The fact that the difference was much greater in Experiment II (castrate females)

than in Experiment I (intact females) may be of importance.

Table 30

Mean Number of Mitoses per 1000 Cells in the Endometrium of Estrogen Treated and Untreated Rats

Experiment	Untreated	3 R.U. Estrogen
I (intact females	11.46	11.95*
II (castrate females)	4.86	10.80**

* diethylstilbestrol
** crystalline estradiol benzoate

INTERACTION. A significant interaction between progesterone and estrogen was found in both Experiments (tables 26 and 27). An important point is with reference to the difference in nature of this interaction in the two experiments. In Experiment I (intact females) estrogen and progesterone in combination resulted in a stimulus to endometrial mitosis above that provided by progesterone or estrogen when given separately, whereas in Experiment II (castrate females) the effect of progesterone and estrogen in combination was to depress mitosis below the level given by either treatment when administered separately (table 31).

There was no significant interaction between thiouracil and estrogen or between thiouracil and progesterone in either experiment (tables 26, 27 and 31).

There was no evidence of a second order interaction, namely thiouracil x estrogen x progesterone, in either experiment (tables 26, 27 and 31).

Figures 2 and 3 are photomicrographs of cross-sections

Section of the Uterus of a Castrate Female That Received Estrogen. Note Arrested Mitoses, a Condition Which Was Typical of Animals in Experiment II With a Normal Thyroid Gland.

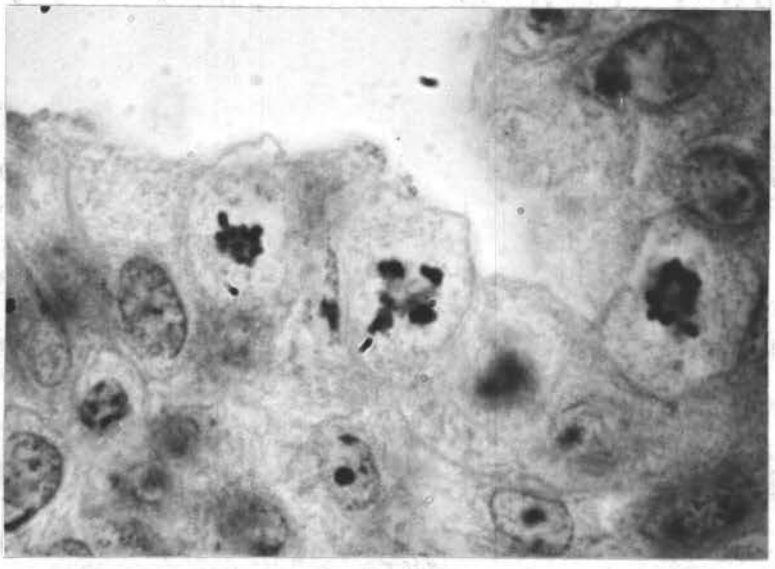


Figure 2.

Section of the Uterus of a Castrate Female That Received Thiouracil and Progesterone. Note Absence of Arrested Mitoses, A Condition Which Was Typical of the Hypothyroid Animals in Experiment II.

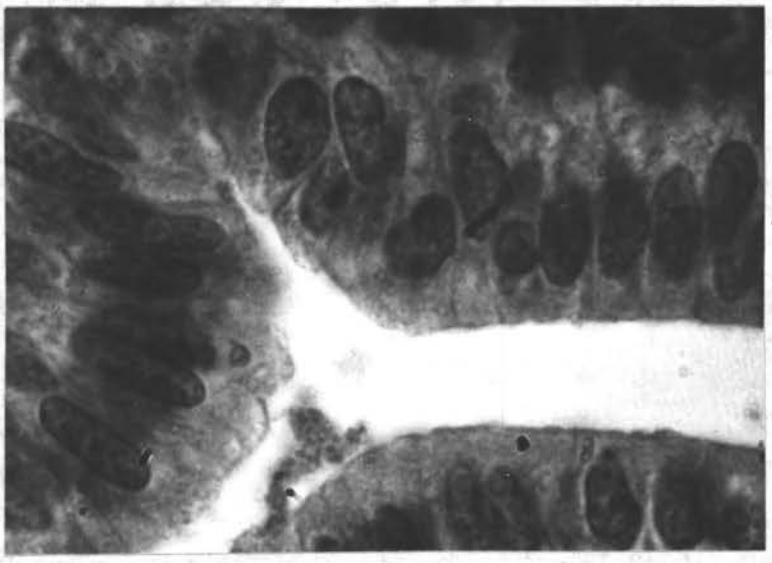


Figure 3.

through the uteri of two of the mature castrate rats of Experiment II. Figure 2 illustrates a high degree of mitotic activity similar to that observed in rats with normal thyroid and those receiving estrogen. In figure 3 there are no arrested mitoses, a condition which was more typical of the hypothyroid rats.

Table 31

Mean Number of Mitoses per 1000 Cells for Each Treatment in Experiments I and II

Treatment	Experiment I	Experiment II
None	16.67	1.75
T	14.53	4.42
P	13.30	9.83
E	14.60	28.83
TP	1.35	3.45
TE	7.18	13.45
PE	16.48	0.58
TPE	9.52	0.33

T - Thiouracil
P - Progesterone
E - Estrogen

Chapter V

DISCUSSION

The estimate for heritability of age at the time of vaginal opening in this study was 16.87 per cent. This figure is the average of heritability estimates obtained for each of the three generations. The average estimate is probably more reliable than any of the estimates for single generations. This indicates that 17 per cent of the variability in age at the time of vaginal opening is due to additive gene action while the remaining 83 per cent of the variability is accounted for by environmental factors, non-additive gene action and environment-gene interactions. It is that portion (17 per cent) of the variability due to additive gene action that can be effectively controlled by selection; and while 17 per cent is not to be considered a high heritability, it is of such magnitude as to offer possibilities of improvement through selection. That age at the time of vaginal opening is partially under genetic control is substantiated by the fact that the progeny of the early and late lines were significantly different with regard to their average ages at the time of vaginal opening. Thus the two lines were separated genetically by selection. Stone and Barker (94, pp.48-50) were also able to change the age of pubescence in rats by selection. After six generations of selection for early and late puberty in both males and females, the females of their two lines differed in age of puberty by an average of 13.9 days. Blunn (19, p.212) concluded that genetic composition was an important factor in determining the age at which rats reached puberty. The

estimate of heritability of age at the time of vaginal opening in rats is in general agreement with estimates of heritability of characters associated with fertility in other species. Thus heritability of litter size in swine has been estimated to be 17 per cent (93, p.366), 22 per cent (35, p.303) and 24 per cent (20, p.97) and heritability estimates for size of litter at weaning have been reported as 22 per cent (20, p.97) and 32 per cent (35, p.303) while estimates for heritability of litter weight at weaning have varied from seven per cent (35, p.303) to 37 per cent (20, p.97).

It should be pointed out that age at the time of vaginal opening is only one of the indications of pubescence and that this must be taken into account especially when making comparisons between species. Further, the estimate of heritability obtained in this study is subject to the usual limitations in application and, like all other heritability estimates, to the errors inherent in the particular method of estimation employed. In the case at hand there are possibilities of over-estimate due to maternal effects and sex-linkage. On the other hand a somewhat higher estimate would be expected if the degree of heterozygosity in the rats studied was more comparable to that normally existing in farm animals such as swine, sheep and cattle.

It is of value to know what effect selection for a given character may have upon other characters. Since selection for early and late age at the time of vaginal opening was successful in this study it was possible by comparison of the performance of the

progeny of the two lines to determine if selection for age at the time of vaginal opening had beneficial, detrimental or no effects on certain productive characters. The characters studied in this connection were number of young born per litter, number of young weaned per litter, weaning weight, weight at the time of vaginal opening, weight at 75 days of age and average daily gains from weaning to the time of vaginal opening. Comparison of the two lines showed that the early line females gave birth to larger litters. The indication is that this greater fertility is most likely the result of a greater functional capacity of the ovary, viz., more eggs shed. If this apparent association between early puberty and high fertility is found to exist in farm animals, age of attaining puberty could then be used as an additional guide in improving fertility. Asdell (7, p.11) is of the opinion that such an association does exist, at least in some species.

The number of young raised per litter and the percentage of litters suckled was the same for the females of the two lines, while the average weaning weight of late-line progeny was greater than that of early-line progeny. In view of the fact that the litters of early-line females were larger at birth it appears that there is a negative relation of fertility to suckling ability. Asdell (8, p.24) found that the weight of the young at birth was inversdy related to the size of the litter and further that the birth weight advantage of the young in smaller litters was maintained through weaning time. Thus the heavier weaning weights of the late-line progeny may be primarily the result of a prenatal advantage which has its origin in the smaller

number of young rather than in any difference in the total amount of food supplied by the females of the two lines either prenatally or postnatally. This does not explain why the average number of young weaned per litter was the same in the two lines even though the average number born was greater in the early line. One explanation that may be offered is that increased gonadotrophic hormone secretion by the anterior pituitary, coincident with early puberty, results in a diminished output of lactogenic hormone and therefore reduced milk production. Asdell and Crowell (9, p.22) have postulated a somewhat similar quantitative inverse relationship between the secretion of the gonadotrophic and growth hormones. This in itself might explain the results obtained here particularly since no direct evidence of a reduced milk secretion was obtained.

There was no difference in the two lines with regard to rate of gain or weight at 75 days of age. The lack of a difference in growth rate from weaning to the time of vaginal opening and weight at 75 days of age indicates that there is no difference between the two lines with regard to potential growth impetus although Cole and Casady (33, p.121) reported that the rats in their early-pubescent strain were heavier at 85 to 90 days of age than were those in their late-pubescent strain.

Selection for several characters must normally be carried out simultaneously. Therefore, it is of importance to know the effect that certain productive characters might have on the progress obtained by selection for early pubescence. This information would be of value

by showing the genetic or physiological correlations of characters and might help in predicting the effectiveness of selection for two or more characters at one time. The effects of number of young born per litter, number of young weaned per litter, weaning weight, weight at the time of vaginal opening and average daily gain from weaning to the time of vaginal opening on age at the time of vaginal opening were determined by use of multiple regression after the line effects were eliminated. The effect of each of the five variates was determined by computing their respective partial regression coefficients while the remaining four variates were held constant mathematically.

Neither the number of young born nor the number of young weaned per litter had an effect on the age at the time of vaginal opening. Thus the size of the litter in which a female was born or reared had no effect in determining her age at the time of vaginal opening. This is in agreement with the findings of Blunn (19, pp.208-210) but contrary to the reports of Engle, Crafts and Zeithaml (42 p.430) who found that females reared in litters of ten or 11 were significantly older at the time of vaginal opening than were those reared in litters of two to five.

The partial regression coefficients for weaning weight (-0.23 days per gram) and daily gain (-4.30 days per gram) were significantly different from zero. Thus the heavier rats at weaning time and those that gained the fastest after weaning were the youngest at the time of vaginal opening. Blunn (19, p.212) also found a negative correlation between daily gain from weaning time to the time of

vaginal opening and age of vaginal opening. Asdell and Crowell (9, pp.22-23) and Evans and Bishop (44, p.333) have likewise found evidence of a relationship between growth rate and age of pubescence. In the present study, each change of one gram in weaning weight brought about a change of 0.23 days in the mean age at the time of vaginal opening. Also, each change of one gram in gain per day resulted in a change of 4.30 days in the mean age at the time of vaginal opening. Therefore, if one selects for high weaning weight and a maximum rate of gain simultaneously he should reduce the age at the time of vaginal opening even though no selection for this trait is practiced.

The multiple correlation coefficient of age at the time of vaginal opening on the five variates studied was 0.89 (R^2= 0.79) indicating that 79 per cent of the variability in age at the time of vaginal opening is accounted for by the variability in the five variates. Thus if one selected for these five characters simultaneously and applied the appropriate selection pressure to each, progress with respect to age at the time of vaginal opening would be expected.

Since average daily gain from weaning to time of vaginal opening was found to be so important in determining the mean age at the time of vaginal opening (partial regression coefficient = -4.30) it seemed important to determine the extent to which the other characters studied were affecting the rate of gain. This was accomplished by the use of multiple regressions. The multiple correlation coefficient obtained was 0.81 and R^2 was 0.66. This indicates that two-thirds of the variability in rate of gain is

accounted for by the variability in the five other variates studied.

Fertility, as indicated by the number of young born per litter, was found to be positively associated with rate of gain after weaning; whereas suckling ability, as indicated by the number of young raised per litter, was not. Thus, the larger the litter at birth the greater were the gains after weaning (partial regression coefficient = 0.02 grams per day). This may be explained on the assumption that only after weaning do the individuals born in large litters overcome the prenatal disadvantage (8, p.24) of lighter birth weight coincident with large litters. It may be seen from the present study that weaning weight is negatively correlated with subsequent daily gains. This lends further to the contention that young in larger litters have a prenatal disadvantage. The partial regression coefficient of daily gain on weaning weight was -0.02 grams per day. Thus for each change of one gram in weaning weight a change of 0.02 grams per day in the mean rate of gain would be expected.

The gonadotrophic hormones of the anterior pituitary and the hormones of the gonads have long been recognized as factors in the development and regulation of the reproductive processes. Since the secretion of the thyroid gland is known to regulate the metabolism of the body in general it seems possible that this hormone may also be a factor in reproduction. Much work has been done which indicates that this is true but the exact effects of thyroxine in this connection and the mechanisms by which it is effective are not known. The work of some investigators (66, pp.760-766; 79, pp.847-849; 98, pp.152-162; 86, p.85; 65, pp.383-384; 67, pp.108-110) indicates that the

thyroid level may alter the response of reproductive tissues to either gonadotrophic or gonadal hormones. Therefore an attempt was made in the present study to determine if thyroid level affected the mitotic response of the endometrium to estrogen and progesterone. Several investigators (66, pp.760-766; 79, pp.847-849; 98, pp.152-162) have presented evidence which indicates that the response of the female reproductive tissues to estrogen is reduced in the hyperthyroid animal and increased in the hypothyroid animal. Perhaps equally as much evidence is available in support of the opposite view. Thus, numerous workers (86, p.85; 65, pp.383-384; 13, p.138; 67, pp.108-110; 90, p.337; 41, p.66; 36, p.453) have reported that the estrous cycle was disturbed in hypothyroid females of various species. Dalton, Morris and Dubrick (36, p.453) also found that the uteri of hypothyroid mice were smaller than those of control animals and Mixner (80, p.578) observed that the normal stimulatory effect of estrogen on the growth of the mammary lobule-alveolar system was inhibited in hypothyroid mice.

The first test in the present study was carried out on immature, intact females after a limited exposure to thiouracil. There was considerable variability in response to all of the treatments as indicated by mitotic rate and no effect of the thyroid could be detected. In the second test ovariectomized females were used so that thiouracil feeding could be continued over a longer period of time without interference from endogenous estrogen or progesterone. The data obtained in this experiment showed that hypothyroidism

resulted in a marked reduction in mitotic activity of the endometrium. The mitotic rate in the endometrium of hypothyroid animals was 5.41 mitoses per 1000 cells while that in the endometrium of normal animals was 10.25 mitoses per 1000 cells. Thus, it is apparent that mitotic activity in the endometrium of hypothyroid animals is lower than normal. This could explain the presence of irregularities or absence of the estrous cycle in hypothyroid animals as observed by several workers as cited above.

Of the remaining treatments tested the only one that had a significant effect on the mitotic rate in the endometrium was the simultaneous administration of estrogen and progesterone. This treatment in the castrate females resulted in a decrease in mitotic activity below the level obtained when either hormone was given separately. Thus under these conditions progesterone exerted a depressing rather than synergistic effect on estrogen. The effect of simultaneous injection of estrogen and progesterone produced in the intact rats of Experiment I a higher rate of mitotic activity than occurred when either was administered alone. Thus in this case there was an apparent synergistic action of the two hormones. Bureau (25, pp.934-936) studied the effects of estrogen and progesterone on mitotic response in the uterine epithelium of ovariectomized rabbits and found that maximum response occurred when progesterone injections followed pretreatment with estradiol benzoate. Williams (99, p.290) noted a similar relationship between estrogen and progesterone with regard to their ability to promote growth of the rat uterus.

Korenchevsky and Hall (63, p.1048) reported that progesterone can prevent or decrease the usual stimulatory effects of estrogen. Gillman (48, p.342) has also shown that the related action of estrogen and progesterone may be synergistic or antagonistic depending upon the time of injections and the amount of each of the hormones. Thus if progesterone administration follows estrogen administration a synergistic effect is noted whereas if both are injected at the same time a depressing or antagonistic effect occurs. A possible explanation for the opposite results obtained in the two experiments of this study is that the inhibiting effect of progesterone on the action of estrogen, as observed in the castrate females, represents the usual situation when the two hormones are administered simultaneously and that the snynergistic action, as observed in the intact females, is typical of the situation when estrogen administration precedes progesterone administration. In the present instance, it is presumed that the intact ovaries of the rats in Experiment I were actively secreting estrogen prior to the actual administration of hormones.

The presence of endogenous estrogen in the intact females prior to and at the time of hormone treatment, a short duration of thiouracil administration in Experiment I and the moderate levels of hormones administered may have accounted for some of the unexplained variability. Another factor which may have contributed to the within treatment variability in the experiment with intact females is that hypothroidism brings about an increase in secretion of the follicle

stimulating hormone (31, pp.90-102). This greater output of follicle stimulating hormone would presumably result in the ovary becoming functional in producing estrogen at an earlier age in the intact rats that received thiouracil.

Bureau (25, pp.933-936), Allen et al. (4, pp.133-181), Allen, Smith and Gardner (5, pp.325-327), Worthington and Allen (102, pp.137-153), Tyslowitz (97, pp.265-273) and Barks and Overholser (14, pp.401-411) have all shown that estrogen stimulates mitosis in the uterine epithelium and/or musculature of ovariectomized animals. Failure to obtain clearcut evidence of such estrogen action in the studies reported here may be due to the small quantity of estrogen given. However, it was considered important not to give massive doses which might bring about a stimulation in excess of that which would occur in the normal animal.

Chapter VI

CONCLUSIONS

(1) Heritability of age of female rats at the time of vaginal opening was estimated to be 17 per cent.

(2) Selection for the extremes in age at the time of vaginal opening was successful and therefore genetically early and late lines were established.

(3) The two divergent lines also differed in average litter size at birth, average weaning weight, and average weight at the time of vaginal opening.

(4) An association between early puberty and prolificacy apparently exists. There is some indication that this association is between early puberty and number of ova shed.

(5) The multiple correlation coefficient for age at the time of vaginal opening with the five other variates studied was 0.89 (R^2= 0.79) indicating that much of the variability in age at the time of vaginal opening is accounted for by the variability in number of young born and raised per litter, weaning weight, weight at the time of vaginal opening and daily gain from weaning to the time of vaginal opening.

(6) Partial regression coefficients show that age at the time of vaginal opening is not affected by the size of the litter either at birth or at weaning, but that it is negatively correlated with weaning weight and daily gain.

(7) Rate of gain from weaning to the time of vaginal opening is influenced by number of young born and raised in the litter in which they are raised, weaning weight and age and weight at the time of vaginal opening.

(8) Partial regression coefficients show that increases in number of young born and weight at the time of vaginal opening are associated with greater gains and that increases in weaning weight and age at the time of vaginal opening are associated with decreased rate of gain.

(9) Hypothyroidism induced by feeding thiouracil reduces mitotic activity in the endometrium.

(10) The effects of estrogen and progesterone on mitotic activity of the endometrium when administered simultaneously to castrate females are antagonistic.

BIBLIOGRAPHY

1. Allen, E. (ed). Sex and internal secretions. 2d ed. Baltimore, Williams, 1939. 529p.

2. _____ and R. N. Creadick. Ovogenesis during sexual maturity. The first stage, mitosis in the germinal epithelium, as shown by the colchicine technique. Anatomical record 69: 191-195. 1937

3. _____ and E. A. Doisy. An ovarian hormone: preliminary report on its localization, extraction and partial purification, and action in test animals. Journal american medical association 81:819-821. 1923.

4. _____, et al. The hormone of the ovarian follicle; its localization and action in test animals and additional points bearing upon the internal secretion of the ovary. American journal of anatomy 34:133-181. 1924.

5. _____, G. M. Smith and W. U. Gardner. Accentuation of the growth effect of theelin on gential tissues of the ovariectomized mouse by arrest of mitosis with colchicine. American journal of anatomy 61:321-329. 1937.

6. Asdell, S. A. Research in animal physiology and genetics in Great Britain. Record of the proceeding annual meeting american society of animal production. 3-7. 1930.

7. _____. Patterns of mammalian reproduction. Ithaca, Comstock publishing co. 1946. 437p.

8. _____, Ralph Bogart and G. Sperling. The influence of age and rate of breeding upon the ability of the female rat to reproduce and raise young. New York (Cornell) agricultural experiment station memoirs 238. 1941. 26p.

9. _____, and Mary F. Crowell. The effect of retarded growth upon the sexual development of rats. Journal of nutrition 10:13-24. 1935.

10. Astwood, E. B. A six-hour assay for the quantitative determination of estrogen. Endocrinology 23:25-31. 1938.

11. _____, and Adele Bissell. Effect of thiouracil on the iodine content of the thyroid gland. Endocrinology 34:282-296. 1944.

12. Astwood, E. B. et al. Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. Endocrinology 32:210-225. 1943.

13. Barker, S. B. The influence of thiouracil on reproduction and growth in the rat. Journal of endocrinology 6:136-143. 1950.

14. Barks, Orville L. and Milton D. Overholser. Hyperplasia and hypertrophy of the uterine musculature in ovariectomized rats following estrone injections. Anatomical record 70: 401-411. 1938.

15. Bevelander, Gerrit. Effect of thiourea on development of the sea urchin Arbacia punctulata. Proceedings of the society of experimental biology and medicine 61:268-270. 1946.

16. Bischoff, Fritz and Georgena J. Clarke. Gonadotropic stimulation in intact and thyroidectomized adult female rats. Endocrinology 29:27-30. 1941.

17. Blandau, Richard J. and William L. Money. The attainment of sexual maturity in the female albino rat as determined by the copulatory response. Anatomical record 86:197-215. 1943.

18. Blaxter, K. L. et al. The role of thyroidal materials and of synthetic goitrogens in animal production and an appraisal of their practical use. Journal of animal science 8:307-352. 1949.

19. Blunn, Cecil T. The age of rats at sexual maturity as determined by their genetic constitution. Anatomical record 74:199-213. 1939.

20. _____ and Marvel L. Baker. Heritability estimates of sow productivity and litter performance. Journal of animal science 8:89-97. 1949

21. Bogart, R., John F. Lasley and Dennis T. Mayer. Influence of reproductive hormones upon growth in ovariectomized and normal female rats. Endocrinology 35:173-181. 1944.

22. _____, R., John F. Lasley and Dennis T. Mayer. Influence of reproductive condition upon growth in the female rat. American journal of physiology 128:355-371. 1940.

23. Bowstead, J. E. The effect of breeding immature ewes. Scientific agriculture 10:429-459. 1930.

24. Brody, S. and R. F. Frankenback. Age changes in size, energy metabolism and cardiorespiratory activities of thyroidectomized cattle. Missouri agricultural experiment station bulletin 349. 1941

25. Bureau, V. L'action de l'estrone et de la progesterone sur la corne uterine de la lapine etudiee par la methode a la colchicine. Comptes rendus societe de biologie 130:933-936. 1939.

26. Burkhart, Elizabeth Z. A study of the early effects of androgenic substances in the rat by the aid of colchicine. Journal of experimental zoology 89:135-165. 1942.

27. Castle, W. E. A further study of size inheritance in rabbits, with special reference to the existence of genes for size characters. Journal experimental zoology 53:421-454. 1929.

28. Chapman, Arthur B. Genetic and nongenetic sources of variation in the weight response of the immature rat ovary to gonadotrophic hormone. Genetics 31:494-507. 1946.

29. _____. Selection in laboratory animals. Presented at the symposium on "The effectiveness of selection for economically important characters" at the 41st meeting of the american society of animal production. 1949. (typewritten 1-9).

30. Chouke, K. S. and Herman T. Blumenthal. Further investigations on the proliferative activity of the thyroid gland of the female guinea pig during the sexual cycle. Endocrinology 30:511-515. 1942.

31. Chu, J. P. Influence of the thyroid gland on pituitary gonadotrophic activity in the rabbit. Endocrinology 34:90-102. 1944.

32. Clark, R. T., Bradford Knapp, A. L. Baker and J. L. Quesenberry. Performance testing of beef cattle. Montana State College bulletin 417. 1943.

33. Cole, H. H. and R. B. Casady. Studies on indices and causes of prolificacy in rats. Endocrinology 41:119-126. 1947.

34. Crandall, William D. A quantitative study of the influence of the ovarian hormones on hyperplasia by mitosis in the rabbit uterus in early pregnacy. Anatomical record 72:195-210. 1938.

35. Cummings, J. N., L. M. Winters and H. A. Stewart. The heritability of some factors affecting productivity of brood sows. Journal of animal science 6:297-304. 1947.

36. Dalton, A. J., H. P. Morris and C. S. Dubrick. Changes in the organs of female C3H mice receiving thiourea. Journal of the national cancer institute 5:451-454. 1945.

37. Dickerson, G. E. and J. C. Grimes. Effectiveness of selection for gain in Duroc swine. Journal of animal science 6:265-287. 1947.

38. _____ and L. N. Hazel. Selection for growth rate of pigs and productivity of sows. Journal of animal science 3:201-212. 1944.

39. Dustin, A. La radiotherapie et la chimiotherapie envisagees a la lumiere des travaux sur les chocs caryoclasiques. Journal of the science medicine de Lille 49:561-573. 1934.

40. _____. L'action de la colchicine sur les tumerus malignes. Leeuwenhoekvereeniging, IV ieme conference, Amsterdam 7-36. 1935.

41. Engle, E. T. The effect of hypothyroidism on mensturation in adult Rhesus monkeys. Yale journal of biology and medicine 17:59-66. 1944.

42. _____, R. C. Crafts and C. E. Zeithaml. First estrus in rats in relation to age, weight and length. Proceeding of the society experimental biology and medicine 37:427-432. 1937.

43. Ershoff, Benjamin H. Effects of thyroid feeding on ovarian development in the rat. Endocrinology 37:218-220. 1945.

44. Evans, H. M. and K. S. Bishop. On the relation between fertility and nutrition. The ovulation rhythm in the rat on a standard nutritional regime. Journal metabolic research 1:319-334. 1922.

45. _____ and K. S. Bishop. On the relation between fertility and nutrition. The ovulation rhythm in the rat on inadequate nutritional regimes. Journal metabolic research 1:335. 1922.

46. Fleishmann, Walter, and I. A. Breekler. Mitotic and wound healing activities of the corneal epithelium in thiouracil treated and thyroidectomized rats. Endocrinology 41:266-268. 1947.

47. Freudenberger, C. B. A comparison of the Wistar albino and the Long-Evans hybrid strain of the Norway rat. American journal anatomy 50:293-349. 1932.

48. Gillman, Joseph. Profound vascular changes induced in the uterus of the castrated rabbit by combinations of estradiol benzoate and progesterone. Endocrinology 29:336-342. 1941.

49. Greenman, M. J. and F. L. Duhring. Breeding and care of the albino rat for research purposes. Wistar Institute 1-109. 1923.

50. Gregory, P. W. The potential and actual fecundity of some breeds of rabbits. Journal experimental zoology 62:271-285. 1932.

51. Guyer, M. F. Animal micrology. 4th ed. Chicago, The University of Chicago press. 1945. 331p.

52. Hetzer, H. O. and G. W. Brier. Extent to which type differences among swine affect litter size. Proceedings american society animal production 135-138. 1940.

53. _____, W. V. Lambert and J. H. Zeller. Influence of inbreeding and other factors on litter size in Chester white swine. United States department of agriculture circular number 570. 1940. 10p.

54. Hunt, Thomas E. Mitotic activity in the anterior hypophysis of female rats. Anatomical record 82:263-275. 1942.

55. ____. Mitotic activity in the thyroid gland of female rats. Anatomical record 90:133-138. 1944

56. ____. Mitotic activity in the anterior hypophysis of ovariectomized rats after injection of estrogens. Anatomical record 97:127-137. 1947.

57. Hunt, Wells E. The effect of size and type of ewe on efficiency of production. Maryland agricultural experiment station bulletin 380. 1935.

58. Jones, G. E., E. Delfs and E. C. Foote. The effect of thiouracil hypothyroidism on reproduction in the rat. Endocrinology 38:337-344. 1946.

59. Keith, T. B. Relation of size of swine litters to age of dam an to size of succeeding litters. Journal of agricultural research 41:593-600. 1930

60. King, Helen Dean. The relation of age to fertility in the rat. Anatomical record 11:269-287. 1916.

61. ____. Life processes in gray Norway rats during fourteen years in captivity. American anatomical memoir 17:5-77. 1939.

62. ____, and H. H. Donaldson. Life processes and size of the body and organs of the gray Norway rat during ten generations in captivity. American anatomical memoirs 14:1-106. 1929.

63. Korenchevsky, V., and K. Hall. Metaplasia and adenoma-like changes in the uterus of rats injected with sex hormones. Nature 144:1048. 1939.

64. Krider, J. L., et al. Effectiveness of selecting for rapid and slow growth rate in Hampshire swine. Journal of animal science 5:3-15. 1946.

65. Krohn, P. L. and H. C. White. The effect of hypothyroidism on reproduction in the female albino rat. Journal of endocrinology 6:375-385. 1950.

66. Langham, Wright and R. G. Gustavson. Effect of level of thyroid activity on response of ovariectomized rats to estrone. American journal of physiology 150:760-767. 1947.

67. Laqueur, G. and L. A. Emge. Thyroid-ovarian relations. III. Morphylogic ovarian changes in estrogen treated thyrohyperplastic rats. Endocrinology 29:108-110. 1941.

68. Levine, Michael. Colchicine and X-rays in the treatment of plant and animal over-growths. Botanical review 11:145-180. 1945.

69. Lits, F. J. Recherches sur les reactions et lesions cellulaires provoquees par la colchicine. Archives international medicine experiment, Paris 11:811-901. 1936

70. Long, J. A. and A. M. Evans. On the attainment of sexual maturity and the character of the first estrus cycle in the rat. Anatomical record 18:244. 1920.

71. Ludford, R. J. The action of toxic substances upon the division of normal and malignant cells in vitro and vivo. Archives fur experimentalle zellforschung 18:411-441. 1936.

72. Lush, J. L. Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. Proceedings of the american society of animal production. 293-301. 1940.

73. Lush, J. L. Animal breeding plans. 3d ed. Ames, The Iowa State College press. 1945. 443p.

74. ____. Heritability of quantitative characters in farm animals. Proceedings of 8th international congress of genetics (Hereditas, suppl.) 356-375. 1949.

75. MacArthur, J. W. Genetics of body size and related characters. II. Satellite characters associated with body size in mice. American naturalist 78:224-237. 1944.

76. MacKenzie, C. G. and J. B. MacKenzie. Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. Endocrinology 32:185-209. 1943.

77. Maqsood, M. and E. P. Reineke. Influence of environmental temperatures and thyroid status on sexual development in the male mouse. American journal of physiology 162:24-30. 1950.

78. McKenzie, Fred F. Growth and reproduction in swine. Missouri agricultural experiment station research bulletin 118. 1928. p.67.

79. Meyer, A. E. and A. Wertz. Metabolism in normal and thyroidectomized rats as influenced by thyroxine and thyroid globulin feeding. Proceeding of the society of experimental biology and medicine 38:847-849. 1938.

80. Mixner, J. P. The influence of thiouracil on mammary lobule-alvoelar growth in mice. Journal of dairy science 30:578. 1947.

81. _____, and C. W. Turner. Influence of thyroxine upon mammary lobule-alvoelar growth. Endocrinology 31:345-348. 1942.

82. Pearson, Oliver P. and Charles F. Bassett. Size of the vulva and its relation to fertility. Anatomical record 89:455-459. 1944.

83. Phillips, Ralph W. and W. M. Dawson. Relation of type and time of birth and birth weight of lambs to their survival, growth and suitability for breeding. Proceeding of american society of animal production. 296-306. 1937.

84. _____, and John H. Zeller. Sexual development in small and large types of swine. Anatomical record 85:387-400. 1943.

85. Rice, V. A. and F. N. Andrews. Breeding and improvement of farm animals. 4th ed. New York, McGraw-Hill book company, inc. 1951. 787p.

86. Richter, C. P. The role of the thyroid gland in the production of gross body activity. Endocrinology 17:73-87. 1933.

87. Silberberg, M. and Ruth Silberberg. Growth and development of the long bones of castrate mice under the influence of thyroxine. Anatomical record 98:181-189. 1947.

88. Slonaker, J. R. The effect of pubescence, oestruation and menopause on the voluntary activity in the albino rat. American journal physiology 68:294-315. 1924.

89. Snedecor, G. W. Statistical methods. 4th ed. Ames, The collegiate press, inc. 1946. 485p.

90. Spielman, A. A., et al. General appearance, growth and reproduction of thyroidectomized bovine. Journal of dairy science 28:329-337. 1945.

91. Stein, K. F., J. Quimby and A. Moeler. Response of germinal epithelium to thyroid tissue or thyroxine in the ovarian capsule of the mouse. Anatomical record 99:249-264. 1947.

92. Stewart, H. A. Appraisal of factors affecting prolificacy in swine. Journal of animal science 4:250-260. 1945.

93. _____. Appraisal of factors affecting prolificacy in swine. Journal of animal science 4:359-366. 1945.

94. Stone, Calvin P. and R. G. Barker. Change of the age of puberty in albino rats by selective mating. Proceeding of the society of experimental biology and medicine 44:48-50. 1940.

95. Trentin, J. J., V. Hurst and C. W. Turner. Thiouracil and mammary growth. Proceedings of the society for experimental biology and medicine 67:461-463. 1948.

96. Tyler, W. J. and A. B. Chapman. Genetically reduced prolificacy in rats. Genetics 33:565-576. 1948.

97. Tyslowitz, Richard. The action of estrogens in inducing mitoses in the muscle, connective tissue and epithelium of the prostate and seminal vesicle of mice as determined by the colchicine technique. Anatomical record 75:265-273. 1939.

98. Van Horn, W. M. The relation of the thyroid to the hypophysis and ovary. Endocrinology 17:152-162. 1933.

99. Williams, Maude F. The vascular architecture of the rat uterus as influenced by estrogen and progesterone. American journal of anatomy 83:247-294. 1948.

100. Wilson, J. G. and W. C. Young. Sensitivity to estrogen studied by means of experimentally induced mating response in the female guinea pig and rat. Endocrinology 29:779-783. 1941.

101. Winters, L. M., et al. Factors affecting productivity in breeding sheep. University of Minnesota agricultural experiment station. Technical bulletin 174. 1946.

102. Worthington, Richard V. and Edgar Allen. Growth of genital tissues in response to estrone as studied by the colchicine technique. Yale journal of biology and medicine 12:137-153. 1939.

APPENDIX

Table 32

Age (days) at Time of Vaginal Opening of Original Females from which Foundation Females Were Selected

Female Number[1]	Age in days[2]	Female Number[1]	Age in days[2]
1H*	50	4H	57
1T*	50	4T	57
1L*	50	4L	57
1R*	50	4HT	57
1HT*	50	4HL	57
1HL*	50	4HR	57
3TL*	57	4TL	57
3TR*	57	4TR	57
5H*	57	4LR	57
5T*	57	5L	57
1HR	57	5R	57
1TL	57	1TR**	57
1LR	57	1HTL**	57
3H	57	3HTL**	57
3T	57	4R**	57
3L	57	2H**	59
3R	57	2L**	59
3HT	57	2R**	59
3HL	57	2HT**	59
3HR	57	2HL**	59
3LR	57	2T**	66

1/Numbers were arbitrarily assigned and have no significance with regard to relationship of the individuals.

2/Shipping and change in environment may have altered the age at time of vaginal opening to some extent.

*Selected as a foundation dam of the early line.

**Selected as a foundation dam of the late line.

Table 33

Performance Record of Female Progeny

Early Line, First Generation.

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
1H	1	2	2	22H	59	39	112	3.79	194	56.5
	2	2	2	H	50	49	-	-		46.5
1T	1	6	4	8H	55	44	124	3.63	124	39.5
				8T	40	51	-	-	169	
				8L	48	47	120	3.27	165	
	2	12	12	H	43	42	96	3.12		42.5
				T	42	46	102	2.86		
				L	39	44	94	2.90		
				R	41	45	101	3.00		
				HT	46	41	98	3.25		
				HL	45	45	110	3.25		
				HR	39	46	102	3.00		
				TL	43	41	97	3.38		
	3	13	6	H	66	43	141	4.17		65.5
				T	61	44	135	3.89		
				L	62	42	135	4.29		
	4	7	0	Female died in parturition						
1L	1	12	10	18H	44	42	103	3.47	180	44.6
				18T	45	43	108	3.50	168	
				18L*	49	41	115	4.12	168	
				18R	41	42	105	3.77	179	
				18HT	45	42	106	3.59	198	
	2	12	9	H	47	40	116	4.60		44.6
				T	47	37	108	5.08		
				L	41	40	97	3.73		
				R	42	40	106	4.27		

*Selected as a dam of the succeeding generation.

1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.

2/Average from weaning (25 days of age) to time of vaginal opening.

3/Average of males and females.

Table 33

Early Line, First Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at V0	Wt. at V0[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
	3	12	12	H	46	44	121	3.95		44.6
				T	45	43	118	4.06		
				L	40	47	109	3.14		
				R	47	41	120	4.56		
				HT	45	42	113	4.00		
				HL	47	44	125	4.11		
				HR	42	44	115	3.84		
	4	7	0							
	5	12	5	H	68	38	127	4.54		65.8
				T	69	32	100	4.43		
				L	56	38	117	4.69		
1R	1	12	10	12H	43	50	114	2.84	163	46.5
				12T	45	47	120	3.41	172	
				12L	44	42	-	-	160	
				12R	45	46	126	3.86	189	
	2	9	5	H	61	37	109	4.00		55.2
				T	55	36	96	3.73		
				L	54	37	100	3.83		
				R	48	43	110	3.44		
	3	9	0							
	4	11	0	Female died in parturition						
1HT	1	13	13	6H	37	44	114	3.42	180	37.2
				6T	32	51	120	2.92	153	
				6L	33	47	100	2.50	162	
				6R	38	46	112	3.52	160	
				6HT	33	46	114	3.86	178	
	2	1	0							
	3	10	10	H	36	43	100	3.56		39.3
				T	36	45	121	4.25		
				L	37	43	107	3.89		
	4	9	9	H	47	42	120	4.29		46.9
				T	44	43	113	3.83		
				L	45	-	-	-		
	5	8	8	H	53	-	-	-		56.1
				T	58	-	-	-		
				L	53	-	-	-		
				R	54	-	-	-		

Table 33

Early Line, First Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at V0	Wt. at V0[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
1HL	1	9	9	20H*	56	40	113	3.80	177	59.4
				20T*	63	42	140	4.53	193	
				20L	56	45	117	3.05	177	
				20R	62	43	130	3.78	201	
				20HT*	57	36	107	4.54	182	
				20HL	62	42	145	4.88	192	
				20HR*	60	40	112	3.47	166	
				20TL	57	42	126	4.06	181	
	2	6	6	H	68	39	-	-		66.3
				T	61	41	-	-		
	3	12	12	H	42	43	117	4.17		43.9
				T	46	39	107	4.36		
				L	39	44	120	4.26		
				R	42	43	119	4.28		
	4	11	0							
	5	11	0	Female died in parturition						
3TL	1	11	10	16H*	53	40	112	3.93	181	54.8
				16T	51	42	118	3.94	173	
				16L	56	44	127	3.74	178	
				16R	51	46	119	3.24	168	
	2	9	0							
	3	9	0							
	4	7	7	H	53	-	-	-		56.0
				T	51	-	-	-		
				L	55	-	-	-		
				R	54	-	-	-		
	5	10	7	H	51	40	114	4.20		51.0
				T	55	38	114	4.54		
				L	53	40	127	4.93		
				R	49	40	108	3.93		
				HT	48	42	127	4.65		
3TR	1	15	13	14H	45	43	113	3.78	158	45.9
				14T*	35	38	91	4.31	168	
				14L	47	42	115	4.00	173	
				14R	43	43	103	3.33	166	
				14HT*	46	41	109	3.94	173	
	2	9	4	H	51	48	129	3.39		57.8
	3	14	12	H	47	41	112	4.06		45.3
				T	45	41	100	3.44		

Table 33

Early Line, First Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt	Age at V0	Wt. at V0[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
3TR Cont.				L	36	45	104	3.40		
				R	43	46	129	4.10		
				HT	50	42	121	4.18		
				HL	45	41	112	4.19		
	4	15	0	Female died in parturition						
5H	1	5	0							
	2	10	8	H	56	40	122	4.40		54.5
				T	56	38	113	4.38		
				L	47	38	99	4.00		
				R	55	37	100	3.75		
	3	9	0							
	4	10	0	Female died in parturition						
5T	1	8	8	10H	58	43	128	3.89	176	55.6
				10T*	51	39	96	3.21	168	
				10L*	57	39	103	3.29	178	
	2	10	9	H	46	51	121	2.88		47.6
				T	47	45	105	2.90		
				L	49	46	115	3.14		
				R	48	43	100	2.89		
				HT	46	43	98	2.89		
				HL	46	46	109	3.00		
				HR	50	47	117	3.05		
	3	10	7	H	50	46	142	4.38		57.0
				T	60	44	157	5.11		
				L	61	-	-	-		
	4	9	9	H	41	45	113	3.60		45.7
				T	44	45	119	3.75		
				L	47	43	136	4.94		
				R	43	44	123	4.21		
				HT	43	-	-	-		
				HL	47	44	118	3.74		
	5	8	5	H	62	36	118	5.09		63.6
				T	67	38	113	3.54		

*Selected as a dam of the succeeding generation.
1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.
2/Average from weaning (25 days of age) to time of vaginal opening.
3/Average of males and females.

Table 33

Early Line, Second Generation

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
10T	1	9	8	32H	47	46	119	3.43	180	48.2
				32T	45	44	110	3.42	171	
				32L	48	44	117	3.63	169	
				32R*	46	42	112	3.88	171	
				32HT	47	48	133	3.74	173	
				32HL	47	44	119	3.79	169	
	2	7	0	Female died soon after parturition						
10L	1	10	10	26H	42	43	102	3.33	176	40.5
				26T	38	45	116	3.90	178	
				26L	39	44	114	3.95	175	
				26R*	42	40	108	4.40	177	
	2	12	12	H	31	49	108	3.21		33.0
				T	31	50	110	3.16		
				L	35	46	113	3.71		
				R	33	49	113	3.33		
				HT	32	45	102	3.50		
				HL	34	-	-	-		
	3	8	8	H	59	36	104	4.09		61.8
14T	1	10	10	28H	49	44	129	4.21	193	47.6
				28T	49	45	123	3.70	183	
				28L*	43	41	104	3.81	193	
				28R	45	43	101	3.11	184	
	2	8	0							
	3	9	6	H	59	35	106	4.70		57.3
					58	33	88	3.75		
					53	37	97	3.67		
14HT	1	11	11	30H*	53	40	115	4.13	167	50.6
				30T*	51	42	111	3.53	181	

*Selected as a dam of the succeeding generation.

1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.

2/Average from weaning (25 days of age) to time of vaginal opening.

3/Average of males and females.

Table 33

Early Line, Second Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at V0	Wt. at V0[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
14HT Cont.				30L*	54	42	123	4.06	181	
				30R	43	47	114	3.23	178	
	2	10	4	H	20	-	-	-		44.8
				T	49	48	129	3.48		
				L	55	-	-	-		
	3	10	7	H	60	41	136	4.75		65.1
16H	1	10	10	34H*	45	40	107	4.13	192	43.7
				34T*	45	39	101	4.00	185	
				34L*	37	42	100	3.71	175	
				34R*	43	40	105	4.13	205	
	2	10	4	H	53	44	119	3.47		59.8
				T	61	-	-	-		
	3	12	7	H	63	37	124	5.08		54.6
				T	60	33	91	3.88		
				L	57	37	111	4.50		
				R	47	36	91	4.00		
				HT	40	44	115	3.95		
18L	1	10	0							
	2	12	3	H	40	47	113	3.32		39.7
	3	13	12	H	43	50	129	3.44		43.1
				T	42	40	100	3.87		
				L	44	35	77	3.30		
				R	43	39	103	4.29		
				HT	44	43	114	3.89		
				HL	40	39	98	4.14		
				HR	42	41	105	3.94		
				TL	43	38	96	4.08		
20H	1	6	2	38H	41	47	138	4.41	180	39.5
	2	9	0							
	3	7	0							
20T	1	10	0	Female died in parturition						

Table 33

Early Line, Second Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
20HT	1	14	0							
	2	12	10	H	40	41	104	4.00		41.0
				T	37	39	89	3.71		
				L	45	39	100	3.93		
				R	41	42	103	3.65		
				HT	45	42	116	4.18		
	3	10	0							
20HR	1	7	0	Female died in parturition						

*Selected as a dam of the succeeding generation.

1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.

2/Average from weaning (25 days of age) to time of vaginal opening.

3/Average of males and females.

Table 33

Early Line, Third Generation

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
26R	1	9	7	50H	52	40	118	4.40	192	54.2
				50T	51	39	104	3.79	173	
				50L	51	43	112	3.39	173	
				50R*	54	37	108	4.50	187	
28L	1	10	0							
30H	1	13	0							
30T	1	9	0							
30L	1	12	12	52H	43	40	107	4.27	159	40.2
				52T	32	-	-	-		
				52L	40	39	93	3.79	169	
				52R	41	44	107	3.47	155	
32R	1	10	10	56H*	50	37	94	3.67	161	47.3
				56T	48	38	105	4.38	175	
				56L*	46	37	98	4.33	174	
				56R	45	45	114	3.45	168	
				56HT*	46	35	83	3.70	155	
				56HL	41	38	95	4.15	162	
34H	1	10	6	62H	47	44	114	3.53	190	51.5
				62T	47	49	121	3.08	170	
				62L	45	43	115	3.89	182	
34T	1	14	13	58H	43	-	-	-		40.9
				58T*	39	38	94	4.23	175	
				58L	39	40	98	3.93	177	
				58R	42	39	110	4.86	183	
				58HT*	38	38	98	4.62	180	
				58HL*	42	38	104	4.77	194	
				58HR	36	39	97	4.36	169	
34L	1	9	9	54H*	53	34	96	4.78	182	54.1
				54T*	53	37	96	3.58	179	

Table 33

Early Line, Third Generation (Continued)

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
34R	1	13	12	60H*	45	35	85	4.00	180	42.7
				60T	44	38	103	4.54	187	
				60L	40	39	105	4.64	183	
				60R	45	39	107	4.43	192	
				60HT	42	41	110	4.25	183	
				60HL	32	46	112	3.81	169	

*Selected as a dam of the succeeding generation.

1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.

2/Average from weaning (25 days of age) to time of vaginal opening.

3/Average of males and females.

Table 33

Late Line, First Generation

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
1TR	1	9	8	13H	58	39	114	4.00	201	59.8
	2	9	0							
	3	6	5	H	67	40	136	4.60		65.6
				T	62	42	137	4.41		
	4	10	0	Female died in parturition						
1HTL	1	8	8	23H	55	44	122	3.53	197	
				23T	53	40	116	4.20	194	
				23L	54	42	134	4.71	191	
				23R	51	41	115	4.00	195	
	2	10	8	H	44	43	106	3.44		46.9
				T	38	48	-	-		
	3	9	9	H	57	46	138	3.86		55.6
				T	55	43	133	4.33		
				L	53	43	127	4.11		
				R	49	45	130	4.05		
	4	9	5	H	23	-	-	-		30.4
				T	33					
	5	9	8	H	50	43	123	4.06		49.4
				T	47	42	106	3.47		
				L	50	38	106	4.31		
2H	1	9	9	9H*	52	47	122	3.18	160	52.7
				9T	49	44	112	3.32	149	
				9L*	50	46	117	3.19	167	
				9R	50	43	110	3.33	161	
				9HT*	52	45	125	3.65	164	
	2	10	8	H	48	45	121	3.65		50.0
				T	47	45	131	4.20		
				L	49	44	125	4.00		
				R	46	45	129	4.15		

*Selected as a dam of the succeeding generation.

1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.

2/Average from weaning (25 days of age) to time of vaginal opening.

3/Average of males and females.

Table 33

Late Line, First Generation (Continued)

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at V0	Wt. at V0[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
2H cont.	3	5	5	H	67	43	135	3.78		66.4
				T	66	43	142	4.22		
				L	68	42	144	4.47		
	4	9	6	H	53	-	-	-		52.0
				T	41	-	-	-		
	5	7	0	Female died in parturition						
2T	1	8	8	15H	55	38	112	4.38	186	53.2
				15T	54	43	121	3.72	181	
				15L	55	48	132	3.35	164	
				15R	50	43	122	4.00	176	
				15HT	52	38	97	3.46	177	
				15HL	52	41	117	4.06	177	
				15HR	56	42	126	4.12	184	
	2	9	8	H	46	43	98	2.89		49.2
				T	47	43	107	3.33		
				L	50	46	115	3.10		
				R	48	41	101	3.31		
				HT	49	44	103	2.84		
	3	9	9	H	48	43	117	3.83		49.3
				T	48	42	117	4.06		
				L	49	39	114	4.64		
				R	53	44	131	4.10		
	4	11	0	Female died in parturition						
2L	1	10	10	19H	51	43	113	3.44	162	51.1
				19T*	53	46	118	3.10	171	
				19L*	52	51	111	2.27	155	
				19R	44	44	101	3.00	159	
				19HT*	51	45	108	2.85	165	
				19HL*	51	45	112	3.05	178	
	2	8	8	H	49	45	117	3.40		46.9
				T	48	46	122	3.52		
				L	49	44	116	3.53		
				R	48	45	120	3.60		
				HT	46	47	116	3.18		
	3	12	10	H	43	49	114	2.96		48.8
				T	45	47	130	3.86		
				L	49	45	128	3.95		
				R	49	48	145	4.17		

Table 33

Late Line, First Generation (Continued)

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
2L cont.	3 (cont.)			HT	48	50	131	3.32		
	4	10	9	H	48	47	131	3.77		46.4
				T	44	49	125	3.38		
				L	47	49	136	3.71		
				R	43	45	113	3.50		
				HT	43	49	127	3.50		
				HL	47	-	-	-		
	6	6	0							
2R	1	12	11	21H	52	42	112	3.53	183	49.6
				21T	52	44	131	4.16	195	
				21L	50	43	114	3.56	132	
				21R	55	44	127	3.79	175	
				21HT	48	44	122	3.89	185	
				21HL	36	44	101	3.42	151	
	2	12	11	H	44	49	-	-		44.7
				T	44	47	-	-		
				L	48	42	-	-		
				R	47	47	-	-		
				HT	43	43	-	-		
				HL	46	40	107	4.07		
	3	9	0	Female died in parturition						
2HT	1	10	10	7H	56	45	-	-	193	56.2
				7T	60	42	139	4.65	144	
				7L	57	44	133	4.00	195	
				7R*	52	46	121	3.29	171	
	2	8	8	H	49	47	134	3.86		53.9
				T	58	46	144	4.10		
				L	52	41	114	3.88		
				R	48	48	131	3.60		
				HT	59	44	147	4.63		
	3	9	9	H	61	40	123	4.13		57.6
					62	42	130	4.00		
					55	50	151	3.84		
					54	43	122	3.78		
					58	43	131	4.06		
	4	9	0	Female died in parturition						

Table 33

Late Line, First Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
2HL	1	6	4	25H	79	43	156	4.28	216	79.5
				25T	69	43	147	4.33	218	
	2	6	0							
	3	9	0							
	4	6	0							
	5	2	0							
3HTL	1	10	10	17H	51	44	129	4.10	176	52.0
				17T*	49	45	129	4.00	183	
				17L	52	44	127	3.95	164	
	2	9	9	H	57	38	94	2.85		54.1
				T	54	43	118	3.56		
				L	51	44	112	3.21		
				R	54	43	116	3.44		
	3	12	11	H	43	50	133	3.60		44.9
				T	44	46	123	3.76		
				L	45	45	117	3.60		
				R	47	49	137	3.75		
				HT	44	48	129	3.70		
				HL	47	45	128	4.05		
				HR	41	47	129	4.00		
	4	8	7	H	52	45	127	3.75		56.1
				T	55	42	122	3.94		
				L	54	45	133	3.95		
	5	6	0							
4R	1	11	8	11H*	59	49	139	3.33	187	58.2
				11T	57	44	128	3.74	186	
				11L	51	44	118	3.54	175	
				11R	55	-	-	-	188	
	2	6	3	H	41	47	106	2.95		38.7
				T	39	46	104	3.10		
	3	9	8	H	57	49	165	4.50		55.8
				T	54	49	154	4.17		
				L	58	51	174	4.46		
				R	58	45	143	4.25		
				HT	51	45	137	4.30		
				HL	55	46	143	4.19		
				HR	54	47	154	4.55		

Table 33

Late Line, First Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
4R cont.	4	4	4	H	62	-	-	-		62.2
	5	9	8	H	39	-	-	-		34.6
				T	36	-	-	-		
				L	33	-	-	-		

*Selected as a dam of the succeeding generation.
1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.
2/Average from weaning (25 days of age) to time of vaginal opening.
3/Average of males and females.

Table 33

Late Line, Second Generation.

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
7R	1	8	7	27H	50	45	130	4.00	176	49.3
				27T	45	45	124	3.95	181	
				27L	51	41	119	4.25	193	
				27R	51	44	141	4.74	195	
	2	10	10	H	44	48	137	4.04		42.8
				T	40	-	-	-		
				L	41	45	118	3.85		
				R	42	48	136	4.09		
				HT	43	47	127	3.82		
	3	7	7	H	63	45	139	3.80		66.0
				T	67	42	137	4.12		
				L	70	48	164	4.09		
				R	68	41	146	4.88		
				HT	60	44	136	4.00		
				HL	64	47	143	3.59		
9H	1	7	5	29H*	52	50	142	3.60	183	56.0
				29T	57	45	133	3.80	172	
				29L	49	46	130	3.86	188	
	2	6	0							
	3	10	0							
9L	1	7	7	31H*	51	48	134	3.61	194	51.1
				31T	53	44	136	4.37	194	
				31L	49	45	136	4.35	207	
	2	11	11	H	41	45	127	4.30		39.7
				T	39	47	113	3.36		
				L	39	46	122	3.95		
				R	42	46	118	3.62		
				HT	38	47	116	3.55		
				HL	39	47	116	3.50		
				HR	42	-	-	-		

*Selected as a dam of the succeeding generation

1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.

2/Average from weaning (25 days of age) to time of vaginal opening.

3/Average of males and females.

Table 33

Late Line, Second Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
9L cont.	3	11	10	H	52	42	119	3.94		49.3
				T	52	48	150	4.26		
				L	45	49	121	3.17		
				R	49	48	140	3.96		
				HT	48	48	130	3.57		
				HL	47	50	150	4.12		
				HR	47	47	128	3.68		
9HT	1	10	10	41H*	48	49	138	3.75	182	46.5
				41T*	48	46	122	3.52	191	
				41L*	47	47	128	3.68	188	
	2	9	0							
	3	8	8	H	59	38	111	4.00		58.5
				T	54	40	120	4.40		
11H	1	9	8	33H*	50	48	135	3.70	172	53.6
				33T	53	45	129	3.80	187	
				33L	54	45	139	4.25	190	
	2	10	8	H	48	52	139	3.37		49.8
				T	50	-	-	-		
				L	52	50	142	3.60		
	3	10	8	H	50	50	142	3.68		50.1
				T	55	48	139	3.65		
				L	51	50	145	3.76		
				R	48	50	141	3.72		
				HT	43	47	121	3.54		
17T	1	3	0							
	2	11	0	Female died in parturition						
19T	1	10	7	37H*	48	47	136	4.00	170	48.1
				37T	49	43	125	4.22	185	
				37L	51	43	117	3.67	167	
	2	4	0							
	3	5	0							
19L	1	6	6	35H	63		133	3.89	196	61.5
				35T	63		140	3.85	183	

Table 33

Late Line, Second Generation (Continued).

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
19L cont.	1 cont.			35L	67		146	3.95	204	
				35R	58		129	3.74	187	
				35HT	58		133	3.57	186	
	2	11	3	H	35	-	-	-		46.7
				T	54	49	151	4.04		
				L	51	-	-	-		
	3	10	0							
19HT	1	11	0							
	2	11	0							
	3	11	11	H	46	41	103	3.56		43.8
				T	43	39	90	3.36		
19HL	1	8	8	39H*	41	48	127	3.74	173	45.9
				39T*	48	51	144	3.69	176	
				39L*	48	46	125	3.67	202	
	2	11	0							
	3	7	0							

*Selected as a dam of the succeeding generation.
1/Age or weight atthe time of vaginal opening; individuals with either value missing were not included in the statistical analysis.
2/Average from weaning (25 days of age) to time of vaginal opening.
3/Average of males and females.

Table 33

Late Line, Third Generation.

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at V0	Wt. at V0[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
29H	1	-	0							
31H	1	11	11	51H	51	-	-	-	-	48.5
				51T	50	42	115	3.82	180	
				51L	46	41	109	3.94	174	
				51R	47	-	-	-	-	
33H	1	10	0							
37H	1	10	0							
39H	1	11	10	63H	51	38	97	3.54	177	52.1
				63T	51	37	95	3.67	159	
				63L	54	37	99	3.75	178	
				63R	51	40	100	3.27	178	
				63HT	50	38	91	3.15	149	
				63HL	54	39	108	3.86	179	
39T	1	8	8	55H	52	41	124	4.50	185	49.6
				55T*	46	45	113	3.35	174	
				55L	47	40	107	4.00	179	
				55R	50	41	116	4.12	191	
				55HT	48	39	104	4.00	165	
39L	1	11	11	53H	53	42	128	4.41	196	47.5
				53T	47	42	126	4.65	180	
				53L*	44	44	126	4.32	211	
				53R	47	41	117	4.38	183	
				53HT*	46	43	127	4.50	207	
41H	1	8	8	59H*	51	48	127	3.30	172	48.6
41T	1	14	13	61H*	38	43	118	4.44	186	38.9
				61T	37	42	94	3.35	158	
				61L*	38	44	107	3.63	162	
				61R	38	42	113	4.41	182	
				61HT*	39	45	118	3.95	173	
				61HL*	40	48	113	3.17	163	
				61HR*	40	46	115	3.57	161	

Table 33

Late Line, Third Generation (Continued)

Dam	Litter	No. born	No. wean	Individual ident.	Wean wt.	Age at VO	Wt. at VO[1]	Gain per day[2]	75 day wt.	Ave. wean wt.[3]
					gms	days	gms	gms	gms	gms
41L	1	9	9	57H*	46	44	127	4.26	189	46.8
				57T	47	43	124	4.28	188	
				57L	44	41	107	3.94	169	
				57R	50	39	107	4.07	184	
				57HT	47	42	126	4.65	188	
				57HL	47	43	117	3.89	189	

*Selected as a dam of the succeeding generation.

1/Age or weight at the time of vaginal opening; individuals with either value missing were not included in the statistical analysis.

2/Average from weaning (25 days of age) to time of vaginal opening.

3/Average of males and females.

Table 34

Mitotic Activity of the Endometrium of Individual Immature Rats With Group Means According to Treatment (number of mitoses per 1000 cells).

Experiment I

Treatment								
Rats	None	P	E	PE	T	TP	TE	TPE
A	43.2	78.8	17.1	9.5	4.2	6.2	0.9	5.0
B	0.0	0.0	16.7	9.2	6.7	0.0	0.4	42.4
C	2.0	0.0	28.0	12.1	8.6	0.6	9.6	1.4
D	2.0	1.0	5.6	34.5	60.8	1.0	0.9	7.4
E	6.8	0.0	4.5	10.4	6.2	0.3	1.3	0.9
F	46.0	0.0	15.7	23.2	0.7	0.0	30.0	0.0
Mean	16.67	13.30	14.60	16.48	14.53	1.35	7.18	9.52

T = 0.2% Thiouracil
E = 3 Rat Units estrogen (dithylstilbestrol)
P = 0.05 mg. progesterone

Table 35

Mitotic Activity of the Endometrium of Individual Castrate Rats With Group Means According to Treatment (number of mitoses per 1000 cells).

Experiment II

Treatment								
Rats	None	P	E	PE	T	TP	TE	TEP
A	2.0	1.5	4.0	1.0	4.0	1.0	6.0	0.0
B	4.0	14.5	74.0	1.0	2.0	2.0	0.0	0.0
C	4.0	1.0	1.0	3.0	0.0	0.0	0.0	0.0
D	0.0	16.0	4.0	0.5	0.0	0.0	2.7	2.0
E	0.0	20.0	26.0	1.0	0.0	16.0	2.0	0.0
F	0.5	6.0	62.0	0.0	20.5	1.0	70.0	0.0
Mean	1.75	9.83	28.83	0.58	4.42	3.45	13.45	0.33

T = 0.2% Thiouracil
E = 3 Rat Units of estrogen (Crystalline estradiol benzoate)
P = 0.05 mg progesterone

Table 36

Formula for Dornfeld's Rat Mix

Ingredient	Percent by Weight
Yellow corn meal (whole corn)	38.0
Whole wheat meal	32.0
Powdered skimmed milk	20.0
Whole ground alfalfa meal	6.0
Cod liver oil	2.0
Yeast (Fleischman's dry type)	1.0
Calcium Carbonate or Lime	0.5
Sodium Chloride	0.5
	100.0