ENDOCRINE AND REPRODUCTIVE DEVELOPMENT OF THE BOVINE FEMALE FROM BIRTH THROUGH PUBERTY

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Claude Desjardins

1966

This is to certify that the

thesis entitled

Endocrine and Reproductive Development of the Bovine Female From Birth Through Puberty

presented by

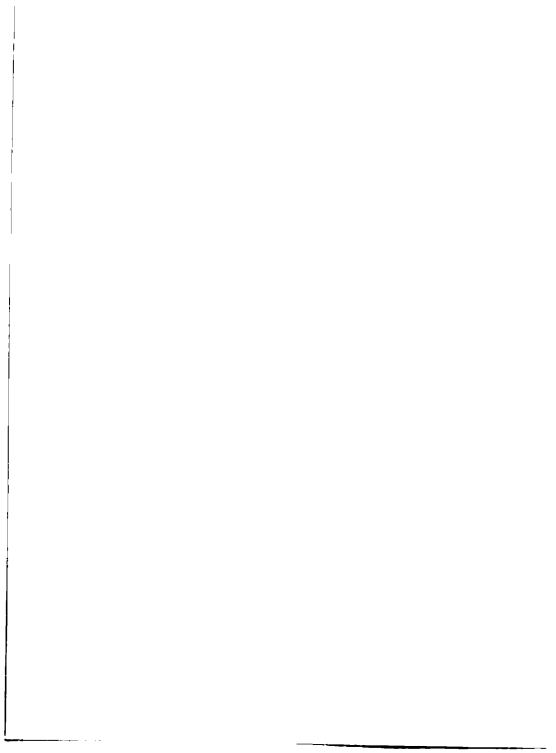
Claude Desjardins

has been accepted towards fulfillment of the requirements for

Ph.D. **degree in** Dairy

Harold A. Hays
Major professor

Date December 14, 1966



ABSTRACT

ENDOCRINE AND REPRODUCTIVE DEVELOPMENT
OF THE BOVINE FEMALE FROM BIRTH
THROUGH PUBERTY

by Claude Desjardins

A total of 65 Holstein calves were purchased between 0 and 5 days of age to study endocrine and reproductive development from birth through puberty. These animals were slaughtered in 13 groups of five animals at 1-month age intervals. For the purposes of this experiment, puberty was defined as the onset of first estrus.

The average age at first estrus was 29.7 $\pm$ 1.3 weeks, and the average estrous cycle length was 20.5 $\pm$ 0.6 days. The average weight of the whole pituitary gland increased 1.1g between birth and 12 months of age. Approximately 90 per cent of this increase was attributable to increases in the anterior lobes.

The average concentration of pituitary luteinizing hormone (LH), measured by ovarian ascorbic acid depletion assays, increased four-fold between birth and 3 months of age, did not change significantly from 3 to 7 months, and declined from 10.3 µg at 7 months to 4.8 µg at 12 months of age ($P < 0.05$). The average

concentratio
(FSH), measu
was 1.67, 2.
respectively
significantl
the differen
groups were
occurred aft
tents parall
gonadotropi
that pituit
the onset o

Pitui
puberal hei
analyses.
before the
to 400 per
Pituitary F
than LH, ar
before estr

Incre
be linear.
and large (
each other
age, declin
constant af
organ weigh

concentration of pituitary follicle stimulation hormone (FSH), measured by ovarian weight augmentation assays, was 1.67, 2.68 and 1.06 μg at 0, 1 and 2 months of age, respectively. These concentration differences were significantly different from each other ($P < 0.05$) while the differences in pituitary FSH among the remaining age groups were not significant, although a small decline occurred after puberty. Total pituitary LH and FSH contents paralleled the concentration values for these gonadotropins. The results were interpreted to indicate that pituitary LH and possibly FSH decreased beginning at the onset of puberty.

Pituitary LH and FSH concentrations of the post-puberal heifers in this experiment were studied in separate analyses. LH concentration decreased 800 per cent just before the time of ovulation and then increased about 300 to 400 per cent within the first six days after ovulation. Pituitary FSH concentration decreased less dramatically than LH, and somewhat before LH, during the last few days before estrus.

Increases in ovarian weight due to age appeared to be linear. Average numbers of small ($\leq$ 5 mm diameter) and large (> 5 mm diameter) ovarian follicles paralleled each other throughout. They increased up to 4 months of age, declined after 4 months of age and became relatively constant after 6 months of age. The relationships between organ weight, total deoxyribonucleic acid (DNA), the

total ribonucleic acid (RNA) and the total protein contents of the uterus, cervix and vagina were determined between birth and 12 months of age. Increases in these four parameters between 0 and 12 months were best described by quadratic growth response curves whereas the increases in these same parameters between 0 and 6 months of age were linear. The results were interpreted to mean that reproductive growth was accelerated after the onset of first estrus. Oviduct, uterine, cervical and vaginal epithelial cell heights were always greater at 0 months than at 1 month of age, probably due to maternal or placental hormones prior to parturition.

Increases in weight, total DNA, total RNA and total protein of adrenal glands between 0 and 12 months of age were linear ($P < 0.01$). The width of the zona glomerulosa remained relatively constant to 12 months of age, but the combined width of the zona-reticularis-fasciculata increased linearly between 2 and 12 months of age. Thyroidal development was measured in these heifers but large variations in thyroid weights prevented meaningful interpretation of these data. Thymus weight and total DNA increased six-fold between birth and 1 year of age, but the RNA/DNA ratio, did not change appreciably ($P > 0.25$).

These data revealed a marked increase in the rate of growth of the reporductive organs beginning at the time of puberty--changes which coincided with and were probably caused by the release of pituitary LH and

possibly FSH into the blood. The data suggested that puberty was precipitated by a sudden release of pituitary LH and that this precipitous LH release apparently occurred regularly on the day of estrus of each estrous cycle for all postpuberal heifers.

ENDOCRINE AND REPRODUCTIVE DEVELOPMENT OF THE BOVINE FEMALE FROM BIRTH THROUGH PUBERTY

By

Claude Desjardins

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy

1966

To

Jane Elizabeth Desjardins

BIOGRAPHICAL SKETCH

of

Claude Desjardins

Born: June 13, 1938, Fall River, Massachusetts

Education:

a. Durfee High School, Fall River, Massachusetts from 1952-1956 (graduated)

b. University of Rhode Island, Kingston, Rhode Island from 1956-1960 (B.S.)

c. Michigan State University, East Lansing, Michigan from 1960-1966 (M.S. and Ph.D.)

Employment: Research Instructor at Michigan State University from 1960 to 1966

The

provided

the cours

preparing

The

Joseph Me

Spe

Ed Conve

Paape, Yo

cal aspec

tance of

research

The

the Upjoh

of the Na

financial

Health (g

ated.

ACKNOWLEDGMENTS

The author gratefully appreciates the assistance provided by his major professor, Dr. Harold Hafs, during the course of this research as well as his guidance in preparing this manuscript.

The help and advice provided by Drs. Allen Tucker, Joseph Meites and Lon McGilliard was greatly appreciated.

Special thanks are due the authors colleagues Mr. Ed Convey, Drs. Kenneth Kirton, Jock Macmillan, Max Paape, Yogi Sinha for their help with the various technical aspects of this work. The devoted and untiring assistance of Mrs. Helga Hulkonen during all phases of this research deserves gargantuan praise.

The gifts of hormones from the Ayrst Laboratories, the Upjohn Company and the Endocrinology Study Section of the National Institutes of Health as well as the financial support provided by the National Institutes of Health (grant number HD01374) are recognized and appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION[1]

Puberty occupies a pivotal position among postnatal developmental events. It signifies the onset of reproductive activity leading to sexual and physical maturity. To the husbandman, the arrival of reproductive activity signifies an increase in an animal's economic potential based upon its ability to produce offspring.

During the past decade, more intensive animal production practices have focused increased attention on aberrant reproductive functions such as infertility and sterility. Reproductive failures may be the major cause of loss of productivity among dairy cattle. Little is known of the direct causes of these reproductive failures, especially of those due to factors other than infectious diseases. However, a considerable body of evidence has accumulated to show that reproductive efficiency of the adult may be drastically affected by the prepuberal environment. Therefore, accurate information on endocrine

---

[1]This thesis research was a portion of a longer study which included mammary growth parameters. The mammary parameters were measured by Y. N. Sinha and will be published under his name.

and reproductive organ development before and during puberty may shed light on the normal function of these organs in the adult.

Although the debut of puberty has been qualitatively recognized through certain behavioral manifestations in most laboratory and farm animals, the physiological changes occurring in the endocrine and reproductive organs during this time have not been extensively quantified; and, consequently, knowledge of controlling mechanisms is meager, especially in the bovine female. For example, the levels of pituitary gonadotropins during prepuberal development have never been described for the bovine. Similarly, quantitative biochemical studies on the growth and development of the other endocrines and reproductive tissues during puberty are not available for this species.

The present study was initiated to quantify endocrine and reproductive organ development of normal heifers from birth through puberty. Levels of pituitary gonadotropins were measured because it was anticipated that changes in these might precipitate puberty. Reproductive organ development was quantified by measurement of DNA as an index of cell numbers, and by measurement of RNA, protein and lipid as indicies of cellular synthetic activity. These hormonal and biochemical parameters were then related to each other and to morphological and micromorphological measurements on each reproductive organ in

an effort to uncover cause and effect relationships, particularly where rapid changes were observed at the time of puberty.

Heifers were very good experimental animals for this research because they provided sufficient quantities of tissues to assess several different parameters on each tissue and, thereby, permitted assessment of functional activity on a within-animal basis. It was anticipated that this "uniformity" study would improve our understanding of the endocrine control of reproductive organ development, as well as providing bases for future research on puberty during specific stages of pre- and post-puberal development.

CHAPTER II

GENERAL LITERATURE REVIEW

Definitions of Puberty

Although there have been many attempts, an exacting definition of puberty has proven difficult. In the first place, the spontaneous onset of puberty and gradual transition from puberty to sexual maturity permits no sharp demarcation of this stage of development. Marshall (1922) suggested that:

> Puberty, or the period at which the organism becomes sexually mature, is marked by the occurrence of those constitutional changes whereby the two sexes become fully differentiated. It is at this period that the secondary sexual characters first become conspicuous, and the essential organs of reproduction undergo a great increase in size.

Asdel (1965) provided a more practical definition when he suggested that: "Puberty is the time at which reproduction first becomes possible, i.e., when germ cells are released." Hammond and Marshall (1952) considered an animal to reach puberty when "the sexual organs had become fully developed, the sexual instincts prominent and reproduction could be completed." Most recently, Donovan and van der Werff ten Bosch (1965) have reviewed several definitions of puberty especially where they may apply to primates and man. During the past years, most authors

have tacitly agreed that the appearance of the first estrus was tantamount to the attainment of puberty. The latter definition will be used throughout this thesis.

Parameters Used for Assessing Puberty in the Female

Since this thesis pertains entirely to females, only those parameters used to study puberty in this gender were reviewed. The time of opening of the vagina has been shown to be highly correlated with the occurrence of the first ovulation and first estrus in rodents. For example, vaginal opening has been the accepted sign of puberty in the mouse (Allen, 1922), rat (Long and Evans, 1922) and guinea-pig (Stockard and Papanicolaou, 1917). Behavioral signs appear to be the best indicators of first estrus and the onset of puberty in most domesticated animals. Several manifestations of estrus in these species include increased vascularity and turgidity of the vulva, mucous discharge from the vulva, frequent bellowing, persistent trailing and attempted mounting of other animals. However, the most conclusive behavioral criterion of estrus in heifers appears to be standing when mounted by other animals in and out of estrus (Tanabe and Almquist, 1960).

Current evidence suggests that there is a specific brain center regulating estrous behavior (Gorski, 1966). The majority of evidence presented so far supports the concept that this center is under the influence of the sex steroids and becomes fully mature at the time of

puberty coincidental with the first maturation of an ovarian follicle and the first ovulation (Gorski, 1966).

General Endocrine and Reproductive Organ Changes Occurring During Puberty

In general, puberty has been associated with two types of changes taking place in the endocrine and reproductive organs. These are: (a) changes occurring in the physiological activity of the endocrine glands and (b) changes occurring in the reproductive and skeletal systems which are brought about by the secretions of the endocrine organs. These changes in the endocrine organs result in a general increase in physiological activity and cause several developmental events to occur which in turn are responsible for securing the most favorable conditions for reproduction. Some of these changes are specific and predictive of a particular stage of puberty (i.e., mammary gland growth in the puberal heifer). Some others, however, may be incidental to a particular period of puberal development. For example, vaginal opening is apparently completely coincidental with the time of the first ovulation in the rat. However, vaginal opening also occurs in rats castrated at infancy, though it is considerably delayed. Generally, puberty accelerates and completes developmental processes which in the absence of a period of rapid endocrine and reproductive organ development would require additional time to mature.

CHAPTER III

MATERIALS AND GENERAL METHODS

Experimental Methods and Slaughter

Two groups of 30 female Holstein calves were obtained from commercial dairy herds located in Dane and Greene Counties in Wisconsin. All calves were sired by registered bulls and born from production tested dams, selection criteria intended to provide a more homogeneous group of experimental animals. Calves in the first group were born between the 11th and 17th of November, 1963. These animals were transported (November 18, 1963) to and reared at the University farm until the time of slaughter. Five heifers were randomly chosen and slaughtered five at a time at 1-month intervals beginning when the animals were 7-months old. Calves in the second group were born between the 5th and 11th of May, 1964. These were transported (May 12, 1964) and reared similar to the calves in the first group. Five calves from the second group were randomly chosen and slaughtered five at a time at 1-month intervals beginning when the animals were 1-month old. An additional group of five calves (born on the 13th to 15th of September, 1964) were

purchased locally and slaughtered on the 16th of September, 1964 to provide the animals here-after referred to as 0 months of age.

The calves were housed in individual pens until they were about 3-months old. Subsequently, calves were reared for about 2-months in pens containing about 10 animals. From 5 months of age, the calves were managed communally in a dry-lot with access to an open shed.

Animals were transported from the farm to the University Meats Laboratory on the morning of slaughter. The five heifers within an age-group were always killed on the same day and by means of a captive bolt immediately followed by exsanguination.

Feeding Procedure

The calves received an average of 3 kg of whole milk per day during the first 3 weeks after arriving at the University farm. From the fourth through sixth weeks, the animals received an average of 5 kg of whole milk daily and from the seventh through sixteenth weeks they received an average of 4 kg daily. During the latter period the calves received a 16 per cent protein calf starter and water and good quality alfalfa hay supplied free choice. During the fourth and fifth months, the calves received an average of 2 kg of ground ear corn grain mix (14 per cent crude protein) along with alfalfa hay provided free choice.

Corn silage or mixed hay was provided from the fourth month of age when it was available and in varying quantities. While the total nutrient intake was not accurately measured, the total intake was intended to provide nutrients sufficient to sustain normal growth for Holstein heifers. That this was achieved was demonstrated by the measured growth characteristics presented in the next chapter.

CHAPTER IV

BODY GROWTH AND ESTROUS CYCLE OCCURRENCE

Review of Literature

Body Growth

The first estrus (puberty) in cattle usually occurred when about 30 per cent of the mature body weight was attained (Brody, 1945). The greatest rate of somatic cell hypertrophy and hyperplasia occurred during prenatal and not during postnatal growth (Brody, 1945), although it has been a popular contention that maximum growth occurred just before or during puberty (Tanner, 1962). Postnatal growth has been divided into two segments which Brody (1945) identified as the "self accelerating phase" and the "self inhibiting phase." The point at which acceleration ceases and a deceleration has not yet begun represents a stage of physiological age equivalence. During this time, most animals seem to pass through puberty and about 30 per cent of the mature body weight has been attained. Thus the inflection point in a graph of body size occurred in female rats at about 30 days, coinciding with the onset of vaginal opening (Kleiber, 1961). In children, the inflection occurs between 12 and 15 years

of age, corresponding to their average age of puberty. For dairy cattle, the inflection occurs at 8-10 months concurring with the time of puberty in this species (Ensminger, 1959).

Additional evidence demonstrating the relationship between body growth and the onset of puberty was provided by Boas (1932) who concluded that when the maximal postnatal growth occurred early in the life of the human female, the onset of puberty was early. This early intense growth rate was of short duration. In contrast, when the period of maximal postnatal growth was delayed, its intensity was slight but its duration was long. Boas concluded that in cases of precocious puberty, skeletal maturation was accelerated, while in hypogonadism, it was delayed.

In general, animals of the smaller breeds within a species attain puberty at an earlier age than the larger breeds. For example, Hammond and Marshall (1952) noted that the small Polish rabbit attained puberty about 6 weeks earlier than the larger Belgian breed. Similarly, Jersey heifers showed signs of estrus about 13 weeks earlier than did the larger Holstein breed of dairy cattle (Eckles, 1915).

Termination of skeletal growth usually coincided with the loss of growth potential of the long bones and has been attributed to calcification of the epiphyseal plates. Greulich (1954) and more recently Morscher,

et al. (1965) gave evidence that closure of the epiphyseal plate of the phalanges coincided extremely well with the occurrence of the first menses in girls. Greulich (1954) even suggested that the skeletal status of the hand permitted the selection, some years before puberty, of children who would mature early and those in whom maturation was delayed. Unfortunately, there seem to be no data on the relationship between puberty and the calcification of the epiphyseal plates in domestic animals.

Estrous Cycle Occurrence

The age at first estrus has received little attention in the heifer. Sorensen et al. (1959) indicated that 10 Holstein heifers exhibited their first estrus at 49 ± 6.3 weeks of age. These same workers indicated that the occurrence of first estrus could be advanced or delayed depending on the level of feeding employed. The data presented by Sorensen et al. (1959) do not indicate whether or not ovulation was concurrent with the appearance of the first estrus. However, in laboratory species like the mouse (Allen, 1922), rat (Long and Evans, 1922) and the guinea pig (Stockard and Papanicolaou, 1917), the first estrus was usually followed immediately by the first ovulation.

Influence of Nutrition on Body Growth and the Occurrence of Puberty

Accelerated growth due to increased energy intake was shown to advance puberty as determined by the age at first estrus (Sorensen et al., 1959). Reid et al. (1964) confirmed and extended the above report indicating that heifers on a low or high plane of nutrition had later or earlier puberal ages, respectively, than heifers on a normal plane of nutrition. In other studies on dairy heifers, Creichton et al. (1959), Hanson (1956) and Joubert (1954) all noted that energy intakes less than that normally recommended delayed puberty in these species (McCance, 1960). Huseby et al. (1945) found that mice which had been reared from weaning on a restricted caloric intake had delayed sexual development. Kennedy and Mitra (1963) observed similar results in rats. The underlying cause for the delay in sexual development resulting from a reduced caloric intake after weaning was probably mediated through the endocrine system and has sometimes been referred to as pseudo-hypophysectomy (Mulinos and Pomerantz, 1940). More recently, Kennedy (1966) advanced the theory that the effects of low or high caloric intake on sexual maturity are under the control of hypothalamic neurohumors. He showed that in rats the maturation of the hypothalamus could be accelerated or decelerated depending on the plane of nutrition.

Hereditary Factors Influencing Body Growth and the Onset of Puberty

Significant variation in the age at which an animal attains puberty has been attributed to hereditary factors. For example, Mirskaia and Crew (1930), studying mice, and King (1915a, 1915b) and Blunn (1939), studying rats, all found hereditary differences in the age at vaginal opening in different strains of animals in these species. Squiers et al. (1952) obtained evidence that crossbred gilts reached puberty at an earlier age than inbred gilts. Foote et al. (1956) reported that puberal age of swine was nonadditively inherited and Warnick et al. (1951) found differences in age at puberty among inbred lines of swine. These workers, as well as Robertson et al. (1951), all observed a negative association between age of puberty and body growth. Hawk et al. (1954) reported that inbreeding delayed puberty in a group of Holstein heifers. Each of these researches with mice, rats, swine and cows indicated hereditary factors can influence the growth rate of these animals and that when puberty was advanced, it was associated with accelerated growth rates.

Materials and Methods

Body Growth

Body weights were measured at 1-month intervals beginning at 30 days of age. Slaughter weight was estimated

by obtaining the average of three weighings made at 24-hour intervals just before slaughter.

Estrous Cycles

Animals were observed twice daily for estrus between 7:30-8:30 AM and between 4:30-5:30 PM beginning when the heifers were 5 months of age. The criteria used to evaluate estrus were (1) standing when mounted by other animals, (2) repeated attempts to mount other animals (in or out of estrus), (3) swollen external genitalia, (4) copious vaginal mucous secretion, and (5) general restlessness.

Results

Body Growth

Body growth was linearly related to age ($P < 0.05$) between 0 and 12 months, as evidenced by the data presented in Figure and in Appendix Table 1. The data presented in Figure 1 also indicated that the growth rates observed in the present experiment paralleled those reported by Morrison (1956) which have been generally accepted as a standard describing the relationship between body weight and age.

Estrous Cycle Occurrence

The data concerning the age at first estrus and the length of consecutive estrous cycles are presented in Table 1. The age at first estrus for the 24 heifers

which had passed puberty ranged from 20.1 to 44.5 weeks with the average age at first estrus occurring at 29.7 ± 1.3 weeks. The average length of 93 estrous cycles from the 22 animals which had completed at least one cycle was 20.5 ± 0.6 days. In general, the data on the length of consecutive estrous cycles indicated that once estrus occurred (puberty), regular and consistent cycles were observed thereafter.

Discussion

The linear relationship between age and body weight demonstrated in the present experiment was in good agreement with the observations of previous researchers using animals of similar age and breed (Morrison, 1956; and Sorensen et al., 1959). Therefore, it was assumed that the growth rate observed in the present experiment exerted the normally expected effects on the growth and development of the endocrine and reproductive systems.

The data from the present experiment indicated that the age at first estrus was 4 to 6 weeks earlier than that recorded by other investigators (Sorensen et al., 1959) for Holstein heifers. The body weight data indicated that accelerated body growth was not the cause of the earlier age at first estrus. The discrepency between the age at first estrus reported here and that reported by Sorensen et al. (1959) may have been due to the differences in housing. Animals in the present study were raised

after 5-months in a loose housing system, whereas Sorensen's animals were stanchioned and allowed out for two 30-minute intervals each day. While objective data are not available, stanchioned heifers may be deprived of adequate opportunity to acquire the behavior pattern associated with estrus in this species and consequently may not fully exhibit the normal behavioral patterns associated with estrus.

Once estrous cycles were begun, they appeared fairly regularly in all of the animals which attained puberty. However, there were a few cycle lengths of either abnormally short or long duration. Some of the apparently long cycles may have involved a missed estrus of very short duration or abnormal estrus behavior. Although the duration of estrus was not specifically measured, interpolation of the morning and afternoon estrous cycle observations suggested an average estrus interval between 12 and 24 hours which appeared to be in agreement with previous reports reveiwed by Asdell (1965). The small standard error of estrous cycle length for the heifers in the present experiment was also in agreement with the value of 20.23 $\pm$ 0.05 days presented by Asdell *et al.* (1949) for unbred heifers. Estrous cycles for cows were of slightly longer duration but with similarly small variation (21.28 $\pm$ 0.06 days).

TABLE 1.--Age at first estrus, stage of estrus at slaughter and length of consecutive estrous cycles.

Months of Age at Slaughter	Heifer Number	Weeks of Age at First Estrus	Stage of Estrous Cycle at Slaughter[a]	Length (days) of Estrous Cycles
7	11	NOE[b]	NOE	
	12	NOE	NOE	
	15	23.6	4	20,24
	16	29.0	3	24
	29	25.5	21	
8	4	NOE	NOE	
	5	NOE	NOE	
	10	NOE	NOE	
	27	20.1	5	22,8,21,22,21
	28	26.1	20	18,19
9	3	26.3	7	14,19,20,17,18,20
	17	29.4	16	25,20
	19	23.6	12	18,19,23
	20	NOE	NOE	
	24	30.0	9	18,18
10	2	26.0	7	20,19,20,17,18,20
	8	44.5	1	18
	22	27.4	3	18,20,19,19,20
	30	40.0	1	
	33	32.3	12	19,18,22
11	6	29.0	11	22,22,21,20,17
	7	35.6	13	28,18,20
	25	28.4	23	22,16,20,22,16,16
	31	29.4	2	23,21,22,20,18,19
	34	32.0	20	21,20,23,21
12	13	24.4	11	26,21,21,17,24,22,21,23
	21	41.2	5	17,13,14,20
	23	28.0	19	18,18,21,22,43,9,14
	32	32.5	17	42,20,17,22,14
	35	28.1	18	25,20,22,26,28,42,8

[a]Number of days following last estrus before slaughter.

[b]Never observed in estrus (NOE).

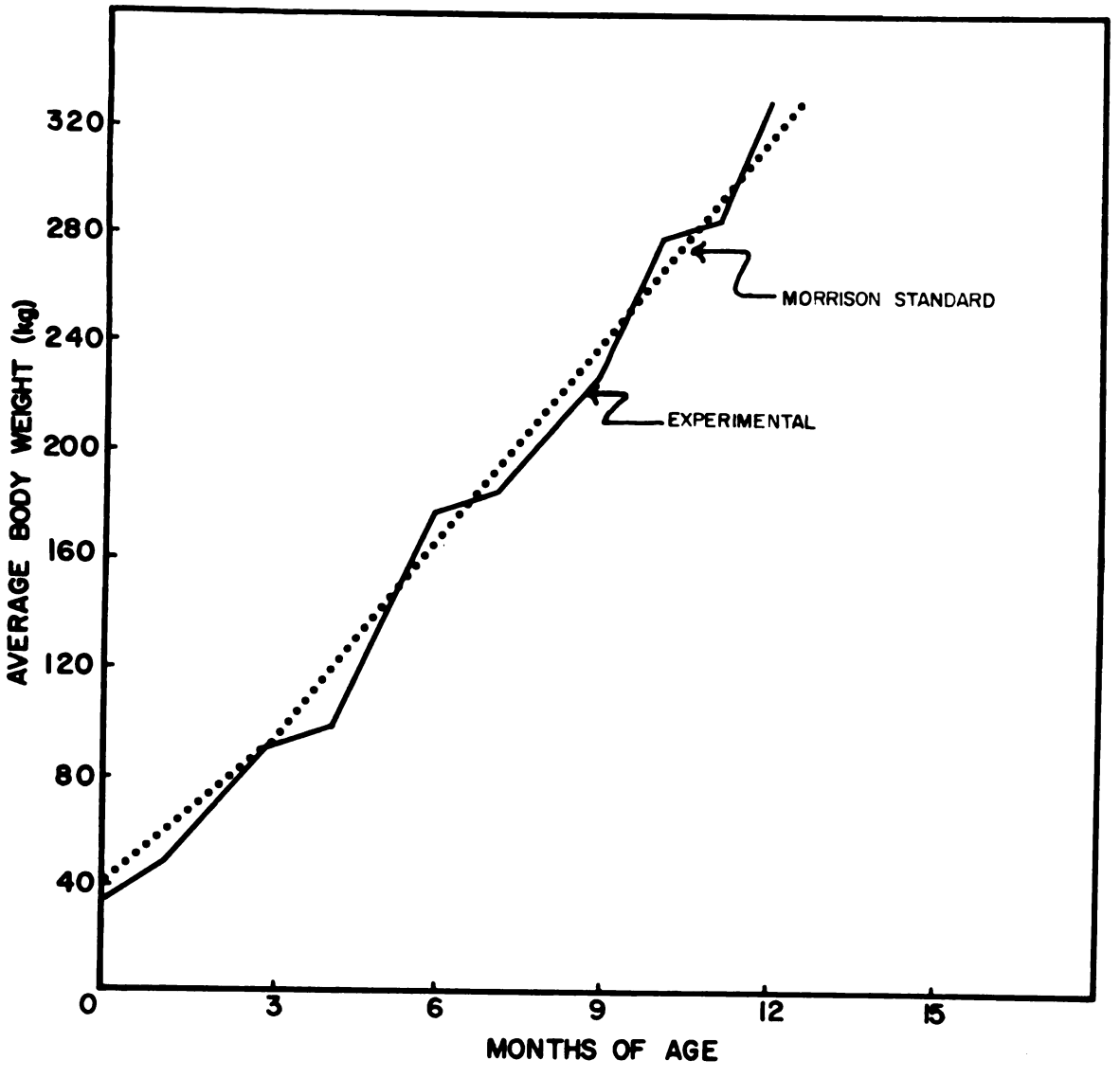


Fig. 1.--Relationship between body weight and age.

CHAPTER V

PITUITARY AND HYPOTHALAMIC FUNCTION

Review of Literature

An enormous body of evidence has accumulated to document the generally accepted negative feed-back relationship between the anterior pituitary hormones and the hormones of their target organs such as the thyroid and the gonads (Harris et al., 1966). An excess of a target gland hormone has an inhibitory effect and a deficiency has a stimulating effect on the synthesis and/or release of its own trophic substance from the pituitary. In recent years, it has become more and more evident that the anterior pituitary-target gland axis is mediated by the central nervous system (Everett, 1964). Consequently, any investigation of a servomechanism like the pituitary-target gland axis should take into account the influence of exterior receptive factors capable of affecting the system.

Exterioceptive Factors and Pituitary Function

That exposure to continuous light initiated precocious gonadal function in prepuberal female rats was

shown by Fiske (1939, 1941) and Luce-Clausen and Brown (1939). Jochle (1956) showed that constant light advanced estrus by as much as 10-days in the rat.

That duration of illumination may be important for normal puberal development was suggested by marked retardation of first estrus in rats reared in complete darkness (Fiske, 1941) or following optic enucleation (Truscott, 1944). After a thorough investigation of the effect of light on the endocrine system, Critchlow (1963) concluded that continuous light caused transmission of a neurohumor from the hypothalamus to the adenohypophysis via the hypophyseal-portal system. The hypothalamic neurohumor, in turn, stimulated release of the pituitary gonadotropin which was directly responsible for the observed precocious puberty.

Among domestic animals, sheep, dogs and horses appear to be markedly influenced by light (Asdell, 1965). Direct evidence for the effect of light on the reproductive cycles of cows and pigs does not appear to be available (Hammond, 1954 and Clegg and Ganong, 1959). However, indirect evidence implicating the influence of light on the reproductive cycle of the cow was provided by Mercier and Salisbury (1947). These workers reported a significant increase in the number of calves born in the spring than during the other seasons of the year, suggesting that ovulations may be more frequent during the last summer and early autumn months, times of the

year which are characterized by decreasing amounts of daylight in the northern hemisphere. Additional evidence provided by Sweetman (1950) indicated that, during the long Alaskan winters, which are characterized by very brief daylight periods, cows responded to supplemental artificial illumination with increased intensity of estrus as well as increased fertility. These data suggested that light may influence the pituitary-ovary relationship in the cow. Unfortunately, no data were available with reference to the effect of light on the age of puberty in the bovine.

Effect of Temperature

The influence of heat upon production traits of farm animals has been studied because of its economic importance. Both cattle (Bonsma, 1949) and sheep (Moule, 1950) attained puberty more slowly in tropical regions than in temperate zones. However, dairy heifers attained puberty at an average age of 13 months whether reared at 27° C. or 10° C. (Dale et al., 1959), indicating that temperatures within this range had no influence on the debut of puberty.

Similar departures from normal ages of puberty in laboratory animals resulted when animals were reared at extreme temperatures. For example, Ogle (1934) observed that vaginal opening and first estrus were delayed when mice were exposed to high temperatures (37° C.). Mice

reared at low temperatures (-3° C.) also exhibited delayed signs of puberty (Barnett and Coleman, 1959). This delay in puberty in both farm and laboratory animals under extreme temperature conditions was associated with and may have been partially due to much slower growth rates, especially since puberty apparently occurs at a certain threshold of body weight (Hafez, 1952). Endocrine measurements in prepuberal rats subjected to extreme temperatures suggested that the pituitary, thyroid, and adrenal glands may be the mediators of the effects of either low or high environmental temperatures (D'Angelo, 1960; Moon, 1937; and Blivaiss et al., 1954).

Effect of Season

The effect of season on the debut of puberty could be partitioned into the effects of light and temperature and the possible interaction of these two factors. For example, Hawk et al. (1954) noted that dairy heifers born during the spring reached puberty at 367 days of age whereas those born during the autumn or summer reached puberty at 425 days of age. Similarly, Joubert (1962), Mounib et al. (1956) and Hafez (1952) noted that ewes attained puberty earlier when they were born in winter than in the autymn. Pomeroy (1960) and Haines et al. (1958) suggested that gilts born in the spring and early summer attained puberty earlier than those born in late summer, autumn or winter. Hammond (1925) observed that

rabbits born during the spring months were older at the first fertile mating than those born during the other months of the year. Clegg and Ganong (1959) postulated that seasonal effects were mediated via the same neural pathways as temperature and light effects and, consequently, affected reproductive activity by virtue of primary effects upon the pituitary gland.

Pituitary Gonadotropins in the Pre- and Postpuberal Animal

In a comprehensive review of the activity of the fetal endocrine glands, Moore (1950) concluded that the gonadotropic hormones were produced during the latter third of fetal life in the guinea-pig, pig and horse. The evidence for gonadotropin activity was provided by the histological appearance of the fetal pituitary as well as the stimulation of the immature mouse reproductive organs by transplanted pituitaries from these three species. These results were later confirmed and extended by Jost (1958) for fetal pituitaries from rats and rabbits.

Evidence that the two-day-old female rat pituitary was capable of functioning in the normal adult manner was provided by Harris and Jacobsohn (1952). These workers noted that estrous cycles began within 10 days of transplanting the two-day-old gland beneath the median eminence of its postpartum and recently hypophysectomized mother. These data suggested that the gonadotropic hormone concentration in the pituitary and the time required for

the maturation of the rat anterior pituitary did not limit reproductive activity in the prepuberal animal.

Smith and Engle (1927) using immature pituitary transplants, were the first to report that the anterior lobe of immature female mouse, cat, rat, guinea-pig or rabbit pituitaries contained significantly larger quantities of gonadotropic hormones than did their adult counterparts. These results were confirmed by Wolfe and Cleveland (1931), by Clark (1935), and by Lauson et al. (1939) who used similar parameters to measure pituitary gonadotropic hormone activity. Histological evidence provided by Wolfe (1934) also indicated that the anterior pituitary of the prepuberal female rat contained a larger number of highly granulated basophil cells (and therefore presumably more gonadotropin) than did normal adult females. Additional cytological evidence for increased basophil cell activity of immature female rats was provided by Siperstein et al. (1954) and by Phillips and Piip (1957).

The response of the immature chick testis indicated that the anterior pituitary glands of immature rabbits and pigs contained larger concentrations of total gonadotropin activity than did glands from mature animals (Bergman and Turner, 1942 and Hollandbeck et al., 1956, respectively). Unfortunately, all of these researches involved bioassay of total gonadotropic activity in the pituitary and did not measure individual gonadotropins.

Since they became available, more specific bioassays capable of distinguishing between follicle stimulating hormone (FSH) and luteinizing hormone (LH) allowed Hoogstra and Paesi (1955), de Jongh and Paesi (1958), Ramirez and McCann (1963) and Parlow et al. (1964) to conclude that the concentration of FSH and LH in the prepuberal anterior pituitary was greater than that found in the postpuberal animal. These reports were in general agreement that the concentrations of pituitary FSH and LH in rats and pigs reached a maximal value before puberty. The tentative conclusion was that gonadotropin was stored in the pituitary prior to puberty and released in large quantities at and after puberty. The problems of major concern in recent years were centered around (1) establishing the mechanism(s) involved in triggering release of large quantities of gonadotropin and (2) correlating pituitary levels of gonadotropin with release on gonadotropin into the systemic blood.

Pituitary levels of FSH and LH during the estrous cycle of adults were recently reported by Rakha and Robertson (1965) for the cow and by Robertson and by Hutchinson (1962) and Robertson and Rakha (1966) for the ewe. These workers concluded that pituitary LH increased continually within an estrous cycle up to the time of ovulation. At the time of ovulation there was a two-fold decrease in pituitary LH. Pituitary FSH levels appeared to parallel pituitary LH levels except that

increases prior to and the decrease following ovulation were not as great as those observed for LH. The LH content of blood plasma obtained from cows at various stages of the estrous cycle (Anderson and McShan, 1966) were approximately inversely related to the levels of pituitary LH reported by Rakha and Robertson (1965).

Hypothalamic Neurohumors Influencing Puberty

The mechanism(s) which cause an alteration of the pituitary gonadotropin concentration in the prepuberal and postpuberal pituitary have received more attention recently, especially after general acceptance of the hypothesis that the hypothalamus exercises a regulatory influence on synthesis and release of hypophyseal gonadotropins (Greep, 1961; Harris et al., 1966).

Most of the early evidence for this hypothesis was provided by experiments in which electrical stimulation and electrolytic lesioning of the hypothalamus resulted in precocious puberty in several different species including man (Sawyer, 1964; Donovan and van der Werff ten Bosch, 1965). Additional evidence for hypothalamic control of the anterior pituitary was based upon the assumption that some measurable biochemical change in hypothalamic function was responsible for initiating puberty by activating the synthesis and/or release of pituitary gonadotropins (Ramirez and McCann, 1963). However, initially, these researchers noted that the

concentration of luteinizing hormone releasing factor (LH-RF) in hypothalami of prepuberal rats was essentially the same as that in postpuberal female rats, suggesting that the "LH-releasing mechanism" was present in the prepuberal animal. These workers also observed increases in both hypophyseal and plasma LH activity following ovariectomy in prepuberal and mature female rats. The increases in LH in the prepuberal castrate suggested that the prepuberal ovary was capable of inhibition of the hypothalamo-hypophyseal axis. That this inhibition by prepuberal ovaries was due to estrogen secretion was suggested by the fact that post-ovariectomy increases in plasma LH could be abolished in the prepuberal animal by injections of small doses of estrogen. Similar injections were without effect on LH levels in mature ovariectomized rats.

The results of this research led Ramirez and McCann (1963) to suggest that the small (relative to the mature rat) quantities of estrogen produced by the ovary immediately before puberty were no longer sufficient to prevent the LH release from the pituitary and, thereby, permitted an augmented gonadotropin release which was directly responsible for precipitating puberty. That the hypothalamo-hypophyseal axis was sensitive to steroids very early after birth was evidenced by several researchers (Gorski, 1966 and Campbell, 1966) who changed the cyclic female hypothalamo-hypophyseal pattern to the constant

pattern typical of the male by injections of testosterone in infant rats at 3 to 5 days of age.

Parlow (1964), who confirmed the work of Ramirez and McCann (1963), also indicated that estrogen inhibited LH synthesis and release. He also showed estrogen had no influence on the pituitary FSH content in prepuberal rats although the precipitous drop in pituitary LH at the time of puberty was accompanied by a similar and concommitant drop in pituitary FSH.

More recently, Ramirez and Sawyer (1965) elicited precocious puberty in female rats by administration of physiological levels of estrogen and suggested that a physiological doses of estrogen were capable of activating the release of pituitary gonadotropin in the immature rat. Long term (chronic) treatment with these levels of estrogen inhibited gonadotropin secretion in the immature rat as it was known to do in the mature animal. These data suggested that the brain-pituitary unit changes suddenly at puberty in the female rat--a conclusion which had been suggested earlier by the data of Byrnes and Meyer (1951); Greep and Jones (1950); Ramirez and McCann (1963); Brown-Grant et al. (1964); and Heim (1966).

With more exacting assay procedures than had been originally used, prepuberal changes in the hypothalamic neurohumor, LH-RF, were observed in female rats by Ramirez and Sawyer (1966). At the time of puberty in the rat, a precipitous drop in LH-RF coincided with a

decrease in pituitary LH and an increase in plasma LH. Gellert et al. (1964) provided more direct evidence when they demonstrated that the pituitary stores of the gonadotropins, FSH and LH, were released into the blood stream when hypothalamic extracts prepared from the pars tuberalis of steers were injected into prepuberal rats, lending further support to the concept that low levels of gonadal steroids act on the hypothalamus (and the pituitary) to prevent the secretion of pituitary gonadotropins in the prepuberal rat.

Recent evidence suggested that two different hypothalamic centers may be responsible for regulating LH release from the rat anterior pituitary (Everett, 1964; Gorski, 1966). These centers in the hypothalamus have been referred to as the cyclic center and tonic center for LH release. The hypothesis may be advanced that the tonic center for LH release becomes functional sometime before birth and that the maturation of the cyclic center for LH release is completed at the time of puberty and is responsible for initiating puberty. Unfortunately, these data for the rat have never been substantiated in farm animals or even in other laboratory animals.

Materials and Methods

The Pituitary

The pituitary glands of each animal were removed by displacing the brain to expose the pituitary stalk.

The stalk was severed and the brain was removed to facilitate removal of the pituitary gland. Once the pituitary was removed it was dissected free of adherent tissue and weighed. The gland was cut longitudinally and the posterior lobe was separated from the anterior lobe. The anterior lobe was then placed in a small plastic bag and this was frozen on Dry Ice and stored at -20° C. until bio-assayed for pituitary FSH and LH. The anterior lobe weight was determined by subtracting the weight of the posterior lobe from the weight of the whole pituitary. This entire procedure usually required no more than 15 minutes from the time the animal was slaughtered. The pituitary FSH and LH contents of each of the 65 pituitaries were bio-assayed in random sequence by the procedures previously outlined from our laboratory (Desjardins et al., 1966).

The Hypothalamus

After removing the brain, from each animal the hypothalamus was immediately removed, weighed, homogenized in cold (5° C.) 0.1N hydrochloric acid, frozen on Dry Ice, and stored at -20° C. until assayed for LH releasing factor (LH-RF) by the Upjohn Company, Kalamazoo, Michigan. This bio-assay for LH-RF consisted of injecting pooled (according to age) fractionated extracts of hypothalami into 32-day-old rats that had been injected with pregnant mare serum gonadotropin at 30 days of age to induce

follicular development. These rats were inspected for ovulation at 33 days of age by determining whether or not ova were released into the oviducts. The number of rats which had ovulated was taken as a measure of the quantity of LH-RF injected.

Results

Pituitary Gland Weights

The average weights of the posterior, anterior and whole pituitary relative to age are summarized in Table 1 and in Figure 2 and the anterior pituitary weights were tabulated in greater detail in Appendix Table 1. The average weight of the whole pituitary increased from 0.69g at birth to 1.80g at 12 months of age--a total increase of 1.11g of tissue during the 12-month period. Increases in anterior pituitary weight paralleled and seemed to account for nearly all of those of the whole pituitary. Orthogonal polynomial analysis of the relationship between whole pituitary or anterior pituitary weight and age indicated that their growths were linear and parallel until 8 or 9 months of age. In contrast, no significant changes were observed in posterior pituitary weight due to age ($P > 0.25$).

Levels of Pituitary FSH and LH

The average concentrations of pituitary FSH and LH from birth through puberty were summarized in Table 2 and

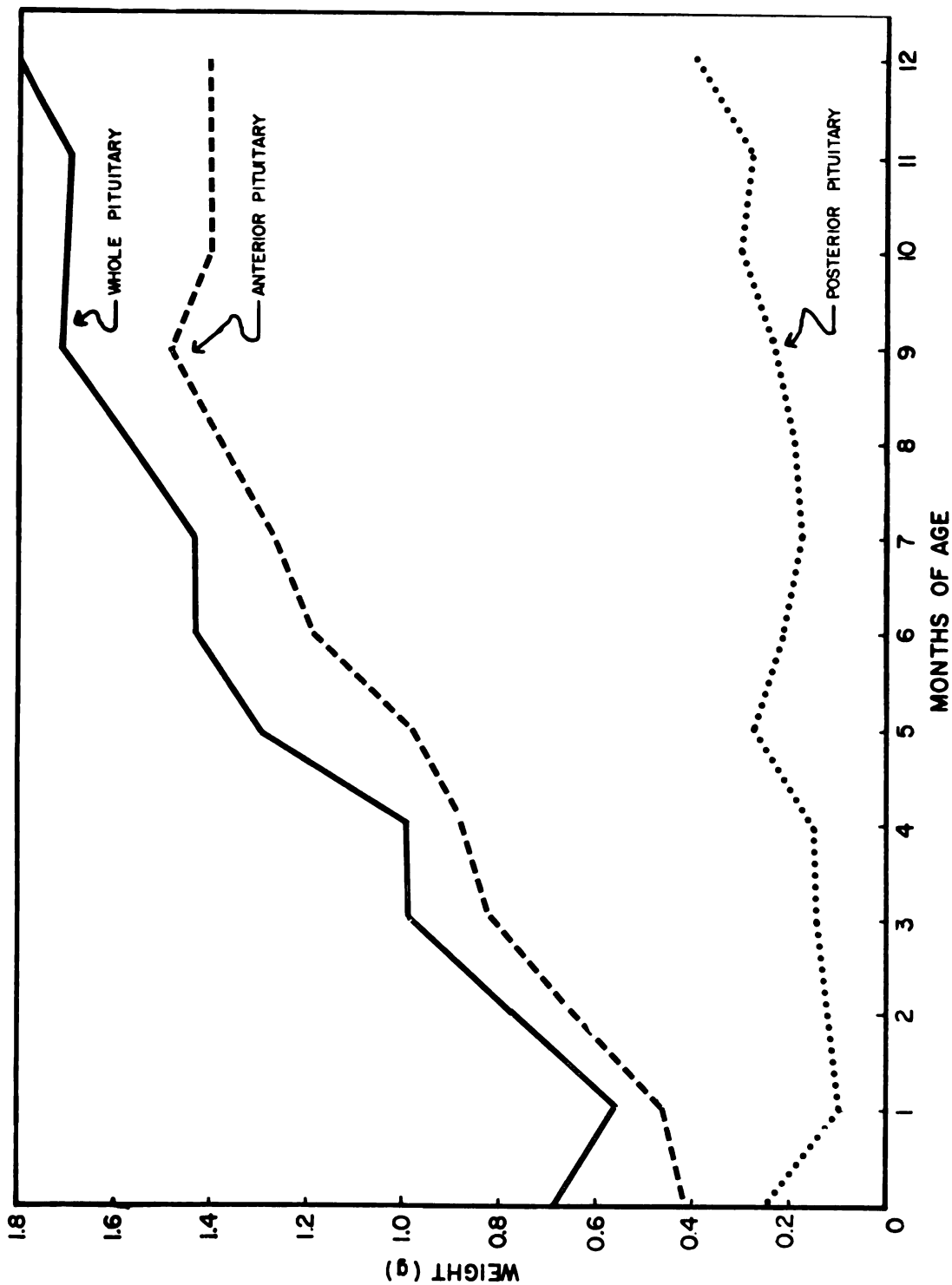


Fig. 2.—Average weights of the posterior, anterior and whole pituitary from birth through puberty.

TABLE 2.--Average weight of whole, anterior and posterior pituitary and the average concentration of LH and FSH from birth through puberty.

Age (Months)	Fresh Pituitary Weights (g)			Concentration of Pituitary Gonadotropin*	
	Whole	Anterior	Posterior	FSH	LH
	(mean ± SE)				
0	0.69 ± 0.07	0.44 ± 0.06	0.25 ± 0.05	1.67 ± 0.23	2.44 ± 0.74
1	0.57 ± 0.05	0.48 ± 0.04	0.09 ± 0.04	2.68 ± 0.42	2.07 ± 0.75
2	0.78 ± 0.04	0.67 ± 0.05	0.12 ± 0.02	1.06 ± 0.06	5.79 ± 0.70
3	1.00 ± 0.06	0.85 ± 0.04	0.15 ± 0.03	0.83 ± 0.12	9.09 ± 3.05
4	1.05 ± 0.10	0.90 ± 0.11	0.15 ± 0.02	0.98 ± 0.08	4.48 ± 1.75
5	1.30 ± 0.11	1.02 ± 0.08	0.28 ± 0.03	0.94 ± 0.06	8.63 ± 1.41
6	1.44 ± 0.25	1.22 ± 0.04	0.22 ± 0.03	0.89 ± 0.17	4.46 ± 1.01
7	1.44 ± 0.09	1.26 ± 0.08	0.18 ± 0.02	0.92 ± 0.19	10.19 ± 3.53
8	1.57 ± 0.11	1.37 ± 0.10	0.20 ± 0.02	1.01 ± 0.08	7.25 ± 1.78
9	1.72 ± 0.06	1.48 ± 0.07	0.24 ± 0.02	0.93 ± 0.23	5.61 ± 2.49
10	1.71 ± 0.09	1.40 ± 0.08	0.31 ± 0.02	0.94 ± 0.15	2.88 ± 1.85
11	1.68 ± 0.10	1.40 ± 0.11	0.28 ± 0.03	0.81 ± 0.07	5.08 ± 1.30
12	1.80 ± 0.03	1.40 ± 0.05	0.40 ± 0.03	0.79 ± 0.05	6.79 ± 1.55

*Potency expressed as µg equivalents of NIH-FSH-S2 or NIH-LH-B2 per mg fresh anterior pituitary tissue.

in Figure 3, while the individual values were tabulated in Appendix Table 1. Inspection of the curves depicted in Figure 3 indicated that pituitary FSH concentration was greatest at 1 month. This deflection in the concentration of pituitary FSH from the average of the 13 age groups was significant ($P < 0.05$). The most striking feature of these FSH averages was their uniformity from 2 to 12 months of age.

Pituitary LH concentrations were much greater than FSH concentrations and appeared to increase rapidly after 1 month of age and declined regularly after 7 months of age. However, the intermediate age group averages varied so widely that the differences observed in the concentration of this gonadotropin were not significant ($P > 0.15$).

As discussed in the review of literature, the concentration of pituitary LH is known to fluctuate with the stage of estrous cycle in some species. Heifers in the present experiment began regular estrous cycles at about 7 months of age and the stage of estrous cycle at the time of slaughter undoubtedly influenced the post-puberal values. Consequently, the pituitary FSH and LH concentrations obtained from heifers which had been cycling previous to slaughter were studied in a separate analysis to determine pituitary levels of gonadotropin at various stages of the estrous cycle. The cylic pattern of pituitary gonadotropin was illustrated in Figure 4. The smallest concentration of LH was observed immediately

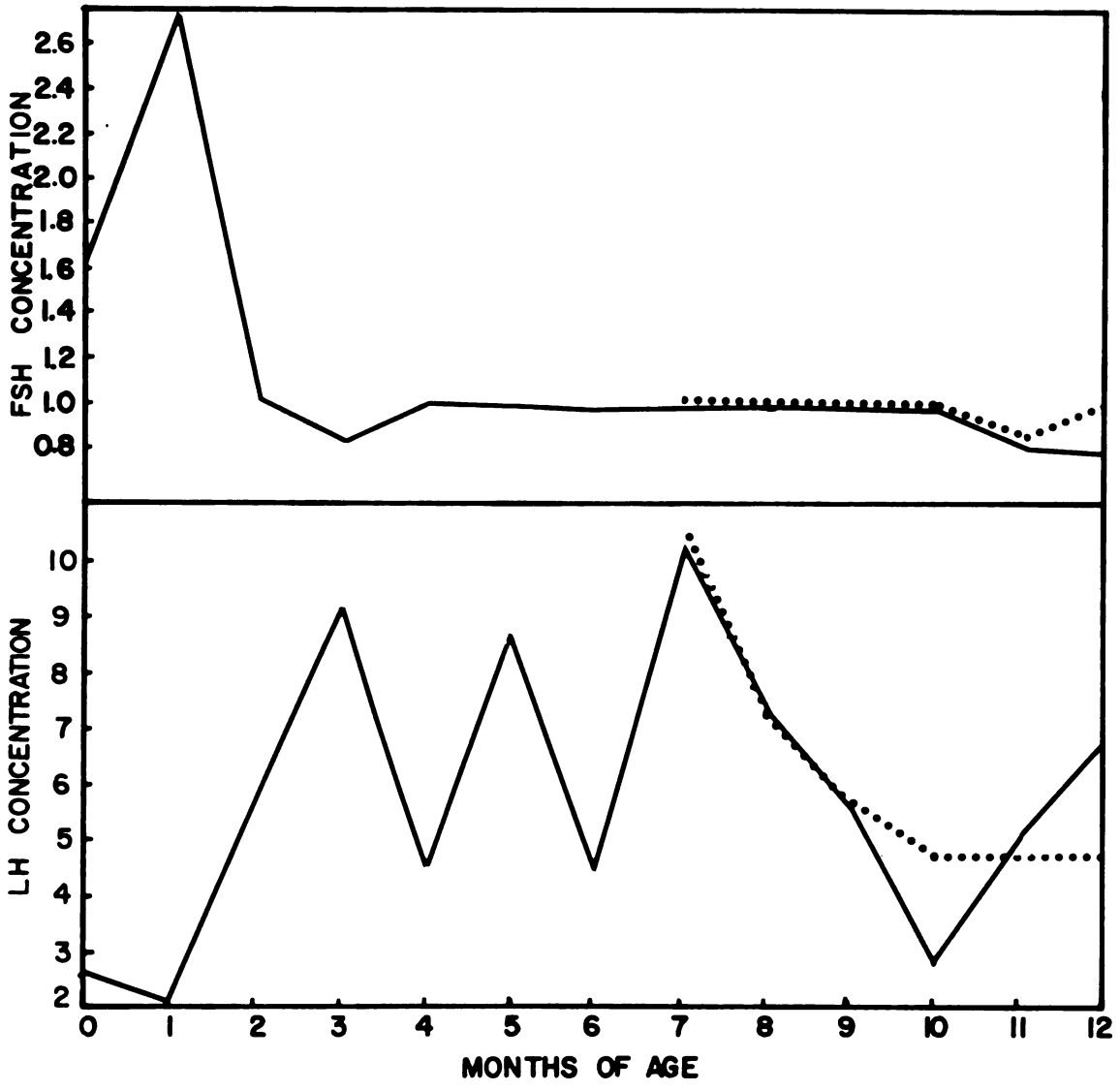


Fig. 3.--Average concentrations (solid lines) of pituitary LH and FSH (µg-equivalents of NIH-LH-B2 or NIH-FSH-S2 per mg of fresh anterior pituitary) from birth through puberty. Dotted lines refer to average concentrations of hormones adjusted for stage of estrous cycle.

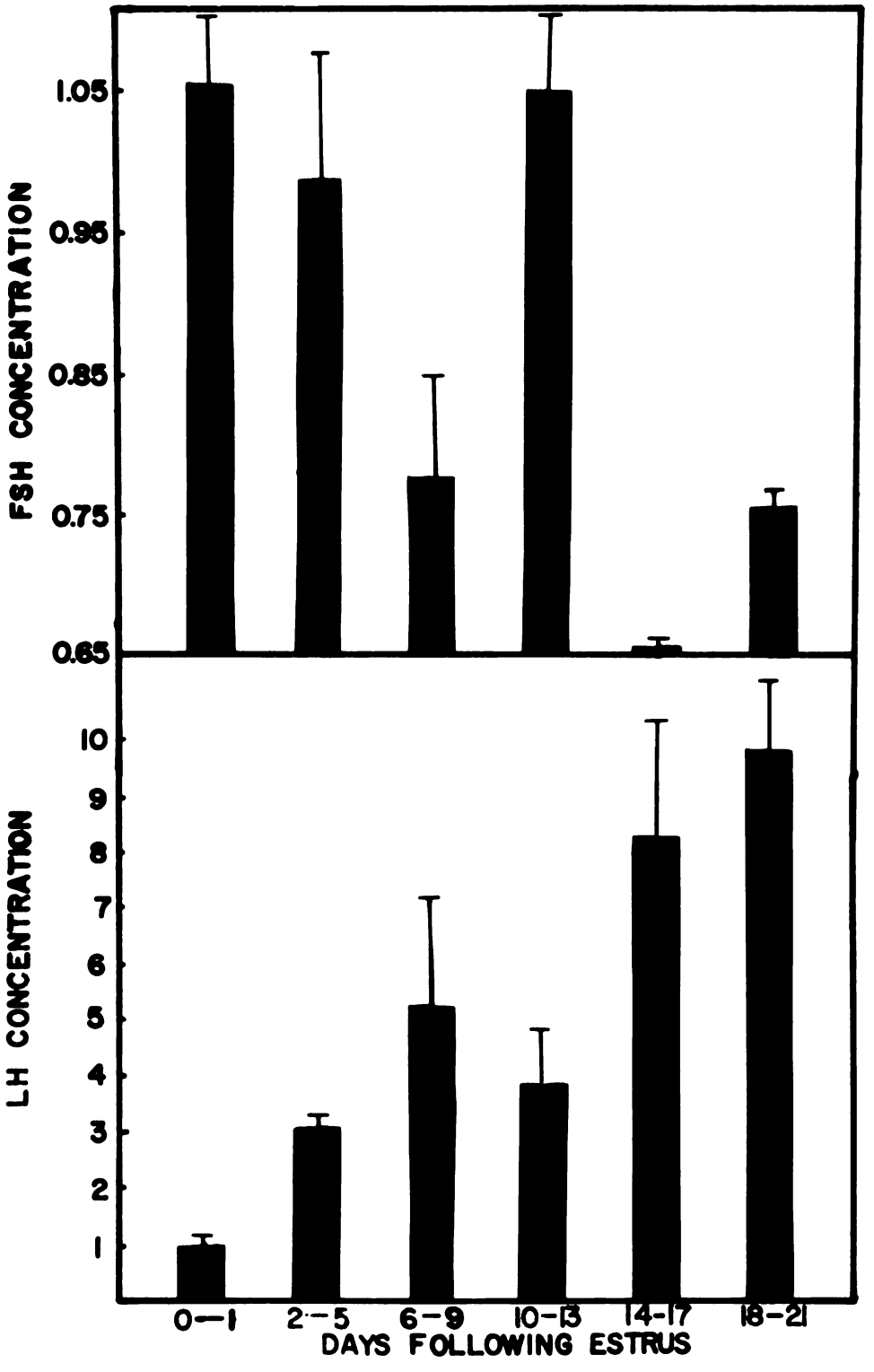


Fig. 4.--Average concentrations of pituitary LH and FSH µg equivalents of NIH-LH-B2 or NIH-FSH-S2 per mg of fresh anterior pituitary) during the estrous cycle. Vertical lines refer to standard errors.

following ovulation. This was followed by a gradual accumulation of pituitary LH and finally a dramatic elevation in the final days before the time of the next ovulation. These cyclic changes represented an eight-fold increase in pituitary LH concentration from the beginning to the end of the estrous cycle. Apparently, about 80 or 90 per cent of pituitary LH was released from the pituitary during the day just before ovulation. No appreciable variation in pituitary FSH concentration was observable between day 0 and day 13 of the cycle (Figure 4). However, the data suggested that pituitary levels of FSH may have declined during the last 4 or 5 days before the next ovulation.

Because of these variations in pituitary FSH and LH concentrations due to stage of estrous cycle at the time of slaughter, the average concentrations of pituitary FSH and LH by age (Figure 3) were probably biased, at least after puberty. In an effort to correct this, the pituitary concentrations of FSH and LH of the cycling animals were adjusted to the average observed on the 11th day of the estrous cycle. Endocrinologically, prepuberal animals probably most closely resemble postpuberal animals at this stage (diestrus) of the cycle. The graph of the adjusted pituitary FSH and LH concentrations (Figure 3--dotted line) indicated that the precipitous decrease in pituitary LH concentration beginning at puberty was not due to variations caused by the stage of

the estrous cycle. Rather, the data suggested that, while the prepuberal pituitary synthesizes LH, it does not release appreciable quantities into the blood until puberty.

The ratio of LH:FSH was determined for each animal using the data corrected for stage of the estrous cycle and these observations are plotted in Figure 5. The ratio LH:FSH increased rapidly until 3 months of age, was characterized by considerable variation from 3 to 7 months of age, and then decreased rather regularly to the lowest level at 12 months of age. The overall change in the ratio of LH:FSH from 3 to 8 months represented an eight-fold decrease in this ratio.

Due to the relative constancy of pituitary FSH during the cycle, the LH:FSH ratio paralleled cyclic variations in the concentration of pituitary LH observed in the postpuberal heifers (Figure 3). The most striking feature of LH:FSH ratios during the estrous cycle was the sudden decline in the ratio just before the time of the next ovulation.

Levels of Hypothalamic LH-RF

Injection of extracts derived from the equivalent of 0.8 calf hypothalami failed to induce ovulation in the test rats. Larger quantities of the extract resulted in severe toxicity and death of the rats. Consequently, it was not possible to determine whether or not changes in the hypothalamic LH-RF content occurred during puberty.

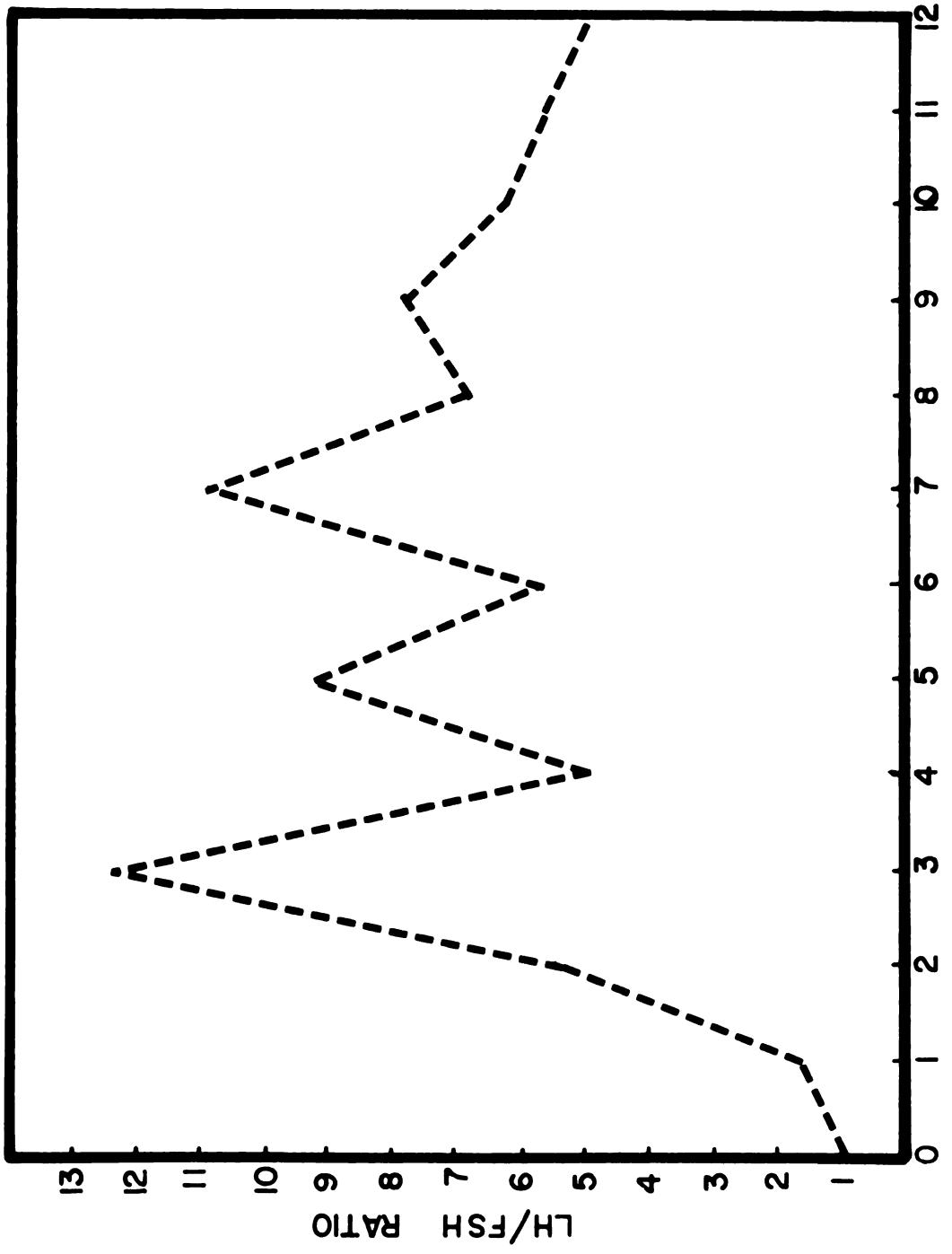


Fig. 5.--Average LH/FSH ratios from birth through puberty calculated from data adjusted for stage of estrous cycle.

Discussion

The increments in weight of the anterior, posterior and whole pituitary with advancing age of the heifers reported here were similar to observations reported by Sorensen et al. (1959) and by Brody and Kibler (1941). Although the absolute weights of the pituitary tissues increased, the weights of these tissues relative to total body weight decreased with advancing age. Surprisingly, the posterior pituitary tissue contributed comparatively little to the total weight increase in the whole pituitary. Most of the increase in the whole pituitary weight was due to the tissue of the anterior lobe.

In fact, the posterior pituitary decreased in weight between birth and 1 month of age, after which period it maintained a slow growth, whereas the weight of the anterior lobe increased continuously from birth. The significance of this early loss of weight of the posterior pituitary was not readily apparent. Perhaps it represented an adjustment on the part of the new-born to its new environment. One possible adjustment could have been in antidiuretic hormone from the posterior pituitary with consequent water conservation in the neonatal calf which is known to have larger water content than older calves. But this hormone was not measured in the heifers studied here.

The slow growth of the posterior pituitary relative to the growth of the anterior pituitary might have been

anticipated because of the neural origin of the posterior pituitary. Neural tissue generally is typified by more prenatal and less postnatal growth than other tissues such as muscle and bone.

The information derived from potency estimates of specific pituitary gonadotropins during pre- and post-puberal development is of necessity subject to interpretation. This is true because the pituitary gland can apparently simultaneously synthesize, store and release a given hormone. Unfortunately, bio-assay of hormone content reflects only the quantity of hormone present in the pituitary at the time the gland was removed from the animal. The measured quantity is a function of the rate of synthesis and of the rate of release of the hormone. Large concentrations of pituitary FSH and LH do not necessarily reflect simultaneously large release rates of the hormones. Despite these difficulties in interpretation, most previous researchers have assumed that large pituitary gonadotropin concentrations represented storage of the hormones in the gland whereas low pituitary concentrations reflected release of the hormones into the blood (Parlow et al., 1964). A notable exception to this generality is the castrate animal which possesses elevated levels of gonadotropin in the pituitary as well as in the blood; and, undoubtedly, other exceptions exist. Nevertheless, the interpretation applied by Parlow et al. (1964) appeared to be correct in most measured cases and

the results pertaining to pituitary LH and FSH were similarly interpreted in this thesis.

Although the levels of pituitary FSH and LH were presented in terms of the concentrations of the hormones, the significance of the total amount of FSH and LH present in the pituitary has not been ignored. Graphs of the total pituitary FSH and LH from birth through puberty and those during the estrous cycle paralleled graphs of the concentration data and revealed no new information; and, consequently, they were not presented here. Large variations in the data for both concentration and total pituitary FSH and LH resulted in similarly large variations in the quantities of these hormones per unit of body weight (mg hormone per kg of body weight). Consequently, the latter data, shed no new light on the initiation of puberal mechanisms.

That the concentration and total pituitary FSH changed relatively little near the time of puberty suggested that this gonadotropin played a permissive, but probably synergistic role in the debut of puberty in the bovine. The average concentration of FSH in prepuberal animals was always higher than the concentrations of FSH observed during the estrous cycle. In fact, the highest quantity of FSH was observed at 1 month of age, and this value was at least twice that observed at any other age or stage of the estrous cycle. This spike in FSH activity was associated with a small but concomitant decrease in LH activity. However, this FSH spike preceded

by 1 month the rapid increase in LH activity which resulted in the largest quantities of LH activity (at 3 months) of any age or stage of estrous cycle. Any attempts to explain the significance of the early alterations of neonatal gonadotropins must include consideration of similar parameters measured prenatally--information which is not available in the literature. Undoubtedly, the level of neonatal pituitary gonadotropins was influenced by maternal hormones.

Similar prepuberal elevations of both FSH and LH in the pituitary glands of rats and pigs were reported by Parlow (1964) and Parlow et al. (1964), respectively. However, Ramirez and McCann (1963) attributed most of the variation in total pituitary gonadotropin to variations in pituitary LH in the prepuberal rat. Ramirez and coworkers expressed the opinion that alterations in pituitary LH were responsible for causing the onset of puberty (Ramirez and McCann, 1963; Ramirez and Sawyer, 1965).

The data for prepuberal rats and pigs, however, showed that while they possessed quantities of pituitary LH, they had undetectable quantities of plasma LH. While no effort was made to measure plasma gonadotropin here, the evidence provided in this report suggested that maturation of the hypothalamo-hypophyseal-gonad axis from birth through puberty resulted in a decrease of pituitary FSH and especially LH which occurred at and

after puberty, and this decrease was presumably due to increased release into the blood.

It is interesting that the level of pituitary LH in post-puberal animals at 12 months of age reported here was about twice that observed in a group of 42 nonpregnant adult cow pituitaries assayed at approximately the same period of time (Desjardins et al., 1966). In contrast, the level of pituitary FSH reported here for the postpuberal animals was very similar to that observed in the adult animals under the experimental conditions described above. The data in Figure 3 suggested that the pituitary level of LH observed in postpuberal animals may continue to decline beyond 12 months of age to the values previously observed for mature cows.

Rakha and Robertson (1965) reported the quantities of pituitary LH and FSH during the bovine estrous cycle. Their values were somewhat lower than those reported in postpuberal heifers in the present experiment, but the cyclic variations in pituitary FSH and LH in the two experiments paralleled each other almost perfectly. Most recently, Anderson and McShan (1966) reported on blood levels of LH during the bovine estrous cycle. Their values for plasma LH described a curve which was approximately the inverse of the pituitary LH values reported here, thus lending further credence to the belief that high pituitary LH levels reflect low plasma LH levels and vice versa.

This relationship between low pituitary LH and high plasma LH during the first 15 days of the estrous cycle coincided with the period of luteal growth and the period during which the bovine corpus luteum contained the most progesterone (Mares et al., 1962), indicating that the ability of the corpus luteum to synthetize progestagins was greatest during the first 15 days of the estrous cycle (Armstrong and Black, 1966). These data lend further support to the hypothesis that LH is the luteotropin in the cow (Hansel, 1966).

CHAPTER VI

ENDOCRINE AND REPRODUCTIVE ORGAN GROWTH AND MORPHOLOGY

Review of Literature

Growth and Morphology

Postnatal growth of the endocrine and reproductive organs has not been extensively studied. In contrast, alterations in growth and morphological appearance of the endocrine and reproductive tissues appear to have been extensively studied (Altman and Dittmer, 1962) during pregnancy and during the estrous cycle in several species including the mouse, rat, cow and human. However, the measurements made during these events consisted largely of changes in organ weight with qualitative morphological data indicating the presence or absence of certain cells. Recently, more quantative estimates of growth have been made available by using the Schmidt-Thannhauser (1945) procedure, for measuring the amount of DNA present in tissues. For example, this method has been extensively used to study adrenal (Branez and Roels, 1961), thyroid (Lindsay and Cohen, 1965), uterus (Lerner, 1964), ovary (Callantine et al., 1965) and the mammary gland (Sinha

and Tucker, 1966) of female rats. Quantitative estimates of tissue DNA have been taken as proportional estimates of cell numbers because of the constancy of DNA among somatic cells. DNA estimates provide information on whether cells are increasing in size (hypertrophy) or whether the cells are increasing in number (hyperplasia). The RNA content of tissues has been used as an index of protein synthetic activity and protein content of tissues (Leslie, 1955). These growth parameters have never been applied to reproductive tissues in the bovine.

The Ovary

The physiology and morphology of the ovary of many species has recently been reviewed by Zuckerman (1962). More pertinent reviews with respect to the physiology and morphology of the bovine ovary have been provided by Hansel (1959), by Rajakoski (1960) and by Salisbury and VanDemark (1961). Several older reports presented growth changes of the bovine ovary based on changes in the weight of this organ mainly during the estrous cycle (Asdell et al., 1949) and Foley and Reece (1953). The left ovary of the young calf appeared heavier than the right (Salisbury and VanDemark, 1961), but there was no significant difference between the number of follicles found on the right or left ovaries of mature cows (Rajakoski, 1960). Foley et al. (1964) reported that bovine ovaries increased in weight parallel to body weight in dairy calves

between 1 and 6 months of age. Similarly, Sorensen et al. (1959) noted that ovarian weights increased up to 48 weeks of age in dairy heifers with no apparent further increase in weight beyond 80 weeks of age. Erickson (1966) indicated that the ovarian weight in beef cows behaved similarly to dairy cows except that gradual increases in ovarian weight continued for as long as 20 years.

Despite changes in ovarian weight, Erickson (1966) demonstrated that there was no appreciable change in the primordial follicle population of the bovine ovary between birth and 24 months of age; thereafter, the primordial follicle population began a steady and gradual decline from 133,000 to 0 follicles at 20 years of age. Additional morphological characteristics of the bovine ovary have been presented by Moss et al. (1954) and by Rajakoski (1960). These reports were principally concerned with gross morphology of the ovary during the estrous cycle.

Hammond (1927) recorded that the follicles of 4 to 5 month old prepuberal calves were equal in weight and appearance to those of puberal and postpuberal animals. A similar report was presented by Casida et al. (1935) who indicated that these follicles responded to exogenous gonadotropin and could subsequently be made to ovulate. In a more extensive study, Marden (1951, 1952, 1953) confirmed and extended the observations of Casida et al. (1935), and indicated that the ovary of a 1-week-old calf can assume morphological growth responses to exogenous gonadotropin

similar to that expected of mature animals. Roberts and Warren (1964) provided evidence that the bovine fetal ovary (8 months of pregnancy) was capable of effecting most of the steroidal conversions seen in the adult. These workers demonstrated that this tissue effected 20α -reduction, 16α -hydroxylation, 17α -hydroxylation, side chain cleavage of progesterone, as well as 17β -reduction and aromatization of androstenedione.

These data on the bovine ovary do not conform to previously published reports in some laboratory animals (Hisaw, 1947). For example, Hertz and Hisaw (1934) and Hisaw (1947) noted that the ovary of newly born laboratory animals failed to respond to exogenous gonadotropin treatment and the authors suggested that the prepuberal ovary must attain a "competence" before it can respond to exogenous hormones. These reported differences in age at which the ovary responded to gonadotropin may have been due to differences in physiological age at birth among the species studied.

The Oviducts

Lombard et al. (1950) and Roark and Herman (1950) investigated the changes in growth and morphological appearance of the bovine oviduct during the various stages of the estrous cycle. They reported that increased epithelial cell heights accompanied an increase in the secretion from the fimbriated region during estrus.

These same workers suggested that nuclei appeared to be extruded from the epithelial cells of the oviduct. Asdell (1965) also considered the origin of these extruded nuclei. Sorensen et al. (1959) noted a gradual increase in the length of this organ from birth to 80 weeks of age. These same workers noted that an increase in cell height of the epithelium occurred at the time of puberty, suggesting that oviduct growth and morphology depended on ovarian function.

The Uterus

Several investigators have reported changes in uterine growth and morphology of adult laboratory and domestic ainmals under various hormonal states (Cole, 1930; Asdell et al., 1949; Roark and Herman, 1950; Foley and Reece, 1953; Velardo, 1959). However, growth and morphological changes occurring in the uterus prior to puberty have received little attention (Reynolds, 1949). Sorensen et al. (1959) indicated that small but gradual increases in uterine weight and length occurred in the calf between birth and 32 weeks of age. Uterine weight doubled between 32 and 48 weeks of age, indicating the marked effect of puberty on uterine growth. The Cornell workers also reported that the uteri of prepuberal calves were typified by shallow endometrial epithelia with very few endometrial glands. They were remimisent of uterine tissues from castrate animals. At puberty, the height

of the uterine luminal epithelium increased from 14 to 36 μ and this change was accompanied by marked increases in the vascularity of the endometrium.

The Cervix and Vagina

Growth and cytological changes in cervical and vaginal tissues during the bovine estrual cycle were investigated by Cole (1930) and Roark and Herman (1950). These authors observed alterations in the heights of cervical and vaginal mucosa which occurred during the estrual cycle. Marion and Gier (1960) confirmed the above reports and suggested that accurate measurements of vaginal epithelium can be made from tissue obtained in close proximity to the cervix. Epithelial cell heights were always greatest during estrus in the above studies. Sorensen et al. (1959) reported similar increases in epithelial cell height in heifers as they approached the age of puberty. Abrupt increases in the length and weight of these tissues were also observed at the time of puberty.

The Adrenal

The first comprehensive reports on the bovine adrenal were provided by Elias (1948) and Weber et al. (1950) who described the three zones of the adrenal cortex: the zona glomerulosa, the zona fasciculata and the zona reticularis, plus the chromaffin tissue of the adrenal medulla. A more comprehensive paper by Nicander (1952) indicated that few morphological changes occurred in the

adrenal cortex from birth to 2 years of age. However, Cupps et al. (1959) reported adrenal cortex degeneration in sterile dairy cattle.

The Thyroid

The only report concerning growth and morphological development of the bovine thyroid from birth through puberty was presented by Sorensen et al. (1959). These workers reported that mean thyroid acinar cell heights increased from birth to 80 weeks of age with no significant change occurring at the time of puberty. Campbell et al. (1949) and Swett et al. (1955) noted that thyroid function of young calves before puberty could be influenced by environmental and genetic factors, respectively.

The Thymus

Changes in the thymus gland during growth of the bovine have not been published, but there are voluminous studies which describe regression of the thymus with advancing age in laboratory animals (Defendi and Metcalf, 1964). Hegyeli et al. (1963) suggested that extracts of prepuberal calf thymus could sterilize adult female mice. However, this report was not supported by Martin (1964) who used male rats. She noted that the ventral prostrates and seminal vesicles of thymectomized rats (6 weeks old) were significantly heavier than sham-operated rats which were atopsied 3 weeks after surgery. The possibility

that the thymus gland exerts a negative or retarding effect on the debut of puberty has never been investigated.

Materials and Methods

Tissue Weights and Morphology

The entire reproductive tract was removed from each animal about 15-25 minutes after slaughter and dissected free of any extraneous tissue. The lengths of the left and right oviduct, uterus, cervix and vagina were determined to the nearest 0.1 cm for each animal. Organ weights were recorded for the left and right ovary, paired oviducts, uterus, cervix and vagina. The posterior demarcation of the vagina was immediately anterior to the suburethral diverticulum.

The number of follicles and corpora lutea appearing on the surface of the ovaries were determined by inspection. Tissue from the upper one-third of the oviduct, the junction of the uterine horn and body, the cervix and the anterior vagina were placed in Bouin's fluid for micromorphological examination. The remaining uterine, cervical and vaginal tissues were placed in 0.25M sucrose for biochemical analysis.

Similarly, immediately after slaughter, the thyroid and adrenal glands were removed, trimmed and weighed. Sample sections of thyroid and adrenal tissue were placed in Bouin's fluid and 0.25M sucrose for micromorphological and biochemical evaluation, respectively. The thymus

gland was weighed and a sample was placed in 0.25M sucrose for biochemical analysis. All samples in 0.25M sucrose for biochemical analysis were quick-frozen at -79° C. and stored at -20° C.

The tissues that were prepared for micromorphological examination were embedded in paraffin, sectioned at 7 microns and stained with hematoxylin and eosin. This entire procedure was adopted from the procedure outlined by the Armed Forces Institute of Pathology (1960). The width of the adrenal zona glomerulosa and the combined width of the zona reticularis and zona fasciculata were determined. Heights of the luminal epithelial cells of the oviduct, uterus, cervix, vagina and thyroid were measured at a magnification of 540 X under oil with a calibrated occular micrometer. Two cells were measured in each of five fields in each of three sections for a total of 30 measurements on each tissue from each heifer. The adrenal cortex measurements were similarly performed except that they were made at a magnification of 20 X.

Biochemical Analysis

Tissue DNA and RNA contents were measured by the procedures outlined by Tucker (1964) for mammary tissues. Lipid content was estimated from the weight of the residue after evaporation of the combined alcohol, methanol-chloroform and ether extractions from the nucleic acid analysis. Protein analyses were performed on tissue

homogenates which were hydrolized with an equal volume of 2N potassium hydroxide at 37° C. for 15 hours according to the procedure of Gornall et al. (1949).

Statistical Analysis

The data from each criterion of response were subjected to analysis of variance. Differences among age groups were tested by within age group variance to determine the significance of age-group differences. Onthogonal polynomial constants were fitted to the age-group means to determine whether growth was linear or quadratic.

Results

The chief aim of the present study was to characterize growth changes occurring in endocrine and reproductive tissues of heifers from birth through puberty. It was recognized at the outset that animals which had passed puberty and begun estrous cycles might warrant special consideration because it was known that estrous cycles would introduce additional variation into many measurements. This was the logic for adjusting pituitary LH concentration for stage of estrous cycle of the postpuberal heifers.

In a more practical sense, puberty does not imply attainment of full reproductive capacity. It is well known, for example, that fertility of heifers increases considerably from the time of puberty at least until 12 to 15 months of age. Consequently, the observations

on the reproductive tract presented in this section were not adjusted for stage of estrous cycle. In support of this decision, inspection of these data indicated that stage of estrous cycle of postpuberal heifers contributed less variation than did their age.

The Ovary and Oviduct

The relationships between follicular development and age and between ovarian weight and age are illustrated in Figure 6 and listed in Table 3. Average ovarian weight increased gradually from birth to 12 months of age and a linear component appeared to describe this increase in ovarian weight.

The ovaries of newborn calves contained no grossly evident follicles. However, a few small ($\leq$ 5mm) and large (> 5mm) follicles were noticed at 1 month of age. A marked increase in the number of both small and large follicles occurred between 1 and 4 months, and this was followed by a decrease between 4 and 8 months of age. Numbers of small and large follicles on the ovary were relatively constant from 8 to 12 months of age, the ages during which estrous cycles were observed in the present experiment.

The average changes with age in oviduct weight, length and epithelial cell height are illustrated in Figure 7 and listed in Table 3. Average oviduct weight increased about 3g between birth and 12 months of age. Although the average oviduct weights for the 13 age groups

TABLE 3.--Ovarian and oviduct development from birth through puberty.

Age (months)	Paired Ovary Weight (g)	Mean No. of Follicles		Oviduct Weight (g)	Oviduct Length (cm)	Oviduct Epithelial Cell Height (μ)
		≤ 5 mm	> 5 mm			
0	0.5 ± 0.1*	0	0	0.5 ± 0.1	11.7 ± 0.4	22.8 ± 0.7
1	1.2 ± 0.1	1.0	1.0	0.6 ± 0.1	11.9 ± 0.6	15.5 ± 0.3
2	3.2 ± 0.4	8.8	0	1.0 ± 0.1	14.7 ± 0.4	30.5 ± 0.3
3	4.2 ± 0.6	11.4	1.0	1.6 ± 0.2	16.1 ± 0.5	30.6 ± 0.3
4	3.5 ± 0.6	22.4	5.4	1.5 ± 0.3	13.3 ± 0.2	31.4 ± 0.3
5	7.1 ± 1.5	13.0	2.0	1.8 ± 0.3	18.2 ± 0.6	30.2 ± 0.3
6	6.8 ± 1.8	8.8	2.4	2.1 ± 0.3	18.1 ± 0.8	29.9 ± 0.3
7	6.9 ± 0.8	14.8	1.4	1.9 ± 0.5	18.3 ± 0.8	30.9 ± 0.3
8	7.1 ± 1.0	5.0	1.2	2.7 ± 0.3	20.0 ± 0.6	29.6 ± 0.2
9	9.0 ± 1.5	5.0	2.0	2.8 ± 0.7	20.3 ± 1.3	31.1 ± 0.4
10	9.4 ± 0.8	6.0	0.4	2.5 ± 0.6	18.3 ± 0.4	30.1 ± 0.4
11	12.6 ± 3.0	5.2	1.0	2.5 ± 0.1	21.2 ± 0.4	29.2 ± 0.2
12	12.4 ± 2.2	4.6	0.4	3.3 ± 0.3	21.7 ± 1.6	28.4 ± 0.2

*Mean ± SE.

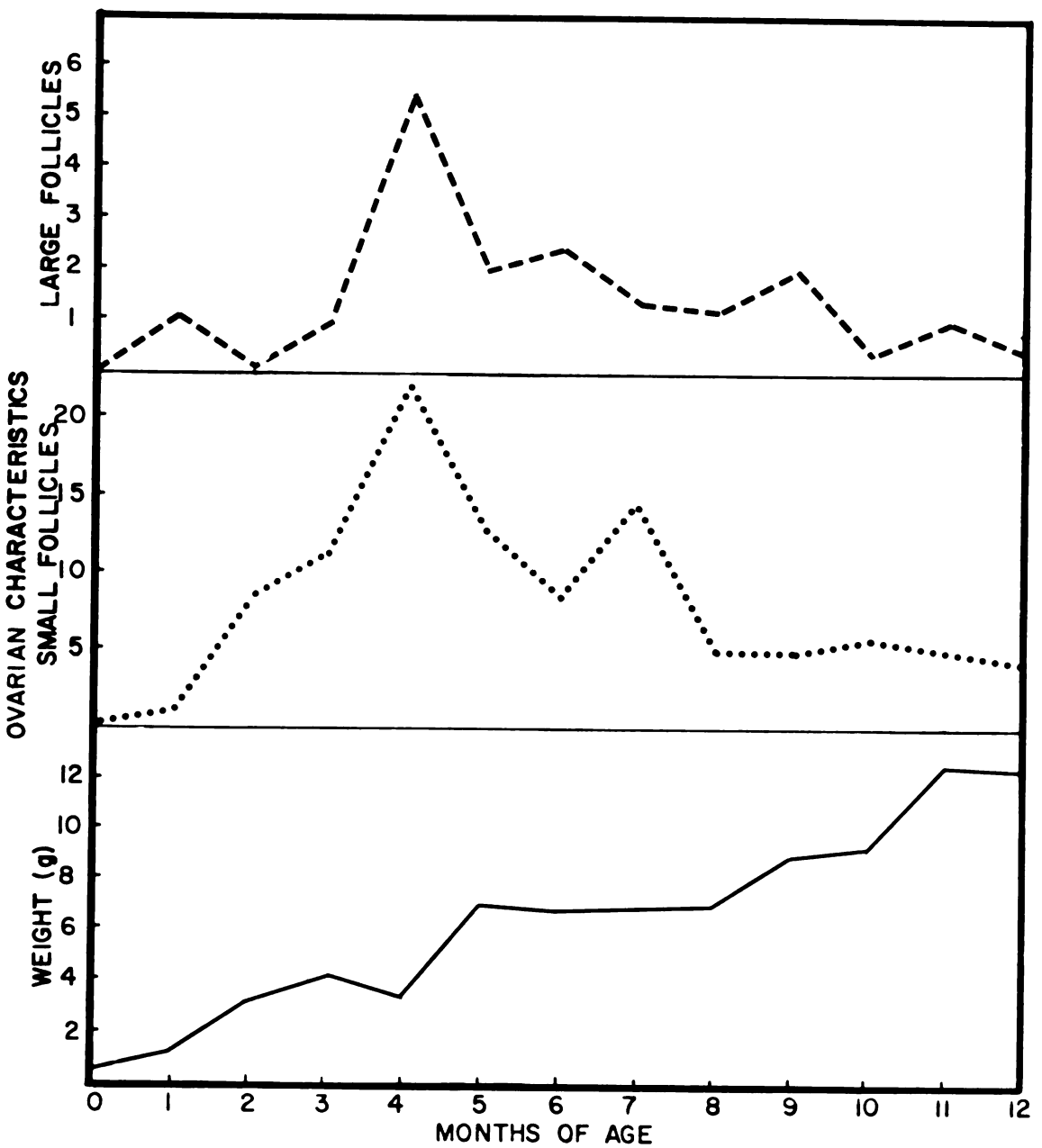


Fig. 6.--Average ovarian weights, numbers of small follicles ($\leq$ 5 mm diameter) and numbers of large follicles ($\geq$ 5 mm diameter) per heifer from birth through puberty.

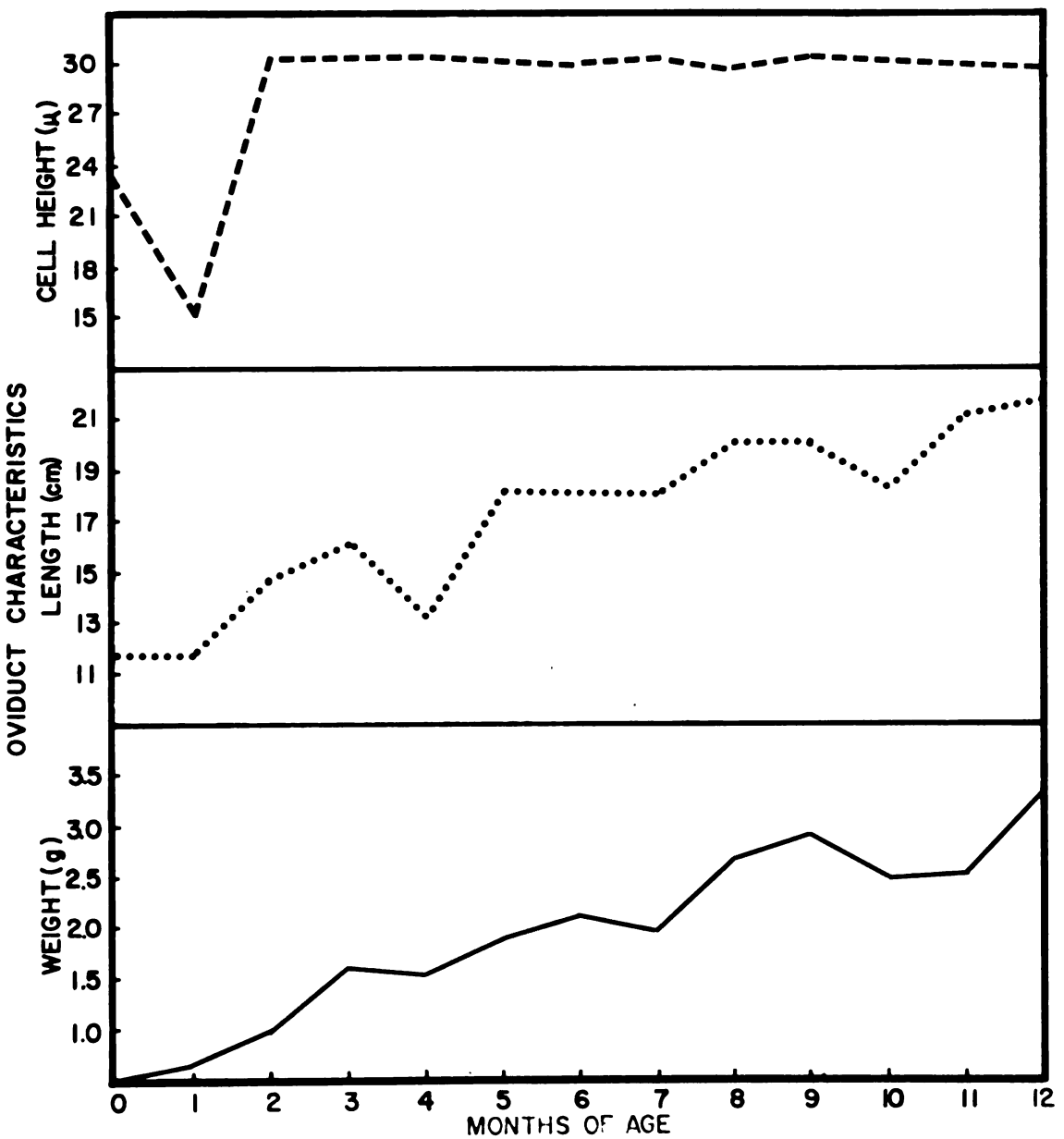


Fig. 7.--Average weights per heifer, lengths and epithelial cell height (μ) of the oviduct from birth through puberty.

were significantly different ($P < 0.10$), the increase in weight with respect to age was not linear ($P > 0.25$).

Average oviduct length increased about 11 cm between birth and 12 months, but the differences did not approach significance ($P > 0.25$). Despite this lack of significance, the data approximated linear growth of oviduct length at least to 8 months of age.

Epithelial cell height measurements from the upper third of the oviduct revealed a marked decrease in epithelial cell height between 0 and 1 month, followed by rapid increase between 1 and 2 months of age. No appreciable changes in this criterion were apparent after 2 months of age. The epithelium in this region of the oviduct was always ciliated even in the very young prepuberal animals. Although the number of cilia were not counted, inspection suggested that fewer cilia were observed in prepuberal animals than in puberal and postpuberal animals. Similarly, more secretion blebs were observed at the luminal extremities of these cells in postpuberal animals than in prepuberal animals. At about the average age of first estrus and thereafter morphological examination suggested the presence of extruded nuclei, which were evident in the photomicrographs, Appendix Figure 1.

The Uterus

The data in Figure 8 and Table 4 indicated that the average uterine weight increased linearly between birth

Table 4.--Uterine development from birth through puberty.

Age (months)	Weight (g)	Length (cm)	Cell Height† (μ)	Total DNA (mg)	Total RNA (mg)	RNA/ DNA	Total Protein (g)	Total Lipid (g)
0	5.7 ± 0.5*	7.7 ± 0.3	20.9 ± 0.7	38.4 ± 2.2	10. ± 1.1	[illegible]	0.6 ± 0.1	1.5 ± 0.1
1	6.4 ± 0.4	6.1 ± 0.4	20.8 ± 0.8	41.3 ± 2.2	7.9 ± 0.4	0.19 ± 0.02	0.7 ± 0.1	1.4 ± 0.2
2	15.6 ± 2.8	11.6 ± 0.6	16.2 ± 0.6	86.8 ± 11.4	[illegible]	[illegible]	1.6 ± 0.2	4.7 ± 1.0
3	23.9 ± 2.1	11.7 ± 0.6	17.1 ± 0.3	119.4 ± [illegible]	[illegible]	[illegible]	2.2 ± 0.2	9.4 ± 0.5
4	25.9 ± 3.1	11.2 ± 0.5	11.5 ± 0.5	167.1 ± 22.3	[illegible]	[illegible]	2.9 ± 0.3	21.9 ± 1.0
5	41.2 ± 3.9	15.8 ± 0.7	16.5 ± 0.6	186.3 ± 19.3	[illegible]	[illegible]	3.7 ± 0.4	12.9 ± 1.2
6	25.0 ± 1.6	12.4 ± 1.1	10.7 ± 0.2	172.9 ± 12.5	[illegible]	[illegible]	2.6 ± 0.2	7.9 ± 0.9
7	54.6 ±10.3	15.1 ± 0.8	20.1 ± 0.8	281.7 ± 38.2	[illegible]	[illegible]	5.4 ± 0.9	9.8 ± 2.0
8	58.0 ±11.9	17.2 ± 0.6	14.8 ± 0.4	315.9 ± 51.[illegible]	[illegible]	[illegible]	6.0 ± 1.2	14.3 ± 2.8
9	78.4 ±14.9	16.7 ± 1.2	24.3 ± 0.6	375.6 ± 51.9	[illegible]	0.32 ± 0.50	7.6 ± 1.4	42.0 ± 26.5
10	128.1 ± 7.7	20.5 ± 0.9	27.9 ± 0.5	402.6 ±122.8	[illegible]	1.03 ± 0.60	15.6 ± 0.7	38.5 ± 5.1
11	132.1 ± 6.7	24.4 ± 0.7	29.3 ± 0.4	434.8 ± 83.6	179.8 ± 14.5	0.49 ± 0.11	13.1 ± 0.9	53.3 ± 6.0
12	149.8 ± 8.6	25.3 ± 2.9	33.0 ± 0.6	557.6 ± 44.0	183.0 ± 9.8	0.33 ± 0.03	16.3 ± 0.9	49.8 ± 8.5

*Mean ± SE.

†Endometrial epithelium cells.

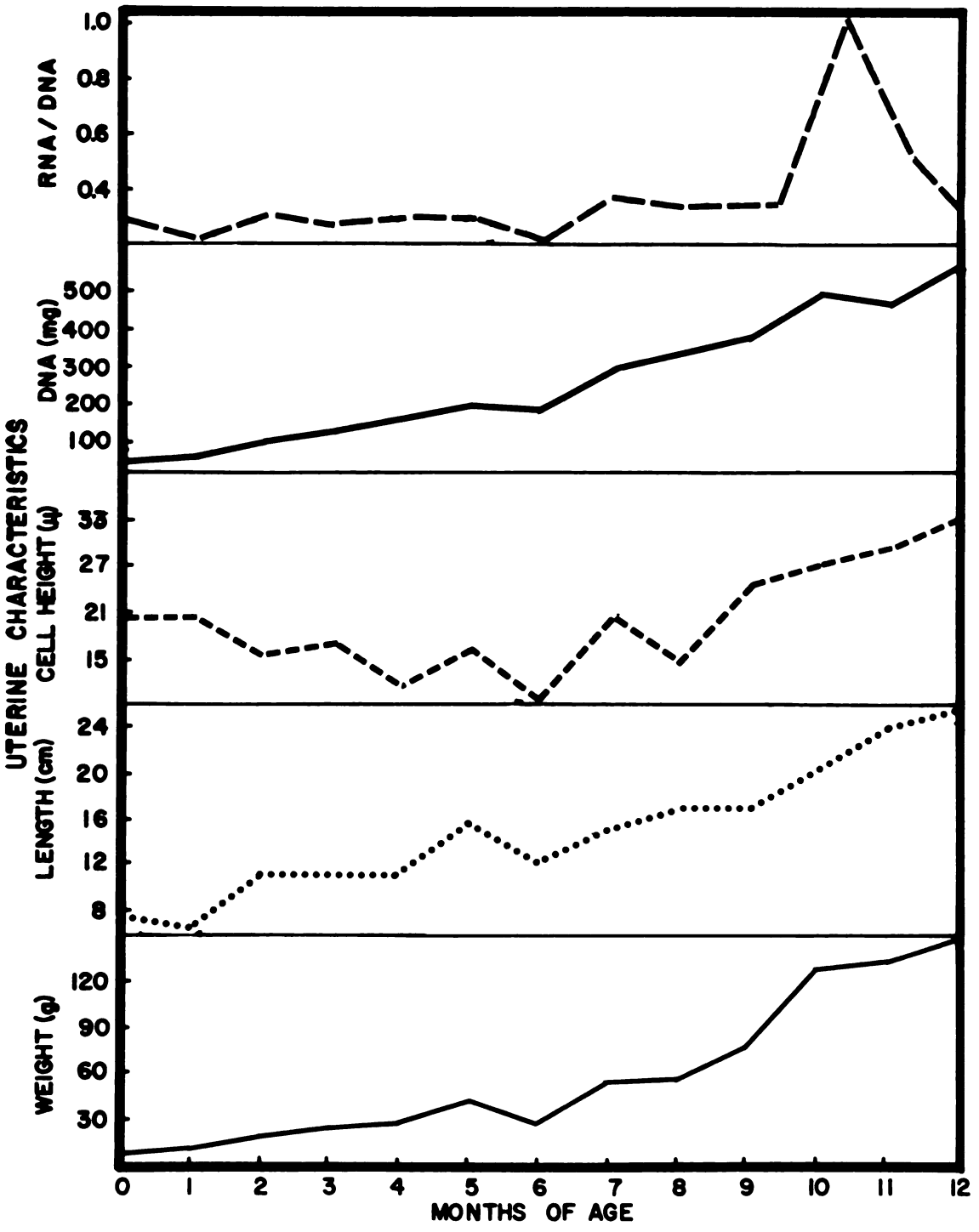


Fig. 8.--Average uterine weights, lengths, epithelial cell heights, DNA (mg), and RNA/DNA ratios per heifer from birth through puberty.

and 6 months of age ($P < 0.01$), but uterine weight increased more rapidly after puberty and consequentry uterine growth from 0 to 12 months was best described by a quadratic curve ($P < 0.01$). Thus, increases in uterine weight were greatest between 6 and 12 months of age. Uterine length increased linearly from birth to 12 months of age ($P < 0.01$), but the rate of increase was greatest after puberty.

A significant linear increase in total uterine DNA, RNA and protein occurred during the first 6 months of life ($P < 0.01$), but subsequent increases in these biochemical components were more rapid with the result that these parameters were best described by a quadratic response ($P < 0.01$). The ratio of uterine RNA/DNA did not change significantly from birth to 12 months of age ($P > 0.25$). Total uterine RNA and paralleled total uterine DNA during all phases of uterine growth. Total uterine lipids varied considerably within the various ages and consequently, the averages for the age groups were not significantly different ($P > 0.25$).

Micromorphological investigation of the endometrial cell heights indicated a significant quadratic growth curve ($P < 0.10$). Epithelial cell heights decreased between 0 and 6 months of age, then generally increased to a maximum height of 33μ at 12 months of age. Both the superficial and basal uterine glands were absent until 4 or 5 months of age but became well developed and lined with tall columnar epithelial cells by 6 or 7 months of age. These changes were evident in the photomicrographs (Appendix Figure 2).

The Cervix

The averages of some of the criteria used to determine cervical growth were graphed (Figure 9) and the average results of all cervical measurements were summarized (Table 5).

Cervical weight increased linearly between birth and 6 months of age ($P < 0.01$), but increases in weight were more rapid after puberty and consequently increases in weight between 0 and 12 months of age were quadratic ($P < 0.01$). Consequently, the greatest changes in cervical weight took place during puberal and postpuberal development. Cervical length also increased with increasing age suggesting a linear trend; however, these data were variable and the age groups did not differ significantly ($P > 0.25$).

Additional data concerning cervical growth between birth and 12 months of age were the total DNA, RNA and protein contents of the cervix. Analysis of these three biochemical criteria revealed that these criteria of cervical growth were similar to cervical weight--linear between birth and 6 months of age but quadratic from 0 to 12 months of age.

Analysis of the RNA/DNA ratio data presented in Figure 9 suggested that this ratio increased at about 8 months of age. This increase in the RNA/DNA ratio at this time paralleled similar increases in cervical weight, total DNA, total RNA and total protein as well as

TABLE 5.--Cervical development from birth through puberty.

Age (months)	Weight (g)	Length (cm)	Cell Height† (μ)	Total DNA (mg)	Total RNA (mg)	RNA/ DNA	Total Protein (g)	Total Lipid (g)
0	3.5 ± 0.3*	2.4 ± 0.3	19.1 ± 0.7	14.5 ± 1.3	7.0 ± 0.9	0.49 ± 0.06	0.8 ± 0.1	0.5 ± 1.0
1	3.9 ± 0.2	2.8 ± 0.1	7.9 ± 0.2	16.5 ± 1.4	8. ± 0.7	0.51 ± 0.05	0.4 ± 0.1	1.8 ± 0.1
2	4.5 ± 0.8	2.8 ± 0.2	8.7 ± 0.2	13.7 ± 2.3	6.1 ± 1.7	0.47 ± 0.03	0.8 ± 0.1	1.0 ± 0.2
3	5.4 ± 0.5	3.4 ± 0.2	9.4 ± 0.2	16.2 ± 2.0	7.9 ± 1.3	0.48 ± 0.02	0.6 ± 0.1	2.4 ± 1.0
4	6.5 ± 0.4	2.1 ± 0.2	11.3 ± 0.3	23.4 ± 2.5	10.3 ± 1.2	0.44 ± 0.02	0.8 ± 0.1	3.2 ± 0.2
5	12.1 ± 2.3	3.3 ± 0.2	11.6 ± 0.3	35.1 ± 7.4	20.7 ± 4.7	0.57 ± 0.02	1.3 ± 0.3	2.9 ± 0.4
6	11.3 ± 0.4	3.4 ± 0.2	10.2 ± 0.3	36.1 ± 1.2	16.9 ± 0.7	0.47 ± 0.03	1.4 ± 0.1	4.0 ± 0.6
7	15.7 ± 1.1	4.0 ± 0.3	12.1 ± 0.3	38.9 ± 4.4	20.5 ± 2.3	0.53 ± 0.04	1.8 ± 0.1	5.3 ± 1.5
8	18.5 ± 2.3	4.2 ± 0.5	12.0 ± 0.3	46.8 ± 8.0	21.1 ± 2.4	0.48 ± 0.03	2.2 ± 0.3	15.9 ± 2.2
9	22.2 ± 1.8	4.4 ± 0.7	15.1 ± 0.5	49.8 ± 6.9	32.1 ± 5.8	0.63 ± 0.04	2.4 ± 0.2	4.3 ± 0.7
10	31.4 ± 6.7	3.9 ± 0.3	24.8 ± 0.6	66.1 ± 12.9	55.0 ± 10.8	0.84 ± 0.07	4.1 ± 0.9	18.4 ± 0.9
11	39.0 ± 2.5	4.9 ± 0.3	24.1 ± 0.7	95.3 ± 12.8	64.8 ± 4.4	0.70 ± 0.06	4.5 ± 0.2	12.5 ± 2.6
12	43.6 ± 5.8	4.9 ± 0.2	23.3 ± 0.6	81.8 ± 8.9	60.6 ± 5.7	0.75 ± 0.04	5.5 ± 0.7	17.9 ± 2.5

*Mean ± SE.

†Epithelium.

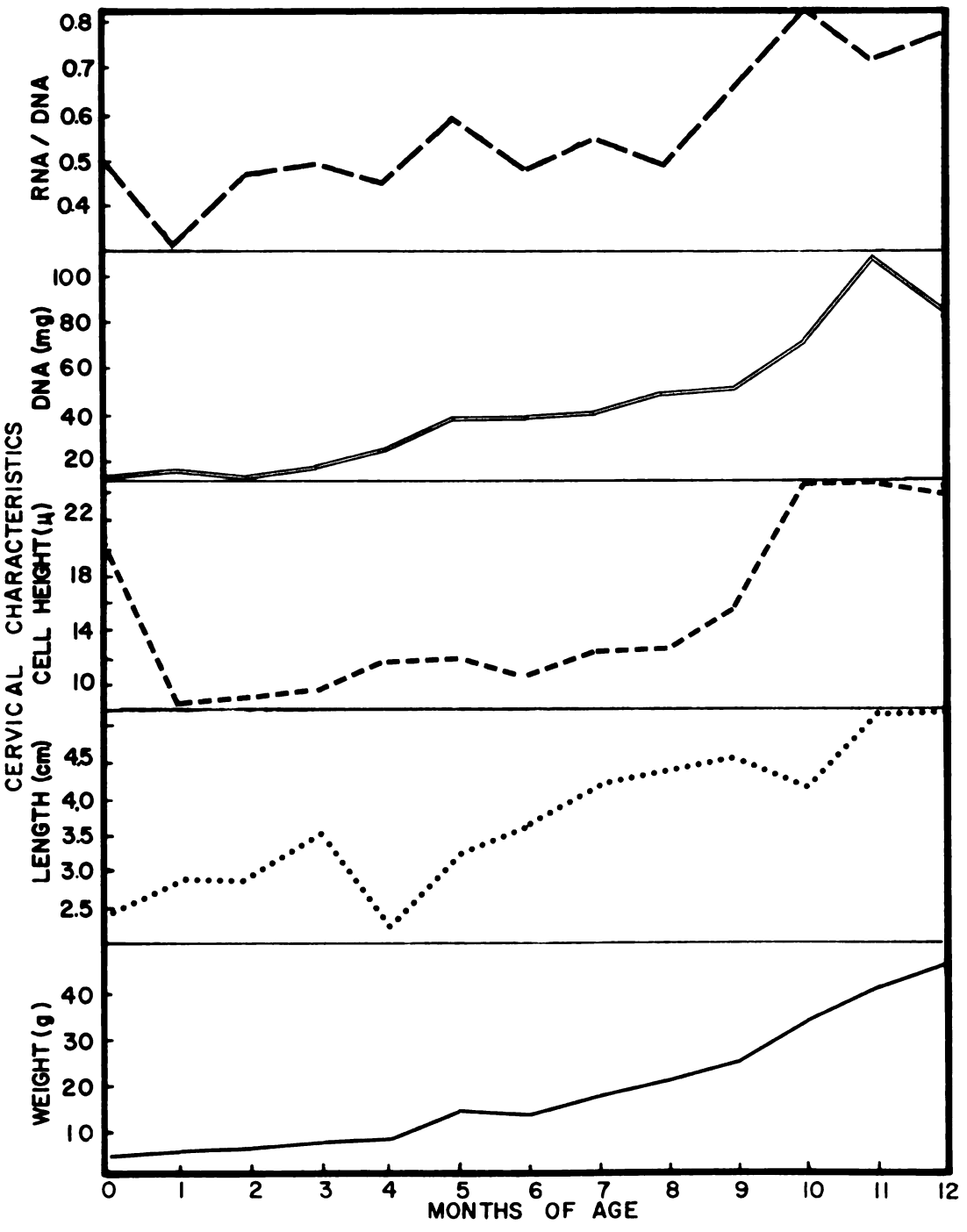


Fig. 9.--Average cervical weights, lengths, epithelial cell heights, DNA, RNA/DNA ratios per heifer from birth through puberty.

epithelial cell height, but differences in RNA/DNA among the age groups were not significant ($P > 0.25$). Results of the lipid analyses of cervical tissue from the 13 different age groups were extremely variable ($P > 0.25$); and, consequently, this was not a useful parameter for evaluating cervical growth.

Cervical epithelial cell heights decreased immediately after birth, suggesting some hormonal stimulation of these cells during gestation. Between birth and 6 months of age, the height of the epithelial cells changed only slightly. After 6 months of age, the height of these cells increased at least 50 per cent to reach a peak at 10 months of age. Cervical epithelial cells were pseudostratified throughout this study. Secretion blebs derived from the cervical epithelium were especially conspicuous during puberal and postpuberal development indicating an increased activity of these cells at this time. In contrast, almost no secretory blebs were observed during prepuberal development.

The Vagina

The averages of all parameters used to determine vaginal growth from birth through puberty were summarized in Table 6. Some of these results were graphed in Figure 10 to facilitate interpretation of the data. The relationships between age and vaginal weight, total DNA, total RNA and total protein were all linear ($P < 0.01$) up to

TABLE 6.--Vaginal development from birth through puberty.

Age (months	Weight (g)	Length (cm)	Epithelial Cell Height (μ)	Total DNA (mg)	Total RNA (mg)	RNA/ DNA	Total Protein (g)	Total Lipid (g)
0	11.9 ± 0.7	5.6 ± 0.3	14.7 ± 0.4	37.1 ± 1.3	31.6 ± 1.7	0.88 ± 0.04	1.4 ± 0.1	3.10 ± 0.5
1	11.3 ± 1.0	9.9 ± 0.5	6.1 ± 0.3	31.3 ± 2.9	12.8 ± 0.6	0.41 ± 0.03	1.4 ± 0.1	3.6 ± 0.4
2	18.1 ± 2.0	7.9 ± 0.5	5.4 ± 0.3	41.0 ± 3.8	30.0 ± 2.6	0.74 ± 0.04	2.0 ± 0.2	4.4 ± 1.1
3	26.1 ± 3.4	9.2 ± 0.8	8.9 ± 0.5	46.3 ± 4.2	26.8 ± 3.4	0.59 ± 0.04	2.0 ± 0.3	9.8 ± 3.1
4	20.3 ± 1.4	6.7 ± 0.6	15.3 ± 0.5	49.2 ± 3.0	33.4 ± 1.9	0.68 ± 0.04	3.6 ± 0.1	14.1 ± 6.3
5	49.7 ± 7.5	9.7 ± 0.5	18.2 ± 0.8	74.3 ± 9.9	64.7 ± 11.0	0.86 ± 0.04	5.6 ± 0.8	16.6 ± 3.9
6	56.4 ± 4.6	10.2 ± 0.7	12.5 ± 0.5	93.6 ± 5.1	80.6 ± 5.7	0.89 ± 0.04	6.6 ± 0.5	18.9 ± 3.6
7	78.4 ± 6.1	11.0 ± 0.7	15.6 ± 0.5	131.2 ± 5.1	95.3 ± 12.4	0.73 ± 0.08	10.8 ± 0.4	22.9 ± 3.2
8	84.3 ± 9.5	11.6 ± 0.7	16.2 ± 0.5	129.8 ± 9.9	89.5 ± 10.3	0.68 ± 0.03	11.3 ± 1.3	74.9 ± 17.6
9	102.5 ± 7.8	11.4 ± 0.4	17.8 ± 0.5	157.4 ± 11.5	122.3 ± 3.2	0.78 ± 0.04	12.4 ± 0.9	39.2 ± 12.8
10	190.3 ± 72.6	14.2 ± 0.9	20.0 ± 0.5	300.2 ± 89.4	280.1 ± 95.4	0.90 ± 0.04	23.7 ± 9.1	46.7 ± 19.0
11	112.5 ± 14.1	10.6 ± 0.6	25.7 ± 0.9	158.3 ± 19.3	151.2 ± 24.2	0.95 ± 0.06	14.0 ± 1.8	47.7 ± 11.5
12	115.3 ± 8.4	10.2 ± 1.0	25.4 ± 1.1	149.5 ± 15.3	146.2 ± 10.2	0.99 ± 0.05	14.7 ± 0.9	46.0 ± 13.2

*Mean ± SE.

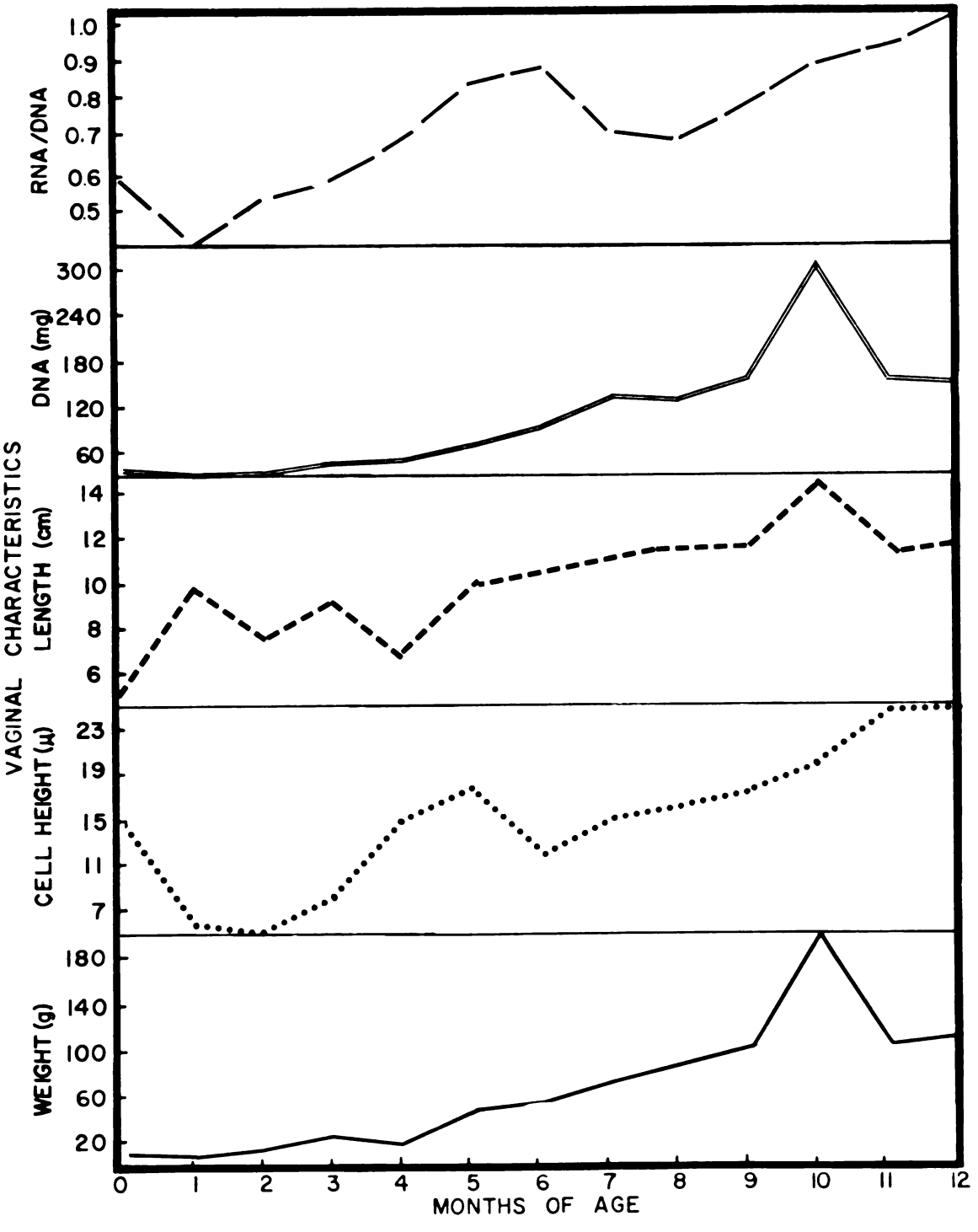


Fig. 10.--Average vaginal weights, lengths, epithelial cell heights, DNA, RNA/DNA ratios per heifer from birth through puberty.

6 months of age but quadratic between 0 and 12 months of age, indicating that vaginal growth was greatest after 6 months of age. Vaginal lipid content, as measured here, varied considerably between age groups ($P > 0.25$) and consequently, was not a satisfactory parameter for evaluating vaginal growth. The average height of the vaginal epithelial cells decreased between birth and 1 month of age, remained relatively low until about 3 months of age, and then began gradually to increase to a maximum at 12 months of age.

An unusually high peak in vaginal weight and DNA occurred at 10 months of age. The observations in this age group were obtained from two animals in mid-cycle and three animals that were close to estrus ($\pm$ 2.5 days). The latter three may have influenced the average inordinately if these criteria varied with stage of estrous cycle. In general, measures of vaginal growth were more variable than similar estimates made on other reproductive tissues. One reason for this was undoubtedly the difficulty encountered in repeatably defining the limits of vaginal tissue at the time of autopsy. This difficulty may have inflated the variability in measuring the growth of the cervix.

The Adrenal

The averages for the various parameters used to evaluate adrenal development from birth through puberty

were summarized in Table 7 and some of these growth measurements were graphed in Figure 11.

Total adrenal weight increased linearly ($P < 0.01$) between birth and 12 months of age. As expected, the increases in total adrenal DNA and total RNA paralleled the increase in adrenal weight and resulted in linear growth through 12 months of age ($P < 0.01$). Therefore, the adrenal did not grow in the same way as the reproductive tissues. The constancy in the RNA/DNA ratio between birth and 12 months suggested that the cells of the prepuberal adrenal were equally capable of synthesizing protein as those cells in the postpuberal animal.

The width of the zona glomerulosa remained relatively constant from birth through 12 months of age ($P > 0.25$). In contrast, the combined width of the zona reticularis-fasciculata decreased between birth and 1 month of age, increased markedly at 2 months of age, remained relatively constant between 2 and 9 months of age, and appeared to increase gradually after 9 months of age.

The changes in total adrenal lipid between the different age groups were not significantly different ($P > 0.25$). Consequently, this biochemical component was not useful in evaluating changes in adrenal growth. Although the changes in total adrenal protein due to age were significant ($P < 0.01$), the changes with advancing age did not fit any of the patterns established for the reproductive tissues or other endocrines.

TABLE 7.--Adrenal development from birth through puberty.

Age (months)	Weight (g)	Width of Zona Glomerulosa (μ)	Combined Width of Zona Reticularis-Fasciculata (μ)	Total DNA (mg)	Total RNA (mg)	RNA/DNA	Total Protein (g)	Total Lipid (g)
0	3.5 ± 0.2*	22.5 ± 0.4	86.6 ± 3.8	20.6 ± 2.1	10.7 ± 1.4	0.49 ± 0.04	2.1 ± 0.2	0.8 ± 0.1
1	3.2 ± 0.2	18.8 ± 0.3	75.4 ± 2.1	20.2 ± 1.3	8.8 ± 1.1	0.43 ± 0.03	2.6 ± 0.2	1.1 ± 0.4
2	4.3 ± 0.5	18.1 ± 0.3	105.2 ± 1.3	26.4 ± 3.1	10.9 ± 0.8	0.43 ± 0.04	1.6 ± 0.1	1.0 ± 0.1
3	4.8 ± 0.3	18.1 ± 0.3	99.4 ± 0.8	27.1 ± 1.9	12.2 ± 1.3	0.45 ± 0.02	2.4 ± 0.5	1.2 ± 0.1
4	7.2 ± 0.5	17.4 ± 0.3	101.2 ± 1.5	40.7 ± 2.4	18.5 ± 1.0	0.57 ± 0.03	5.0 ± 0.8	2.2 ± 0.7
5	7.7 ± 0.4	14.7 ± 0.2	107.3 ± 1.5	37.4 ± 3.2	17.7 ± 3.3	0.46 ± 0.05	3.2 ± 0.7	1.6 ± 0.2
6	7.5 ± 0.3	14.7 ± 0.2	103.9 ± 1.8	38.6 ± 1.7	15.8 ± 1.8	0.41 ± 0.04	2.8 ± 0.7	1.4 ± 0.1
7	9.1 ± 0.6	15.3 ± 0.3	96.7 ± 1.7	46.7 ± 3.3	21.1 ± 1.7	0.45 ± 0.03	2.4 ± 0.4	1.1 ± 0.1
8	9.4 ± 0.6	17.1 ± 0.6	104.3 ± 2.1	51.4 ± 4.4	24.6 ± 2.2	0.48 ± 0.01	4.1 ± 0.9	2.1 ± 0.3
9	9.8 ± 1.0	17.7 ± 0.4	104.9 ± 2.0	52.6 ± 5.5	28.5 ± 2.9	0.54 ± 0.02	3.6 ± 0.8	1.9 ± 0.3
10	12.0 ± 0.7	17.9 ± 0.5	114.2 ± 2.3	61.0 ± 4.6	29.3 ± 3.6	0.48 ± 0.04	4.3 ± 0.7	3.1 ± 1.2
11	12.9 ± 0.4	18.9 ± 0.5	119.8 ± 2.1	62.4 ± 2.3	28.6 ± 1.5	0.46 ± 0.03	4.3 ± 0.3	2.0 ± 0.2
12	14.5 ± 1.2	22.2 ± 0.5	121.7 ± 1.9	68.8 ± 5.7	32.3 ± 5.2	0.46 ± 0.04	8.0 ± 0.7	2.8 ± 0.4

*Mean ± SE.

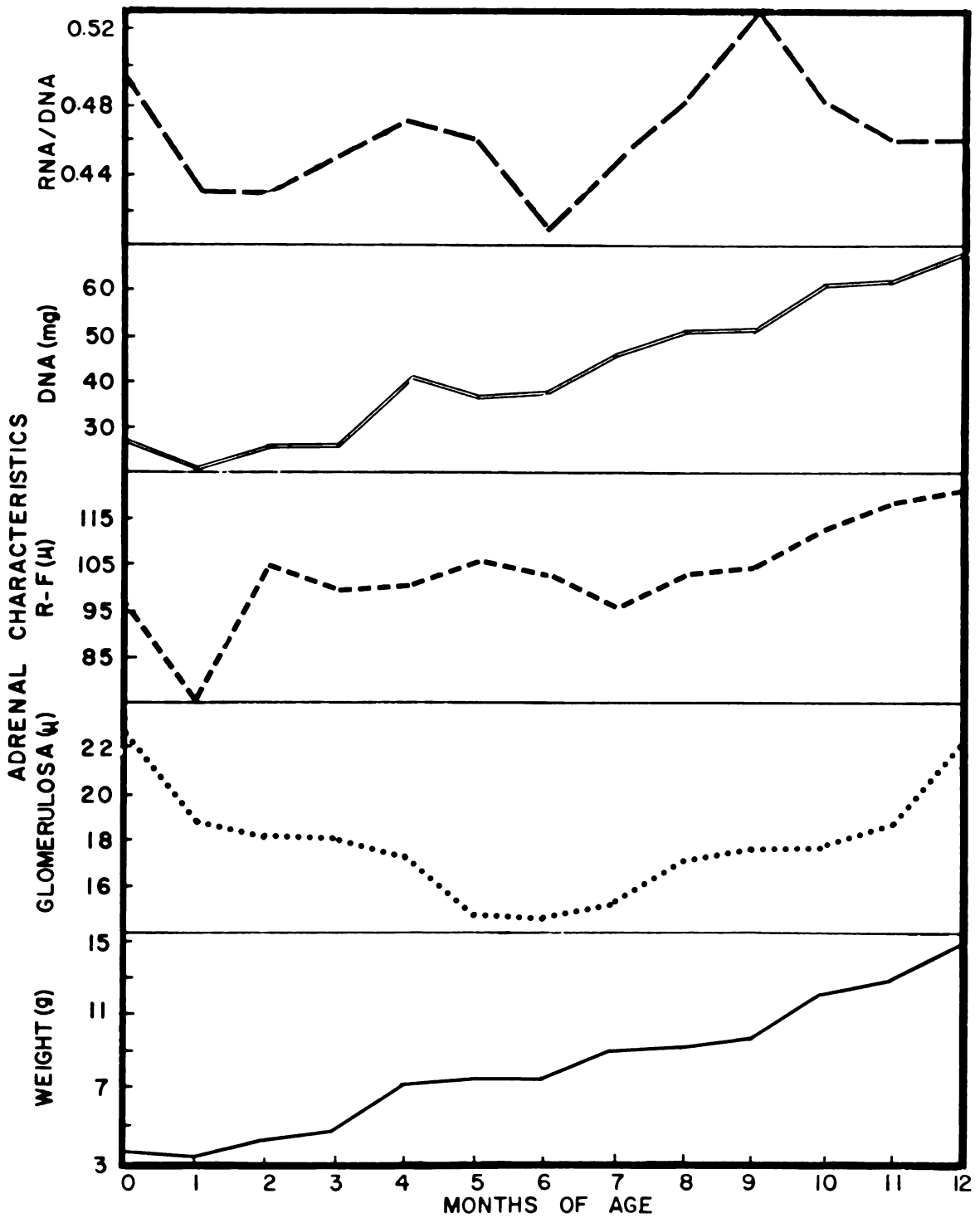


Fig. 11.--Average adrenal weights, widths of zona glomerulosa, combined widths of zona reticularis-fasciculata (R-F), DNA and RNA/DNA ratios per heifer from birth through puberty.

The Thyroid

The average values for the seven different parameters used to evaluate thyroid growth during the year after birth were summarized in Table 8. A portion of these data were illustrated in Figure 12.

The differences among age groups for thyroid weight, total DNA and total RNA were not significant ($P > 0.25$). Thyroid weight, total thyroid DNA and total thyroid RNA varied considerably from birth to 6 months of age, but only slightly from 6 to 12 months of age. The differences in the RNA/DNA ratio due to age were not significant ($P > 0.20$). Total thyroid RNA paralleled total thyroid DNA from birth to 12 months of age. The changes in total protein due to age were significant ($P < 0.01$).

Thyroid acinar cell heights decreased at least 50 per cent between birth and 1 month of age. The height of these epithelial cells remained relatively constant between 1 and 5 months of age. Between 5 and 12 months of age, the height of the cells gradually increased, reaching maximal heights at 11 and 12 months of age.

The Thymus

The average weight, total DNA, total RNA, RNA/DNA ratio, total protein and total lipid content of the thymus gland from birth through 1 year of age was summarized in Table 9, and some of these parameters were illustrated in Figure 13.

TABLE 8.--Thyroid development from birth through puberty.

Age (months)	Weight (g)	Acinar Cell Height (μ)	Total DNA (mg)	Total RNA (mg)	RNA/DNA	Total Protein (g)	Total Lipid (g)
0	8.6 ± 0.7*	12.4 ± 0.2	[illegible]	[illegible]	[illegible]	0.9 ± 0.1	[illegible]
1	15.6 ± 5.3	5.4 ± 0.2	103.6 ± [illegible]	[illegible]	[illegible]	1.5 ± 0.1	[illegible]
2	9.7 ± 0.6	5.7 ± 0.2	[illegible]	[illegible]	[illegible]	0.7 ± 0.1	[illegible]
3	12.0 ± 2.1	4.8 ± 0.2	[illegible]	[illegible]	[illegible]	1.0 ± 0.2	0.5 ± 0.1
4	25.0 ± 6.5	6.9 ± 0.2	129.3 ± [illegible]	[illegible]	[illegible]	1.1 ± 0.1	0.8 ± 0.1
5	16.5 ± 2.1	8.4 ± 0.2	[illegible]	[illegible]	[illegible]	1.3 ± 0.1	1.0 ± 0.1
6	13.3 ± 1.8	11.3 ± 0.2	[illegible]	[illegible]	[illegible]	1.3 ± 0.1	1.0 ± 0.1
7	12.7 ± 1.0	9.9 ± 0.3	[illegible]	[illegible]	[illegible]	1.5 ± 0.1	1.0 ± 0.1
8	13.3 ± 1.1	9.2 ± 0.3	[illegible]	[illegible]	[illegible]	1.8 ± 0.2	1.3 ± 0.1
9	15.3 ± 3.1	10.2 ± 0.2	[illegible]	[illegible]	[illegible]	1.7 ± 0.2	1.2 ± 0.2
10	16.6 ± 1.0	10.6 ± 0.3	[illegible]	[illegible]	[illegible]	2.0 ± 0.2	1.5 ± 0.2
11	15.0 ± 2.6	13.0 ± 0.2	[illegible]	[illegible]	[illegible]	2.3 ± 0.2	1.6 ± 0.1
12	19.1 ± 2.9	12.1 ± 0.3	65.8 ± 15.2	56.7 ± 16.6	0.84 ± 0.06	2.4 ± 0.4	1.9 ± 0.3

*Mean ± SE.

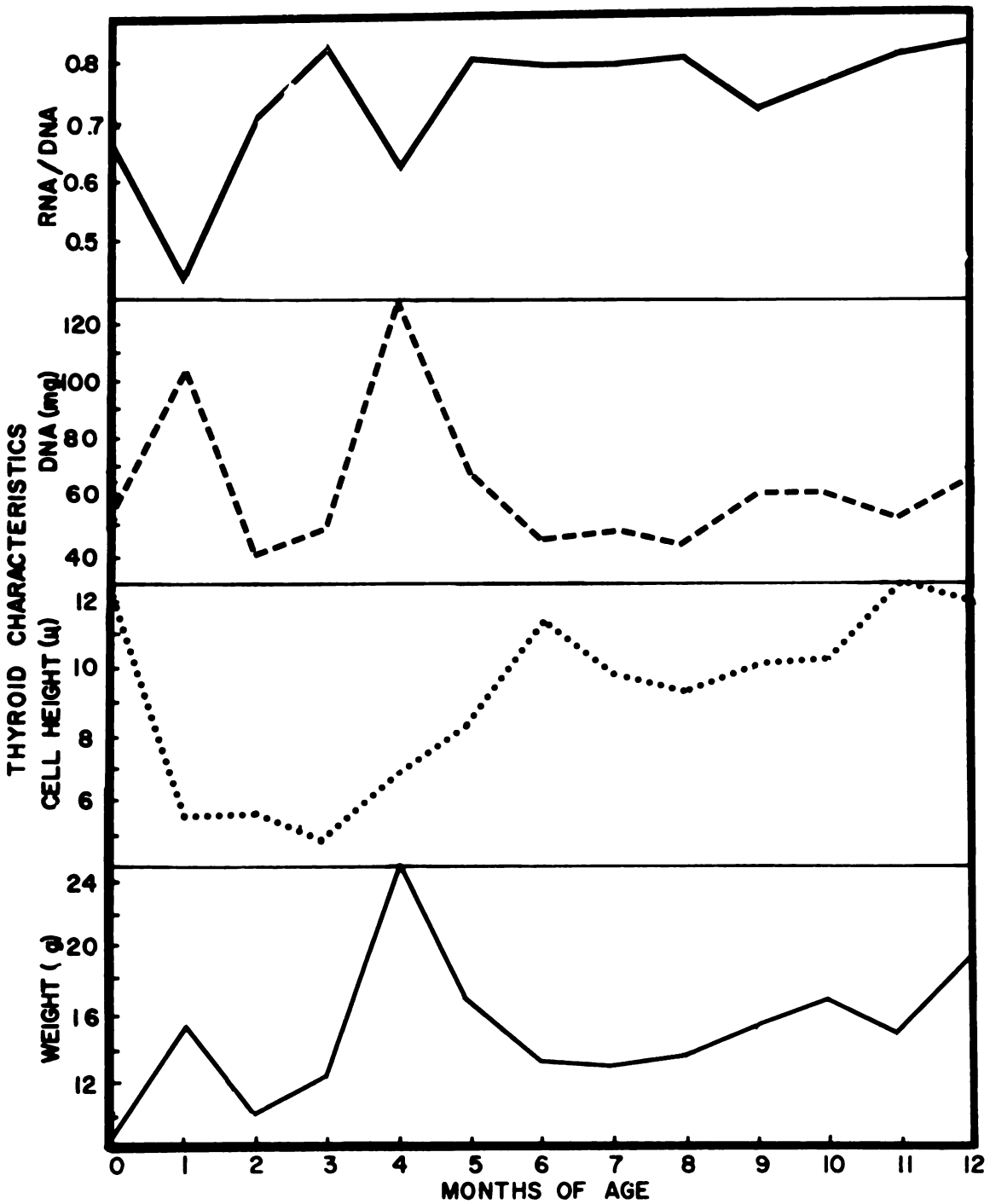


Fig. 12.--Average thyroid weights, acinar cell heights, DNA and RNA/DNA ratio per heifer from birth through puberty.

TABLE 9.--Thymus development from birth through puberty.

Age (months)	Weight (g)	Total DNA (g)	Total RNA (mg)	RNA/DNA ($x10^{-3}$)	Total Protein (g)	Total Lipid (g)
0	109.5 ± 13.4*	2.9 ± 0.5	[illegible] ± 57.3	5 ± 0.5	11.6 ± 1.7	22.8 ± 3.6
1	66.7 ± 9.9	2.1 ± 0.4	[illegible] ± [illegible]	6 ± 0.6	8.2 ± 1.3	14.1 ± 1.3
2	153.3 ± 24.5	3.2 ± 1.1	[illegible] ± [illegible]	7 ± 0.2	17.7 ± 2.9	37.9 ± 4.8
3	291.8 ± 64.1	9.3 ± 1.9	[illegible] ± [illegible]	7 ± 0.4	36.9 ± 10.3	83.2 ± 20.9
4	232.5 ± 24.4	8.2 ± 1.1	[illegible] ± [illegible]	8 ± 0.4	26.8 ± 3.9	50.3 ± 7.5
5	340.8 ± 36.7	12.2 ± 1.5	[illegible] ± [illegible]	8 ± 0.3	43.3 ± 4.4	118.1 ± 15.6
6	377.0 ± 51.4	13.1 ± 1.9	1,[illegible]13.9 ± 149.1	8 ± 0.3	47.5 ± 6.4	119.7 ± 16.3
7	350.2 ± 31.7	12.2 ± 1.5	86[illegible].3 ± [illegible]	7 ± 0.1	40.3 ± 3.4	76.0 ± 5.1
8	325.5 ± 31.4	11.1 ± 1.2	691.6 ± 61.3	6 ± 0.2	36.6 ± 3.4	101.9 ± 46.4
9	366.8 ± 23.4	11.8 ± 0.8	856.2 ± 88.9	7 ± 0.4	44.1 ± 4.1	83.1 ± 9.2
10	553.3 ± 101.8	19.6 ± 3.9	1,414.6 ± 279.5	7 ± 0.2	68.5 ± 13.6	119.3 ± 28.3
11	392.9 ± 79.6	13.8 ± 3.0	1,002.8 ± 221.7	7 ± 0.1	49.1 ± 10.6	139.5 ± 35.2
12	590.6 ± 91.3	19.6 ± 3.8	1,497.6 ± 291.1	8 ± 0.1	76.3 ± 14.3	192.8 ± 28.6

*Mean ± SE.

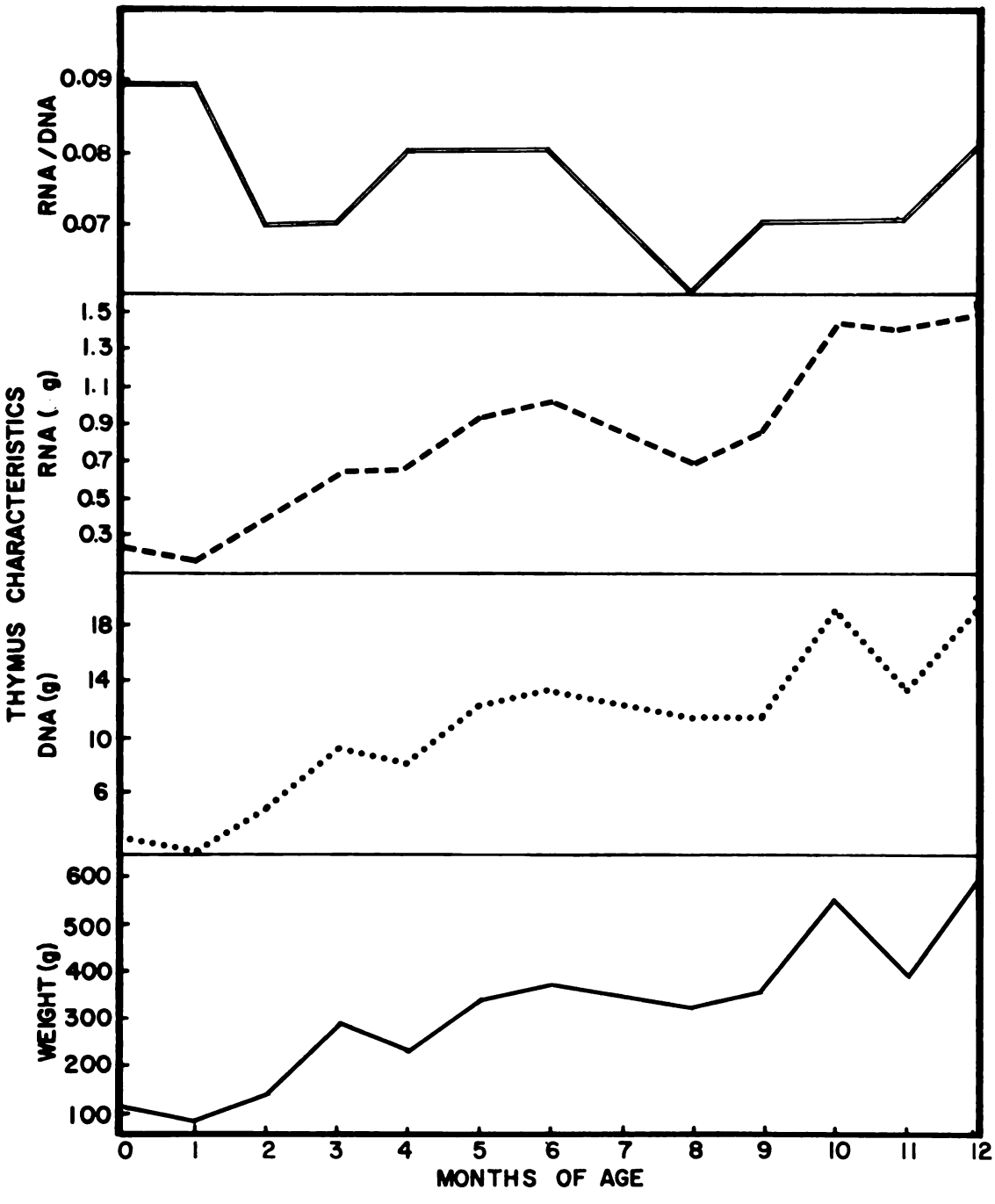


Fig. 13.--Average thymus weights DNA, RNA and RNA/DNA ratios per heifer from birth through puberty.

A five-fold increase in thymus weight was observed between birth and 12 months of age. The increases in thymus weight, total DNA and total RNA also lipid and protein due to age were significant ($P < 0.01$); however, no attempt was made to try to describe the growth response curve because it was too complex. The changes in the RNA/DNA ratio due to age were not significant ($P > 0.35$). A close parallel existed between total thymus DNA and total thymus RNA during the entire experiment.

Discussion

The Ovary and Oviduct

Ovarian weight increased from birth to 12 months of age, and the data appeared to suggest a linear increase in weight with respect to age, but the data were too variable to confirm this trend statistically. Although absolute ovarian weight increased with age, ovarian weight per kilogram of body weight increased from 13.6 mg per kg to 37.6 mg per kg from 0 to 12 months of age, respectively. No drastic changes in ovarian weight were observed at the time of first estrus. This observation was not in accord with that reported by Sorensen et al. (1959) who observed a marked change in ovarian weight at the time of estrus. However, these workers used groups of animals that differed in age at the time of puberty by 112 days, whereas the animals used in the present experiment differed only by 30 days, and this difference may account for the failure

to observe marked increases in ovarian weight at the time of puberty in the present data.

The increases in ovarian weight that occurred during the first 7 months were chiefly influenced by the number and size of follicles. In contrast, corpora lutea largely contributed to increases in ovarian weight after 7 months of age.

Although no histological observations were performed on the ovarian tissues, direct gross morphological follicle counts indicated a peak in the number of small follicles occurred at 4 months of age, and this peak was followed by a gradual decline to normal numbers of small follicles at about 8 months of age. Although Sorensen et al. (1959) did not report direct follicle counts, these researchers noted a very large number of atretic follicles in the ovaries of prepuberal heifers. Consequently, their data suggested that the eventual fate of the small prepuberal follicle is atresia. Additional support for this hypothesis was provided by Rajakoski (1960) who noted that the fate of the majority of small follicles in normal cycling adult cows was also atresia. Unfortunately, at present no data are available to indicate whether or not the small and large prepuberal follicles are capable of producing steroids. The evidence provided by Roberts and Warren (1964) suggested that the fetal bovine ovary was capable of certain steroidal transformations. If ovarian steroids

are produced, it would be attractive to advance the theory that steroids from the ovary influenced the prepuberal growth and maturation of the endocrine and reproductive systems.

The results reported on oviduct length in the present study were in agreement with those reported by Sorensen et al. (1959). The majority of the increase in oviduct weight and length occurred after 6 months of age, illustrating that the rapid phase of growth of this organ occurred during puberal and postpuberal development. Gross inspection of the oviduct suggested that the majority of the increase in the weight of the oviduct was due to increased thickness of the muscle and connective tissue of the lamina propria.

Epithelial cell heights of the oviduct varied only slightly during the various stages of the estrous cycle of the postpuberal heifers, but a precipitous decrease in epithelial cell height occurred between 0 and 1 month of age. These observations suggested that the epithelium of the oviduct of the neonatal calf may be under the influence of maternal and/or placental hormones at birth but came under full influence of endogenous hormones within 2 months.

The presence of extruded nuclei in puberal and postpuberal animals made in this study confirmed the report of Sorensen et al. (1959). These workers

suggested that these nuclei were derived from the luminal epithelial cells of the oviduct because their size, shape and tinctorial properties were similar to those observed in normal positions within the cell. Recently, Asdell (1965) suggested that "extruded nuclei: were globules of cytoplasm containing centers which stained intensely with hematoxylin. These bodies appeared to be secreted protein which were inbibing fluid at the periphery and, thus, stained less intensely in that region than at the center. The exact nature of these bodies remains an enigma.

The Uterus

The several parameters used to characterize development of the uterus between birth and 12 months of age paralleled each other. In general, uterine length and weight, as well as total DNA, total lipid, and total protein contents indicated that uterine growth was relatively slow and linear during the first 6 months of life. However, these same measures indicated that uterine growth was more rapid during the puberal and postpuberal phases. During this time uterine growth was best described by a quadratic curve. The marked acceleration in uterine growth, which began at puberty, was probably due to higher levels of pituitary and gonadal hormones associated with the onset of puberty. The greatest changes in uterine weight, DNA and protein occurred between 6 and 7 months of age, indicating that the stimulus for uterine growth was greatest just before the onset of first estrus.

An indication of the total protein synthetic activity of the uterus was provided by total uterine RNA. Since total uterine RNA paralleled total uterine DNA very closely, it was concluded that uterine protein synthetic activity was considerably elevated just prior to the onset of first estrus. That the RNA/DNA ratio remained relatively constant throughout the different phases of uterine growth suggested that the cells of the prepuberal uterus were just as capable of synthesizing protein as those cells present in the postpuberal uterus. Thus, these data were interpreted to mean that the protein synthetic activity per cell remained constant in the pre- and postpuberal animal. It is proposed that the relationship between total uterine DNA and age represents the normal growth curve of the bovine uterus between birth and 12 months of age. Uterine weight, total uterine protein, and total uterine RNA described similar curves, and these criteria of response were no more variable than total uterine DNA.

Although a linear increase in total uterine DNA and uterine protein was observed between birth and 6 months of age, the height of the uterine endometrial epithelium declined during this same period. In contrast, uterine epithelial cell height paralleled uterine growth between 7 and 12 months of age. Because of the known influence of ovarian steroid hormones on uterine tissues, these data suggested that the steroidal output

of the prepuberal bovine ovary was small relative to its output during postpuberal uterine development. This conclusion, based upon the data on epithelial cell heights, was supported by the absence of superficial and basal endometrial glands in prepuberal heifers. The invaginations into the endometrium which were lined by epithelial cells and comprised the endometrial glands became fully apparent after the onset of puberty.

The Cervix

The estimates of cervical growth provided by measuring changes in cervical weight, total DNA, total RNA and total protein revealed increases which paralleled each other between birth and 12 months of age. In addition, graphs of these different measurements with age indicated that growth was linear between 0 and 6 months of age and that a significant increase in growth occurred after this time resulting in the quadratic response between 0 and 12 months of age. That the RNA/DNA ratio did not differ between age groups suggested that the protein synthetic activity per cell was similar in all age groups.

Since it is known that the epithelial cell height was responsive to estrogen, the findings on the heights of the cervical epithelial cells suggested that steroidal stimulation provided by the ovary during postpuberal development was greater than that provided during prepuberal development. These results agree well with those

provided by Roark and Herman (1950) for mature cows in various physiological states.

Thus, it is proposed that the growth estimates reported here present the normal growth curve of the bovine cervix from birth through puberty. In addition, these data on cervical development appeared to parallel closely similar data presented above for uterine growth from birth through puberty.

The Vagina

The measures used to evaluate vaginal growth between birth and 12 months were in general agreement with each other. Estimates of total DNA, total RNA and total protein all suggested that vaginal growth was linear between birth and 6 months of age and quadratic between 0 and 12 months of age.

Although the vaginal RNA/DNA ratio did not differ ($P > 0.25$) between age groups, trends in these data were evident which suggested two growth waves. An almost identical response in epithelial cell heights coincided with the changes in the RNA/DNA ratio. Unfortunately, any explanation of these growth waves does not conform to the previous patterns described for the development of other reproductive tissues.

Vaginal epithelial cell height, like that of the other reproductive tissues previously described, decreased between birth and 1 month of age lending further support

to the hypothesis that the reproductive tissues of the new-born calf may be under the influence of maternal or placental hormones. Once removed from this hormonal stimulus, the epithelial cell height decreased to levels which were presumably more indicative of the endocrine capacity of the neonatal calf.

The Adrenal

Adrenal growth between birth and 12 months of age was linear ($P < 0.10$), as measured by adrenal weight, total adrenal DNA and total adrenal RNA and was in marked contrast to the quadratic response reported above for the reproductive tissues. These data suggested that the control of adrenal gland growth during the first year of life may be quite different from that of reproductive tissues during this same time.

The width of the zona glomerulosa did not appear to be influenced by the onset of puberty. In contrast, the combined width of the zona reticularis-fasciculata apparently increased markedly after puberty. This observation suggested an increased function in either the zona reticularis or the zona fasciculata after the time of puberty. Because the zona fasciculata has been shown to be associated with sex steroid production (Turner, 1966), it would be attractive to suggest that postpuberal growth of this tissue represented increases in function after the time of puberty. However, the staining procedures

used did not allow differentiation of the zona reticularis from the zona fasciculata and consequently the increase in the width of the combined zones could also be attributed only to the zona reticularis which is thought to be responsible for glucocorticoid production.

The Thyroid

Few changes were observed in thyroid growth between 7 and 12 months of age when growth was assessed by thyroid weight or total thyroidal DNA and RNA. In contrast, marked variance in these growth parameters were observed between 0 and 6 months of age. The large variation associated with thyroid development during the first 6 months after birth may have in part been caused by an iodine deficient diet for these heifers. This suggestion was provoked by the fact that thyroid acinar cell heights were uniformly lower during the first 6 months of life than during the last 6 months of life. Unfortunately, there exist many exceptions to this histologic finding and this evidence alone was not sufficient to establish the functional state of the gland between birth and 6 months of age. The iodine content of the ration was not measured.

The Thymus

According to observations made on rats and humans (Turner, 1966), the thymus gland attained its greatest size at the time of puberty and regressed thereafter. In contrast to humans and rats, the bovine thymus gland

weight, total DNA and total RNA each continued to increase after puberty to 12 months of age. These data indicated that the bovine thymus gland does not involute at the appearance of the first estrus, an age which is the accepted sign of puberty in this species. Whether or not the thymus involutes in the adult bovine could not be determined from these data because the present study continued only about 5 months beyond puberty.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The normal growth and development of the endocrine and reproductive systems of Holstein heifers was studied in 13 groups of five animals which were slaughtered at monthly intervals between 0 and 12 months of age in an effort to detect and quantify some of the changes occurring in these systems at the time of puberty.

Body Growth and Estrus

Body growth, as determined by body weight, conformed to that listed by Morrison (1956). Age at first estrus was 29.7 $\pm$ 1.3 weeks, and the average length of 93 estrous cycles was 20.5 $\pm$ 0.6 days. These estrous cycle data agreed with those provided by Asdell (1965), and suggested that the animals used in this study were overtly normal.

The Pituitary

The most important observation on the pituitary relative to puberty was a progressive decline in LH concentration beginning at the time of puberty. Differences in pituitary FSH concentration due to age were

only significant ($P < 0.05$) during the first 3 months of age. Thereafter, pituitary FSH remained relatively constant although a small decrease was noted at puberty. These changes in pituitary gonadotropin suggested that puberty was initiated by a sudden release of LH and possibly a smaller release of FSH into the blood. Assays of peripheral plasma for LH and FSH are needed for confirmation of this hypothesis. Also, experiments designed to discern the location and function of hypothalamic cyclic and tonic LH release centers appear warranted to determine the relative function of these centers in prepuberal and postpuberal animals. In addition, experiments should be designed to determine whether or not some ovarian steroidal substance may be responsible for blocking the release of pituitary gonadotropins in the prepuberal heifer.

The Reproductive Organs

The growth of both large and small ovarian follicles appeared to lag about 1 month behind the marked decreases in pituitary FSH which occurred at 2 and 3 months of age. After 4 months of age, the number of large and small follicles decreased and subsequently were relatively constant. This observation appeared to coincide with the relatively constant level of pituitary FSH during this period of growth. These observations on the prepuberal ovary, particularly at 3 and 4 months of age and thereafter, suggest that it may possess some steroidogenic

capacity and that experimental comparison of steroidogenesis in prepuberal ovaries with that in postpuberal ovaries may contribute to our knowledge of any role of prepuberal ovaries in inhibiting puberty.

Growth (as measured by weight and DNA) and function (as measured by protein and RNA) of the uterus, cervix and vagina slowly increased in a linear manner until puberty. However, each of these criteria was accelerated beginning at puberty, presumably because of the influence of elevated levels of ovarian steroids. Assays of peripheral blood plasma for steroids could confirm this hypothesis. RNA/DNA ratios for each of the reproductive tissues remained relatively constant throughout the period of growth studied in this thesis. Consequently, the accelerated puberal growth (weight and DNA) and total function (RNA and protein) was largely attributable to hyperplasia rather than to hypertrophy.

In general, the height of the oviduct, uterine, cervical and vaginal epithelia appeared to parallel each other from birth to 12 months of age. Epithelial cell heights of each of these tissues decreased between 0 and 1 month of age, suggesting that the maternal environment may have exerted a stimulating effect. The height of the endometrial epithelial cells of the uterus decreased to 50 per cent of their size at birth by 6 months of age, but the birth height was restored by 12 months of age. These data were interpreted to mean that the

stimulatory steroid environment of the reproductive organs was minimal just before puberty.

Adrenal, Thyroid, and Thymus

In contrast, to the quadratic growth observed for reproductive tissues, the adrenal grew linearly ($P < 0.01$) as determined by increases in its weight, total DNA, total RNA and total protein. Changes in thyroid growth from birth to 7 months of age were difficult to evaluate because of large variations in thyroid weights observed between these ages. However, after 7 months of age thyroid weight, acinar cell height, total DNA, total RNA all increased, and these increases appeared to parallel each other.

Thymus growth was not retarded by the onset of puberty in the present experiment. Rather a five-fold increase was observed in thymus weight, total thymus DNA and total thymus RNA between 6 and 12 months of age. The present results must be extended beyond 12 months of age to determine whether or not the bovine thymus regresses with advancing age beyond 12 months of age. The present data suggested that the thymus appeared to have no primary relationship with the onset of puberty.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allen, E. 1922. The oestrous cycle in the mouse. Am. J. Anat., 30:297.

Altman, P. L. and Dittmer, D. S. 1962. Growth., Federation of Am. Soc. for Exp. Biol., Washington, D. C., p. 145.

Anderson, R. R. and McShan, W. H. 1966. Luteinizing hormone levels in pig, cow and rat blood plasma during the estrous cycle. Endocrinology, 78:976.

Armed Forces Institute of Pathology. 1960. Manual of histologic and special staining techniques. 2nd Ed. McGraw-Hill Co., Inc., New York, N. Y., p. 28.

Armstrong, D. T. and Black, D. L. 1966. Influence of luteinizing hormone on corpus luteum metabolism and progesterone biosynthesis throughout the bovine estrous cycle. Endocrinology, 78:937.

Asdell, S. A. 1965. Patterns of mammalian reproduction. 2nd Ed. Cornell Univ. Press, Ithaca, N. Y., p. 514.

Asdell, S. A., deAlba, J. and Roberts, S. J. 1949. Studies on the estrous cycle in dairy cattle: cycle length, size of corpus luteum, and endometrial changes. Cornell Vet., 39:389.

Barnett, S. A. and Colemen, E. M. 1959. The effect of low environmental temperature on the reproductive cycle of female mice. J. Endocrin., 19:232.

Bergman, A. J. and Turner, C. W. 1942. Gonadotropic hormone in AP of male and female rabbits during growth. Endocrinology, 30:11.

Blivaiss, B. B., Hanson, R. O., Rosenzweig, R. E. and McNiel, K. 1954. Sexual development in female rats treated with cortisone. Proc. Soc. Exp. Biol. Med., 86:678.

Blunn, C. T. 1939. The age of rats at sexual maturity as influenced by their genetic constitution. Anat. Record, 74:199.

Boas, F. 1932. Studies in growth. Human Biology, 4:307.

Bonsma, J. C. 1949. Breeding cattle for increased adeptability to tropical and subtropical environments. J. Agric. Sci., 39:204.

Branez, E. and Roels, H. 1961. Variations in the nuclear deoxyribonucleic acid content in the adrenal cortex of the female white rat during the oestrual cycle. Nature, 192:1043.

Brody, S. 1945. Bioenergetics and growth. Reinhold, New York, N. Y., p. 499.

Brody, S. and Kibler, H. H. 1941. Growth and development LII. Relation between organ weight and body weight in growing and mature animals. Missouri Agr. Exp. Sta. Res. Bull., 328.

Brown-Grant, K., Quinn, D. L. and Zarrow, M. X. 1964. Superovulation in the androgen-treated immature rat. Endocrinology, 74:811.

Byrnes, W. W. and Meyer, R. K. 1951. The inhibition of gonadotrophic hormone secretion by physiological doses of estrogen. Endocrinology, 48:133.

Callantine, M. R., Humphrey, R. R. and Lee, S. 1965. Effect of follicle-stimulating hormone on ovarian nucleic acid content. Endocrinology, 76:332.

Campbell, H. J. 1966. Cyclic activity in the male hypothalamus. Nature, 210:1060.

Campbell, J. L., Holland, M. G. and Flux, D. S. 1949. Thyroid weights of cattle. New Zealand J. of Sci. and Tech., 31:29.

Casida, L. E., Chapman, A. B. and Rupel, I. W. 1935. Study of ovarian development in calves. J. Agr. Res., 50:953.

Christiam, J. J. 1964. Effect of chronic ACTH treatment on maturation of intact female mice. Endocrinology, 74:669.

Clark, H. M. 1935. A prepubertal reversal of sex difference in the gonadotropic hormone content of the pituitary gland of the rat. Anat. Record, 61:175.

Clegg, M. T. and W. F. Ganong. 1959. Environmental factors other than nutrition affecting reproduction, p. 229, Vol. 2. In H. H. Cole and P. T. Cupps (eds.) Reproduction in domestic animals. Academic Press, New York, N. Y.

Cole, H. H. 1930. A study of the mucosa of the genital tract of the cow with special reference to cyclic changes. Am. J. Anat., 46:261.

Creichton, J. A., Aitken, J. N., and Boyne, A. W. 1959. The effect of plane of nutrition during rearing on growth, production and reproduction and health of dairy cattle. I. Growth to 24 months. Anim. Production, 1:145.

Critchlow, V. 1963. The role of light in the neuro-endocrine system, p. 377. In A. V. Nalbandov (ed.), Advances in Neuroendocrinology, Univ. of Illinois Press, Urbana, Illinois.

Cupps, P. T., Laben, R. C. and Mead, S. W. 1959. Histology of pituitary, adrenal and reproductive organs of normal cattle and cattle with lowered reproductive efficiency. Hilgardia, 29:383.

Dale, H. E., Ragsdale, A. D. and Cheng, C. S. 1959. Effect of constant environmental temperatures of 50° and 80° F. on appearance of puberty in beef calves. J. Anim. Sci., 18:1362.

D'Angelo, S. A. 1960. Adenohypophyseal function in the guinea-pig at low environmental temperature. Federation Proc., 19:Suppl. 5, 51.

Defendi, V. and Metcalf, D. 1964. The thymus. The Wistar Inst. Symp. Monograph No. 2, The Wistar Inst. Press, Philadelphia, Pennsylvania.

Desjardins, C., Sinha, Y. N., Hafs, H. D. and Tucker, H. A. 1966. Follicle-stimulating hormone, luteinizing hormone and prolactin potency of bovine pituitaries after various methods of preservation. J. Animal Sci., 25:223.

Donovan, B. T. and van der Werff ten Bosch, J. J. 1965. Physiol of Puberty, E. Arnold Publishers Ltd., London, p. 113.

Eckles, C. H. 1915. The ration and age of calving as factors influencing the growth and dairy qualities of cows. Missouri Agr. Exp. Sta. Bull., 135.

Elias, H. 1949. Growth of the adrenal cortex in domesticated ungulata. Am. J. Vet. Res., 9:173.

Ensminger, M. E. 1959. The Stockmans Handbook, Interstate Publishers, Inc., Danville, Illinois, p. 2.

Erickson, B. H. 1966. Development and senescence of the postnatal bovine ovary. J. Animal Sci., 25:800.

Everett, J. W. 1964. Central neural control of reproductive functions of the adenohypophysis. Physiol. Reviews, 44:373.

Fiske, V. M. 1939. Effects of light and darkness on activity of the pituitary of the rat. Proc. Soc. Exp. Biol. Med., 40:189.

Fiske, V. M. 1941. Effect of light on sexual maturation, estrous cycles, and anterior pituitary of the rat. Endocrinology, 29:187.

Foley, R. C. and Reece, R. P. 1953. Histological studies of the bovine uterus, placenta and corpus luteum. Massachusetts Agr. Exp. Sta. Bull., 468.

Foley, R. C., Black, D. L., Black, W. G., Damon, R. A. and Howe, G. R. 1964. Ovarian and luteal weights in relation to age, breed, and live weight in nonpregnant and pregnant heifers and cows with normal reproductive histories. J. Animal Sci., 23:752.

Foote, W. C., Waldorf, D. P., Chapman, A. B., Self, H. L., Grummer, R. H. and Casida, L. E. 1956. Age at puberty of gilts produced by different systems of mating. J. Animal Sci., 15:959.

Gellert, R. J., Bass, E., Jacobs, C., Smith, R. and Ganong, W. F. 1964. Precocious vaginal opening and cornification in rats following injections of extracts of steer median eminence and pars tuberalis. Endocrinology, 75:861.

Gornall, A. G., Bardawill, C. J. and David, M. M. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177:751.

Gorski, R. A. 1966. Localization and sexual differentiation of the nervous structures which regulate ovulation. J. Reprod. Fertil., Suppl. 1, 67.

Greep, R. O. 1961. Physiology of the anterior hypophysis in relation to reproduction, p. 240. In W. C. Young (ed.), Sex and internal secretions. Williams and Wilkins, Co., Baltimore, Maryland.

Greep, R. O. and Jones, I. C. 1950. Steroid control of pituitary function. Recent Progr. in Hormone Res., 5:197.

Greulich, W. W. 1954. The relationship of skeletal status to the physical growth and development of children, p. 212. In Boell, E. J. (ed.), Dynamics of Growth Processes, Princeton Univ. Press, Princeton, New Jersey.

Hafez, E. S. E. 1952. Studies on the breeding season and reproduction of the ewe. I. The breeding season in different environments. II. The breeding season in one locality. J. Agr. Sci., 42:189.

Haines, C. E., Warnick, A. C. and Wallace, H. D. 1958. The effect of exogenous progesterone and level of feeding on prenatal survival in gilts. J. Animal Sci., 17:879.

Hammond, J. 1925. Reproduction in the rabbit. Oliver and Boyd, Edinburgh, p. 111.

Hammond, J. 1927. Physiology of reproduction of the cow. Cambridge Univ. Press, Cambridge, p. 103.

Hammond, J. and Marshall, F. H. A. 1952. The life cycle, p. 793, Vol. 2. In A. S. Parkes (ed.), Marshall's Physiology of Reproduction. Longmans, Green and Co., London.

Hammond, J. Jr. 1954. Light regulation and hormone secretion. Vitamins and Hormones, 12:157.

Hansel, W. 1959. The estrous cycle of the cow, p. 223, Vol. 1. In H. H. Cole and P. T. Cupps (eds), Reproduction in domestic animals. Academic Press, New York, N. Y.

Hansel, W. 1966. Luteotrophic and luteolytic mechanisms in bovine corpora lutea. J. Reprod. Fertil., Suppl. 1, 33.

Hanson, A. 1956. Influence of rearing intensity on body development and milk production. Proc. Brit. Soc. Animal Prod., p. 51.

Harris, G. W. and Jacobsohn, D. 1952. Functional grafts of the anterior pituitary gland. Proc. Roy. Soc. (B), 139 :263.

Harris, G. W., Reed, M. and Fawcett, C. P. 1966. Hypothalamic releasing factors. British Med. Bull., 22:266.

Hawk, H. W., Tyler, W. J., and Casida, L. E. 1954. Some factors affecting age at puberty in Holstein-Friesian heifers. J. Dairy Sci., 37:252.

Hegyeli, A. McLaughlin, J. A. and Szent-Gyorgyi, A. 1963. On the chemistry of the thymus gland. Proc. Natl. Acad. of Sci., 49:230.

Heim, L. M. 1966. Effect of estradiol on brain maturation: Dose and time response relationships. Endocrinology, 78:1130.

Hertrz, R. and Hisaw, F. L. 1934. Effect of follicle-stimulating and luteinizing pituitary extracts on ovaries of infantile and juvenile rabbits. Am. J. Physiol., 108:1.

Hisaw, F. L. 1947. Development of the Graafian follicle and ovulation. Physiol. Reviews, 27:95.

Hollandbeck, R., Baker, B., Norton, H. W., and Nalbandov, A. V. 1956. Gonadotrophic hormone content of swine pituitary glands in relation to age. J. Animal Sci., 15:418.

Hoogstra, M. J. and Paesi, F. J. A. 1955. A comparison between the FSH and ICSH-contents of the hypophysis of adult and immature rats. Acta. Physiol. Pharm. Neerl., 4:395.

Huseby, R. A., Ball, Z. B. and Visscher, M. B. 1945. Further observations on the influence of simple calorie restriction on mammary cancer incidence and related phenomena in C3H mice. Cancer Res., 5:40.

Jochle, W. 1956. Uber den emflusz des lichtes uf sexual-entwicklung and secualperiodik bei saugern. Endokrinologie, 33:129.

deJongh, S. E. and Paesi, F. J. A. 1958. The I.C.S.H.-concentration in the hypophysis of immature and adult rats. Acta Endocrin., 29:413.

Jost, A. 1958. Embryonic sexual differentiation, p. 15. In H. W. Jones and W. W. Scott, (eds.). Hermaphroditism genital anomalies and related endocrine disorders. The Williams and Wilkins Co., Baltimore, Maryland.

Joubert, D. M. 1954. The influence of winter nutritional depressions on the growth reproduction and production of cattle. J. Agr. Sci., 44:5.

Joubert, D. M. 1962. Sex behaviour of purebred and cross-bred Merino and Blackhead Persian ewes. J. Reprod. Fertil., 3:41.

Kennedy, G. C. 1966. Food intake, energy balance and growth. British Med. Bull., 22:216.

Kennedy, G. C. and Mitra, J. 1963. Body weight and food intake as initiating factors for puberty in the rat. J. Physiol., 166:408.

King, H. D. 1915a. On the weight of the albino rat at birth and the factors that influence it. Anat. Record, 9:213.

King, H. D. 1915b. The growth and variability in the body weight of the albino rat. Anat. Record, 9:751.

Kleiber, M. 1961. The fire of life. John Wiley, New York, N. Y., p. 223.

Lauson, H. D., Golden, J. B. and Sevringhaus, E. L. 1939. The gonadotropic content of the hypophysis throughout the life cycle of the normal female rat. Am. J. Physiol., 125:396.

Lerner, L. J. 1964. Hormone antagonists: Inhibitors of specific activities of estrogen and androgen. Recent Progr. in Hormone Res., 20:435.

Leslie, I. 1955. The nucleic acid content of tissues and cells, p. 1, Vol. 2. In E. Chargaff and J. N. Davidson, (eds.), The Nucleic Acids. Academic Press, New York, N. Y.

Lindsay, R. H. and Cohen, P. P. 1965. Nucleic acid synthesis in normal and goitrous rat thyroid. Endocrinology, 76:737.

Lombard, L., Morgan, B. B. and McNutt, S. H. 1950. The morphology of the oviduct of virgin heifers in relation to the estrous cycle. J. Morph., 86:1.

Long, J. A. and Evans, H. M. 1922. The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif., 6:1.

Luce-Clausen, E. M. and E. F. Brown. 1939. The use of isolated radiation in experiments with the rat. III. Effects of darkness, visible and infra-red radiation on three succeeding generations of rats, (b) reproduction. J. Nutr., 18:551.

Marden, W. G. R. 1951. The hormone control of ovulation in the calf. J. Physiol., 115:22.

Marden, W. G. R. 1952. The hormone control of ovulation in the calf. Endocrinology, 50:456.

Marden, W. G. R. 1953. The hormone control of ovulation in the calf. J. Agr. Sci., 43:25.

Mares, S. E., Zimbelman, R. G. and Casida, L. E. 1962. Variation in progesterone content of the bovine corpus luteum of the estrual cycle. J. Animal Sci., 21:266.

Marion, G. B. and Gier, H. T. 1960. Histological and cytological changes in the bovine vaginal epithelium. J. Animal Sci., 19:1328.

Marshall, F. H. A. 1922. The physiology of reproduction. 2nd ed. Longmans, Green and Co., London, p. 1.

Martin, C. R. 1964. Influence of thymectomy on growth of secondary reproductive structures in rats. Am. J. Physiol., 206:193.

McCance, R. A. 1960. Severe undernutrition in growing and adult animals (1) Production and general effects. Brit. J. Nutr., 14:59.

Mercier, E. and Salisbury, G. W. 1947. Fertility level in artificial breeding associated with season, hours of daylight, and age of cattle. J. Dairy Sci., 30:817.

Mirskaia, L. and Crew, F. A. E. 1930. On the genetic nature of the time of attainment of puberty in the female mouse. Quart. J. Exp. Physiol., 20:299.

Moon, H. D. 1937. Inhibition of somatic growth in castrate rats with pituitary extracts. Proc. Soc. Exp. Biol. Med., 37:36.

Moore, C. 1950. The role of the fetal endocrine glands in development. J. Clin. Endocrin. and Metab., 10:942.

Morrison, F. B. 1956. Feeds and feeding. 22nd ed. Morrison Publishing Co., Clinton, Iowa, p. 680.

Morscher, E., Desaulles, P. A., Schenk, R. 1965. Experimental studies on tensile strength and morphology of the epiphyseal cartilage at puberty. Ann. Paediat., 205:112.

Moss, S., Wrenn, T. R. and Sykes, J. F. 1954. Some histological and histochemical observations of the bovine ovary during the estrous cycle. Anat. Record, 120:409.

Moule, G. R. 1950. Some problems of sheep breeding in semi-arid tropical Queensland. Aust. Vet. J., 26:29.

Mounib, M. S., Ahmed, I. A. and Hamada, M. K. D. 1956. A study of the sexual behaviour of the female Rahmany sheep. Alexandria J. Agr. Res., 4:85. Cited from Animal Breed Abst., 25:1936.

Mulinos, M. G. and Pomerantz, L. 1940. Pseudo-hypophesectomy: a condition resembling hypophectomy produced by malnutrition, J. Nutr., 19:493.

Nicander, L. 1952. Histological and histochemical studies on the adrenal cortex of domestic and laboratory animals. Acta. Anat., Suppl. 16, 14:1.

Ogle, C. 1934. Adaptation of sexual activity to environmental stimulation. Am. J. Physiol., 107:628.

Parlow, A. F. 1964. Differential action of small doses of estradiol on gonadotrophins in the rat. Endocrinology, 75:1.

Parlow, A. F., Anderson, L. L. and Melampy, R. M. 1964. Pituitary follicle-stimulating hormone and luteinizing hormone concentrations in relation to reproductive stages of the pig. Endocrinology, 75 365.

Phillips, J. B. and Piip, L. K. 1957. A cytochemical study of pituitary glands of 1 to 15-day-old rats utilizing the aldehyde-fuchsin staining technique. Anat. Record, 129:415.

Pomeroy, R. W. 1960. Infertility and neonatal mortality in the sow. IV. Further observations and conclusions. J. Agric. Sci., 54:57.

Rakha, A. M. and Robertson, H. A. 1965. Changes in levels of follicle stimulating hormone and luteinizing hormone in the bovine pituitary gland at ovulation. J. Endocrin., 31:245.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta Endocrin., 34:Suppl. 52.

Ramirez, V. D. and McCann, S. M. 1963. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology, 72:452.

Ramirez, V. D. and Sawyer, C. H. 1965. Advancement of puberty in the female rat by estrogen. Endocrinology, 76:1158.

Ramirez, V. D. and Sawyer, C. H. 1966. Changes in hypothalamic luteinizing hormone releasing factor (LHRF) in the female rat during puberty. Endocrinology, 78:958.

Reid, J. T., Loosli, J. K., Trimberger, G. W., Turk, K. L., Asdell, S. A. and Smith, S. E. 1964. Causes and prevention of reproductive failures in dairy cattle. IV. Plane of nutrition during early life on growth, reproduction, production, health and longevity of Holstein cows. Cornell Univ. Agr. Exp. Sta. Bull. 987.

Reynolds, S. R. M. 1949. Physiology of the uterus. 2nd ed. Harper and Bros., New York, N. Y., p. 183.

Roark, D. B. and Herman, H. A. 1950. Physiological and histological phenomena of the bovine estrual cycle with special reference to vaginal-cervical secretions. Missouri Agr. Exp. Sta. Res. Bull. 445.

Roberts, J. D. and Warren, J. C. 1964. Steroid biosynthesis in the fetal ovary. Endocrinology, 74:846.

Robertson, G. L., Grummer, R. H., Casida, L. E., Chapman, A. B. 1951. Age at puberty and related phenomena in outbred Chester White and Poland China gilts. J. Animal Sci., 10:647.

Robertson, H. A. and Hutchinson, J. S. M. 1962. The levels of FSH and LH in the pituitary of the ewe in relation to follicular growth and ovulation. J. Endocrin., 24:143.

Robertson, H. A. and Rakha, A. M. 1966. The sequence, time and duration, of the release of follicle-stimulating hormone and luteinizing hormone in relation to oestrus and to ovulation in the sheep. J. Endocrin., 35:177.

Salisbury, G. W. and VanDemark, N. L. 1961. Physiology of reproduction and artificial insemination of cattle. W. H. Freeman and Co., San Francisco, California, p. 23.

Sawyer, C. H. 1964. Control of secretion of gonadotropins, p. 113. In H. H. Cole, (ed.), Gonadotropins. W. H. Freeman and Co., San Francisco, California.

Schmidt, G. and Thannhauser, S. J. 1945. A method for the determination of desorybibonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. J. Biol. Chem., 161:83.

Sinha, Y. N. and Tucker, H. A. 1966. Mammary gland growth of rats between 10 and 100 days of age. Am. J. Physiol., 210:601.

Siperstein, E., Nichols, C. W., Griesbach, W. E., Chaikoff, I. L. 1954. Cytological changes in the rat anterior pituitary from birth to maturity. Anat. Record, 118:593.

Smith, P. E. and Engle, E. T. 1927. Experimental evidence regarding the role of the anterior pituitary in development and regulation of the genital system. Am. J. Anat. 40:159.

Squiers, C. D., Dickerson, G. E. and Mayer, D. T. 1952. Influence of inbreeding, age and growth rate of sows on sexual maturity, rate of ovulation fertilization and embryonic survival. Missouri Agr. Exp. Sta. Res. Bull. 494.

Sorensen, A. M., Hansel, W., Hough, W. H., Armstrong, D. T., McEntee, K. and Bratton, R. W. 1959. Causes and prevention of reproductive failures in dairy cattle. I. Influence of underfeeding and overfeeding on growth and development of Holstein heifers. Cornell Univ. Agr. Exp. Sta. Bull. 936.

Stockard, C. R. and Papanicolaou, G. N. 1917. The existance of a typical oestrous cycle in the guinea pig with a study of its histological and physiological changes. Am. J. Anat., 22:225.

Sweetman, W. J. 1950. Artificial breeding in Alaska and the effect of extra light during the short winter days. J. Dairy Sci., 33:391.

Swett, W. W., Mathews, C. A. and Fohrman, M. H. 1955. Weight of thyroid in dairy cows from different goegraphical areas within the United States. Dairy Hus. Res. Branch, U. S. D. A. Tech. Bull. 1123.

Tanabe, T. Y. and Almquist, J. O. 1960. The nature of subfertility in dairy heifers. I. Estrus and estrual cycles. Pennsylvania Agr. Exp. Sta. Bull. 672.

Tanner, J. M. 1962. Growth at adolesence. 2nd ed. Oxford: Blackwell Sci. Publ., London, p. 28.

Tucker, H. A. 1964. Influence of number of suckling young on nucleic acid content of lactating rat mammary gland. Proc. Soc. Exp. Biol. Med., 116: 218.

Turner, C. D. 1966. General endocrinology. 4th ed. W. B. Saunders Co., Philadelphia, Pennsylvania, p. 342.

Truscott, B. L. 1944. Physiological factors in hypophyseal-gonadal interaction. I. Light and the follicular mechanism of the rat. J. Exptl. Zool., 95:291.

Velardo, J. T. 1959. The Uterus. Annals N. Y. Acad. Sci., 75:385.

Warnick, A. C., Wiggins, C. L., Casida, L. E., Grummer, R. H. and Chapman, A. B. 1951. Variation in puberty phenomena in inbred gilts. J. Animal Sci., 10:479.

Weber, A. F., McNutt, S. H., and Morgan, B. B. 1950. Structure and arrangement of zona glomerulosa cells in the bovine adrenal. J. Morph., 87:393.

Wolfe, J. M. 1934. The normal level of the various cell types in the anterior pituitaries of mature and immature rats and further observations on cyclic histologic variations. Anat. Record, 61:321.

Wolfe, J. M. and Cleveland, R. 1931. Comparison of the capacity of anterior hypophyseal tissue of mature and immature female rabbits to induce ovulation. Anat. Record, 51:213.

Zuckerman, S. 1962. The Ovary. Vols. 1 and 2. Academic Press, New York, N. Y.

APPENDICES

APPENDIX TABLE 1.--Body weight, anterior pituitary weight, and pituitary concentration of FSH and LH from birth through puberty.

Age Group (months)	Animal Number	Body Weight[a]	Anterior[b] Pituitary Weight	FSH Concentration		LH Concentration	
				Potency ± SE[c]	95% C.I.[d]	Potency ± SE[c]	95% C.I.[d]
0	71	39.0	0.69	1.39 ± 0.47	0.43 - 3.81	5.03 ± 2.34	1.56 - 10.53
	72	37.6	0.28	2.11 ± 0.71	0.48 - 3.18	2.68 ± 0.71	1.10 - 4.44
	73	30.4	0.44	1.34 ± 0.33	0.68 - 1.84	0.84 ± 0.24	0.33 - 1.66
	74	37.2	0.36	1.73 ± 0.33	0.97 - 2.44	1.11 ± 0.28	0.61 - 1.79
	75	33.4	0.52	1.47 ± 0.34	0.77 - 2.13	2.65 ± 0.84	1.36 - 4.11
	Avg	36.7 ± 1.6	0.44 ± 0.06	1.67 ± 0.13[e]	0.46 - 3.81	2.44 ± 0.74[e]	1.10 - 6.31
1	41	51.2	0.49	2.55 ± 0.57	1.40 - 3.68	0.30 ± 0.09	0.15 - 0.52
	44	46.6	0.41	4.15 ± 0.64	3.78 - 5.52	2.02 ± 0.44	1.37 - 3.11
	49	52.8	0.66	1.50 ± 0.30	0.85 - 2.14	4.81 ± 1.14	2.78 - 8.56
	58	46.1	0.48	2.63 ± 0.48	1.46 - 3.80	1.26 ± 0.33	0.63 - 2.23
	69	44.7	0.42	2.59 ± 0.47	1.44 - 3.75	1.98 ± 0.86	0.94 - 3.74
	Avg	48.3 ± 1.6	0.48 ± 0.04	2.68 ± 0.41[e]	1.59 - 3.78	2.07 ± 0.75[e]	1.15 - 3.63
2	47	70.0	0.72	0.89 ± 0.23	0.43 - 1.35	7.99 ± 1.88	4.60 - 14.99
	56	76.2	0.65	1.24 ± 0.31	0.61 - 1.87	6.33 ± 1.72	3.38 - 12.86
	60	68.9	0.80	0.93 ± 0.23	0.46 - 1.40	4.86 ± 1.02	3.01 - 8.00
	61	66.1	0.65	1.15 ± 0.25	0.65 - 1.66	4.31 ± 0.98	2.51 - 7.50
	64	68.2	0.50	1.09 ± 0.94	0.77 - 2.96	5.48 ± 0.74	3.04 - 8.49
	Avg	69.9 ± 1.7	0.66 ± 0.05	1.06 ± 0.06[e]	0.58 - 1.85	5.79 ± 0.70[e]	3.31 - 10.37

3	37	92.3	0.85	0.77 ± 0.[illegible]	0.34 - 1.[illegible]	4.36 ± 0.[illegible]	1.69 - 7.[illegible]
	38	92.4	0.98	1.39 ± 0.[illegible]	0.76 - 1.98	5.69 ± 1.18	3.04 - 9.71
	46	82.4	0.76	0.63 ± 0.[illegible]	0.35 - 1.[illegible]	7.38 ± 1.[illegible]	4.[illegible] - 15.[illegible]
	50	90.8	0.80	0.[illegible] ± 0.[illegible]	0.89 - 0.[illegible]	6.49 ± 1.48	3.93 - 11.88
	57	107.8	0.84	0.[illegible] ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	11.[illegible] - 44.[illegible]
	Avg	91.6 ± 4.4	0.85 ± 0.04	[illegible].[illegible] ± [illegible].[illegible][a]	0.39 - 1.37	[illegible].[illegible] ± [illegible].[illegible][b]	[illegible].[illegible] - 18.[illegible]
4	43	82.4	0.68	2.[illegible] ± 0.[illegible]	[illegible].[illegible] - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	1.[illegible] - [illegible].[illegible]
	48	110.5	1.29	0.[illegible] ± 0.[illegible]	0.44 - 1.[illegible]	[illegible].[illegible] ± 1.[illegible]	[illegible].[illegible] - [illegible].[illegible]
	49	109.4	0.80	0.[illegible] ± 0.[illegible]	0.32 - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	1.[illegible] - [illegible].[illegible]
	59	109.3	1.08	0.[illegible] ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] - [illegible].[illegible]
	66	98.1	0.[illegible]	1.[illegible] ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	1.[illegible] - [illegible].[illegible]
	Avg	101.9 ± 5.4	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible][a]	0.48 - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible][b]	[illegible].[illegible] - [illegible].[illegible]
5	36	117.6	0.89	1.12 ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± 1.[illegible]	3.[illegible] - 11.[illegible]
	40	133.2	0.86	0.[illegible] ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± 1.[illegible]	[illegible].[illegible] - [illegible].[illegible]
	62	151.8	1.17	0.[illegible] ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] - [illegible].[illegible]
	68	157.8	0.[illegible]	0.[illegible] ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] - [illegible].[illegible]
	70	156.0	1.27	1.[illegible] ± 0.[illegible]	0.[illegible] - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] - [illegible].[illegible]
	Avg	143.3 ± 7.6	1.02 ± 0.08	[illegible].[illegible] ± [illegible].[illegible][a]	0.48 - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible][b]	[illegible].[illegible] - [illegible].[illegible]
6	42	189.1	1.45	0.[illegible] ± 0.[illegible]	0.28 - 1.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] - [illegible].[illegible]
	51	176.6	1.03	0.77 ± 0.[illegible]	0.36 - 1.17	3.09 ± 0.[illegible]	1.99 - 4.73
	52	174.4	1.54	0.[illegible] ± 0.[illegible]	0.33 - 1.[illegible]	3.[illegible] ± 1.01	1.[illegible] - 7.13
	55	171.3	1.00	0.[illegible] ± 0.31	0.26 - 1.[illegible]	2.97 ± 0.81	1.49 - 5.67
	67	180.9	1.08	1.[illegible] ± 0.[illegible]	0.91 - 2.[illegible]	4.14 ± 0.79	2.[illegible] - 6.48
	Avg	178.5 ± 3.1	1.22 ± 0.04	0.[illegible] ± 0.17[b]	0.35 - 1.44	4.[illegible] ± 1.01[b]	2.[illegible] - 7.01
7	11	188.0	1.22	0.[illegible] ± 0.[illegible]	0.19 - 0.[illegible]	8.[illegible] ± 0.21	5.[illegible] - 16.26
	12	176.3	1.12	1.22 ± 0.44	0.39 - 2.[illegible]	22.56 ± 0.14	5.90 - 129.10
	15	186.2	1.29	1.43 ± 0.[illegible]	0.45 - 2.41	3.01 ± 0.58	1.89 - 4.66
	16	205.9	1.56	0.83 ± 0.22	0.40 - 1.27	4.19 ± 1.27	1.96 - 9.03
	29	177.6	1.09	0.47 ± 0.[illegible]	0.10 - 0.84	12.68 ± 4.22	6.06 - 33.46
	Avg	186.8 ± 5.3	1.26 ± 0.08	0.92 ± 0.[illegible][b]	0.31 - 1.53	10.19 ± 3.53[b]	4.16 - 38.50

APPENDIX TABLE 1.--(Continued)

Age Group (months)	Animal Number	Body Weight[a]	Anterior Pituitary Weight[b]	FSH Concentration		LH Concentration	
				Potency ± SE[c]	95% C.I.[d]	Potency ± SE[c]	95% C.I.[d]
8	4	314.7	1.28	0.79 ± [illegible]	0.35 - 1.20	13.83 ± 1.89	10.57 - 18.60
	5	203.3	1.20	1.07 ± 0.26	0.62 - 1.67	3.44 ± 0.94	1.75 - 6.62
	10	210.6	1.75	0.95 ± 0.22	0.51 - 1.41	4.99 ± 0.93	3.29 - 6.43
	27	211.0	1.32	1.24 ± 0.28	0.68 - 1.79	6.14 ± 0.13	0.38 - 10.11
	28	197.9	1.30	0.83 ± 0.23	0.43 - 1.33	7.84 ± 1.67	4.91 - 15.19
	Avg	207.3 ± 3.1	1.37 ± 0.10	1.01 ± 0.09[e]	0.62 - 1.48	7.25 ± 1.78[e]	4.34 - 11.39
9	3	227.2	1.65	0.98 ± 0.24	0.49 - 1.46	4.73 ± 2.07	1.25 - 20.14
	17	234.3	1.44	0.57 ± 0.19	0.19 - 0.94	6.27 ± 1.44	3.74 - 10.98
	19	202.3	1.26	1.80 ± 0.61	0.58 - 3.03	1.38 ± 0.75	0.07 - 5.11
	20	219.2	1.57	0.58 ± 0.19	0.20 - 0.95	14.72 ± 7.47	4.56 - 134.41
	24	259.7	1.48	0.73 ± 0.23	0.26 - 1.19	0.97 ± 0.21	0.57 - 1.52
	Avg	228.5 ± 9.6	1.48 ± 0.07	0.93 ± 0.23[e]	0.34 - 1.51	5.61 ± 2.49[e]	2.04 - 34.43
10	2	261.2	1.17	0.64 ± 0.20	0.24 - 1.05	10.17 ± 2.24	6.33 - 17.58
	8	302.3	1.52	0.75 ± 0.21	0.34 - 1.16	1.92 ± 0.89	0.32 - 6.35
	22	311.4	1.53	0.73 ± 0.21	0.31 - 1.15	0.48 ± 0.11	0.26 - 0.79
	30	258.8	1.42	1.37 ± 0.47	0.42 - 2.31	0.21 ± 0.35	1.01 - 2.66
	33	279.5	1.27	1.21 ± 0.42	0.36 - 2.05	1.60 ± 0.85	0.10 - 6.04
	Avg	282.6 ± 10.6	1.40 ± 0.08	0.94 ± 0.15[e]	0.33 - 1.54	2.88 ± 1.85[e]	1.60 - 6.68
11	6	276.8	1.20	0.74 ± 0.20	0.34 - 1.13	4.50 ± 0.10	2.66 - 7.69
	7	288.3	1.47	0.65 ± 0.19	0.26 - 1.03	4.10 ± 1.09	2.13 - 7.91
	25	284.0	1.49	0.98 ± 0.24	0.49 - 1.47	5.09 ± 0.72	2.93 - 9.30
	31	295.8	1.13	0.70 ± 0.20	0.30 - 1.10	2.81 ± 0.54	1.80 - 4.30
	34	288.6	1.72	0.97 ± 0.22	0.53 - 1.41	8.92 ± 2.20	5.24 - 17.23
	Avg	286.7 ± 3.1	1.40 ± 0.11	0.81 ± 0.07[e]	0.38 - 1.23	5.08 ± 1.13[e]	2.95 - 9.29

12	13	291.7	1.29	0.71 ± 1.20	0.38 - 1.18	7.10 ± 3.03	2.00 - 37.00
	21	351.6	1.36	0.93 ± 0.37	0.78 - 2.64	2.48 ± 0.99	1.38 - 4.20
	23	329.2	1.52	0.86 ± 1.11	0.44 - 1.99	3.91 ± 2.98	0.11 - 17.56
	32	349.2	1.37	0.71 ± 0.00	0.38 - 1.11	10.20 ± 3.34	4.98 - 35.34
	35	386.5	1.44	1.04 ± 0.14	0.87 - 1.44	4.19 ± 1.11	0.10 - 9.08
	Avg	329.7 ± 11.8	1.40 ± 0.06	0.77 ± 1.07[e]	0.44 - 1.48	6.77 ± 1.9	1.34 - 19.76

[a]Body weight in kg.

[b]Fresh weight of anterior pituitary tissue in g.

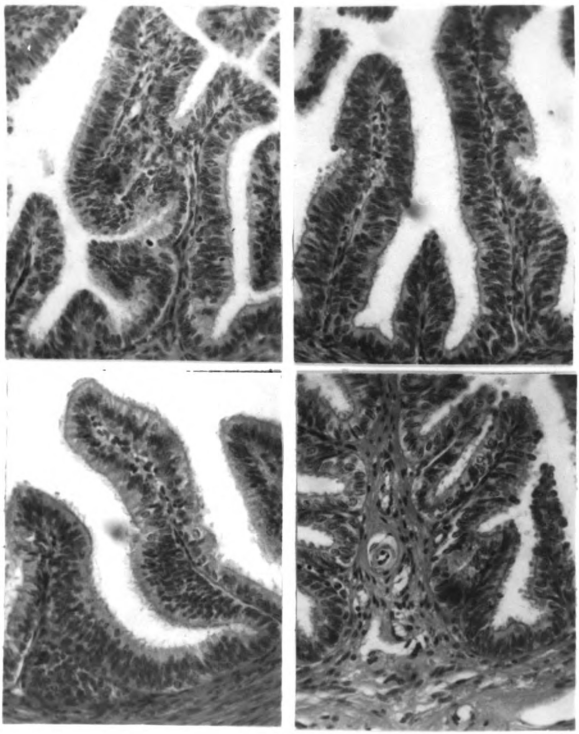
[c]Potency ± standard error of potency expressed as µg - equivalents of either NIH-FSH-S2 or NIH-LH-S1 per mg of fresh anterior pituitary tissue.

[d]95% conficence interval of potency estimate.

[e]Average ± standard error of potency estimate.

APPENDIX FIGURE 1

Photomicrographs (x340) of oviducts from 0 (upper left), 1 (upper right), 5 (lower left) and 12 (lower right) month old heifers showing changes in the luminal epithelial cell development from birth through 12 months of age. After puberty, the mucosa was thrown into a greater number of secondary and tertiary folds. Ciliated epithelia were present in all age groups. Large numbers of extruded nuclei were observed after the onset of puberty (see lower right).



APPENDIX FIGURE 2

Photomicrographs (x340) of uteri from 0 (upper left), 1 (upper right), 5 (lower left), and 12 (lower right) month old heifers showing endometrial changes from birth through 12 months of age. Uterine glands were absent in prepuberal animals; a few developing uterine glands could be observed at 5 months of age and well developed uterine glands were noted by 12 months of age. A large uterine gland was sectioned (lower right) through the wall of the gland neck and through the lumen of the basal portion of the gland.