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Horizons of Early Uterine Growth in the Rat

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HORIZONS OF EARLY UTERINE GROWTH IN THE RAT

by

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LIFE

Barbara Anne Kasprow was born in Hartford, Connecticut on April 23, 1936, the daughter of Mr. and Mrs. Stephen G. Kasprow.

She attended local elementary and high schools in New Britain, Connecticut, and was graduated from Albertus Magnus College in New Haven, Connecticut, where she received the A.B. degree cum laude in June, 1958. In the fall of 1958, she entered the Yale University Graduate School, and pursued numerous courses while working first as a laboratory assistant and subsequently as a U.S.P.H. Service Graduate Training Scholar. In 1961 she accepted a position as Research Associate in Anatomy, and in 1962 was appointed Senior Research Associate and Administrative Assistant at the Institute For The Study Of Human Reproduction, the first such institute of its kind in the free world. She remained at this position until the academic year 1967-1968, at which time she came to Stritch School of Medicine of Loyola University of Chicago as Senior Research Associate in Anatomy, and enrolled in the Graduate School of Loyola University, in the Department of Anatomy. Here she continued her scientific investigations for the doctorate of philosophy degree in anatomy.

During the period 1960-1968, Miss Kasprow attended and participated in several international congresses in New York, The Netherlands, Italy, Germany and Washington, D.C. She has also attended and participated in numerous annual meetings of the American Association of Anatomists, American Society of Zoologists (Section on Comparative Endocrinology), American Physiological Society, and the American Association for the Advancement of Science.

Miss Kasprow is a member of the American Association for the Advancement of Science, American Society of Zoologists, New York Academy of Sciences and Sigma Xi.

Actively engaged in research since 1958, Miss Kasprow is the co-author of 13 abstracts, the majority of which were presented as scientific papers at national meetings and international congresses (anatomy, endocrinology and physiology). Her most current contribution (number 14) is an abstract stemming from her original research conducted in 1967-1969, based on the early uterotropic responses of young adult, albino rats to estradiol-17beta.

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I. INTRODUCTION

There are many references concerning uterine growth in the literature of the past half century, but among them one cannot find the comprehensive details of the sequential, morphological changes which occur in the uterus as it passes from immaturity through maturity. Physiological and biochemical studies of the uterus of the intact and surgically and hormonally manipulated adult animal and the immature animal are comparatively numerous; however, in these two areas of study also the details of the sequential changes incident to approaching maturity are lacking in the literature. It therefore seemed pertinent to initiate studies which could provide detailed information concerning the nature of uterine growth under physiological conditions during the period of transition from immaturity to maturity. The major treatises which have been written on the subject of the uterus have been most helpful to this study. The classical monograph on the subject of the physiology of the uterus is clearly the thoroughly documented account presented by S.R.M. Reynolds (1949, rev. 1965). A conference monograph, highlighting comparative and biological aspects of uterine growth, edited by J.T. Velardo (1959a), supplements in a helpful way Reynold's scholarly treatise. Recently, an additional compilation of studies on the uterus has been edited by Wynn (1967). Much of the available information on how the uterus can grow under various hormonal manipulations and in mature adult mammals is encompassed in these three

works; yet, the information as to how early growth occurs stepwise in nature is lacking.

Some pertinent questions could be asked concerning the possible courses of events leading to uterine maturity: what documentable, morphological, transitional steps might precede the patterns seen in the established estrous cycle in the mature rat; might the mode of growth be linear and gradual, or occur in spurts, or as a function of time or total body growth; do the endometrium and myometrium exhibit similar developmental growth characteristics with approaching maturity; are there distinctively recognizable stages in the growth process with approaching maturity; and, for the sake of quantitative understanding of the maturation process, can one experimentally duplicate the natural phenomena observed in ovariectomized subjects of different ages given known amounts of either estradiol-17β, progesterone, or combinations of the two ovarian sex steroid hormones.

Most of the uterine studies heretofore pursued concerning prepubertal rats have either been of very small numbers of animals and for one age period (and some of animals of unknown status as regards the maturation history for the strain under study) (Williams, 1948; Pritchard, 1949; Augustin et al., 1954) or have had their emphasis placed on very young ages of animals rather than on those ages nearer to maturity as judged by appearance of vaginal patency (Price and Ortiz, 1944; Price and Harvey, 1947). Ovarian development has been studied from birth through puberty in a strain of rats which matured

at approximately 60 days of age (Sneider, 1940) and this study afforded additional encouragement that the careful study of the uterus during these periods should be quite revealing in helping to explain the biology of reproduction in the rat.

Certain other uterine growth studies have pioneered the way for the many investigations which have followed them, as well as for the present study. E. Allen (1928) demonstrated that the immature animal is responsive to hormones normally found only in the adult; Allen et al. (1937) and Reynolds (1938) pioneered studies of myometrial growth responses. Also among cornerstone work in the field of uterine growth are the contributions of the early schools of F.L. Hisaw, C. Hartman, G.W. Corner and H. McL. Evans (cf. Velardo, 1958), each of which has given particular impetus to research in this area.

The need for the study of the transitional steps from uterine immaturity to maturity obviously existed. The value of extension of the study into the realm of experimental quantification was equally obvious. Therefore, these studies were undertaken, and are the subject of this dissertation.

A significant emphasis of this investigation has been to analyze the sequential morphological changes of endometrial and myometrial aspects of uterine growth in detail. Intact, albino rats, of immature, young adult and adult ages were studied with a minimum of physical handling and without the bodily introduction of any foreign material or chemical, so as not to disturb any of the normal, vital functions

of the growing and maturing rat. By these methods, it has been possible to study natural growth phenomena extensively without any imposed artificial or pharmacological conditions. This report is the first such attempt to record by short time intervals the normal horizons of uterine growth. The benefits to be gained by such a study are quite evident: a perfect, natural control animal whose reproductive tract history has been learned in detail is being compared with an experimentally manipulated animal of the same strain and natural history. Such rigid base-line controls add infinitely to the meaning and interpretability of the experimental data. Such a control can make a more significant contribution than the often-read conclusions "greater than the ovariectomized controls." The last section of these studies concerns such a comparison of the experimentally manipulated animal with the intact, non-disturbed animal of the same strain. A method of administering ovarian sex steroid hormones in a programmed, physiological protocol, known as the experimental model system (Velardo, 1964), was chosen as the means of elucidating the sequelae of the early horizons of uterine growth in a quantitative way, after uterine stimulation with known amounts of ovarian sex steroid hormone.

II. REVIEW OF SIGNIFICANT LITERATURE AND ANALYSIS

A review of the historical surveys of the literature pertaining to the cyclical phenomena of the reproductive tract and the general subject of biologic actions of sex steroid hormones provides the following pertinent information: 1) the ovaries, the female sex organs, are responsible in the main for the cyclical changes which occur in the accessory reproductive organs, especially the uterus and the vagina; specifically, the major phases of the ovarian cycle can be correlated with the cycle of changes that takes place in the accessory organs; 2) surgical removal of the sex organs, termed ovariectomy, is followed by cessation of cyclical activity of the reproductive accessory organs; and 3) proper sex steroid hormonal manipulation of ovariectomized animals results in varying degrees of reproductive "cyclical repair," i.e. stimulated growth spurts. (Allen, Hisaw and Gardner, 1939; Burrows, 1949; Velardo, 1958 and 1963; and Corner, 1965).

Regarding the biological actions of estrogens, the literature is replete with references indicating that the entire female reproductive tract and the mammary glands are estrogen-stimulable (cf. Velardo, 1958). It should also be mentioned that there are numerous other bodily effects, general and specific, caused by estrogens. Numerous compounds other than the naturally occurring estrogenic hormones and those synthetically produced also manifest estrogenic activity. Biological

and pharmacological studies reveal that androgens and progestins are also estrogenic, although only slightly so (Butenandt, 1936; Selye, 1942).

The biological effects of the naturally occurring estrogens (chiefly estradiol-17beta and estrone, female hormones secreted by ovarian follicles, primarily by the theca interna) can be characterized as a function of activity on specific end-organs, eliciting responses. The vagina and uterus are two such end-organs in which estrogenic responses can be measured with great facility. The subcutaneous administration of estrogen or extracts of ovaries containing estrogenic substances in bilaterally ovariectomized animals stimulates vaginal repair and uterine growth following a period of vaginal and uterine atrophy as a consequence of surgical removal of the ovaries. This has been shown in numerous species, including rabbits (Allen and Heckel, 1936), mice (Allen, Smith and Gardner, 1937), rats (Allen and Doisy, 1923; Astwood, 1938; Bulloughs, 1955) monkeys (Hisaw, 1950) and the human female (DiPaola and Del Castillo, 1942). For completeness, it should also be pointed out that estrogens have a growth-promoting effect on the cervix and uterine tubes also. Velardo (1958) in an extensive chapter entitled "The Anatomy and Endocrine Physiology of the Female Reproductive System," has exhaustively reviewed this subject. Reports on the biological actions of progestins (predominantly on progesterone, a female sex steroid hormone primarily secreted by the corpora lutea of the ovaries) are likewise numerous, and deal with a wide array of

end-organ responses. These reports are of two major categories: those dealing with progesterone alone, and those describing the combined actions of estrogen plus progesterone. Much less is known concerning the action of progesterone without the simultaneous, prior, or sequential administration of estrogen.

Regarding the action of progesterone per se, it can be said that progesterone has the ability to produce progestational endometria in castrated monkeys (Hisaw, Greep and Fevold, 1937; Hartman and Speert, 1941). Selye (1940) and Selye, Browne and Collip (1936) reported that progestational uteri could be induced in spayed female rats which were not pretreated with estrogen. Selye and his colleagues reported, however, that large daily doses of progesterone (10.0 milligrams) had to be given for prolonged periods of time (10 to 20 days) to accomplish such an effect. Hooker (1940), utilizing 0.25 to 1.0 milligrams progesterone daily, was able to elicit mitoses and decidual reactions in adult, ovariectomized mice.

The normal, physiological status of the ovary during prograviđ conditions within the uterus reveals that the ovary at these times secretes both estrogens and progestins. Therefore, the studies utilizing combinations of these two ovarian sex steroid hormones provide us with a more meaningful analysis of the progestational type reaction within the uterus during the prograviđ phase (previously referred to as the "secretory" phase, an erroneous concept expunged by the meticulous research of Bartelmez, 1953; the term secretory is misleading in that

endometrial secretory function is seen in the uterus during the follicular phases of the ovarian cycle also). As early as 1930, Hisaw and Leonard showed that a relationship existed between the follicular and corpus luteum hormones in the production of progestational proliferation of the uterus of the rabbit. Undoubtedly, the decidual reaction stands as a bona fide end-organ response to estrogen plus progesterone. In this connection, the pioneering efforts of Loeb (1908, 1909) should be highlighted. It was the classical work of Loeb which elucidated the role of the corpora lutea in providing the chemical (hormonal) stimulus for decidual development. The report by Velardo, Dawson, Olsen and Hisaw (1953) provided a thorough analysis of the physiological amounts of estradiol-17beta and progesterone required in bilaterally ovariectomized, pseudopregnant rats to approximate the functional capacity of the corpora lutea of pseudopregnancy in the development and maintenance of decidual reactions. Their work showed that 1.5 to 4.5 milligrams of progesterone daily plus minute amounts of estradiol-17beta (0.08 microgram daily) produced decidua in the ovariectomized, pseudopregnant rat that were almost identical to those found in the intact rat.

It is now generally believed that combinations of estrogen and progesterone are required for the true progravid response to occur within the uterus of numerous species (Courrier, 1950; Hisaw, 1950). This same concept likewise applies for the human female (Phelps, 1958). Regrettably, this basic information is derived from a wide array of

published reports; many detailed studies requiring extensive investigation which the extant information suggests to the investigator are lacking; therefore, one must resort to piecing together the important contributions so as to obtain a composite picture for a given species.

Among the many studies which led to the conclusions concerning the modus operandi of reproductive processes there cannot be cited one detailed report focussed on the early and maturing responses of the uterus of the rat to endogenous ovarian sex steroid hormones, that is, a detailed account extending from the juvenile state by short time periods through maturity and showing the variations in the uterus that can be correlated extensively with the maturing ovary, and correlated with sex steroid manipulation in ovariectomized rats. No such study, that firmly attempts to quantify the hormonal relationships that typify and elicit the establishment and continuation of such cyclical patterns under rigidly standardized conditions, is published in the literature. The published studies varied widely in method: in species and strains of animals studies, age and maturity of test animals, extent of study and reporting, experimental design, controls sustained, and, especially, as noted above, in studies utilizing hormonal manipulation, in dosages of hormones used. These studies answer, in the main, isolated questions. They do present a worthwhile departure for studies that should yield information which can bridge the several gaps in our knowledge. The published studies most pertinent to this investigation are herewith

reviewed.

Reports of studies on the uterus of the prepubertal rat are few in number. Regrettably, the much quoted, and, indeed, classic study of Long and Evans, "The Oestrous Cycle in the Rat and Its Associated Phenomena" (1922) provided only a cursory account of the uterus of the rat, and that account describes the estrous cycle of mature rats. A composite of the work of Long and Evans on uterine morphology during the estrous cycle can be summarized in tabular form:

Stage of Estrous Cycle	Uterine Histology and Gross Appearance
Proestrus	Luminal fluid abundant; vascular engorgement very evident; uterine distention begins.
Early Estrus	Maximal distention and vascular congestion early in phase; luminal epithelium appears cuboidal during this state. Later in early estrus the luminal fluid distending the uterus is released and epithelium appears columnar. Vacuolar degeneration of luminal epithelium may begin.
Late Estrus	Uterus not distended; uterine luminal fluid has been released; vacuolar degeneration of uterine luminal epithelium typical. Leucocytic infiltration may reach epithelium.
Metestrus	Vacuolar degeneration of luminal epithelium reaches maximum. Simultaneously, regeneration proceeds, and uterus is never denuded at lumen.
Diestrus	Uterus of smallest diameter seen at any stage in the cycle; regeneration of epithelium continues; uterine lumen is slit-like and the lining epithelium simple columnar; leucocytic infiltration seen, but not in lining epithelium.

An extension of the Long and Evans study was undertaken by W. M. Allen (1931). Allen's main aim was to describe the histology of the uterus of the rat in pregnancy and pseudopregnancy, but he also

included an account of the histology of the uterus during the estrous cycle as a prelude to the other studies. Adult rats having regular cycles as recorded by vaginal smears were utilized. At necropsy the entire reproductive tract of the animal was removed intact and immersed in Bouin's fluid. The usual formula for Bouin's fluid prescribes 25 per cent formalin, and presumably this is the fluid Allen used. The uterine states described for the period one to four days post-estrus were said to be the same in the normal cycling rat as in the pseudopregnant, and one description is given to cover both. The data obtained by Allen which add to that already observed by Long and Evans (1922) are summarized in the following table:

Stage of Estrous Cycle	Uterine Histology
Early Proestrus	Luminal epithelial cell height 24-30 micra; basement membrane present at stromal border of epithelium; very few mitoses in surface epithelium and none in glands; extensive leucocytic infiltration of muscle and stroma.
Late Estrus	Luminal epithelial cell height 30 micra; some areas of surface epithelium show degeneration, accompanied by loss of basement membrane; leucocytic infiltration as in proestrus.
1-3 Days Post-Estrus	Luminal epithelial cell height 15-18 micra; nuclei of epithelial cells large and ovoid; many mitoses in epithelia, surface and glandular; glands empty and collapsed; leucocytic infiltration decreased.
4 Days Post-Estrus	Luminal epithelial cells columnar with granular cytoplasm at surface border and small, round or oval nuclei located in mid-cell; epithelial mitoses rare; epithelial cell walls indistinct and basement membrane absent; stromal mitoses present; stromal nuclei large, oval and vesicular; stromal cells crowded beneath the surface epithelium; leucocytic infiltration slight.

Changes in the connective tissue of the uterus of the rat have been studied by Burack et al. (1941). In very young rats, five to seven days of age, only reticular connective tissue was found in the endometrium and myometrium, while some collagen fibers were observed in the mesometrium. By ten days of age, uteri showed some transformation of reticulum into collagen, but the finding was not universal; at 15 days of age, uteri of all the animals studied showed some transformation of reticulum into collagen. In uteri of rats 30 to 60 days of age, collagen areas were expanded to occupy 50 to 80 per cent of the endometrium, and trabeculae in the longitudinal muscle and intermuscular stroma were collagenous. By the age of 90 days, the collagen zone in the uterus occupied from 75 to 95 per cent of the endometrium, and collagenous fibers began appearing between muscle cells of the myometrium. It is thus clear that a substantial portion of the weight increase of the uterus of the rat with increasing age is due to deposition of collagen. The transitional stages of the uterus from immaturity to maturity were not studied in correlation with the entire histological patterns in the uterus in this investigation.

Using an inbred strain of rats at the University of Chicago, a strain of rats which showed vaginal opening typically at 50 days of age, Price and Ortiz (1944) studied intact juveniles with and without gonadotropin and estrogen administration. They reported that uterine glands regularly began to show development at ten days of age in normal, intact, female rats. However, their work was concerned in the main with

treated rats and the associated ovarian phenomena, and only a brief description of uteri of non-treated animals was provided. Gravimetric data for uteri of non-treated, intact rats were obtained at six, 18 and 36 days of age; the uterine weights in milligrams were 3.4, 19.0 and 25.8, respectively. Histologically, the uterus of the 18 day old rat was seen to be small in size, to possess a lumen with shallow inpocketings, a dense stroma, and somewhat low luminal epithelium.

The uteri of intact, immature rats responded to one rat unit (1 R.U.), the 0.083 microgram equivalent of estradiol-17beta, by weight gain from birth, i.e. when rats were injected daily for six days. Percentage weight gain increased with increasing age and approaching maturity, at least for groups studied at the ages of six through 36 days; thus the uterus became increasingly competent to respond to the sex steroid hormone. Advanced differentiation of endometrium was not seen until after the usual age of vaginal opening, even in estrogen-treated rats.

In a subsequent study, Price and Harvey (1947) reported the response of the uterus of the rat to different amounts of estrogen given to rats commencing at birth and for seven subsequent days. Of especial interest is the fact that although all dosages used produced accelerated uterine growth as measured by weight gain criterion, only the lowest daily dosage, which was the biological equivalent of 0.0105 microgram estradiol-17beta, produced precocious development of uterine glands, i.e. the pattern seen in uteri of normal ten day old

rats. The other dosages, equivalent to 0.083, 0.415 and 0.83 microgram estradiol-17beta, can be regarded as overdosages.

Pritchard (1949) studied uteri of immature and mature rats of different physiologic states. All rats were of the Lister strain, both piebald and albino. Age for vaginal opening was not stated, but was apparently between the ages of 60 days (none showing introitus) and 90 days (all showing introitus). Pritchard fixed these uteri in absolute alcohol, which has a marked shrinking effect on tissues. Cell height in uterine luminal epithelium and location and intensity of histochemical reaction for alkaline phosphatase were the main points studied. Unfortunately, only seven prepubertal rats were studied. Two rats at 42 days of age and one rat at 60 days of age showed a diestrous pattern for alkaline phosphatase, which was also similar to that which Pritchard found in uteri of rats castrated at maturity and studied at eight to nine days and four months after castration, but not at 14 to 15 days after castration. However, the epithelial cell height was greater in the maturing juveniles than in mature diestrous or castrated rats, which indicated a difference in reproductive state; of two 42 day old rats, uterine luminal epithelium of one averaged 11 micra, the second 16 micra; in a rat near puberty, 60 days of age, the uterine luminal epithelium averaged 23 micra. Pritchard obtained his average cell heights by measuring six cells chosen at random. The luminal epithelial cell heights that he obtained during the estrous cycle from uteri of mature rats are substantially lower than those

found in the present study; factors which are undoubtedly concerned in this difference are fixation of tissue and manner of disposing tissue for fixation, measuring technique - since cell heights often vary widely in one uterus,-and physiological and nutritional state of the rat.

Augustin, Heidenreich and Thilo (1954) also studied alterations in alkaline phosphatase activity in the rat uterus in different physiological states. Unfortunately, only a few juvenile animals were studied, and neither their age nor the age on onset of maturity in the strain used were stated. The body weights of the juvenile rats used indicate that they were probably weanlings, i.e. about 21 days old. Using morphological, histochemical and biochemical criteria, these authors found that although morphological development of maturity had not yet been reached, alkaline phosphatase in uteri of the juveniles was as high as that of mature rats in some stages of the estrous cycle, and several times the average values for castrates of 41 days post-ovariectomy.

The immature rat has been widely used as a supposedly negative control for studies of the action of ovarian sex steroid hormones. These studies reveal that the uterus of the immature rat is competent to respond to sex steroid stimulation in many ways. But they do not indicate how maturation occurs in the intact animal. Dosages of hormones used have often been excessive. It is a fact in pharmacological phenomena that concerning substances which are biologically active, smaller dosages tend to stimulate, while larger

dosages suppress action. This was seen in the work of Price and Harvey (1947) reviewed above: approximately 1/12 R.U. of estrogen produced a physiological effect, whereas 1 R.U. was an overdosage for the infantile rat. Normally, one observes that 1 R.U., or approximately 0.1 microgram estradiol-17beta equivalent, is physiological for the adult rat (Hisaw, Velardo and Goolsby, 1954). Unfortunately, the literature reports results mostly from studies utilizing from six to 200 times this amount in both immature and mature intact and castrated rats. Therefore, such data can be used only with caution.

Owing to the paucity of physiological studies and the lack of solid base-line, intact control studies, it appeared both timely and necessary to perform the work presented in this dissertation to provide a platform from which all future uterine studies could be translated into and equated with physiological terms.

In this regard, using rats of the same strain origin, the works of Hisaw, Velardo and Goolsby (1954) on effects of different dosages of different estrogens on the uterus of the mature, ovariectomized rat; of Hisaw, Jr. (1959) similarly in the immature rat; of Velardo (1964) on the development of an effective protocol for administration and programming of ovarian sex steroid hormones in the ovariectomized, young adult rat; of Velardo et al. (1967) on the extending of this protocol; and Velardo et al. (1968) and Kasprow (1969) on certain results from its use to date, present a firm base upon which the present work can build to provide physiological information pertaining

to the several horizons related to uterine growth and morphological patterns.

This review of the literature has focussed in the main on the cornerstone papers which are both pertinent and critical for the scientific understanding of the work documented in this dissertation.

III. MATERIALS AND METHODS

The investigations carried out for this dissertation utilized 1,261 female, albino rats of Sprague-Dawley origin. They were obtained from Charles River Breeding Laboratories, Incorporated, Wilmington, Massachusetts. Of this number of animals, 378 served as intact control animals: 108 for day by day age studies, 126 for studies of first estrous cycles, and 144 for studies of young adult and adult estrous cycles; 883 were utilized as bilaterally ovariectomized, young, adult subjects for the determination of effects of short term exposure to exogenously administered ovarian sex steroid hormones. The rats were housed in standard cages according to their experimental groups with the exception that no more than nine animals were housed together at any time. Purina Lab Chow and water were available ad libitum.

A. Experiments utilizing intact rats. Rats for studies previous to and through the time of first estrus were assigned by age in groups of six to 13 and housed six to seven rats per cage. Rats were obtained for the study of ages 23, 26, 33, 34, 35, 36, 37, 38, 39, 40, 41 and 42 days. At the termination of this experiment, the observation was made that body weights for 36 day old rats were subnormal, and upon investigation of notes concerning the animals at their arrival the fact was discovered that one of these six animals had been hyperactive and had aggressive and vicious tendencies. Therefore, this group was

removed from the study. The animals for this section of investigation were weighed daily and on day of necropsy. At weighing time inspection was made of the vagina to determine patency. Samples of vaginal exfoliative cytology were taken at day of vaginal opening and once daily thereafter up through day of necropsy.

B. Experiments utilizing bilaterally ovariectomized rats. Rats were obtained when 49 days of age or younger, and maintained as noted above. They were ovariectomized as noted, in C below, at 50 days of age, and allowed seven days of rest. Subcutaneous injections were given starting at 57 days of age in the suprascapular region. Each time group included four subgroups: Sesame Oil (S.O.) subgroup received 0.2cc sesame oil; Estradiol-17β (E) subgroup received 0.1μg estradiol-17β in 0.1cc sesame oil; Progesterone (P) subgroup received 1.5 mg progesterone in 0.1cc sesame oil; E + P subgroup received injections of E and P at separate sites for each injection time. Groups received either single injections, or multiple injections according to the Experimental Model System (EMS) (Velardo, 1964). The EMS protocols call for four dosages during the first week of treatment, and five per week during each of the next three weeks of treatment. Rats given single injections were killed five, ten, 15, 30, 60 and 90 minutes after injection, also at two, three, four, five, six, seven, eight, nine, ten, 11, 12, 15, 18, 21, 24, 48 and 72 hour intervals after injection. All other animals used in this study, i.e. for treatment periods of two, three, five, ten and 17 days received multiple dosages.

Dosages were given once per day on the appointed days and all of these animals were killed 24 hours after the last injection.

C. General procedures. Body weights were taken to the nearest gram on a direct reading torsion balance so as to minimize handling. Samples of vaginal exfoliative cytology were made by means of small pipettes with warm water. Bilateral ovariectomies were under ether anesthesia by the dorsolumbar approach. At necropsy date, rats were killed by cervical dislocation (young rats) or decapitation (older rats). Reproductive and endocrine organs were dissected from the body and freed from adhering adipose and other adhering tissue (with the exception of a few ovaries seen to be near ovulation, which were taken with adhering tissue for special study). Ovaries were left attached to and weighed with the uterine tube; uteri were cut above the cervical junction and below the uterine tubal junction, nicked with scissors and luminal fluid gently expressed on bibulous paper. Reproductive and endocrine organs were weighed on a Roller-Smith design torsion balance to the nearest 0.2 mg. The tissues used for cyto- and histological study reported here were placed in neutral formalin and weak Bouin's fixatives, and were fixed in the cold for approximately 24 hours. They were then washed in water, dehydrated through graded alcohols to 70% and held briefly for processing.

Uteri and ovaries were dehydrated in graded alcohols, cleared in xylol and embedded in paraffin of melting point 52.5°C ± 0.5°C. Blocks were sectioned at 5 micra and tissues stained with Harris

hematoxylin and eosin.

All photomicrographs are of cross sections. The photomicrographs were made on 4x5 inch size film with the Zeiss Ultraphot II microscope. The measurements which appear in table 2 were made with a filar micrometer standardized with a stage micrometer and with a simple ocular micrometer brought to read in micra with a Bausch & Lomb zoom microscope. Scanning powers for counting of mitoses were 500 diameters to 625 diameters, since these proved to be the most reliable.

IV. RESULTS

Prefatory Remarks

Inasmuch as the major effort of this dissertation involves the description of the cyto- and histomorphological changes within the uterus of the rat during numerous conditions, it should prove helpful to first consider the general anatomy of the uterus of the rat.

The uterus of the rat is suspended by the mesometrium. Gross and histological examination reveals that the myometrium lies directly beneath the serous covering. The myometrium consists of an outer longitudinal layer and an inner circular layer of smooth muscle. The external layer surrounds not only the uterus per se, but also much of the mesometrium. The blood vessels which enter through the mesometrium course between the two muscle layers of the myometrium. These blood vessels form numerous ramifications as they approach the uterus, and the mesometrial area is seen to become gradually wider as it approaches the uterus. On histological cross sections of the uterus the area is seen as a triangle limited on two sides by the extension of longitudinal muscle and on the third by the inner circular muscle of the uterus; this area is known as the mesometrial triangle. The part of the uterus diametrically opposed to the mesometrial triangle is known as the antimesometrial aspect of the uterus. Extensive studies of the uterine vasculature in immature, and mature intact rats and in mature, ovariectomized and hormonally manipulated rats, as well as of rats in

various other states, have been made (Williams, 1948). Therefore the entire description of vascular phenomena of the uterus of the rat is not given among these results.

The endometrium of the uterus is clearly outlined at the lumen by a distinctive covering of epithelial cells, called the uterine luminal epithelium. Underlying this covering is the stroma, which extends to the boundary of the inner, circular muscle of the myometrium. Glandular structures may be seen in varying numbers in the stroma; these are likewise conspicuous by their epithelial lining component. The epithelial components of the endometrium of the uterus of the rat are by far the most active of its components; this is reflected morphologically by changing cellular architectural arrangements and by high mitotic indices.

A. Intact, Control Rats: Gravimetric and Uterine Morphological Data at Different Time Periods and Stages of the Estrous Cycle.

1. The Prepubertal Rat.

a. Gravimetric Data.

Gravimetric analyses of body, uterine and endocrine gland weights of prepubertal and near-pubertal rats reveal that as the body weights of immature rats 23 through 33 days of age increase approximately 100 per cent, the uteri likewise do so, showing an almost parallel correlation (table 1). This similar magnitude of uterine weight increase, i.e. in 23 day old rats from 26.73 milligrams (range 22.8 to 31.8) to 57.17 milligrams (range 30.2

TABLE 1

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF PREPUBERTAL RATS NOT SHOWING VAGINAL OPENING

Age in Days	Item	Body Wt.	Ovarian Wt.	Uterine Wt.	Thyroid Wt.	Adrenal Wt.	Pit.[c] Wt.	Descrip. of Rats[d]
23	g, mg[a]	44.3±0.9[b]	24.03±1.09	26.73±1.39	4.33	15.57	2.87	6/6
	Range	41-47	20.0-28.0	22.8-31.8	3.2-5.8	14.0-16.8	2.0-3.6	
	mg%		54.24±3.35	60.34±2.77	9.77	35.15	6.48	
	Range		42.55-68.29	49.57-67.66				
26	g, mg	49.5±1.2	24.77±0.89	37.60±2.61	6.60	16.60	2.63	6/6
	Range	46-54	23.0-29.0	27.4-46.2	5.2-8.8	12.2-20.8	2.2-4.0	
	mg%		50.04±2.33	75.96±6.02	13.33	33.53	5.31	
	Range		45.00-60.42	55.92-100.43				
33	g, mg	80.8±3.5	34.03±2.94	57.17±9.36	7.43	19.83	2.90	6/6
	Range	70-92	27.4-47.4	30.2-96.0	4.6-9.6	15.8-22.8	2.6-3.2	
	mg%		42.16±2.20	70.75±9.03	9.19	24.54	3.59	
	Range		35.13-51.52	38.72-104.35				
34	g, mg	112.7±4.1	52.10±4.69	144.53±15.07	8.23	24.87	5.48	6/6
	Range	94-124	37.0-72.4	91.8-189.6	6.2-11.0	17.4-29.0	3.6-8.2	
	mg%		46.23±3.61	128.24±10.69	7.30	22.07	4.86	
	Range		32.17-58.39	97.66-160.68				

- continued -

TABLE 1--Continued

Age in Days	Item	Body Wt.	Ovarian Wt.	Uterine Wt.	Thyroid Wt.	Adrenal Wt.	Pit. Wt.	Descrip. of Rats
35	g, mg	113.4±8.7	59.68±9.18	138.88±29.99	12.80	33.44	5.48	5/6
	Range	79-127	29.8-85.2	54.5-228.0	10.0-15.4	27.0-39.8	4.4-7.8	
	mg%		52.63±5.90	122.47±22.86	11.29	29.49	4.83	
	Range		37.72-72.20	68.86-191.60				
37	g, mg	97.7±2.4	47.25±2.99	152.88±16.17	10.27	26.63	5.53	12/13
	Range	80-111	34.0-69.2	45.0-228.6	7.2-12.2	19.2-34.6	3.8-8.2	
	mg%		48.36±2.85	156.48±16.30	10.51	27.26	5.66	
	Range		35.79-65.17	47.37-238.13				
38	g, mg	99.6±2.3	47.47±3.53	111.47±16.80	10.40	27.82	6.33	12/13
	Range	81-108	35.4-82.4	45.6-213.2	9.0-13.2	20.4-42.2	4.2-9.2	
	mg%		47.66±2.98	111.92±15.95	10.44	27.93	6.34	
	Range		37.04-76.30	51.13-206.99				
39	g, mg	11.0±4.4	44.13±1.59	159.07±21.09	11.62	29.52	6.50	8/13
	Range	97-130	36.4-50.0	52.4-238.2	8.2-13.8	23.0-38.0	4.6-9.0	
	mg%		39.76±1.41	143.31±17.21	10.47	26.59	5.85	
	Range		34.67-45.55	54.02-208.95				
40	g, mg	117.0±1.9	50.05±5.14	173.40±25.74	12.46	31.00	6.65	
	Range	110-125	33.6-79.6	52.2-260.8	9.4-16.4	25.8-38.2	4.4-8.0	
	mg%		45.34±3.99	148.21±22.43	10.65	26.49	5.68	
	Range		30.00-64.19	41.76-224.83				

- continued -

TABLE 1--Continued

Age in Days	Item	Body Wt.	Ovarian Wt.	Uterine Wt.	Thyroid Wt.	Adrenal Wt.	Pit. Wt.	Descrip. of Rats
41	g, mg	116.5±3.3	60.93±10.36	191.33±12.89	10.87	31.80	7.10	6/13
	Range	102-127	43.8-111.6	153.6-221.6	8.4-12.6	27.2-36.4	6.2-8.2	
	mg%		52.30±8.56	164.23±14.52	9.33	27.30	6.09	
	Range		38.09-94.58	124.25-217.25				
42	g, mg	119.0±10.8	39.25±5.47	135.05±35.86	11.00	29.70	6.10	4/13
	Range	.88-137	23.6-48.2	30.4-189.4	7.6-14.2	25.2-34.0	4.0-7.4	
	mg%		32.98±2.73	113.49±25.00	9.24	24.96	5.13	
	Range		26.82-39.83	34.55-145.69				

[a]Body weights are shown in grams (g), organ weights in milligrams (mg).

[b]In the case of body weights in grams and ovarian and uterine weights in milligrams and milligrams per cent, the arithmetic mean is followed by the standard of the mean.

[c]Pit. = pituitary.

[d]The figure before the diagonal represents the number of rats of the total group studied(closed vaginae at time of necropsy); the figure after the diagonal represents the total number of rats studied in the age group.

to 96.0) in 33 day old rats; and body weight increase from 44.3 grams (range 41 to 47) to 80.9 grams (range 70 to 92) in 23 and 33 day old, immature rats, respectively, is of true biological significance. In broad perspective, and on a weight relationship basis, one could point to a gravimetrically related horizon in uterine growth. It should be pointed out here that such a weight increase as a biological end-point criterion is of tremendous importance as a discriminative index in biological assay (Dorfman and Dorfman, 1954; Rubin, Dorfman, Black and Dorfman, 1951). Such end-organ increases of 100 per cent, or approximately 100 per cent have been repeatedly utilized as end-organ responses of significance for the analyses of hormones and numerous other metabolic agents.

The uterine weights of immature rats studied progressively to near puberty, from ages 34 through 42 days reveal interesting changes, which, however, within the group are not as dramatic as those seen in the first group for uteri of rats 23 to 33 days of age.

Immature rats at ages 34, 35 and 37 days had uteri with two-fold weight increases over uteri of 33 day old rats; those of the next age group studied, at 38 days of age, had less-well defined increases, and those of age groups closer to the advancing stages of maturity, 39 to 42 days of age, had uteri that revealed the most, but statistically acceptable, biological variation: uteri averaged 159 milligrams in 39 day old rats, 173 in 40 day

olds, 191 in 41 day olds, and 135 milligrams in 42 day olds (table 1). A good linear relationship revealed itself from ages 39 to 41 days, but at age 42 days a marked decrease was clearly evident. Note also that the figures discussed in this section are only for animals of each age group that were truly sexually immature at necropsy, as manifested by the animals having had closed vaginae. From the tabular data, one observes the fact that almost 100 per cent of the animals were sexually immature through the thirty-eighth day of life, and thereafter with the advancing of age, on a day to day basis, the percentage of immature animals decreased from the 100 per cent figure to 60 60 per cent at ages 39 and 40 days, 46 per cent at age 41 days, and finally, down to 30 per cent in the group at age 42 days, cf. table 1.

The variations in weight and age at which maturity is attained are to be expected, especially since it is well known that with the advancing stages of maturity, resulting from a biological crescendo of endocrinological activity - particularly at the C.N.S.-hypothalamico-adenohypophyseal-ovarian axis - the animal commences to adapt to new biological rhythms. Prior to the establishment of the mature pattern, there is much necessary reorganization of biological actions and interactions (Young, 1961).

Further analyses of the gravimetric data reported in table 1 clearly depict a noted increase in ovarian weight,

approximately 100 per cent, but occurring at age 34 rather than at age 33 as observed for a similar increase in uterine weight. Thereafter, up to age 41 days, the ovarian weights seem to fluctuate about a central arithmetic mean, thereafter decreasing in comparable magnitude as observed for the uterine weights at age 42 days, thus substantiating the aforementioned concept of biological crisis attendant upon the emerging roles of the C.N.S.-endocrine systems and end-organ responses in and for physiological accommodation for sexual maturity.

Additional highlights from the tabular data indicate some rather remarkable features for the weights of the thyroid, adrenal and pituitary glands. The thyroid gland grows almost linearly through the 35th day, with a doubling of weight being observable on day 34, and a tripling on day 35 over the 23 day old weight; thereafter, the thyroidal weights show only minimal fluctuation, i.e. within 1 to 2 milligrams.

The adrenal glands in these immature rats are of further interest: a notable 100 per cent increase appears on day 35, after which time the weights tend to reveal a measurable and statistically significant decrease, ranging from 1.64 to 6.81 milligrams. Irrespective of the noted decrease, the adrenal glands reveal an almost 100 per cent increase at age 42 days over the control data recorded for animals 23 days of age, i.e. 15.57 milligrams at 23 days of age, and 29.70 milligrams at 42 days of age.

Finally, the analyses of the weights of the pituitary glands offer a rather interesting point for summarization of this large body of data on the comparisons of body, uterus and endocrine gland weights of prepubertal rats not showing vaginal opening: (a) notable increases of pituitary weights reaching approximately 100% on day 34-35, similar to the increases for the adrenal glands to day 35; whereas such increases of 100 per cent occur on day 33 for the body and uterine weights, and on day 34 for the ovarian and thyroidal weights, and (b) these data point-up, in a definitive way, that parallel increases exist for (1) body and uterine weights up to day 33, (2) ovarian and thyroidal weights up to day 34, and (3) pituitary and adrenal weights up to day 35 (table 1).

b. Cytological and Histomorphological Data, Table 2, Plates I-XIX, Figures 1-48.

In assessing the uteri of immature rats, commencing at 23 days of age, the starting age of rats utilized in these studies, it became obvious quite soon that the quiescent uterine pattern of prepubertal animals is typically that found in animals with seemingly nonactivated ovaries (plates I-III, figures 1-8). Uteri are generally of the class I type, cf. table 2, section A. 1.; some show one mitotic figure per cross section in one of the three designated areas for study, as is the case of the stromal mitotic figure seen in figure 6, plate II. Typically, these uteri possess low surface epithelium, up to a mean of 20 micra, and for the most part lack mitotic figures in the uterine luminal epithe-

TABLE 2

COMPARATIVE CLASSIFICATION OF UTERI OF PREPUBERTAL RATS
(WITH CLOSED VAGINAE)
ACCORDING TO CYTOLOGICAL DETAILS

A. Description of classifications:

I. Uteri possessing low columnar luminal epithelium (up to a mean of 20 micra). Mitotic figures in luminal epithelium, stroma and circular muscle absent for the most part; only rarely does one observe a mitotic figure in the three designated areas.

II. Uteri possessing low columnar luminal epithelium (up to a mean of 20 micra), as described in I, above, but exhibiting substantial mitotic activity in one, two or all of the designated areas for study, cf. I, above.

III. Uteri possessing columnar luminal epithelium of moderate height, (i.e. up to a mean of 20-30 micra) which does not exhibit vacuolar degeneration.

IV. Uteri possessing high columnar luminal epithelium (mean greater than 30 micra) and showing either no vacuolar degeneration or only small vacuoles in a small minority of cells.

V. Uteri possessing luminal epithelium exhibiting moderate or extensive vacuolar degeneration, irrespective of height of luminal epithelial cells.

- continued -

B. Summary of Distribution of Prepubertal Rats 23-37 Days of Age by Age and Uterine Class.

Age in Days	No. Studied	CLASS I	II	III	IV	V
23	5	4	1			
26	6	2	4			
33	6	2	4			
34	5	3		1		1
35	5	1			1	3
37	9		2	4	2	1

C. Detailed Account of Cytology of Uteri of Prepubertal Rats 23 to 37 Days of Age Correlated with Uterine Weight and Body Weight.

Class	Age in Days	Height of Lum. Epith.[a] in micra Mean	Range	No. of Mitoses/ Cross Section Lum. Epith.	Stroma	Circ. Muscle[b]	Ut. Wt. mg.	mg%	Body Wt. gm
I	23	12	(11-15)	0	0	0	23.6	54.9	43
		14	(10-17)	0	0	0	29.2	64.9	46
		15	(11-16)	0	1	0	31.8	67.7	48
		18	(14-21)	0	0	0	26.0	63.4	41
	26	13	(10-17)	0	0	0	27.4	55.9	49
		13	(10-18)	0	0	0	34.0	70.8	48
	33	11	(8-13)	0	0	0	30.2	38.7	78
		12	(8-16)	1	0	0	49.6	70.9	70
	35	12	(9-14)	0	0	0	54.4	68.9	79
II	23	18	(13-22)	4	9	0	22.8	49.6	46
	26	14	(13-20)	8	24	6	46.2	100.4	46
		15	(11-17)	3	4	1	41.0	78.9	52
		15	(12-18)	5	0	0	38.2	70.7	54
		16	(13-18)	1	0	3	38.8	80.8	48

- continued -

TABLE 2--Continued

Class	Age in Days	Height of Lum. Epith.[a] in micra Mean	Range	No. of Mitoses/ Cross Section Lum. Epith.	Stroma	Circ. Muscle[b]	Uterine Wts. mg	mg%	Body Wt., gm
II, cont'd	33	11	(7-15)	0	7	0	42.6	54.6	78
		14	(12-16)	3	2	1	68.2	75.8	90
		15	(9-17)	0	2	1	56.4	73.3	77
		19	(16-20)	13	13	5	96.0	104.3	92
	34	14	(12-19)	6	28	6	91.8	97.7	94
		19	(15-23)	8	3	2	166.2	149.7	111
		19	(17-20)	7	2	3	189.6	160.7	118
	37	19	(12-21)	5	9	1	45.0	47.4	95
		19	(15-23)	0	5	5	167.4	170.8	98
III	34	21	(18-23)	18	12	7	139.8	122.6	114
	37	23	(19-30)	25	14	1	175.4	184.6	95
		24	(20-34)	14	7	3	139.8	141.2	99
		25	(21-28)	6	5	3	139.0	125.2	111
		28	(22-39)	10	4	1	196.4	188.0	104
IV	35	41	(30-54)	2	3	0	228.0	191.6	119
	37	42	(31-53)	0	4	0	228.6	238.1	96
		45	(31-57)	1	4	0	228.0	235.1	97
V	34	41	(26-56)	0	3	2	167.0	134.7	124
	35	24	(20-51)	4	0	0	107.4	86.6	124
		30	(21-35)	10	0	0	124.4	97.9	127
		40	(35-50)	0	3	0	180.2	152.7	118
	37	35	(32-47)	1	0	0	133.8	123.9	108

[a] Lum. Epith. = uterine luminal epithelium.

[b] Circ. Muscle = inner circular muscle. Mitoses which gave the appearance of being in connective tissue areas in the muscle are not included in these figures.

lium, endometrial stroma, and inner circular muscle. Interestingly enough, histological study of the ovaries of these young immature rats revealed inconstant, sporadic follicular development, cf. plate III, figures 7 and 8.

The uteri of immature rats 26 days of age predominantly inclined toward class II in appearance, i.e. four of six rats studied were of class II, whereas two of six were of class I, cf. table 2.B. Histologically, the class II uteri exhibited low columnar luminal epithelium, up to a mean of 20 micra, as described for class I, but exhibited substantial mitotic activity throughout the endometrium and inner circular muscle, cf. plates IV and V, figures 9-15. Ovarian histology of rats in this group revealed slightly activated follicular activity, cf. plate VI, figures 16 and 17.

Previously, it was indicated that the uteri of rats 33 days of age showed sufficient weight increases to categorize this stage of development as an horizon. Cytologically, these uteri were seen to have been predominantly also of class II, as were the uteri of 26 day old rats, in spite of the pronounced increase in weight. Mitotic figures were more regularly seen in the stroma of uteri of the 33 day old rats than in the 26 day old rats, and were very abundant also in the luminal epithelium and circular muscle of one uterus which was very close to being of the next, more mature class, and shown in plates VII and VIII, figures 18-22. Ovaries of the 33 day old animals revealed a large vesicular follicular

population, thus pointing up a true morphological horizon from the point of view of ovarian stimulation and its end organ response as seen in the uterus, cf. plate IX, figure 23.

The follow-up age group of 34 days is of further interest: histologically, the appearance of the cross section of the uterus is quite reminiscent of that of preproestrus in the mature cycling animal, cf. plate X, figures 24 and 25. Study of the ovaries of these animals reveals that as the uterus begins its climb toward adulthood, the ovary in large measure begins to exhibit increased follicular-metabolic activity (plate XI, figures 26-28). Uteri of animals necropsied on day 35 were less revealing of growth patterns than those of the 34 day old group (table 2.B.).

The uterine growth period between days 33 and 37 represents a second early horizon in uterine growth. Especially noteworthy of the uteri of the 37 day old rats are the facts that seven of the nine uteri so studied had luminal epithelial cells of average heights greater than 20 micra; four of the nine, which displayed luminal epithelial average heights of between 20 and 30 micra, the criterion for class III, also displayed numerous mitotic figures and manifested marked stromal and muscular cellularity (cf. table 2.A,B,C; plate XII, figures 29-31; and plate XIV, figures 34 and 35). That a distinctively second early uterine horizon is typical of this age group is borne out by the histological observations on the ovaries, which show enlarged vesicular follicles with near- and preovulatory swelling (plate XIII, figure 32). It is approximately

at this stage that meiotic figures begin to be found in some maturing egg cells (plate XIII, figure 33, also, in a rat very close to this uterine Class III stage, plate XI, figures 27 and 28).

The cyto- and histological observations of prepubertal rats between the ages of 37 and 42 days of age indicate that this is the period during the early history of the reproductive cycle when the events of follicular maturation and uterine growth race toward the initiation of the first estrous cycle. Uteri of 41 day old rats were typically of classes IV and V. A typical example of the fully developed class IV pattern is shown on plates XV and XVI, figures 36-38, and figure 39, respectively. The uterine luminal epithelium is of high columnar type without vacuoles, the luminal epithelium is thrown into deep folds, and the overall appearance of the uterus is strikingly similar to that of the uterus of the mature rat in proestrus. A notable exception is that this uterus of the 41 day old rat does not show the stromal edema typical of proestrus in the mature uterus. The histological findings of the ovary corroborate the concept that these animals are closely approaching sexual maturity, note the marked follicular development and maturing egg cell (plate XVI, figures 40 and 41).

That not all animals respond uniformly to the intrinsic biological patterns of an "awakening" endocrine system is apparent from the variations one observes among rats in the age groups 34 to 42 days (table 2.B and C). This is reflected in the young age

of the immature animal illustrating uterine class V, cf. plates XVII-XIX, figures 42-47. The uterus of this immature, 34 day old rat shows an advanced pattern typical of late estrus. The uterine luminal epithelium is tall columnar with nuclei predominately basally-oriented, and the epithelial cells cells frequently exhibit small vacuoles containing material. The luminal epithelium is mitotically quiescent, as is usual of estrous uteri, while there is activity in the stroma and inner, circular muscle, cf. figure 44. Examination of the ovary of this rat gives indisputable proof of the advanced reproductive state of this 34 day old rat, cf. follicle in process of ovulation, figure 48.

2. The Pubertal Rat.

a. Gravimetric Data.

The majority of animals utilized for these studies attain puberty as manifested by vaginal opening variously between the ages of 37 to 42 days of age, although exceptions on either side of this age range are found. A summary of body, uterine and endocrine gland weights of rats necropsied at specific ages and found to have open vaginae at necropsy are presented in table 3. The ovarian and uterine weights are quite variable; thyroid weights are less variable, whereas those of the adrenal gland show marked fluctuation between rats of the same age group as well as between age groups. Pituitary gland weights also show considerable variation.

TABLE 3

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS OF PUBERTAL RATS SHOWING VAGINAL OPENING BY AGE GROUP

Age in Days	Item	Body Wt.	Ovarian Wt.	Uterine Wt.	Thyroid Wt.	Adrenal Wt.	Pit.[b] Wt.	Desc. of Rats[c]	No./ E.C. Stage[d]
35	g, mg[a]	128	71.4	268.6	11.4	34.6	7.0	1/6	E
	mg%		55.78	209.84	8.91	27.03	5.47		
37	g, mg	126	66.4	160.8	12.2	34.4	6.4	1/13	E
	mg%		52.70	127.62	9.68	27.30	5.08		
38	g, mg	108	56.0	153.0	10.6	29.8	7.6	1/13	E
	mg%		51.85	141.67	9.81	27.59	7.04		
39	g, mg	120.6±3.5[e]	60.88±3.65	262.12±79.92	11.88	34.08	7.28	5/13	PE: 2
	Range	111-132	53.0-72.4	113.0-547.4	10.2-13.8	31.8-36.6	5.8-8.2		PE-E: 1
	mg%		50.48±3.31	217.35±62.50	9.85	28.26	6.04		E: 2
	Range		40.61-58.39	101.80-441.45					
40	g, mg	127.2±2.5	67.64±5.88	161.80±20.67	13.08	27.72	8.12	5/13	PPE-PE: 1
	Range	118-132	54.0-86.2	122.8-241.8	10.2-17.6	19.8-32.4	6.6-9.8		E: 2
	mg%		53.18±4.52	127.20±16.32	10.28	21.79	6.38		ME: 1
	Range		42.73-68.41	104.07-191.90					DE: 1
41	g, mg	118.0±3.7	69.09±6.10	198.57±21.71	13.80	32.51	7.91	7/13	PE: 4
	Range	102-135	48.2-93.6	136.2-296.4	10.0-17.0	24.4-39.4	6.4-9.8		E: 3
	mg%		58.55±3.84	168.28±18.46	11.69	27.55	6.71		
	Range		41.20-70.25	122.81-255.52					

- continued -

TABLE 3--Continued

Age in Days	Item	Body Wt.	Ovarian Wt.	Uterine Wt.	Thyroid Wt.	Adrenal Wt.	Pit. Wt.	Desc. of Rats	No./ E.C. Stage	
42	g, mg	133.4±3.1	55.80±3.85	183.31±22.51	10.31	31.69	6.85	9/13	PPE:	3
	Range	123-148	42.8-76.2	96.4-274.0	8.6-12.2	26.2-41.6	5.0-10.2		PE:	1
	mg%		41.46±2.50	137.41±18.34	7.73	23.75	5.13		PE-E:	1
	Range		32.92-55.22	74.73-217.46					E:	2
									ME:	1
									DE:	1

[a]Body weights are shown in grams (g), organ weights in milligrams (mg).

[b]Pit. = pituitary.

[c]The figure before the diagonal represents the number of rats of the total group studied which had open vaginae at time of necropsy; the figure after the diagonal represents the total number of rats studied in the age group.

[d]Rats necropsied which had open vaginae were staged by vaginal smear at time of necropsy. The abbreviation for the estrous cycle stage is followed by the number of rats found to be in that stage. The abbreviations are: PPE: preproestrus; PE: proestrus; E: estrus; ME: metestrus; and DE: diestrus.

[e]In the case of body weights in grams, and ovarian and uterine weights in milligrams and milligrams per cent, the arithmetic mean is followed by the standard error of the mean.

In gravimetric studies of pubertal rats undergoing reproductive cyclicity for the first time, however, one can see the emergence of a pattern of weight changes for uteri and endocrine glands consistent with the individual phases of the estrous cycle (cf. table 4). Briefly stated, the uterine weights during the first vaginal cycle were heaviest during proestrus preceding first estrus, decreased in late estrus, decreased again approximately 20 per cent in metestrus, and reached a low in diestrus. The heaviest uterine weights in the estrous cycle of the adult rat are normally found at estrus; the rats presented in this table representing first estrus were those necropsied on the first day of vaginal opening at the appearance of an all cornified cell vaginal smear of exfoliated cells. It is probable that these rats were in late estrus from the lowered uterine weights; this may be a characteristic of the first vaginal-opening pattern. This finding agrees with that of Long and Evans (1922) that when vaginal opening occurs very near the time of first ovulation, it does so in late estrus after the time of heat has passed. On the other hand, many rats show vaginal opening with exfoliative cytology typical of diestrus, preproestrus or proestrus, and when these rats are necropsied at the first appearance of the proestrus smear (consisting all of large epithelial cells) their uteri are of the typically high proestrus weight. As the animals approached their second estrous phase, uteri increased in weight over diestrous uteri, and at second estrus, attained a high weight. Ovarian weights followed the trends of

TABLE 4

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS OF PUBERTAL RATS DURING THE FIRST ESTROUS CYCLE STAGES FROM TIME OF VAGINAL OPENING

Estrous Cycle Stage	Item	Body Weights	Ovarian Weights	Uterine Weights	Thyroid Weights	Adrenal Weights	Pit.[c] Weights
Proestrus Before First Estrus	g, mg[a]	122.6±2.6[b]	57.56±6.72	260.60±15.35	12.72	34.16	7.48
	Range	117-132	44.6-83.6	216.4-311.6	8.2-20.0	31.2-39.4	6.4-8.2
	mg%		46.95±5.95	212.56±8.40	10.37	27.86	6.10
	Range		36.56-70.25	184.96-236.06			
First Estrus	g, mg	120.8±3.7	70.12±5.57	154.84±10.20	11.72	30.64	7.84
	Range	108-126	56.0-89.4	122.2-186.0	10.2-15.4	19.8-37.4	6.4-9.8
	mg%		58.05±4.06	128.18±8.97	9.70	25.36	6.49
	Range		51.85-73.88	102.69-153.72			
Met-estrus After First Estrus	g, mg	130[d]	42.8	123.0	9.2	26.2	6.0
	mg%		32.92	94.61	7.08	20.15	4.61
Diestrus After First Estrus	g, mg	128.7±2.6	59.50±2.62	122.23±7.31	8.73	29.53	5.70
	Range	122-139	49.10-69.0	96.4-146.0	6.0-11.8	23.0-35.8	4.2-6.4
	mg%		46.23±2.79	94.97±6.15	6.78	22.94	4.43
	Range		40.16-55.65	74.73-110.00			

- continued -

TABLE 4--Continued

Estrous Cycle Stage	Item	Body Weights	Ovarian Weights	Uterine Weights	Thyroid Weights	Adrenal Weights	Pit. Weights
Prepro-estrus	g, mg	136.5±8.0	57.20±2.24	151.50±9.66	7.40	30.50	5.40
	Range	119-152	53.4-63.2	137.10-180.0	5.6-10.4	24.0-41.6	4.8-6.0
Before Second Estrus	mg%		41.90±1.32	110.99±6.87	5.20	22.34	3.96
	Range		39.19-45.55	92.57-124.03			
Second Estrus	g, mg	139.4±5.7	94.30±14.09	258.48±54.11	8.12	39.24	7.92
	Range	121-150	62.6-122.2	158.2-457.0	6.0-13.8	30.0-46.6	6.8-8.8
	mg%		65.49±8.72	185.42±33.77	5.82	28.15	5.68
	Range		47.79-83.13				

[a]Body weights are shown in grams (g); organ weights in milligrams (mg).

[b]In the case of body weights in grams and ovarian and uterine weights in milligrams and milligrams per cent, the arithmetic mean is followed by the standard error of the mean.

[c]Pit. = pituitary.

[d]Only one animal was observed in classical metestrus.

uterine weights with the exception that the ovarian weights were highest in estrus for both of the two estrous phases considered. The thyroid weights were quite variable during the first estrous cycle; adrenal weight trends followed those of the uteri, while pituitary weights followed the ovarian pattern (table 4).

b. Cytological and Histomorphological Data, Plates XX-XXVIII, Figures 49-73.

The animals studied during their first estrous cycle were examined daily for vaginal opening. The predominant vaginal patterns were those of proestrus, estrus, or an intermediate of these two, although exceptions were found. Ovaries for the great majority of animals necropsied during the first estrous cycles exhibited the expected lack of or presence of corpora lutea, depending upon whether an estrous smear had ever been observed for the animal. However, there were exceptions. One 35 day old rat, necropsied on the first day of vaginal patency manifested vaginal and uterine patterns typical of early preproestrous and displayed large corpora lutea in the ovary. A second animal, with a first vaginal opening had vaginal cytology of proestrus and also showed one generation of corpora lutea in the ovary, along with large vesicular follicles (plates XX and XXI, figures 49-51 and 52-54, respectively). The uterus of the second animal, however, was more of preproestrus pattern than would be expected. Thus, it can be seen that in some individual animals, all of the components of the reproductive system may not respond to the "awakening" endocrine system in the

coordinated manner seen in the adult.

Examination of many pubertal rats, however, revealed that in the great majority, the uterus on the first instance of vaginal opening is one that has gained competence to respond to the ovarian hormones typical of the follicular, preovulatory and ovulatory phases of the estrous cycle. Uteri examined in the preproestrous-proestrous-estrous phases of the estrous cycle at this time period in the life of the rat have attained the first appearance of endometrial growth typical of the proliferative phase of the estrous cycle. Additional studies on the uteri and ovaries of pubertal rats 38 to 43 days of age reinforce and fully confirm these findings, cf. plates XXII to XXVIII, figures 55 to 73. These data point-up the third, early horizon in uterine growth as occurring coincident with the first opening of the vagina in the pubertal rat. At this time the uterus is typically one showing a characteristic proliferative pattern: a highly cellular endometrium, and numerous uterine glands within the endometrial stroma, tall columnar uterine luminal epithelium, numerous mitotic figures in luminal epithelium, stroma and inner circular muscle in uteri of early proliferative state, diminishing with approaching estrus.

3. The Young Adult, Cycling Rat.

a. Gravimetric Data.

Studies of uterine weights of rats 55 to 66 days of age experiencing normal, repetitive estrous cycles revealed that those

of preproestrus attained a raw weight of 378.68 milligrams (200.57 milligrams per cent), a sizable increase over that seen in a comparable stage during the first estrous cycle (151.5 milligrams; 110.99 milligrams per cent), cf. tables 4 and 5. The uterine weights of animals in proestrus were somewhat heavier than those for the earlier phase of estrus, while those of estrus appeared as the heaviest in the group, averaging 427.24 milligrams and 222.99 milligrams per cent. The uterine weights of rats in metestrus and early and late diestrus showed a marked reduction in weight, having dropped to 346.80, 234.60 and 246.04 milligrams, respectively. The ovarian weights likewise appeared heaviest during estrus-early metestrus and lightest during early and late diestrus. Thyroid, adrenal and pituitary glands were heaviest during proestrus and estrus and lightest also during early and late diestrus (table 5).

b. Cyto- and Histomorphological Data, Plates XXIX to XXXII, Figures 74 to 84.

Cytological and histomorphological studies on the uteri and ovaries of cycling, young adult rats indicated quite clearly that endometrial and ovarian cycles became regularized between the ages of 42 and 55 days. Commencing with preproestrus, the endometrium manifests tall columnar epithelium, numerous mitotic figures, indented luminal epithelium, stromal edema and developing glands in stroma (plate XXIX, figures 74-76). Progressing to estrus, one observes an endometrium in high metabolic activity: markedly indented and stellate-shaped lumen, marked stromal edema, and very

TABLE 5

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF RATS 55 TO 66 DAYS OF AGE
DURING THE STAGES OF THE ESTROUS CYCLE

Estrous Cycle Stage[a]	Item	Body Weight	Ovarian Weights	Uterine Weights	Thyroid Weights	Adrenal Weights	Pituitary Weights
PPE	g, mg	188.8±5.0	92.92±5.75	378.68±22.54	15.24	53.16	11.84
	Range	174-202	74.6-109.8	307.4-440.0	9.8-20.4	47.4-58.6	8.2-16.2
	mg%		49.22±3.78	200.57±11.44	8.07	28.16	6.27
	Range		37.68-59.67	176.67-239.13			
PE	g, mg	199.4±8.1	103.16±6.70	406.4±9.37	17.52	55.00	12.8
	Range	178-226	90.0-128.2	375.2-428.4	12.6-21.8	48.6-64.2	10.2-16.6
	mg%		51.73±3.00	203.81±6.81	8.79	27.58	6.42
	Range		44.51-61.93	195.27-225.51			
E: early	g, mg[b]	191.6±6.6[c]	110.60±6.44	427.24±15.98	16.28	57.00	13.72
	Range	168-208	92.4-124.6	388.6-482.4	12.4-20.4	41.8-62.4	9.2-17.0
	mg%		57.72±2.36	222.99±15.83	8.50	29.75	7.66
	Range		49.90-63.63	201.35-287.14			
ME	g, mg	188.8±5.2	117.08±4.42	346.80±7.25	12.96	53.92	10.72
	Range	174-205	101.8-127.8	327.8-363.8	12.4-14.0	41.6-63.8	10.6-11.0
	mg%		62.01±3.45	183.69±1.95	6.86	28.56	5.68
	Range		49.66-70.23	177.46-188.39			

- continued -

TABLE 5--Continued

Estrous Cycle Stage	Item	Body Weight	Ovarian Weights	Uterine Weights	Thyroid Weights	Adrenal Weights	Pituitary Weights
DE: early	g, mg	195.2±8.1	95.80±3.15	234.60±14.00	14.96	50.28	9.68
	Range	169-216	87.2-103.0	197.4-281.6	13.8-16.6	43.8-55.2	9.0-10.6
	mg%		49.08±1.61	120.18± 7.02	7.66	25.76	4.96
	Range		44.81-53.25	105.56-139.05			
DE: late	g, mg	173.2±3.9	94.24±4.97	246.04±12.67	14.6	50.64	10.92
	Range	163-184	79.8-105.6	208.6-273.4	13.2-15.8	42.4-61.2	8.0-15.6
	mg%		54.41±2.71	142.05±8.26	8.43	29.24	6.30
	Range		48.07-60.34	122.83-166.38			

[a]Abbreviations for stages: PPE: preproestrus; PE: proestrus; E: estrus; ME: metestrus; DE: diestrus. Estrous animals in this table were necropsied after the "C^4" smear of cornified cells was first seen. Early diestrus encompasses the first 48 hours after first manifestation of estrous smear; late diestrus the next 24 hour period, extending to 72 hours.

[b]Body weights are shown in grams (g); organ weights in milligrams (mg).

[c]The arithmetic mean is followed by the standard error of the mean in the case of body weights in grams and ovarian and uterine weights in milligrams and milligrams per cent.

tall columnar luminal epithelium (plate XXX, figures 77 and 78). The uterus during this stage in the 55 day old animal is truly representative of estrus, and is clearly delineated as the fourth distinctive early horizon in uterine growth. The following stage of metestrus appears as an aftermath of vigorous uterine metabolic activity. During this time the uterine luminal epithelium undergoes reduction in size and simultaneously becomes quite ragged. The reduction in size and breaking open of cells at the luminal border does not proceed in all areas of the lumen at the same time, and the cells comprising the uterine luminal epithelium are of varying sizes and shapes (plate XXXI, figures 79 and 80). The uterus of the diestrous animal typically appears quiescent when a cross section is viewed at lower powers of magnification: especially noteworthy are the reduced uterine size, cuboidal luminal epithelium, narrowed uterine lumen, and a marked reduction in uterine glands within the stroma (plate XXXII, figures 81 and 82). Early diestrus, however, was seen to be a time of high mitotic activity in the luminal epithelium, stroma and in the circular muscle, reminiscent of the class III stage seen in uteri of prepubertal rats (plate XXXII, figures 83 and 84).

4. The Cycling, Adult Rat at Reproductive Prime.

An extension of the work concerned with the early horizons of uterine growth was included with the purpose of ascertaining the nature of the cyclical changes in animals of age often used

TABLE 6

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF RATS 124 TO 133 DAYS OF AGE
DURING THE STAGES OF THE ESTROUS CYCLE

Estrous Cycle Stage[a]	Item	Body Weight	Ovarian Weight	Uterine Weight	Thyroid Weight	Adrenal Weight	Pituitary Weight
PPE	g, mg[b]	245.8±6.1[c]	110.28±3.95	462.84±34.13	14.60	56.36	13.12
	Range	232-263	98.2-119.4	361.6-545.0	10.8-20.2	50.2-66.6	8.2-17.8
	mg%		44.87±1.73	188.30-17.52	5.94	22.93	5.34
	Range		41.79-51.47	140.70-226.72			
PE	g, mg	256.2±11.9	115.64±6.40	502.36±9.06	14.88	57.96	13.52
	Range	226-291	96.4-134.6	479.0-518.0	12.0-18.0	46.8-69.2	11.6-16.8
	mg%		45.14±3.98	196.08±11.20	5.81	22.62	5.28
	Range		35.44-59.56	176.10-229.03			
E	g, mg	279.0±13.5	123.60±6.58	519.20±35.55	15.08	69.92	15.36
	Range	242-298	104.6-145.8	442.4-624.4	12.2-18.4	58.2-85.0	13.0-20.0
	mg%		44.30±2.75	186.09±12.17	5.41	25.06	5.51
	Range		35.98-51.65	152.03-226.74			
ME	g, mg	291.3[d]	124.27	513.20	15.6	50.93	16.53
	Range	259-316	93.0-142.8	474.2-545.4	14.8-16.4	48.4-53.8	14.6-18.4
	mg%		42.66	176.17	5.35	17.48	5.67
	Range		31.10-52.89	158.59-200.77			

- continued -

TABLE 6--Continued

Estrous Cycle Stage	Item	Body Weight	Ovarian Weight	Uterine Weight	Thyroid Weight	Adrenal Weight	Pituitary Weight
DE							
12 Hr.[e] $_1$	g, mg	255	121.2	528.4	15.2	57.0	13.6
	mg%		47.53	207.22	5.96	22.35	5.33
12 Hr.[f] $_2$	g, mg	281.5	124.0	374.4	18.2	53.9	12.9
	Range	260-303	115.6-132.4	330.2-418.6	15.6-20.8	51.0-56.8	12.6-13.2
	mg%		44.05	133.00	6.47	19.5	4.58
	Range		38.15-50.92	127.00-138.15			
DE, Cont'd							
24 Hr.	g, mg	264.0±8.9	113.93±7.39	362.23±34.24	17.40	56.77	14.70
	Range	233-288	98.2-146.0	313.4-530.2	9.6-21.0	49.2-61.6	12.2-16.4
	mg%		43.16±2.14	137.21±16.23	6.59	21.50	5.57
	Range		38.67-51.96	111.53-213.79			
48 Hr.	g, mg	269.6±9.0	126.6±11.69	375.0±16.71	15.35	66.2	15.28
	Range	241-296	91.8-154.6	323.2-404.8	12.6-18.0	51.8-93.6	10.2-20.4
	mg%		46.96±4.67	139.09±8.48	5.84[g]	24.55	5.67
	Range		34.77-55.61	120.15-165.48			

[a]Abbreviations for stages: PPE: preproestrus; PE: proestrus; E: estrus; ME: metestrus; DE: diestrus.

[b]Body weights are shown in grams (g); organ weights in milligrams (mg).

[c]The arithmetic mean is followed by the standard error of the mean in the case of body weights in grams and ovarian and uterine weights in milligrams and milligrams per cent.

- continued -

TABLE 6--Continued

[d]Only three observations of classical metestrus were obtained from a large group of animals; therefore, the standard error is not shown for this group. It is also not shown for diestrus of 12 hours, group subscript 2, in which there were only two animals, or for diestrus of 12 hours, group subscript 1, for which only one animal was observed.

[e]Observation of an animal which exhibited an "L^4" or total leucocytic vaginal smear 12 hours after the last estrous smear was observed, and during the same daylight period.

[f]Observation of two animals which exhibited an "L^4" or total leucocytic vaginal smear 12 hours after the last estrous smear was observed, but after a dark period (estrous smear in the evening, diestrus smear the following morning).

[g]Represents four observations and is based on the average body weight of the four rats represented.

for breeding. Animals at least twice the age of the young adult, cycling animals were thus studied. This proved quite rewarding inasmuch as it was possible to corroborate the findings reported and analyzed for the animals 55 to 66 days of age. Gravimetric data obtained from rats 124 to 133 days of age revealed very similar trends for uteri and ovaries as observed in the group of rats 55 to 66 days of age. The thyroids, however, were found to be heaviest during diestrus. The adrenal glands in both groups were heaviest during estrus (tables 5 and 6). Most interestingly, the pituitary glands showed a continued increase from preproestrus through metestrus, thereafter showed a marked decrease in diestrus of approximately 12 hours post estrus, but not appreciably so during late diestrus, cf. also the milligram per cent values (table 6).

Cytological and histomorphological assessments revealed uterine and ovarian data of a very similar nature to that already described for animals 55 to 66 days of age.

B. Ovariectomized, Experimental Rats Receiving Single or Multiple Dosages of Female Sex Steroid Hormones, Estradiol-17β, Progesterone, or a Combined Treatment of Estradiol-17β and Progesterone.

1. Uterine Data, Plates XXXIII to XXXV, Figures 85 to 96.

These studies revealed that the vehicle used for the ovarian sex steroid hormones, sesame oil, is without any immediate mitogenic or growth promoting activity during the treatment periods

five minutes to three hours after single dosages (tables 7-9, 17; plate XXXIII, figures 85-88; plate XXXIV, figure 89), thus providing an excellent base-line for the correct interpretations of ovarian sex hormonally induced effects on the uterus and endocrine glands of bilaterally ovariectomized rats treated one week after surgical castration.

Concerning the effects of separate, single or multiple-daily injections of either estradiol-17β or progesterone alone, or dual injections of estradiol-17β and progesterone for treatment periods of time ranging from five minutes through 17 days, several facts are of noteworthy importance. First, the hormonal vehicle, sesame oil, does not induce growth promoting effects throughout the experimental period. Secondly, estradiol-17β, an active growth promoter of the several components of the reproductive tract of the rat, elicited stromal edema, a feature of early uterine awakening, within three hours after subcutaneous administration of a 0.1 microgram dosage. Similar time exposure to 1.5 milligrams of progesterone did not produce any uterine stimulation, whereas the combined administration of 0.1 microgram estradiol-17β and 1.5 milligrams progesterone also induced stromal edema (table 8; plate XXXIV, figures 89-92). It is also at the three hour time level that uterine weights for both the estradiol-17β and combined treatment groups become significantly greater than the uterine weights of the concommitant sesame oil controls (table 17).

Footnotes to Tables 7 - 16, common to more than one table.

[a]Ses. Oil = Sesame oil, hormonal vehicle.

[b]The controls (rats receiving only sesame oil) were a single group for the time periods five minutes through one hour.

[c]Figures in parentheses are always the range observed for the given group under consideration.

[d]Est.-17beta = estradiol-17beta, 0.1 microgram per dosage.

[e]The mean is followed by the Standard Error of the mean.

[f]Prog. = progesterone, 1.5 milligram per dosage.

[g]Est.-17beta + prog. = groups receiving one dosage of each of the two steroids at separate sites per dosage.

Milligrams per cent were determined in each case by calculating against the body weights represented by the organ samples in the particular organ group being considered.

TABLE 7

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
5 TO 15 MINUTES AFTER SINGLE DOSAGE OF ESTRADIOL-17β, PROGESTERONE,
OR ESTRADIOL-17β AND PROGESTERONE ADMINISTERED SUBCUTANEOUSLY

	Time After Injection					
	5 Minutes		10 Minutes		15 Minutes	
BODY WEIGHTS	in grams		in grams		in grams	
Treatment						
Ses. Oil[a]			179.7±3.8[b]	(134-215)[c]		
Est.-17β[d]	194.0±8.7[e]	(163-224)	169.9±6.8	(144-198)	203.1±7.3	(178-228)
Prog.[f]	191.0±4.6	(176-209)	190.0±4.2	(169-203)	201.7±3.6	(194-222)
Est.-17β[g] + Prog.	193.7±2.9	(182-204)	181.6±3.44	(170-193)	193.9±3.5	(181-211)

UTERINE WTS.	in mg	in mg%	in mg	in mg%	in mg	in mg%
Treatment						
Ses. Oil			79.0±3.7[b] (46.2-126.6)	43.9±1.8 (31.1-68.4)		
Est.-17β	81.6±8.6 (55.2-108.0)	42.1±3.3 (33.9-54.8)	76.8±5.3 (54.2-94.6)	45.2±2.9 (32.9-56.0)	88.4±4.5 (70.6-102.6)	43.5±2.1 (34.6-51.0)
Prog.	85.6±6.6 (51.6-108.2)	44.8±3.6 (25.9-54.1)	81.0±3.3 (70.6-93.6)	42.7±2.1 (36.8-48.9)	96.0±5.1 (73.8-110.8)	47.6±2.9 (37.3-57.1)
Est.-17β + Prog.	96.9±9.0 (72.2-144.2)	50.1±4.2 (39.7-72.1)	79.3±8.2 (53.8-120.4)	43.7±4.1 (29.6-62.4)	100.5±6.8 (64.4-117.8)	51.8±3.3 (35.6-60.7)

- continued -

TABLE 7--Continued

	Time After Injection								
	5 Minutes			10 Minutes			15 Minutes		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	----------			13.3	(9.0-20.0)	7.4	----------		
Est.-17β	15.1	(12.2-18.6)	7.8	11.7	(9.6-14.0)	6.9	14.9	(12.6-18.4)	7.3
Prog.	19.2	(14.6-25.6)	10.1	13.7	(9.2-17.2)	7.2	14.5	(12.2-18.2)	7.2
Est.-17β + Prog.	13.5	(9.2-21.2)	7.0	12.7	(10.8-14.8)	7.0	16.0	(13.2-19.4)	8.2
ADRENAL WTS. Treatment									
Ses. Oil	----------			45.3	(30.4-63.8)	25.2	----------		
Est.-17β	51.2	(40.8-67.6)	26.4	45.0	(37.0-49.0)	26.5	47.4	(35.6-53.6)	23.3
Prog.	50.6	(45.8-59.6)	26.5	48.0	(38.8-53.8)	25.3	50.5	(43.8-55.6)	25.1
Est.-17β + Prog.	51.0	(42.2-59.2)	26.3	46.2	(38.0-56.8)	25.4	48.9	(43.6-55.2)	25.2
PITUITARY WTS. Treatment									
Ses. Oil	----------			7.8	(5.8-11.6)	4.3	----------		
Est.-17β	8.7	(6.2-11.6)	4.5	6.3	(4.8-9.0)	3.7	8.8	(6.2-10.2)	4.3
Prog.	10.9	(9.0-13.6)	5.7	8.3	(6.6-9.4)	4.4	10.5	(8.8-12.8)	5.2
Est.-17β + Prog.	9.5	(8.2-10.6)	4.9	8.3	(6.0-9.8)	4.5	9.6	(7.8-12.4)	4.9

a,b,c,d,e,f,g Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

TABLE 8

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
30 TO 60 MINUTES AFTER SINGLE DOSAGE OF ESTRADIOL-17β, PROGESTERONE,
OR ESTRADIOL-17β AND PROGESTERONE ADMINISTERED SUBCUTANEOUSLY

	Time After Injection					
	30 Minutes		45 Minutes		60 Minutes	
BODY WEIGHTS	in grams		in grams		in grams	
Treatment						
Ses. Oil[a]			179.7±3.8[b]	(134-215)[c]		
Est.-17β[d]	200.7±6.7[e]	(179-227)	197.7±5.6	(172-220)	184.3±4.2	(165-214)
Prog.[f]	184.6±8.3	(142-212)	189.1±4.0	(174-200)	201.5±7.3	(174-233)
Est.-17β[g] + Prog.	204.9±4.5	(193-225)	193.7±5.4	(166-209)	187.3±3.7	(170-205)
UTERINE WTS.	in mg	in mg%	in mg	in mg%	in mg	in mg%
Treatment						
Ses. Oil			79.0±3.7[b] (46.2-126.6)	43.9±1.8 (31.1-68.4)		
Est.-17β	96.7±7.6 (65.6-122.4)	48.2±4.5 (28.9-64.4)	100.1±5.0 (70.8-117.2)	50.9±2.9 (32.5-62.2)	99.2±6.1 (65.0-149.2)	53.8±2.5 (39.4-73.1)
Prog.	106.7±9.1 (80.6-151.8)	57.8±6.9 (45.0-87.9)	108.0±9.9 (77.8-140.6)	57.1±4.9 (41.8-71.4)	110.4±7.1 (81.6-152.2)	54.8±2.6 (37.3-65.3)
Est.-17β + Prog.	112.7±4.4 (101.8-135.0)	55.0±1.4 (50.2-60.0)	97.2±7.2 (64.2-126.0)	50.2±3.2 (38.7-65.6)	103.0±7.8 (68.4-132.2)	55.0±3.9 (36.4-68.5)

- continued -

TABLE 8--Continued

	Time After Injection								
	30 Minutes			45 Minutes			60 Minutes		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	----------			13.3	(9.0-20.0)	7.4	----------		
Est.-17β	13.9	(11.8-16.4)	6.8	15.7	(12.4-19.2)	7.9	11.3	(7.8-16.0)	6.1
Prog.	15.4	(13.6-19.0)	8.3	17.4	(15.6-20.4)	9.2	13.0	(10.4-16.4)	6.4
Est.-17β + Prog.	16.4	(13.8-19.4)	8.0	17.1	(12.4-21.2)	8.8	11.3	(9.6-14.2)	6.1
ADRENAL WTS. Treatment									
Ses. Oil	----------			45.3	(30.4-63.8)	25.2	----------		
Est.-17β	53.3	(48.4-59.4)	26.6	53.2	(43.0-64.2)	26.9	47.6	(39.2-54.8)	25.8
Prog.	56.7	(47.4-63.2)	30.7	55.9	(48.0-63.2)	29.6	51.4	(42.0-55.2)	25.5
Est.-17β + Prog.	61.6	(54.4-68.4)	30.1	59.7	(50.4-67.4)	30.8	50.8	(46.2-58.6)	27.1
PITUITARY WTS. Treatment									
Ses. Oil	----------			7.8	(5.8-11.6)	4.3	----------		
Est.-17β	8.5	(7.0-10.4)	4.2	10.4	(8.4-11.6)	5.3	7.7	(5.2-10.4)	4.2
Prog.	7.9	(6.6-9.0)	4.3	8.2	(7.2-9.6)	4.3	9.4	(8.2-10.6)	4.6
Est.-17β + Prog.	8.5	(6.2-9.4)	4.1	10.0	(7.2-12.2)	5.2	9.1	(7.8-10.2)	4.9

a,b,c,d,e,f,g Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

TABLE 9

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
90 MINUTES TO 3 HOURS AFTER SINGLE DOSAGE OF ESTRADIOL-17β, PROGESTERONE,
OR ESTRADIOL-17β AND PROGESTERONE ADMINISTERED SUBCUTANEOUSLY

	Time After Injection					
	90 Minutes		2 Hours		3 Hours	
BODY WEIGHTS	in grams		in grams		in grams	
Treatment						
Ses. Oil[a]	191.7±6.5[e]	(175-225)[c]	198.3±1.7	(195-208)	190.7±3.6	(183-204)
Est.-17β[d]	189.0±4.9	(168-216)	203.9±2.7	(198-215)	192.9±2.7	(183-207)
Prog.[f]	182.0±3.8	(164-210)	200.0±2.8	(188-212)	193.7±6.2	(172-217)
Est.-17β + Prog.[g]	175.6±5.7	(154-194)	201.6±3.9	(191-222)	201.0±3.4	(189-209)
UTERINE WTS.	in mg	in mg%	in mg	in mg%	in mg	in mg%
Treatment						
Ses. Oil	81.1±4.8 (65.6-101.0)	42.3±3.0 (32.5-57.4)	96.8±6.0 (75.4-118.2)	48.8±3.0 (38.7-59.7)	95.9±9.6 (76.0-138.4)	50.3±5.2 (40.2-74.4)
Est.-17β	89.6±5.8 (60.0-120.4)	47.4±3.3 (33.4-68.8)	108.4±5.5 (87.6-123.8)	52.9±2.4 (44.5-58.1)	139.1±10.3 (111.8-200.4)	72.1±5.3 (59.8-103.3)
Prog.	96.7±5.2 (76.4-133.4)	53.1±4.6 (43.4-74.9)	105.1±4.5 (84.4-119.0)	52.5±2.4 (43.3-60.1)	113.5±8.0 (77.0-147.6)	58.6±3.8 (44.8-76.1)
Est.-17β + Prog.	87.9±7.8 (59.0-111.6)	50.1±4.0 (35.5-64.9)	124.2±9.8 (96.6-163.8)	61.6±4.3 (49.3-73.8)	134.1±7.9 (112.0-159.6)	66.7±3.7 (54.9-78.2)

- continued -

TABLE 9--Continued

	Time After Injection								
	90 Minutes			2 Hours			3 Hours		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	15.4	(10.8-18.2)	8.0	13.4	(10.0-16.6)	6.7	13.2	(10.8-18.2)	6.9
Est.-17β	15.6	(11.8-19.4)	8.2	15.1	(10.8-18.8)	7.4	14.9	(10.2-19.4)	7.7
Prog.	13.4	(9.0-19.6)	7.3	15.6	(10.4-19.0)	7.8	13.5	(10.6-18.2)	7.0
Est.-17β + Prog.	15.7	(13.0-18.8)	8.9	13.5	(10.6-17.4)	6.7	15.4	(12.2-20.0)	7.6
ADRENAL WTS. Treatment									
Ses. Oil	49.1	(35.2-56.4)	25.6	59.0	(48.2-65.6)	29.7	52.9	(34.6-64.6)	27.7
Est.-17β	52.5	(42.4-66.4)	27.8	54.0	(46.4-61.0)	26.5	48.0	(37.0-60.4)	24.9
Prog.	49.8	(40.4-60.8)	27.3	55.4	(49.4-62.2)	27.7	51.4	(42.6-61.0)	26.6
Est.-17β + Prog.	46.8	(40.4-52.4)	26.6	52.6	(47.8-58.2)	26.2	62.2	(51.8-67.6)	30.9
PITUITARY WTS. Treatment									
Ses. Oil	9.3	(7.4-11.4)	4.8	9.9	(7.4-18.6)	5.0	6.9	(6.2-7.8)	3.6
Est.-17β	10.3	(8.2-13.6)	5.4	10.5	(8.8-14.4)	5.1	9.9	(7.4-14.4)	5.1
Prog.	9.8	(7.6-14.8)	5.4	10.8	(8.8-12.2)	5.4	10.3	(5.6-15.2)	5.3
Est.-17β + Prog.	9.4	(7.0-11.4)	5.4	10.2	(8.6-12.4)	5.1	11.5	(6.6-13.8)	5.7

a,c,d,e,g Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

Gravimetrically, it appears that the uterine response is more marked at three hours than at six hours (tables 9 and 10). At eight and 11 hours the response to estradiol-17β and estradiol-17β plus progesterone appears quite demonstrable (tables 11 and 12). Likewise, at 15 and 18 hours the response to a single injection of 0.1 microgram estradiol-17β is markedly obvious (table 13). The combined effects of estradiol-17β and progesterone are significantly greater at 15 and 18 hours than at 21 hours. Progesterone, however, is not a vigorous uterine growth promoter, and for the most part elicited only questionable and erratic effects on uterine weight, slight effects appearing at six, eight, nine and 21 hours after administration of a single injection of 1.5 milligrams progesterone (tables 10, 11 and 13; and 17).

In the extended treatment injection schedules ranging from one day through 17 days, it appears that three daily injections of 0.1 microgram estradiol-17β produced a marked uterine growth promoting action over a 72 hour period, inducing a 100 per cent increase in weight over the sesame oil control, and thus containing the hallmark of uterine horizon I; progesterone administered alone elicited the least amount of weight increase during this time period (tables 14-16). On the other hand, on examination of data for the time periods through five, ten and 17 days for rats treated with estradiol-17β or the combination of estradiol-17β plus progesterone, it appeared obvious that the longer the exposure of the animal to these treatments, the more distinctive was the

TABLE 10

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
4 TO 6 HOURS AFTER SINGLE DOSAGE OF ESTRADIOL-17β, PROGESTERONE
OR ESTRADIOL-17β AND PROGESTERONE ADMINISTERED SUBCUTANEOUSLY

	Time After Injection					
	4 Hours		5 Hours		6 Hours	
BODY WEIGHTS Treatment	in grams		in grams		in grams	
Ses. Oil[a]	204.7±16.4[e]	(178-282)[c]	206.6±4.9	(180-220)	198.7±5.0	(175-210)
Est.-17β[d]	196.4±6.2	(180-220)	183.0±2.9	(175-199)	205.4±4.8	(191-228)
Prog.[f]	200.6±6.9	(190-240)	204.7±4.5	(185-221)	206.4±6.1	(185-225)
Est.-17β + Prog.[g]	196.3±6.5	(170-220)	207.9±2.3	(192-211)	187.2±2.7	(175-207)
UTERINE WTS. Treatment	in mg	in mg%	in mg	in mg%	in mg	in mg%
Ses. Oil	87.8±5.4 (72.4-104.4)	42.9±2.1 (35.6-49.7)	95.9±3.8 (83.2-108.4)	46.4±2.3 (39.6-56.1)	110.5±3.2 (97.4-117.0)	56.6±2.0 (46.4-59.5)
Est.-17β	103.3±7.0 (84.2-135.2)	52.6±2.4 (46.8-64.4)	132.2±8.6 (85.8-151.4)	72.1±4.1 (67.7-86.5)	125.3±10.3 (105.8-166.2)	61.0±5.0 (49.8-79.9)
Prog.	91.9±5.3 (63.8-102.8)	45.8±2.5 (33.6-53.2)	106.3±7.3 (77.2-136.4)	51.9±2.9 (41.1-64.1)	87.3±2.9 (80.0-100.0)	42.3±2.1 (37.4-52.1)
Est.-17β + Prog.	88.9±8.9 (53.2-132.0)	45.3±4.2 (26.6-60.0)	128.8±8.9 (112.6-189.0)	62.0±4.2 (53.7-89.6)	107.6±6.3 (89.8-154.8)	57.5±2.8 (48.1-79.4)

- continued -

TABLE 10--Continued

	Time After Injection								
	4 Hours			5 Hours			6 Hours		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	12.3	(10.4-14.2)	6.2	13.3	(8.0-15.6)	6.4	15.1	(11.8-20.4)	7.7
Est.-17β	9.4	(7.2-12.6)	4.8	12.7	(12.2-14.4)	6.9	15.5	(12.4-21.0)	7.6
Prog.	12.6	(10.2-17.2)	6.3	13.8	(10.6-17.4)	6.8	14.5	(11.8-19.4)	7.0
Est.-17β + Prog.	11.5	(9.8-14.0)	5.9	14.3	(11.4-18.0)	6.9	16.5	(12.0-23.6)	8.8
ADRENAL WTS. Treatment									
Ses. Oil	48.4	(41.6-53.8)	24.0	49.3	(36.8-59.8)	23.9	51.2	(37.6-57.8)	26.2
Est.-17β	47.5	(38.4-58.2)	24.1	51.1	(44.6-60.4)	28.1	52.9	(44.2-65.4)	25.8
Prog.	48.4	(44.2-59.2)	24.1	49.3	(44.2-55.4)	24.1	54.7	(49.8-58.4)	26.5
Est.-17β + Prog.	43.0	(35.4-48.8)	21.9	54.5	(42.4-71.6)	26.2	57.1	(48.6-64.4)	30.5
PITUITARY WTS. Treatment									
Ses. Oil	4.7	(3.4-6.0)	2.3	7.0	(4.4-11.0)	3.4	8.4	(7.0-9.4)	4.3
Est.-17β	4.5	(3.6-5.8)	2.3	7.0	(5.8-8.4)	3.8	9.7	(7.8-11.6)	4.7
Prog.	6.1	(3.6-11.2)	3.0	8.6	(6.4-12.6)	4.2	9.6	(8.2-11.4)	4.6
Est.-17β + Prog.	4.9	(2.4-7.2)	2.5	9.1	(6.4-12.4)	4.4	10.2	(8.6-14.6)	5.4

a,c,d,e,f,g Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

TABLE 11

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
7 TO 9 HOURS AFTER SINGLE DOSAGE OF ESTRADIOL-17β, PROGESTERONE,
OR ESTRADIOL-17β AND PROGESTERONE ADMINISTERED SUBCUTANEOUSLY

	Time After Injection					
	7 Hours		8 Hours		9 Hours	
BODY WEIGHTS Treatment	in grams		in grams		in grams	
Ses. Oil[a]	189.2±5.7[e]	(168-210)[c]	176.2±3.3	(163-184)	187.0±6.2	(165-207)
Est.-17β[d]	184.3±4.9	(167-207)	177.9±5.4	(159-198)	187.0±7.5	(152-210)
Prog.[f]	177.2±5.1	(153-186)	178.6±3.9	(161-189)	175.7±7.9	(151-204)
Est.-17β + Prog.[g]	189.0±4.0	(180-209)	179.9±3.4	(165-191)	180.6±7.5	(140-198)
UTERINE WTS. Treatment	in mg	in mg%	in mg	in mg%	in mg	in mg%
Ses. Oil	96.1±1.6 (92.0-102.2)	50.8±1.3 (47.1-55.8)	90.2±5.1 (65.8-101.6)	51.2±2.3 (40.4-56.1)	92.3±6.5 (65.4-115.0)	49.3±2.6 (39.2-56.1)
Est.-17β	134.0±9.2 (95.2-159.6)	72.7±4.4 (50.9-84.9)	152.3±10.8 (118.4-185.5)	85.6±4.0 (71.3-99.9)	146.5±8.6 (104.6-174.6)	78.4±3.9 (68.8-94.1)
Prog.	93.7±10.6 (73.6-140.2)	52.9±5.3 (41.4-77.0)	113.4±6.8 (84.4-136.2)	63.5±4.5 (49.4-84.6)	104.7±8.0 (65.8-136.6)	59.6±3.8 (38.7-68.0)
Est.-17β + Prog.[g]	126.1±6.3 (110.4-160.8)	66.7±3.8 (59.9-88.3)	136.6±7.3 (100.6-160.4)	75.9±3.2 (59.2-87.2)	122.4±7.8 (96.4-155.2)	67.8±4.9 (52.4-89.0)

- continued -

TABLE 11--Continued

	Time After Injection								
	7 Hours			8 Hours			9 Hours		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	15.2	(12.2-17.8)	8.0	16.3	(13.6-19.4)	9.2	17.9	(15.0-20.6)	9.6
Est.-17β	16.3	(14.2-19.2)	8.8	16.0	(12.8-19.4)	9.0	18.5	(12.8-23.0)	9.9
Prog.	15.2	(12.2-17.8)	8.6	13.3	(10.0-17.2)	7.5	18.8	(16.8-21.6)	10.7
Est.-17β + Prog.	20.4	(16.4-25.6)	10.8	16.2	(12.0-19.6)	9.0	19.3	(13.6-25.6)	10.7
ADRENAL WTS. Treatment									
Ses. Oil	50.2	(40.8-62.2)	26.5	48.8	(37.2-63.0)	27.7	49.6	(41.2-56.6)	26.5
Est.-17β	48.8	(41.0-60.0)	26.5	41.4	(31.6-48.0)	23.3	53.7	(51.0-56.4)	28.7
Prog.	47.8	(40.2-50.0)	27.0	47.8	(42.4-57.4)	26.7	50.9	(42.6-63.6)	29.0
Est.-17β + Prog.	55.9	(50.0-74.6)	29.6	50.2	(43.0-55.6)	27.9	51.7	(40.8-58.6)	28.6
PITUITARY WTS. Treatment									
Ses. Oil	9.2	(6.4-11.0)	4.9	9.3	(7.6-10.4)	5.3	11.7	(9.2-14.6)	6.3
Est.-17β	10.3	(8.6-12.0)	5.6	9.3	(7.4-11.2)	5.2	12.1	(10.6-13.6)	6.5
Prog.	10.0	(9.0-11.0)	5.6	9.2	(6.4-11.2)	5.1	11.0	(8.2-13.4)	6.3
Est.-17β + Prog.	13.2	(12.2-14.6)	7.0	10.5	(8.2-12.0)	5.8	12.6	(10.2-15.4)	7.0

a,c,d,e,f,g Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

TABLE 12

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
10 TO 12 HOURS AFTER SINGLE DOSAGE OF ESTRADIOL-17β, PROGESTERONE,
OR ESTRADIOL-17β AND PROGESTERONE ADMINISTERED SUBCUTANEOUSLY

	Time After Injection					
	10 Hours		11 Hours		12 Hours	
BODY WEIGHTS Treatment	in grams		in grams		in grams	
Ses. Oil[a]	186.6±7.1[e]	(165-217)[c]	209.4±8.0	(195-237)	192.8±4.3	(179-208)
Est.-17β[d]	183.0±9.8	(126-198)	207.0±3.7	(189-219)	185.7±7.0	(155-202)
Prog.[f]	177.7±3.2	(167-190)	199.9±8.4	(156-217)	184.0±3.9	(173-195)
Est.-17β + Prog.[g]	171.6±4.4	(155-191)	202.1±4.0	(186-214)	194.4±5.8	(173-212)

UTERINE WTS. Treatment	in mg	in mg%	in mg	in mg%	in mg	in mg%
Ses. Oil	93.7±3.8 (76.8-107.4)	50.2±1.7 (45.3-56.3)	102.0±3.9 (90.0-114.4)	48.7±2.9 (41.3-56.9)	81.2±8.0 (48.2-107.0)	42.1±4.0 (26.9-57.5)
Est.-17β	124.1±9.9 (95.2-174.0)	67.8±11.7 (50.4-138.1)	142.1±6.8 (109.2-165.8)	68.7±3.1 (51.7-75.7)	129.9±6.3 (103.0-149.2)	69.9±5.1 (60.2-93.5)
Prog.	106.0±10.2 (72.2-139.2)	59.7±5.3 (40.3-73.3)	93.1±9.6 (54.4-132.6)	46.6±3.4 (34.9-62.3)	72.0±5.6 (57.8-91.6)	39.1±2.7 (30.6-47.0)
Est.-17β + Prog.	127.3±7.7 (112.0-166.0)	74.2±3.2 (65.9-86.9)	134.0±4.0 (95.6-168.4)	66.3±4.8 (46.4-80.6)	126.1±6.6 (103.8-157.4)	68.5±2.9 (55.9-74.3)

- continued -

TABLE 12--Continued

	Time After Injection								
	10 Hours			11 Hours			12 Hours		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	15.2	(12.6-18.4)	8.1	13.5	(11.0-15.4)	6.5	14.6	(12.4-18.4)	7.6
Est.-17β	16.3	(12.4-19.4)	8.9	18.3	(13.2-21.4)	8.8	13.5	(11.6-16.2)	7.3
Prog.	16.0	(12.8-19.6)	9.0	15.7	(11.6-18.6)	7.9	14.0	(13.0-16.2)	7.6
Est.-17β + Prog.	14.0	(8.6-18.6)	8.2	15.9	(14.0-17.6)	7.9	13.6	(11.4-15.8)	7.4
ADRENAL WTS. Treatment									
Ses. Oil	53.5	(44.6-63.2)	28.7	58.5	(50.2-73.8)	27.9	49.0	(42.0-58.0)	23.4
Est.-17β	53.1	(48.4-59.2)	29.0	55.7	(47.0-61.4)	26.9	51.7	(38.8-62.4)	27.8
Prog.	53.9	(46.0-66.0)	30.3	53.1	(42.6-66.0)	26.5	49.8	(46.4-53.2)	27.0
Est.-17β + Prog.	47.5	(40.2-52.8)	27.7	54.2	(49.4-60.4)	26.8	54.7	(45.6-63.8)	28.1
PITUITARY WTS. Treatment									
Ses. Oil	7.2	(6.2-7.8)	3.8	8.7	(7.8-9.6)	4.2	9.3	(8.2-10.0)	4.8
Est.-17β	8.5	(6.0-12.4)	4.7	11.2	(9.2-13.2)	5.4	9.7	(8.4-10.6)	5.2
Prog.	9.8	(7.2-13.2)	5.5	10.2	(9.2-11.6)	5.1	9.5	(9.2-10.0)	5.1
Est.-17β + Prog.	9.2	(6.6-12.6)	5.4	10.3	(7.6-12.8)	5.1	10.0	(8.2-12.0)	5.4

a,c,d,e,f,g Explanation for all footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

TABLE 13

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
15 TO 21 HOURS AFTER SINGLE DOSAGE OF ESTRADIOL-17β, PROGESTERONE,
OR ESTRADIOL-17β AND PROGESTERONE ADMINISTERED SUBCUTANEOUSLY

	Time After Injection					
	15 Hours		18 Hours		21 Hours	
BODY WEIGHTS	in grams		in grams		in grams	
Treatment						
Ses. Oil	194.7±5.7[e]	(170-217)[c]	204.7±4.5	(182-217)	187.2±3.7	(177-201)
Est.-17β[d]	186.7±4.2	(173-199)	202.3±4.2	(189-218)	199.0±1.7	(193-205)
Prog.[f]	181.1±7.0	(155-201)	206.1±5.7	(178-221)	202.6±5.7	(178-220)
Est.-17β + Prog.[g]	185.3±4.1	(162-194)	208.9±2.4	(202-222)	190.5±4.3	(159-213)
UTERINE WTS.	in mg	in mg%	in mg	in mg%	in mg	in mg%
Treatment						
Ses. Oil	98.3±5.2 (78.8-117.8)	50.5±2.4 (40.0-54.7)	101.7±4.3 (85.4-119.4)	49.7±2.7 (40.1-61.0)	97.3±5.1 (75.6-111.4)	52.0±2.7 (41.1-58.2)
Est.-17β	133.5±14.2 (87.0-194.4)	71.5±7.6 (43.7-97.7)	137.1±9.9 (112.8-168.2)	67.7±5.7 (50.8-83.7)	130.6±13.01 (85.0-166.4)	65.6±6.7 (43.1-86.2)
Prog.	88.9±8.3 (54.6-122.2)	49.1±4.0 (31.9-62.2)	123.4±10.1 (74.6-156.0)	59.9±4.0 (41.9-72.2)	90.8±5.9 (69.0-116.0)	44.8±1.8 (38.8-53.5)
Est.-17β + Prog.	124.5±4.1 (112.6-143.6)	67.2±2.4 (59.7-76.8)	159.5±8.1 (124.8-191.6)	76.4±3.4 (60.3-86.3)	113.6±6.1 (66.6-155.6)	59.6±2.8 (38.7-78.6)

- continued -

TABLE 13--Continued

	Time After Injection								
	15 Hours			18 Hours			21 Hours		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	16.3	(11.0-20.4)	8.4	15.2	(14.2-16.4)	7.4	16.4	(11.8-22.0)	8.8
Est.-17β	17.1	(14.4-20.6)	9.1	15.2	(10.6-18.6)	7.5	15.5	(10.4-20.2)	7.8
Prog.	17.1	(13.6-20.6)	9.5	15.5	(11.0-18.0)	7.5	14.1	(12.6-17.4)	6.9
Est.-17β + Prog.	16.9	(13.6-20.0)	9.1	16.9	(15.2-19.2)	8.1	14.7	(8.6-19.4)	7.7
ADRENAL WTS. Treatment									
Ses. Oil	55.3	(45.6-60.2)	27.8	58.0	(51.0-64.8)	28.3	52.5	(42.4-58.6)	28.0
Est.-17β	51.0	(37.6-63.6)	27.3	55.7	(48.2-63.6)	27.5	53.4	(38.4-61.0)	26.9
Prog.	54.5	(42.6-75.0)	30.1	56.9	(49.8-65.8)	27.6	52.4	(46.4-59.8)	25.9
Est.-17β + Prog.	49.7	(45.0-53.6)	26.8	62.4	(54.4-75.6)	29.9	51.0	(44.0-67.2)	26.7
PITUITARY WTS. Treatment									
Ses. Oil	11.5	(5.8-15.6)	5.9	10.1	(8.0-11.6)	4.9	11.8	(8.2-16.0)	6.3
Est.-17β	13.4	(9.4-18.2)	7.2	10.6	(10.0-11.4)	5.2	10.5	(9.0-12.2)	5.3
Prog.	13.9	(12.4-17.0)	7.7	11.1	(8.4-12.4)	5.4	10.0	(9.0-10.8)	4.9
Est.-17β + Prog.	13.9	(12.8-16.2)	7.5	11.0	(8.6-14.0)	5.3	9.9	(8.2-12.0)	5.2

a,c,d,e,f,g Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

TABLE 14

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS OF YOUNG, ADULT OVARIECTOMIZED RATS GIVEN EITHER ESTRADIOL-17β, PROGESTERONE, OR A COMBINATION OF ESTRADIOL-17β AND PROGESTERONE FOR DAILY PERIODS: 1 TO 2 DAYS[h]

	Duration of Treatment Period					
	1 Day		2 Days[i]		2 Days[j]	
BODY WEIGHTS	in grams		in grams		in grams	
Treatment						
Ses. Oil[a]	194.4±5.8[e]	(165-240)[c]	208.4±3.3	(195-220)	190.1±6.1	(165-223)
Est.-17β[d]	190.5±2.7	(169-210)	204.1±2.4	(190-221)	201.6±5.4	(175-220)
Prog.[f]	183.1±3.1	(165-200)	196.2±4.6	(181-206)	201.5±4.6	(180-221)
Est.-17β + Prog.[g]	187.0±3.9	(169-210)	199.3±6.9	(171-226)	201.3±3.1	(189-214)
UTERINE WTS.	in mg	in mg%	in mg	in mg%	in mg	in mg%
Treatment						
Ses. Oil	82.6±7.4 (50.0-123.0)	42.5±2.9 (28.8-60.6)	102.8±7.5 (80.6-129.2)	49.3±3.3 (39.1-60.1)	99.1±7.9 (68.8-126.6)	52.1±3.5 (40.9-66.2)
Est.-17β	122.8±5.4 (78.6-146.6)	64.5±2.8 (43.7-75.8)	121.6±4.9 (99.6-154.2)	59.6±2.2 (49.3-75.1)	168.5±16.7 (103.0-238.4)	83.6±8.1 (54.2-119.5)
Prog.	87.3±3.8 (65.0-124.0)	47.7±1.8 (38.7-63.6)	86.5±6.3 (73.8-105.2)	44.1±2.5 (39.1-52.7)	101.7±4.5 (75.8-114.6)	50.5±2.4 (39.7-60.2)
Est.-17β + Prog.	112.9±6.6 (85.6-156.2)	60.4±3.1 (46.1-84.4)	114.1±6.1 (90.2-136.8)	57.3±4.8 (41.6-80.0)	155.7±9.2 (110.8-199.4)	77.4±4.1 (57.1-93.2)

- continued -

TABLE 14--Continued

	Duration of Treatment Period								
	1 Day			2 Days[i]			2 Days[j]		
THYROID WTS.	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Treatment									
Ses. Oil	13.0	(7.2-19.4)	6.7	12.7	(10.0-18.0)	6.1	14.8	(7.8-20.0)	7.8
Est.-17β	13.0	(8.0-17.6)	6.8	12.5	(7.8-12.6)	6.1	14.0	(12.6-16.6)	6.9
Prog.	12.1	(7.8-16.6)	6.6	11.2	(9.4-11.8)	5.7	11.0	(7.8-14.6)	5.5
Est.-17β + Prog.	12.7	(9.4-17.2)	6.8	12.3	(9.4-14.1)	6.2	13.1	(5.4-17.6)	6.5
ADRENAL WTS.									
Treatment									
Ses. Oil	50.4	(38.8-57.8)	25.9	54.6	(44.0-61.0)	26.2	51.6	(34.4-60.0)	27.2
Est.-17β	50.8	(43.0-64.8)	26.7	52.4	(37.2-69.6)	26.7	49.2	(39.4-60.6)	24.4
Prog.	45.0	(34.4-57.4)	24.6	49.0	(37.4-57.6)	25.0	51.1	(41.4-60.0)	25.3
Est.-17β + Prog.	50.5	(35.8-61.6)	27.0	49.7	(40.6-61.6)	25.0	54.0	(45.2-68.6)	26.8
PITUITARY WTS.									
Treatment									
Ses. Oil	9.6	(6.2-16.2)	4.9	9.7	(6.6-12.2)	4.7	8.7	(5.8-10.2)	4.6
Est.-17β	7.9	(5.0-10.4)	4.1	8.8	(7.0-11.2)	4.3	9.1	(7.2-12.4)	4.5
Prog.	8.6	(5.8-16.4)	4.7	7.2	(5.8-8.2)	3.7	8.0	(7.0-9.6)	4.0
Est.-17β + Prog.	8.9	(5.2-15.0)	4.7	8.0	(7.0-8.6)	4.0	9.3	(7.6-13.4)	4.6

[a,c,d,e,f,g]Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

[h]Animals were necropsied 24 or 48 hours after last unit dosage, cf. footnotes i and j.
[i]A single unit dosage given 48 hours before necropsy.
[j]Two daily dosages given, the last 24 hours before necropsy.

TABLE 15

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS
OF YOUNG, ADULT OVARIECTOMIZED RATS
GIVEN EITHER ESTRADIOL-17β, PROGESTERONE, OR
A COMBINATION OF ESTRADIOL-17β AND PROGESTERONE OVER THREE DAYS:
DAILY DOSAGE ON DAYS ONE, ONE AND THREE OR DAYS ONE THROUGH THREE[h]

	Dosage Schedule					
	One Dosage		Two Dosages		Three Dosages	
BODY WEIGHTS	in grams		in grams		in grams	
Treatment						
Ses. Oil[a]	211.3±8.1[e]	(183-232)[c]	199.6±3.8	(188-219)	217.1±3.9	(210-235)
Est.-17β[d]	212.3±4.7	(190-226)	195.4±5.6	(174-222)	208.4±13.8	(134-241)
Prog.[f]	211.7±7.0	(180-234)	196.7±5.0	(181-218)	210.1±4.7	(194-226)
Est.-17β + Prog.[g]	217.0±7.6	(216-248)	201.2±3.5	(191-211)	213.3±11.3	(178-251)

UTERINE WTS.	in mg	in mg%	in mg	in mg%	in mg	in mg%
Treatment						
Ses. Oil	86.2±4.9 (71.2-101.0)	40.8±1.8 (36.0-47.3)	61.4±2.9 (53.0-73.0)	30.7±1.2 (26.4-35.8)	97.6±7.2 (62 - 118)	44.9±3.0 (29.5-52.7)
Est.-17β	97.5±3.6 (82.8-111.8)	45.9±2.1 (39.2-52.8)	128.2±6.0 (112.0-152.8)	65.6±4.1 (55.5-83.6)	194.7±15.2 (131.0-250.6)	93.4±5.1 (69.5-112.3)
Prog.	93.3±6.7 (71.8-118.2)	44.1±2.8 (34.7-54.3)	78.5±7.8 (55.0-108.6)	39.9±4.5 (26.9-55.1)	99.3±5.9 (78.0-118.6)	47.2±2.6 (37.9-57.6)
Est.-17β + Prog.	98.5±5.9 (76.2-116.4)	45.4±2.7 (31.5-52.0)	136.8±3.0 (124.8-144.6)	68.0±1.9 (62.1-75.3)	163.9±12.9 (116.6-222.2)	76.8±4.3 (64.1-88.5)

- continued -

TABLE 15--Continued

	Dosage Schedule								
	One Dosage			Two Dosages			Three Dosages		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	15.1	(12.6-17.6)	7.2	12.6	(9.8-18.0)	6.3	----------		
Est.-17β	18.4	(11.6-24.0)	8.7	9.7	(7.2-13.8)	5.0	12.6	(9.6-17.4)	6.1
Prog.	20.0	(13.8-32.2)	9.5	10.6	(7.8-13.4)	5.4	16.0	(13.4-20.0)	7.6
Est.-17β + Prog.	17.1	(14.2-23.4)	7.7	12.3	(9.2-19.2)	6.2	16.0	(13.6-18.8)	7.5
ADRENAL WTS. Treatment									
Ses. Oil	56.0	(45.0-84.4)	26.5	46.2	(35.4-52.8)	23.1	----------		
Est.-17β	57.3	(45.4-69.0)	27.0	43.9	(31.8-51.2)	22 5	54.2	(37.6-64.8)	26.0
Prog.	58.5	(46.2-67.8)	27.6	48.6	(42.6-56.6)	24.7	54.8	(47.4-66.6)	26.3
Est.-17β + Prog.	60.1	(44.6-79.4)	27.7	53.0	(47.2-60.8)	26.5	57.2	(48.4-69.8)	26.8
PITUITARY WTS. Treatment									
Ses. Oil	11.2	(9.6-14.2)	5.3	6.1	(4.4-7.8)	3.1	----------		
Est.-17β	13.6	(9.6-16.0)	6.4	6.9	(5.8-8.6)	3.5	11.1	(6.6-18.6)	5.3
Prog.	14.6	(10.8-18.2)	6.9	6.3	(3.6-8.6)	3.2	11.5	(9.6-16.2)	5.5
Est.-17β + Prog.	11.5	(6.0-15.6)	5.3	7.7	(6.4-9.8)	3.9	11.3	(9.4-13.0)	5.3

[a,c,d,e,f,g]Explanation for footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

[h]Animals in groups receiving one daily unit dosage were necropsied 72 hours after injection; those in groups receiving two or three daily dosages were necropsied 24 hours after the last injection.

TABLE 16

GRAVIMETRIC COMPARISONS OF BODY, UTERUS AND ENDOCRINE GLANDS OF YOUNG, ADULT OVARIECTOMIZED RATS GIVEN ESTRADIOL-17β, PROGESTERONE, OR A COMBINATION OF ESTRADIOL-17β AND PROGESTERONE FOR PERIODS OF FROM 5 TO 17 DAYS IN A PATTERN BASED ON THE EXPERIMENTAL MODEL SYSTEM[h]

	Duration of Treatment Period					
	5 Days		10 Days		17 Days	
BODY WEIGHTS	in grams		in grams		in grams	
Treatment						
Ses. Oil[a]	219.7±2.8[e]	(209-229)[c]	236.0±7.8	(211-267)	279.3±10.3	(249-302)
Est.-17β[d]	217.3±4.1	(202-235)	222.1±9.0	(188-258)	253.5±8.2	(222-301)
Prog.[f]	214.0±4.5	(199-229)	233.3±10.2	(191-263)	276.0±6.4	(252-304)
Est.-17β + Prog.[g]	222.3±12.7	(168-272)	225.0±5.6	(205-248)	264.1±4.6	(240-281)
UTERINE WTS.	in mg	in mg%	in mg	in mg%	in mg	in mg%
Treatment						
Ses. Oil	64.1±4.1 (49.4-78.8)	29.2±1.9 (21.9-35.5)	78.2±8.1 (57.2-121.4)	33.1±3.1 (27.0-49.5)	81.4±4.7 (60.4-96.2)	29.1±1.9 (24.0-38.6)
Est.-17β	176.8±8.4 (135.0-209.6)	81.4±3.3 (66.8-96.1)	173.2±10.5 (130.8-214.0)	78.0±2.7 (69.6-90.3)	219.3±10.0 (180.8-264.6)	86.5±4.0 (73.5-102.8)
Prog.	96.4±7.6 (71.0-119.0)	45.0±3.7 (34.3-59.8)	66.8±7.4 43.8-97.2)	28.7±2.6 (21.4-38.7)	98.7±6.9 (71.6-130.4)	35.7±2.5 (25.4-44.7)
Est.-17β + Prog.	165.5±8.1 (138.0-208.6)	74.5±2.1 (64.4-82.1)	154.5±6.8 (126.2-174.6)	68.7±2.7 (61.6-78.3)	213.0±12.4 (169.4-280.0)	80.7±4.1 (64.2-100.0)

- continued -

TABLE 16--Continued

	Duration of Treatment Period								
	5 Days			10 Days			17 Days		
THYROID WTS. Treatment	in mg	mg range	in mg%	in mg	mg range	in mg%	in mg	mg range	in mg%
Ses. Oil	16.6	(13.8-20.8)	7.5	16.8	(11.2-20.2)	7.1	15.9	(13.0-23.8)	5.7
Est.-17β	15.7	(12.4-17.4)	7.2	18.1	(12.6-20.4)	8.1	15.7	(14.4-17.4)	6.2
Prog.	13.9	(12.2-16.2)	6.5	17.1	(12.6-20.6)	7.3	16.5	(14.0-19.4)	6.0
Est.-17β + Prog.	12.0	(8.2-17.4)	5.4	17.0	(13.6-20.0)	7.5	16.1	(12.8-21.0)	6.1
ADRENAL WTS. Treatment									
Ses. Oil	54.7	(41.2-71.6)	24.9	56.9	(43.4-67.2)	24.1	63.1	(51.2-78.0)	22.6
Est.-17β	53.0	(45.2-61.0)	24.4	65.5	(52.2-78.4)	29.5	57.0	(46.6-64.6)	22.5
Prog.	52.6	(47.8-56.4)	24.6	60.7	(41.8-67.8)	26.0	56.8	(44.4-68.0)	20.6
Est.-17β + Prog.	53.2	(43.2-63.4)	23.9	66.2	(48.2-77.0)	29.4	64.8	(57.2-71.0)	24.5
PITUITARY WTS. Treatment									
Ses. Oil	10.5	(9.0-12.0)	4.8	11.9	(9.2-14.4)	5.1	10.7	(7.2-15.6)	3.8
Est.-17β	12.8	(9.6-17.2)	5.9	12.4	(8.8-15.6)	5.6	13.2	(9.6-15.0)	5.2
Prog.	10.1	(8.0-10.6)	4.7	10.9	(8.6-13.2)	4.7	12.7	(9.8-14.4)	4.6
Est.-17β + Prog.	6.2	(3.8-8.6)	2.8	12.5	(10.4-13.8)	5.5	13.1	(11.2-14.8)	5.0

a,c,d,e,f,g Explanation of footnotes common to all tables 7 to 16 inclusive appear on a single sheet preceding table 7.

- continued -

TABLE 16-- Continued

[h]According to the Experimental Model System during the first four weeks of steroid hormone administration, four daily dosages are given during the first week and five are given per week during the next three weeks (Velardo, 1964). In this series, animals in the five and ten day treatment period groups were necropsied 24 hours after the last of three consecutive daily unit dosages; animals in the 17 day period 24 hours after the last of four consecutive daily unit dosages.

TABLE 17

SIGNIFICANCES OF DIFFERENCES OF UTERINE WEIGHTS IN MILLIGRAMS PER CENT FOR GROUPS OF OVARIECTOMIZED RATS GIVEN ESTRADIOL-17β, PROGESTERONE, OR A COMBINATION OF ESTRADIOL-17β, PROGESTERONE FOR ALL TIME PERIODS IN WHICH ONE OR MORE COMPARISONS SHOWED A STATISTICALLY SIGNIFICANT DIFFERENCE[a]

	Compared with Controls[b]			Expt'ls Compared with Each Other		
Time Group	E-17β[c]	Prog.[d]	E-17β + Prog.	E-17β vs. Prog.	E-17β vs. E-17β + Prog.	Prog. vs. E-17β + Prog.
30 Min. °F[e]	27	27	27	12	12	12
t[f]	0.861	1.938	4.789	1.164	1.436	0.397
P[g]	<0.400	<0.100	<0.001	<0.400	<0.200	>0.500
45 Min. °F	29	27	27	12	34	12
t	2.039	2.511	1.712	1.091	0.166	1.185
P	<0.100	<0.025	<0.100	<0.400	>0.500	<0.400
60 Min. °F	34	14	13	21	20	15
t	3.215	1.218	1.080	0.267	0.254	0.044
P	<0.005	<0.400	<0.400	>0.500	>0.500	>0.500
2 Hrs. °F	11	12	12	10	10	12
t	1.070	0.955	2.427	0.119	1.766	1.840
P	<0.400	<0.400	<0.050	>0.500	<0.200	<0.100
3 Hrs. °F	12	11	9	13	11	10
t	2.939	1.293	2.575	2.080	0.834	1.542
P	<0.025	<0.400	<0.050	<0.100	<0.500	<0.200
4 Hrs. °F	11	11	12	12	13	13
t	3.035	0.879	0.507	1.939	1.524	0.106
P	<0.025	<0.400	>0.500	<0.100	<0.200	>0.500
5 Hrs. °F	13	13	13	14	14	14
t	5.456	1.479	3.268	3.996	1.734	1.969
P	<0.001	<0.200	<0.010	<0.005	<0.200	<0.100
6 Hrs. °F	11	11	16	12	17	17
t	1.047	4.607	0.609	3.475	0.618	4.379
P	<0.500	<0.001	>0.500	<0.005	>0.500	<0.001
7 Hrs. °F	11	10	11	11	12	11
t	4.747	0.373	3.959	2.860	1.033	2.111
P	<0.001	>0.500	<0.005	<0.025	<0.400	<0.100

- continued -

TABLE 17--Continued

Time Group	Compared with Controls			Expt'ls Compared with Each Other		
	E-17β	Prog.	E-17β + Prog.	E-17β vs. Prog.	E-17β vs. E-17β + Prog.	Prog. vs. E-17β + Prog.
8 Hrs. °F	11	11	11	12	12	12
t	7.413	2.414	6.300	3.638	1.883	2.241
P	<0.001	<0.050	<0.001	<0.005	<0.100	<0.050
9 Hrs. °F	12	12	12	12	12	12
t	6.164	2.216	3.304	3.435	1.676	1.317
P	<0.001	<0.050	<0.010	<0.005	<0.200	<0.400
10 Hrs. °F	12	11	12	11	12	11
t	1.489	1.691	6.536	0.635	0.523	2.339
P	<0.200	<0.200	<0.001	>0.500	>0.500	<0.050
11 Hrs. °F	10	10	10	12	12	12
t	4.636	0.477	3.141	4.771	0.418	3.371
P	<0.001	>0.500	<0.025	<0.001	>0.500	<0.010
12 Hrs. °F	11	9	11	10	12	10
t	4.287	0.615	5.339	5.320	0.236	7.372
P	<0.005	>0.500	<0.001	<0.001	>0.500	<0.001
15 Hrs. °F	12	12	12	12	12	12
t	2.645	0.293	4.922	2.615	0.544	3.883
P	<0.025	>0.500	<0.001	<0.025	>0.500	<0.005
18 Hrs. °F	11	12	12	11	11	12
t	2.881	2.124	6.121	1.133	1.299	3.128
P	<0.025	<0.100	<0.001	<0.400	<0.400	<0.010
21 Hrs. °F	10	11	17	11	17	18
t	1.878	2.217	1.957	2.982	0.822	4.423
P	<0.100	<0.050	<0.100	<0.025	<0.500	<0.001
24 Hrs. °F	26	26	24	28	26	26
t	5.489	1.516	4.190	5.066	0.968	3.500
P	<0.001	<0.200	<0.001	<0.001	<0.400	<0.005
2 Days (1 Dose) °F	20	11	12	19	20	11
t	2.454	1.241	1.322	4.618	0.438	2.412
P	<0.025	<0.400	<0.400	<0.001	>0.500	<0.050
2 Days (2 Daily Dosages) °F	14	14	14	14	14	14
t	3.586	0.403	4.713	3.945	0.691	5.701
P	<0.005	>0.500	<0.001	<0.005	>0.500	<0.001

- continued -

TABLE 17--Continued

Time Group	Compared with Controls: E-17β	Prog.	E-17β + Prog.	Expt'ls Compared with Each Other: E-17β vs. Prog.	E-17β vs. E-17β + Prog.	Prog. vs. E-17β + Prog.
3 Days °F	12	12	11	12	11	11
(2 Daily t	8.063	1.943	16.859	4.173	0.530	5.723
Dosages) P	<0.001	<0.100	<0.001	<0.005	>0.500	<0.001
3 Days °F	12	12	12	12	12	12
(3 Daily t	12.001	5.733	10.269	8.086	2.489	5.860
Dosages[h]) P	<0.001	<0.001	<0.001	<0.001	<0.050	<0.001
5 Days °F	12	11	12	11	12	11
t	13.621	3.824	16.133	7.311	1.757	6.962
P	<0.001	<0.005	<0.001	<0.001	<0.200	<0.001
10 Days °F	12	12	12	12	12	12
t	11.025	1.118	8.746	13.244	2.476	10.760
P	<0.001	<0.400	<0.001	<0.001	<0.050	<0.001
17 Days °F	13	13	13	14	14	14
t	12.980	2.114	11.421	10.820	1.025	9.401
P	<0.001	<0.100	<0.001	<0.001	<0.400	<0.001

[a]Pairs of observations were tested in the formula $t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S.E._1^2 + S.E._2^2}}$ in which $\bar{x}_1$, $\bar{x}_2$ are the means for the two series of observations and $S.E._1$ and $S.E._2$ their respective Standard Errors. The resulting t value was matched with values in Table 2.7.1, Snedecor, G.W., 1956, Statistical Methods, Ames, Iowa, The Iowa State College Press, 46, of the distribution of "Student's" t.

[b]Control Uteri were those of rats which had received 0.2cc Sesame Oil, vehicle for the steroidal hormones for the indicated times before necropsy.

[c]E-17β = Estradiol-17β, 0.1µg/dosage.

[d]Prog. = Progesterone, 1.5mg/dosage

[e]°F = Degrees of freedom, equivalent to n-1 for each of the series of observations, where n equals the number of observations averaged to obtain the mean.

[f]t = value calculated for "Student's" t by the formula given in footnote a, above.

[g]P = the probability that the difference observed in milligrams per cent mean values being compared would occur only by chance.

- continued -

TABLE 17--Continued

[h]The Sesame Oil controls used for comparison for this group were those of the preceding group, three day treatment period, two daily dosages.

response of the uterus. The highest response in terms of actual milligram weight was obtained when the treatment of estradiol-17β or estradiol-17β plus progesterone was extended through 17 days (table 16; plate XXXV, figures 93-96). Results from these experiments help to establish the early uterine horizon II and the early aspects of horizon III as characteristic of responses to estradiol-17β and progesterone when administered over a 17 day period according to the protocol of the Experimental Model System.

2. Endocrine Organ Gravimetric Responses.

In general, analyses of the gravimetric responses of the thyroid, adrenal and pituitary glands to the ovarian sex steroid hormones disclose that (a) the thyroids were the least responsive to the exogenously administered steroid hormones; (b) the adrenal glands were most responsive to the combined treatments of estradiol-17β and progesterone, and somewhat less so to the single effects of estradiol-17β and progesterone alone (tables 7-16); (c) the pituitary glands were most responsive to the combined administration of estradiol-17β and progesterone, and equally, slightly less so to the administration of estradiol-17β alone and progesterone alone (tables 7-16).

3. Body Weight Responses.

Early in these studies it was determined that the recording of body weights could prove of further enlightenment in at least two major ways: (a) to help ascertain facts regarding growth curves,

especially the relationship of organ weights to body weights, and (b) to further delineate the importance of the exogenously administered hormones on body weights and overall metabolism. Thus, it became possible to obtain sufficient data on the overall effects of endogenous and exogenous hormones on end-organ responses. Grossly, these data can be interpreted as an effective correlate to the hormonal responses. Specifically, these data reveal that the hormonal vehicle, sesame oil, interferred the least with the promotion of body weight, whereas progesterone > estradiol-17β + progesterone > estradiol-17β in inducing the promotion of body weight of bilaterally ovariectomized animals so treated for periods of time up to 17 days. Finally, it should be re-emphasized that while gravimetric figures have been given for raw weights and adjusted to body weights, i.e. in terms of milligrams per 100 grams body weight, much emphasis has been placed on raw data, and the milligrams per cent data has been included for the sake of completeness; in the case of uterine studies, the milligrams per cent were made the basis of the probability studies since in hormonally manipulated animals milligrams per cent has been shown previously to have further significance.

To summarize, these data clearly reveal that the early uterine horizons observed in the intact, prepubertal and pubertal rat can in large measure be duplicated in the bilaterally, ovariectomized, young adult rat, especially with the combined adminis-

tration of 0.1 microgram estradiol-17β and 1.5 milligrams progesterone (plate XXXV, Figure 96).

V. DISCUSSION

During the last seven decades, many researchers concerned with reproductive mechanisms have studied some aspect of uterine growth in intact, or ovariectomized, or in hormonally-modified, surgically manipulated animals, much of this culminating from the contributions of early workers such as Heape (1900). Recently, several investigators have utilized the uterus to study end-organ response with a view toward ascertaining how estrogen induces certain of its effects, notably Fishman (1951), Huggins and Jensen (1954, 1955a, b), Hisaw, Velardo and Goolsby (1954), Bever, Velardo and Hisaw (1953 a, b, and c; 1956), Leathem (1959), Talalay (1965), Villee (1967), Villee, Hagerman, and Joel (1960), Velardo (1958a, 1959, 1960, 1963, 1964a and 1965 a, b, c and d), Velardo and Kasprow (1965 a, b, c, and d; 1966); Mc Kerns (1967), and Segal and Scher (1967). It should be highlighted here that prior to this time only a few contributions in this specific area were regarded as informational, chiefly the contribution of Alden (1947) on the alteration in osmiophilic epithelial lipids of the uterus of the rat, and that of Pritchard (1947) on a detailed analysis of the distribution of alkaline phosphatase in the uterus of the pregnant rat.

In attempting to ascertain some critical lines of evidence pertaining to structure-function relationship between ovarian sex steroid hormones and uterine growth, one or a combination of three principal experimental (surgical) approaches were utilized, namely ovariectomy,

adrenalectomy, hypophysectomy. These surgical maneuvers were utilized in specifically pin-pointed experiments designed to ascertain the uterine growth promoting properties of naturally-occurring estrogens and synthetically-produced estrogenic substances. Most of these studies have focused mainly on some physiological or biochemical aspect of the uterine growth process, i.e., gravimetric changes, uterine-body weight relationships, or changes in protein, lipid, or carbohydrate content, or changes in uterine concentrations of specific enzymes (cf. Velardo, 1963; Segal and Scher, 1967; Mc Kerns, 1967).

Comparatively, uterine morphological studies of true significance have been very few in number. It appears that much more is known concerning certain of the molecular events associated with the overall uterine growth process than that of the cyto- and histomorphological sequelae of uterine growth from immaturity to maturity, i.e., relative quiescence to dynamic activity. Furthermore, all-encompassing, age-progression studies on uterine growth in any animal have not been undertaken. Consequently, the literature is devoid of any such information that would allow a detailed explanation of the cytological and histological factors involved in the transitional stages from immaturity to maturity.

Giving renewed interest in the specific area of uterine morphology are the landmark, scientific contributions of Professor S.R.M. Reynolds. His critical analysis of the known and non-explored areas of uterine physiology clearly pointed-up the fact of our lack of understanding of

the structural and cytodynamic aspects of the uterus. His monograph, Physiology of the Uterus (1949, revised 1965), in a true sense and in very large measure, gave impetus to the renewed interest for much of the recent work that has appeared in the last two decades. Picking up the Reynolds' inspired threads of inquiry and piercing them into specific questions of uterine morphology, Rosa and Velardo (1954 a, b, 1959) and Velardo and Rosa (1963), in a number of scientific contributions-including a monograph-survey, reported on uterine energy cycles by means of cellular localizations of succinic and lactic dehydrogenases. Their contributions are quite significant; even more important are their critiques which emphasize that their work could be more significantly interpreted if one really knew the details of uterine morphology in the transition from immaturity to maturity. Their restatement of our lack of information stimulated new attention on the problem. How much do we really know about the structural relationships of uterine growth; how much do we really know about the emerging patterns of uterine growth, from a period of immaturity through the first few estrous cycles in albino rats?

Several exhaustive surveys of the literature in this area pointed up one reference in a most repetitive manner: Long and Evans' 1922 monograph (memoir) on the estrous cycle of the rat and associated phenomena, an excellent pioneering work of great strength. This memoir from the University of California Press presents a very detailed account of the ovary of the rat during the estrous cycle, but unfortunately, only a very brief description of the morphology of the uterus,

as has been recounted in this dissertation in the Review of the Literature.

Like most pioneering studies, all of the details are not present, owing largely to limitations of time, technology, or specifically-narrowed interests. Obviously, it is not possible to relate cyto-histo-and biochemical results to the uterine histomorphological data in the literature. The need for such a detailed study on uterine cyto-and histomorphology was clearly evident.

Inasmuch as there are numerous cytochemical and biochemical studies on the uterus of the rat in progress here and throughout the world, and inasmuch as it is not yet possible to correlate such studies with highly standardized cyto-and histomorphological uterine patterns of intact controls, it appeared of exciting interest to undertake such a study. The results, from the aforementioned study, make it possible to appreciate the fact that control, morphological studies are not only desirable, but are of critical importance. Both the gravimetric and cytologic analyses and the photomicrographic documentations make it possible for the first time to gain a comprehensive view of the transitional changes within the uterus, i.e. from immaturity to maturity. Furthermore, this dissertation reports for the first time the quantitative relationships between the uterus and the endocrine organs, as well as the uterine-body weight relationships throughout the most dynamic growth periods emanating from prepubertal states and progressing through maturity. From such information, it has been possible

to establish the horizons in early uterine morphological response of the rat to ovarian sex steroid hormones, estradiol-17β and progesterone. Such experimental studies conducted and reported herein gived added significance to this dissertation.

As one envisages the cyto- and histomorphological characteristics of the growing uterus of immature, albino rats, in a sequential and time-lapse manner, one receives the running, visual impression that the following uterine alterations are associated with the initial growth characteristics: increased uterine vascularity, hypertrophy, and imbibition of water. Both the gravimetric results and the photomicrographic documentation give credence to the affirmation of these facts. Studies dealing with the immature rats, ages 23 through 37 days, provide a scaffold of information that allows for a stepwise account of the sequential gravimetric and cyto- and histomorphological events associated with uterine growth. Specifically, one observes an emerging, changing pattern from quiescence to rapid metabolic activity. Chronologically, commencing with the intact immature rat of 23 days of age, the uterus is reminiscent of the surgical castrate; the endometrial stroma is relatively non-activated at this early age, and the lumen does not show deep inpocketings. The uterine luminal epithelium, however, although of cuboidal type in diestrus in cycling animals, is yet apparently lower in the uterus of the 23 day old rat. The uteri of the 26 day old rats studied, however, provide evidence of "awakening" and mitotic activity is becoming quite obvious. Thus, it

has been possible to classify these uteri as class I and class II, respectively, cf. table 2, A, B, C.

Progressing to the immature animals of 33 days of age, one captures the impact of a true, early horizon in uterine growth. It is at this stage that one observes a doubling in uterine weight, over that seen in the group of 23 day old, immature rats. The uterine cyto- and histomorphological criteria likewise reveal active growth of the uterine proliferative type, i.e. uterine enlargement and mitotic figures becoming increasingly noticeable, especially in the uterine stroma. Based on these studies, one can point to the marked changes as indicative of the first early horizon in uterine growth. It is noteworthy to emphasize at this point that the ovarian follicular population of the 33 day old animals is composed of numerous, well-formed, vesicular follicles, the morphologic hallmark of estrogenic secretions.

The second noteworthy early uterine horizon appears on day 37, an event heralded by the more frequent appearance of moderately tall to tall uterine columnar epithelium, numerous mitotic figures and marked stromal and muscular development. Concordantly, the ovarian studies reveal further maturational signs: enlarged vesicular follicles, pre-ovulatory swelling of several follicles nearing ovulation, and one follicle in process of ovulation as detected by presence of a meiotic figure.

The third early uterine horizon is coincident with the opening of the vagina, variously at 38 to 43 days. At this time, a full prolif-

erative type endometrium becomes obvious, and is remarkably characteristic of that normally associated with the proestrous phase. Ovarian histology further corroborates the attainment of such an horizon: the ovarian follicles are responsive to adenohypophyseal gonadotropic hormones, follicle stimulating hormone (F.S.H.) and luteinizing hormone (L.H.), and one observes preovulatory and ovulatory follicles in the several different stages of ovulation.

In sequential, chronological progressions, permitting of characterizations of the uterus during the normal estrous cycle of young adult, cycling animals, 55 to 66 days of age, one further observes the zenith of uterine end-organ responses to the changing titers of ovarian hormones. Gravimetrically, rather dramatic increases in uterine weight become established in young, cycling rats as early as 55 days of age. Cytological and histomorphological observations of the uteri of these animals, necropsied during the different stages as revealed by vaginal cytology, indicate beyond question that one <u>can</u> diagnose each of the six stages of the estrous cycle. The endometrium of the proestrous animal manifests tall columnar epithelium, numerous mitotic figures, highly infolded uterine luminal epithelium, stromal edema and developing stromal glands. The next phase of the cycle, estrus, however, reveals that it is the most dynamic of all such phases, one of increased metabolic activity. Tissue studies on the uteri of estrous animals indicate that increased growth has taken place within the endometrium, the uterine luminal epithelium appears to be organized into a highly infolded pattern, the uterine surface columnar epithelium is quite tall

and highly cellular, stromal edema is quite marked, and the uterine glands within the stroma, are numerous and well-developed. Thus, during this stage, early estrus, the uterus has reached the peak of horizons: the fourth early uterine horizon. During proestrus, and estrus, the ovary shows great competence to respond physiologically to the adenohypophyseal gonadotropic hormones, F.S.H. and L.H., as evidenced by the appearance of enlarged follicles of several types: vesicular, preovulatory, ovulatory. In these animals, newly formed corpora lutea were found. Consequently, the conclusion is quite justifiable that these are mature, cycling animals, and that they are well on their way towards attaining full reproductive vigor.

The following phase, metestrus, appears as one following much vigorous activity. During metestrus, the uterine luminal epithelium undergoes marked reduction in size, becomes somewhat ragged in appearance, and is comprised of cells of varying sizes and shapes. The ovaries reveal the presence of corpora lutea and follicles of several different stages, but not of the preovulatory stage.

The intermediate phase of diestrus, the phase appearing between periods of estrus, in the young cycling animals, 55 to 66 days of age, shows great inactivity from a physiological point of view, but mitoses are often present. The uterus is of lowest weight and smallest size during this phase. The uterine lumen is small; likewise, the uterine luminal surface epithelium is markedly reduced in height. The uterine stromal glands are fewer in number and lack secretory material for the most part but, as just stated, mitoses are often present in the

endometrium. An interesting highlight that bears pointing-up is that irrespective of the reduced endometrial activity, the surrounding myometrial component, the inner circular muscle, appears activated and one can usually observe some mitotic figures within the inner circular muscle. The ovaries of these animals contain corpora lutea and follicles of increased size and development.

Thus, from the above discussion, it is clear that one can readily observe the sequential changes associated with the transitional stages within the uterus from (a) immaturity to maturity, and (b) from the first estrous cycle to the time when the cycle becomes regularized. Also, such observations make it possible to dually establish a classification pattern of the uteri based on the aforementioned criteria, and one based on the early horizons in uterine growth.

Of added significance are the parallel studies concerning the endocrine organs of these animals. In an effort to adhere to the major effort at hand, suffice it to say that the chronological investigation and gravimetric determinations pertaining to the endocrine organs point-up numerous, specific endocrinological relationships between the uterine end-organ responses and the endocrine organs. It is of specific biological interest to recount here that although the first uterine horizon is associated with the thirty-third day of life of the rat, noteworthy biological patterns for the body weights also occur on day 33, while those of ovarian and thyroid weights are characteristic of day 34, and those of pituitary and adrenal weights of 35 days of

age. The tabular data and text are replete with such relationships, cf. tables 1-6.

As an extension of the cited and discussed data, a large number of highly related experiments were undertaken with a view toward ascertaining the early horizons of uterine growth in ovariectomized animals given physiological dosages of estradiol-17β and progesterone. The experiments were designed in such a way so as to permit a capturing of the cyto- and histomorphological events within the uterus associated with single injections of estradiol-17β or progesterone, as well as following the administration of estradiol-17β and progesterone concurrently, but at different subcutaneous sites. The duration of the experiments was from five minutes through 17 days of treatment, thus affording bona fide opportunities to determine the early effects as well as those elicited after a time interval commensurate with three plus estrous cycles.

The observations from the hormonally treated, ovariectomized animals are of meaningful significance when analyzed in terms of the horizonal patterns. Such analyses clearly reveal that the first early uterine horizon (I) can be duplicated with estradiol-17β, and that the early uterine horizons II and the beginning aspects of III can in fact be elicited by estradiol-17β and by the combined administration of estradiol-17β and progesterone according to the experimental model system. The combined dosage showed a more marked effect in eliciting a horizon III-like pattern than estradiol-17β alone.

These data now make it possible to pursue a wide array of experimental procedures utilizing techniques from several disciplines that can add significantly to these findings. This dissertation extends the original observations of Long and Evans (1922) and of W.M. Allen (1931) on the uterus of the rat by furnishing a detailed analysis of the cyto- and histomorphological sequelae associated with the transition from immaturity to maturity, and by providing a statistical and photomicrographic documentation of the uterine changes associated with growth both in intact, cycling young adult rats, and in hormonally-treated, bilaterally ovariectomized, young adult rats. For the first time, such a large body of data now makes it possible to classify the uteri according to a number of diagnostic cyto- and histomorphological criteria. Also, and of added significance, these data yield distinctive patterns that permit of establishing the early horizons of uterine growth.

The specific value of this work is to provide two highly documented frameworks: the first is the furnishing of a detailed analysis of the growth characteristics of the uterus in intact rats 23 through 66 days of age, and the second is the demonstration that carefully planned experiments utilizing physiological dosages of estradiol-17β, progesterone and combinations of the two ovarian sex steroid hormones can elicit many of the uterine growth characteristics observed in the intact series. Such statistically valid and photomicrographic documentations herein presented create myriad possibilities for facilitating the extension of this work in numerous disciplines, including

cyto- and biochemistry and electronmicroscopy. In this regard, it should be recalled that the classic papers of E. Allen (1922) on the estrous cycle of the mouse and of Long and Evans (1922) on the rat have provided the bulk of information utilized during the last five decades. Further work is required to help provide additional lines of information.

The work of Nilsson (1958a, b, c; 1959a, b, c; 1962) on the ultrastructure of the mouse uterine surface epithelium under different estrogenic influences, is a definitive contribution, and can truly be identified as a remarkable extension of the work of E. Allen (1922). Nilsson's contributions provide specific data showing that the uterine surface epithelium in (a) castrated mice measures approximately one-half of that observed in estrous mice; (b) in estrogenized mice (1.0µg estradiol-17β) it increases to about 75% of that observed during estrus, and (c) in castrated mice after two days treatment with estradiol had uterine luminal heights that returned to that observed in non-hormonally treated, castrated mice.

Utilizing C3H mice and a long-acting estrogen, poly-estradiol phosphate, Nilsson (1959a) in a subsequent paper showed that such treatment, over a period ranging from one and one-half to three days had profound effects on the uterine epithelial cells. In a comparison of estrogenized animals with those of non-treated groups, it was ascertained that the uterine epithelial cells had a triple height, longer profiles of the cellular membranes, and a larger Golgi region with many small vesicles and vacuoles. It was also noted that a

two-times higher proportion of 0.06-0.12 micron profiles of dense vesicles was localized within 1 micron under the luminal cell surface. The microvilli grew longer also, thus increasing the uterine luminal surface of cells of treated mice by approximately fifty per cent. Nilsson also observed rounded structures with a diameter of about 300 Ångstroms in the cytoplasm and at the luminal aspect of the surface of the cell. In continuing these investigations in spayed and estrogenized animals, Nilsson (1959b) made some additional observations on the appearances of uterine epithelia 15 and 30 days post-estrogenization (with the long-acting substance, poly-estradiol phosphate). Lipid granules were observed in the animals on prolonged treatment, but were usually absent immediately after treatment. Some circular bodies, about 0.2μ in size, with an outer layer of thin, irregular membranes and an anterior amorphous substance were observed. Mitochondria were frequently present. Of further interest is the fact that such continuous administration of estrogen did not induce squamous metaplasia of the columnar epithelial cells. In a follow-up study, Nilsson (1962) concluded that the appearance of membranes around the lipid granules represented the outset of their decomposing, and further, that the granulated bodies participated in the lipid decomposition process.

A companion paper, dealing with changes in some cellular components by Nilsson (1959c) presented evidence showing that the injection of a long-acting estrogen into spayed mice resulted in the process of secretion by the uterine luminal epithelial cells. These ultrastructural studies showed that the epithelium of animals on long-term treatment

was similar to that of mice given the same estrogenic substance for two days. Comparisons with epithelium from spayed animals indicated that the luminal cell surface area was approximately 50 per cent larger in cells showing secretion. The ratio of Golgi region with vesicles and vacuoles to the area with Golgi membranes increased about two and one-half times at the two-day stage and during uterine secretion. The increase in the number of circular profiles was higher than the increase in the area with Golgi membranes. Also, it was noted that the number of profiles per square micron of Golgi region was one-half lower. The proportion of 0.06-0.12 micron large profiles in the area (one micron) under the cell surface changed from 25 per cent to 54 per cent, with an associated increase in density of the interior of the profiles.

Regarding extensions of Long and Evans (1922) work, very little has been done along comparable lines to further delineate uterine growth patterns with the exception of the physiological and physio-pathological studies on steroid hormonal interactions on uterine growth (cf. Velardo, 1963). Regrettably, uterine morphological studies in the rat are comparatively very few in number, as compared with the recent impetus to uncover biochemical information pertaining to many different aspects of hormonal receptor sites within the uterus. Of great morphological significance as a contribution to the body of knowledge of the uterus of the rat is the study by Bertalanffy and Lau (1963). They reported extensive data on mitotic rates and turnover times of all epithelia lining the female genital system of adult female rats, weighing 200-250 grams, of the Holtzmann-Sprague-Dawley strain. Their

results indicate that 75 per cent of the cells of the uterine surface epithelium are formed during one estrous cycle, with a turn-over time of 5.9 days. The epithelial lining of the endometrial glands appears to have only 43 per cent of cells formed during one estrous cycle, with a turn-over time of 10.4 days. Their studies did not include the age spectrum from immaturity through the several transitional periods leading up to maturity.

The recent biochemical studies are for the most part concerned with uterine nuclear receptor sites for estradiol and other sex steroid hormones, and the action of estrogens of the genetic potentials of the uterus. Mueller (1968) has made some tremendous advances in each of these areas. While it had been shown that hormone responses depended on the synthesis of new metabolic regulators, the comparatively new experiments with antibiotics, puromycin and actinomycin-D revealed this dependence beyond doubt. Simultaneously, it became possible for him to point-up the role of estrogens in modifying genetic expression mechanisms at the cellular level of the uterus. Mueller, Gorski and Aizawa (1961) reported that the levels of puromycin which inhibited 90 per cent of the protein synthesis in the uterus in vivo blocked the estrogen induced alterations of RNA and phospholipid synthesis as well as the imbibition of water. Subsequently, utilizing cycloheximide, another inhibitor of protein synthesis, Gorski and Axman (1964), confirmed the work performed in Mueller's laboratory, which showed that the early hormonal effects depend on protein synthesis. Ui and Mueller (1963) showed that levels of actinomycin-D that reduce the in vivo synthesis

of RNA in the uterus to below 10% of the control value, also prevented the acceleration of protein and phospholipid synthesis by estradiol. The imbibition of water, however, was markedly less affected by the antibiotic and about 30 per cent of the wet weight response remained, irrespective of the massive doses of actinomycin-D which abolished RNA synthesis. Regarding receptor sites and estrogenic action, Mueller (1968) maintains that the initial step in hormonal action is seen as the combination of estrogenic molecules with a specific receptor protein. He postulates that this protein is itself a product of specific gene action and that a separate group of factors govern the expression of this gene in selectively differentiated cells. The binding studies suggest that some receptors are complexed with RNA, since RNA plays a role in the hormone binding process. The contemporary work of Gorski, Toft, Shyamala, Smith and Notides (1968) on hormone receptors amplifies Mueller's (1968) findings; Gorski et al. speculate that their theory is in accord with the concept of Monod, Changeux and Jacob (1963). Specifically, Gorski et al. feel that their model depicts estrogen as traveling via the blood stream to the target tissue, probably in association with serum protein. The estrogen then migrates into the cell, where it interacts with a cytoplasmic protein to form the large (200,000 molecular weight) 9.5 S designated receptor-estrogen complex, which results in a protein conformation capable of migrating into the nucleus. Other changes occur in the nucleus that result in the appearance of estrogen bound to a 5 S designated protein. In their model, the 9.5 S protein is shown as composed of subunits which separate after

entering the nucleus. The large size of the 9.5 S receptor indicates that it might be composed of subunits, possibly of more than one type. Thus, it appears that this theory might well be in accord with the concept of Monod et al. (1963), that allosteric proteins are composed of subunits and are involved in regulatory processes. More recently, Rochefort and Baulieu (1969), utilizing incubated uterine tissue, studies the interaction of estradiol with rat uterus receptors. They confirmed the reversibility of estradiol binding and two types of binders were observed: the first, possessing great affinity, limited capacity and high specificity; the second, manifesting lesser affinity and non-limited capacity in the range of concentration used (up to 1×10^{-5}M).

It is quite apparent that much of the work in the area of biochemistry could be more meaningfully interpreted on a structure-function level if the morphological studies could be incorporated concurrently. The information provided in this dissertation lays the groundwork for cooperative studies, of a wide multidisciplinary nature, to gain impetus so as to catch up on the lag-time of the contributions in this area.

VI. SUMMARY AND CONCLUSIONS

1. This dissertation was concerned with a gravimetric analysis and a photomicrographic documentation of the horizons in early uterine morphological growth responses of the uterus of the rat to intrinsic hormones during transitional stages from immaturity through maturity, and to exogenously administered ovarian sex steroid hormones, estradiol-17β and progesterone, in bilaterally ovariectomized, young adult albino rats 57 days of age at the time of first hormone administration.

2. Gravimetric analyses revealed that as the body weights increased with increasing age of rats from 23 through 33 days of age the uterine weights likewise increased, both showing increases at 33 days of age of approximately 100 per cent over weights at 23 days of age.

3. Uteri of immature rats 34, 35 and 37 days of age showed two-fold increases in weight over that seen at age 23 days.

4. Uteri of immature rats 38-42 days of age showed the expected biological variation, especially as the rats neared maturity; uterine weights ranged upwards from 111.5 milligrams at 38 days of age to 191.3 milligrams at 41 days of age, then dropped to 135 milligrams at 42 days of age.

5. Of the groups of animals studied at ages 23 through 42 days of age, almost 100 per cent were sexually immature through the thirty-

eighth day as manifested by the appearance of closed vaginae. In contrast, only 60 per cent were immature at ages 39 and 40 days, 46 per cent at age 41 days and 30 per cent at age 42 days.

6. Gravimetric studies on the endocrine organs of animals 23 through 42 days of age disclosed notable increases for ovarian and thyroid weights up to day 34 and for pituitary and adrenal weights up to day 35.

7. Analysis of the cytological and histomorphological data gave rise to a comparative classification of uteri of prepubertal rats, i.e. rats showing vaginae:

Class I. Uteri possessing low columnar luminal epithelium (up to a mean of 20 micra). Mitotic figures in luminal epithelium, stroma and circulat muscle absent for the most part; only rarely does one observe a mitotic figure in the three designated areas.

Class II. Uteri possessing low columnar luminal epithelium (up to a mean of 20 micra), as described in I, above, but exhibiting substantial mitotic activity in one, two or all of the designated areas for study, cf. I, above.

Class III. Uteri possessing columnar luminal epithelium of moderate height, (i.e. up to a mean of 20-30 micra) which does not exhibit vacuolar degeneration.

Class IV. Uteri possessing high columnar luminal epithelium (mean greater than 30 micra) and showing either no vacuolar degeneration or only small vacuoles in a small minority of cells.

Class V. Uteri possessing luminal epithelium exhibiting moderate or extensive vacuolar degeneration, irrespective of height of luminal epithelial cells.

8. Utilizing this new comparative classification based on uterine cytological details in the main, it was determined that:

a) Uteri of 23 day old immature rats were typically class I;

b) A majority of uteri of 26 day old immature rats were of class II, with a minority of class I; body and uterine weights of rats with uteri of class II were moderatley elevated above those of class I;

c) A majority of uteri of 33 day old immature rats likewise were of class II with the minority of class I; however, uteri of class II showed a marked increase in weight over those of class I within this age group;

d) Uteri of 34 day old immature rats were also class II for the most part (although over 100 per cent higher in weight than the class II uteri of 33 day old rats) while one showed a class III and one a class V characterization;

e) Uteri of 35 day old immature rats were typically of class V, exceptions were one uterus of class I and one of class IV:

f) Uteri of 37 day old immature rats showed quite a range of development: classes II, III, and IV for the most part, with only one in class V.

9. Gravimetric and cyto-and histomorphological data on pubertal rats ranging in age from 35 through 42 days of age disclose several noteworthy facts:

a) Gravimetric data of rats necropsied at the time of attaining puberty indicate great variability in uterine and ovarian weights. The thyroid weights are less variable; the adrenal glands show marked fluctuation and the ptiuitary gland weights show rising and falling fluctuations.
Gravimetric studies of pubertal rats showing early cyclicity tend toward regularized patterns, i.e. weights bearing some relationship to the stage of the estrous cycle.

b) Uterine morphological studies reveal that coincident with vaginal opening a preproestrous-proestrous-estrous pattern appears in the uterine endometrium.

10. Extending these studies to cycling, young adult rats, 55 to 65 days of age provided additional opportunities for comparisons and an overview of events leading up to adulthood.

a) Gravimetric data showed that the uterine weights follow a cyclical pattern, heaviest during estrus and lightest during diestrus. The ovarian weights likewise appeared heaviest during estrus-metestrus while the light weight displayed during early and late diestrus extended into preproestrus. In similar pattern, the weights of the thyroid, adrenal and pituitary glands were heaviest during proestrus and estrus, and lightest during early and late diestrus.

b) Uterine morphological analyses indicated that the endometrial cycles become regularized between ages 42 and 55 days. On the fifty-fifth day, it was possible to diagnose the endometrium in terms of estrous cycle phases. These data, consequently, provided a morphological characterization of the uterus during each phase of the estrous cycle.

11. These studies were further extended to rats in full reproductive prime, 124-133 days of age, thus affording a basis for comparison spanning 100 days. Tabular and text sections give analyses of these data.

12. In a series of studies utilizing hormonally-treated, bilaterally ovariectomized young cycling rats 57 days of age during a time span of five minutes through 17 days it was observed that:

a) The hormonal vehicle sesame oil, was without any uterine growth promoting effects;

b) Estradiol-17β (0.1μg per unit dose) was quite effective in eliciting a uterine proliferative type reaction, the early awakening pattern being seen within three hours after subcutaneous injection. The uterine response to estradiol-17β was more marked at three hours than at six hours and progressing with time the response becomes enhanced. The most marked response among the short term studies was seen when three daily injections were given over a 72 hour period.

c) Progesterone (1.5mg per unit dose) did not seem to have marked uterine growth promoting properties; comparatively, it gave the poorest uterotropic response of the three treatment groups studied i.e. 1) estradiol-17β, 2) progesterone, and 3) estradiol-17β plus progesterone.

d) Combination treatments of estradiol-17β (0.1μg) and progesterone (1.5mg) appeared to induce a peak reaction at the very end of the 17 day period, but it was much less than that observed with three daily injections of estradiol-17β, giving a uterine weight of 80.7 versus 93.4 milligrams per cent.

13. Analysis of all the criteria studied for all of the intact and ovariectomized and hormonally manipulated rats used in this work led to the recognition of four distinctive horizons in uterine growth. All four of these horizons appear in the intact series of rats, and

three in the hormonally-treated, bilaterally ovariectomized series.

a) Intact series.

(1) The first early uterine horizon is represented by the criteria observed for the uteri of 33 day old intact, prepubertal rats, and in this group was highlighted by a 100 per cent weight increase over immature uteri of 23 day old rats;

(2) The second early uterine horizon is represented by the criteria observed for the uteri of 37 day old intact, prepubertal rats, which exhibited emerging patterns of maturity;

(3) The third early uterine horizon is represented by the proliferative type of endometrium observed coincident with the opening of the vagina in rats 38 to 43 days of age;

(4) The fourth early uterine horizon is characterized by the criteria observed in the uterus of a mature rat in estrus, i.e. during high metabolic activity typical of that seen in this series first in rats 55 days of age.

b) Hormonally-treated, bilaterally ovariectomized series.

(1) The first uterine horizon, typical of the events

associated with the uterus of the 33 day old rat was elicited with estradiol-17β, given in three daily doses over a three day period;

(2) The second and initial aspects of the third early uterine horizons were seen after administration of the combination of estradiol-17β and progesterone and also with estradiol-17β alone over a 17 day period.

14. These data clearly indicate that it is possible to duplicate many of the uterine responses observed in the intact series in bilaterally ovariectomized rats given ovarian sex steroid hormones according to carefully planned experimental protocols.

15. Finally, these data provide an extensive analysis of the uterine growth factors observed during the transitional stages from immaturity to maturity, and furnish direct evidence that phenomena observed in uteri of the intact series can, in large measure, be reproduced in bilaterally ovariectomized rats with physiological amounts of estradiol-17β and progesterone.

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Introductory Note To Plates

All histological sections are cross sections, cut at five micra and stained with Harris hematoxylin and eosin. Magnifications are indicated at each figure. N.B.: All figures depicting entire cross sections of uteri are uniformly represented at x25.

PLATE I

Cross section of uterus of immature rat, 23 days of age (class I, i.e. relatively quiescent, cf. table 2A).

EXPLANATION OF FIGURES

Fig. 1. Uterus, x25. Specific detail reveals a relative lack of endometrial glandular development. Note also the lack of pronounced infolding of uterine luminal area, which becomes prominent as a function of stages leading to and including the several stages of estrus.

Fig. 2. Uterus depicted in figure 1, x 250, revealing a typically immature and quiescent histological pattern.

Fig. 3. Uterus, x25. Specimen from another rat of the same age, showing a similar pattern as described in figure 1.

Fig. 4. Uterus depicted in figure 3, x250. Histological appearance is comparable to that seen in figure 2. Of especial significance is the luminal epithelium, showing an early "awakening" pattern, but at this time still devoid of noticeable mitoses.

PLATE I

Figure 1

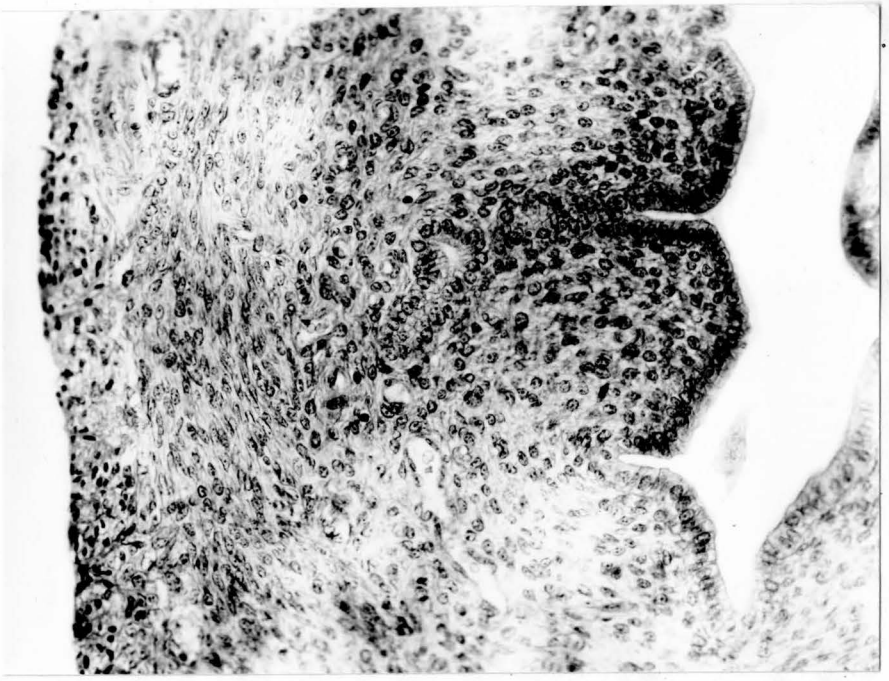


Figure 2

Figure 3

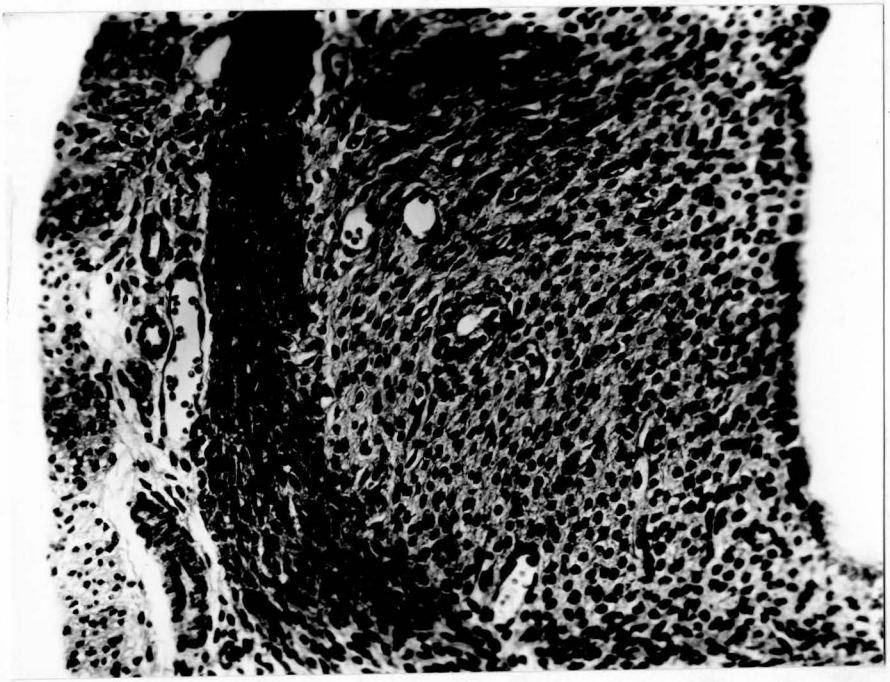


Figure 4

PLATE II

Luminal epithelium of uterus of 23 day old rat shown in figures 1 and 2, x1000.

EXPLANATION OF FIGURES

Fig. 5. Note juxtanuclear vacuolization, a feature typically observed in uteri of immature and mature, diestrous rats.

Fig. 6. Different focal areas of same uterus, cf. figure 5, revealing the single stromal mitotic figure at center of figure. Note also lack of juxtanuclear vacuolization in the luminal epithelium of these areas.

PLATE II

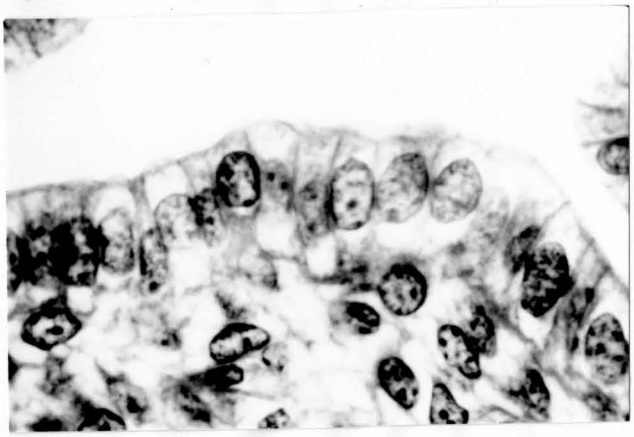


Figure 5

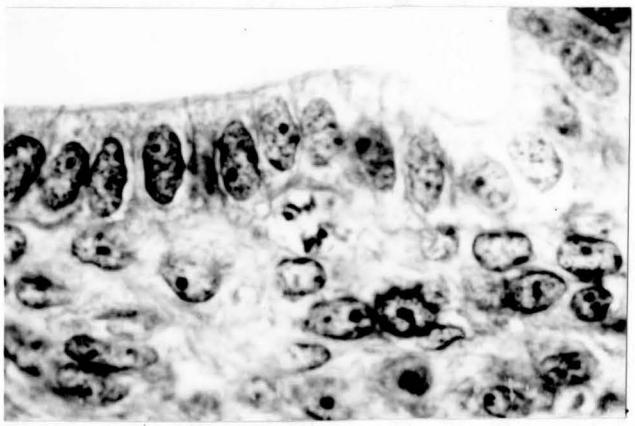


Figure 6

PLATE III

Ovaries of immature rats, 23 days of age, x50.

EXPLANATION OF FIGURES

Fig. 7. Ovary of rat whose uterus is depicted in figure 1, showing sporadic follicular development.

Fig. 8. Ovary of rat whose uterus is depicted in figure 3, showing a spurt of follicular activity.

PLATE III

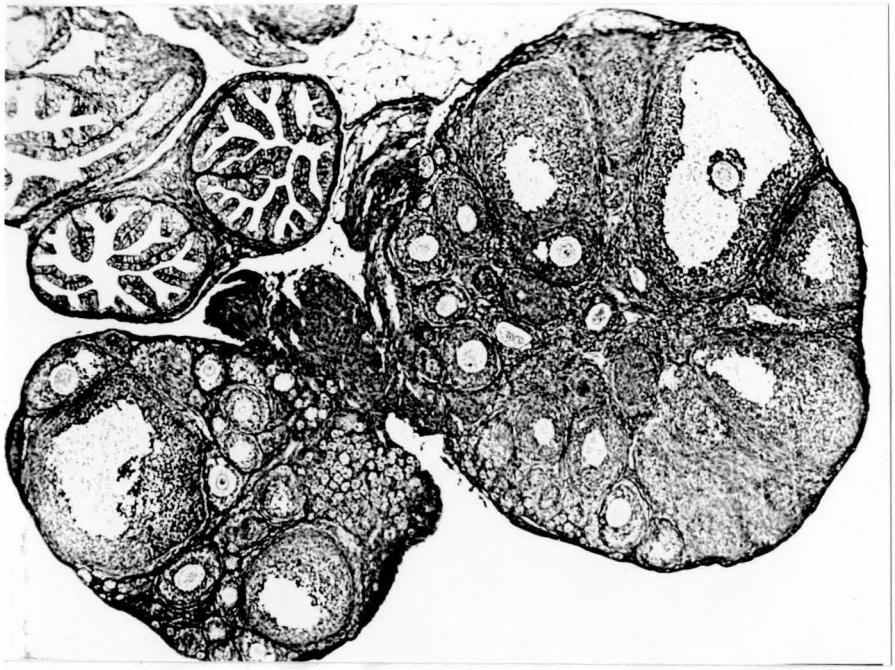


Figure 7

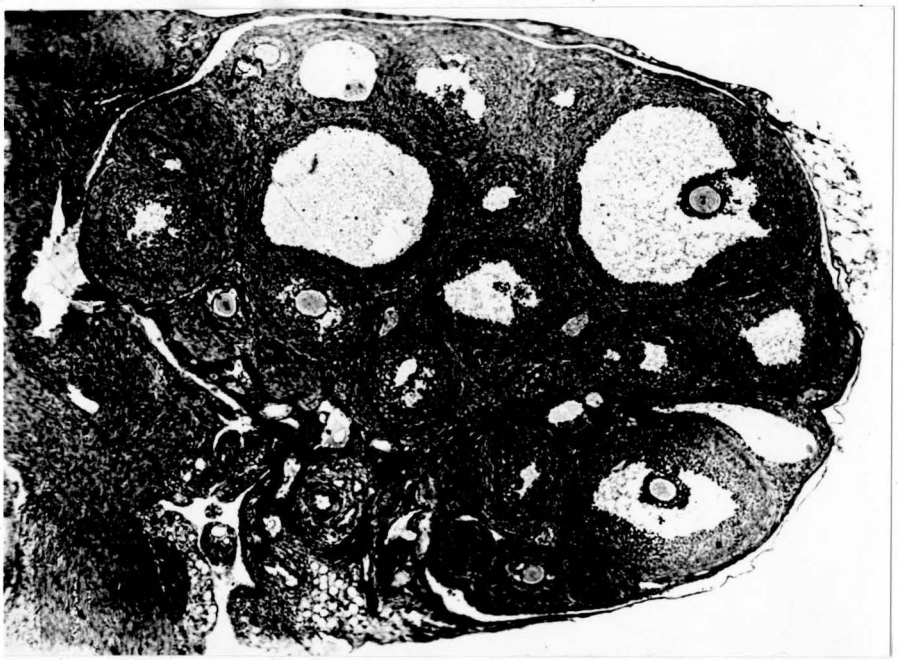


Figure 8

PLATE IV

Uterus of immature rat, 26 days of age (class II, i.e. mitotically active, cf. table 2.A.)

EXPLANATION OF FIGURES

Fig. 9. Uterus, x25. Note slight increase in size and uterine luminal epithelial infolding.

Fig. 10. Uterus depicted in figure 9, x250. Note mitotic figures; uterine luminal epithelium truly "awakening' with varying degrees in increased height, but not yet columnar.

PLATE IV

Figure 9

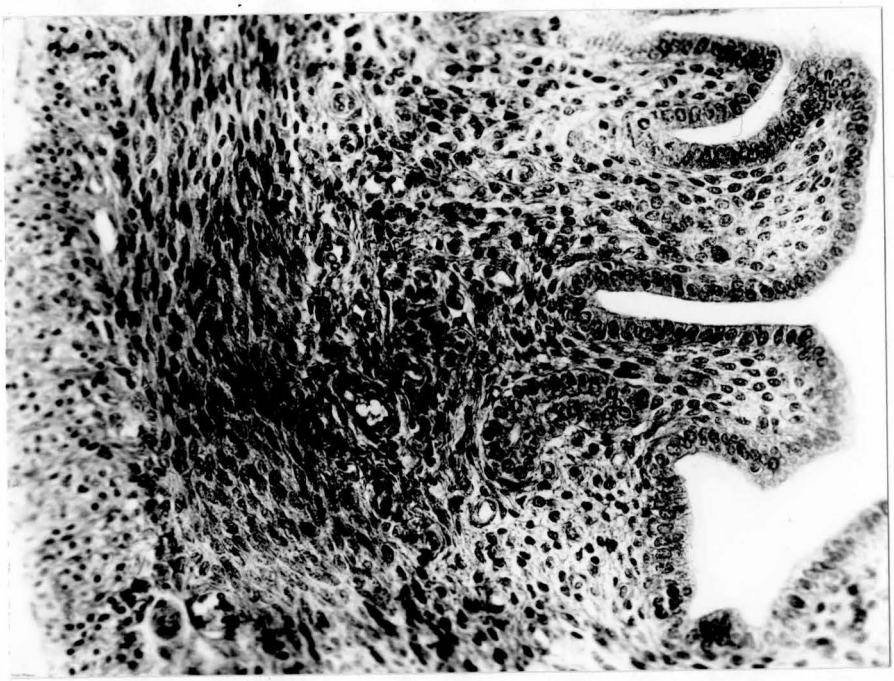


Figure 10

PLATE V

Uterus of immature rat, 26 days of age, of low uterine weight range for this class (class II, showing high mitotic activity).

EXPLANATION OF FIGURES

Fig. 11. Uterus, x25. Note deep infolding of uterine luminal epithelium, and highly cellular stroma.

Fig. 12. Higher power of uterus shown in figure 11, x250. High degree of cellularity indicates uterus in active metabolic state. Uterine luminal epithelium is yet low.

Fig. 13. Higher magnification of uterus shown in figure 11, x400. Stroma appears at 10:00 o'clock whereas circular muscle extends subadjacently. Note high mitotic index in stroma and circular muscle.

Fig. 14. Increased magnification of uterus shown in figure 13, x1000. Note mitotic figure in metaphase near center of photomicrograph.

Fig. 15. High power view of uterus shown in figure 11, x1000. Note mitotic figure in late prophase in circular muscle.

PLATE V

Figure 11

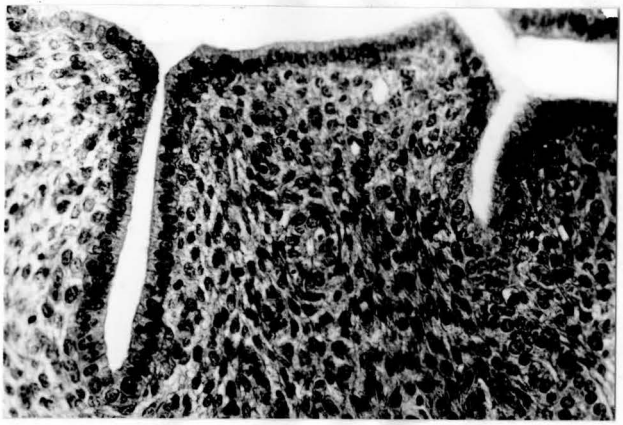


Figure 12

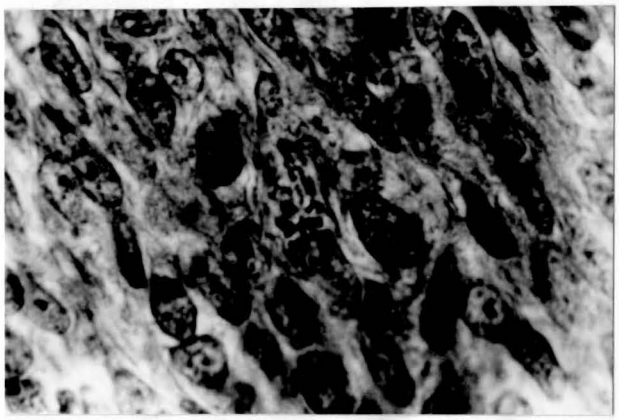


Figure 14

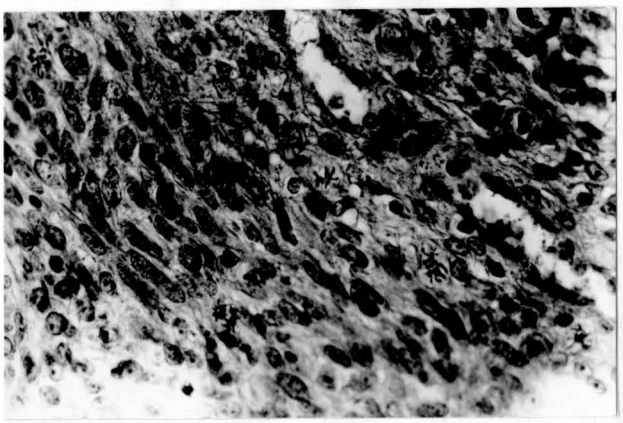


Figure 13

Figure 15

PLATE VI

Ovaries of immature rats, 26 days of age, of rats whose uteri are typically class II, x25.

EXPLANATION OF FIGURES

Fig. 16. Ovary of rat whose uterus is depicted in figure 9. Follicular activity more intense than that seen in figures 7 and 8, plate III.

Fig. 17. Ovary of rat whose uterus is depicted in figure 11. Follicular activity not as intense as that seen in figure 16, but with a single dominant follicle in this section.

PLATE VI

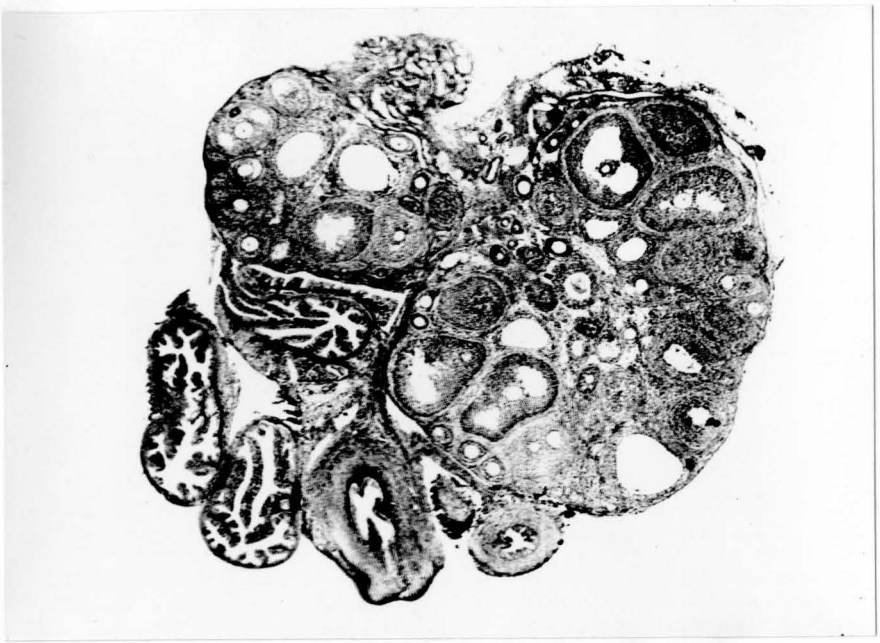


Figure 16

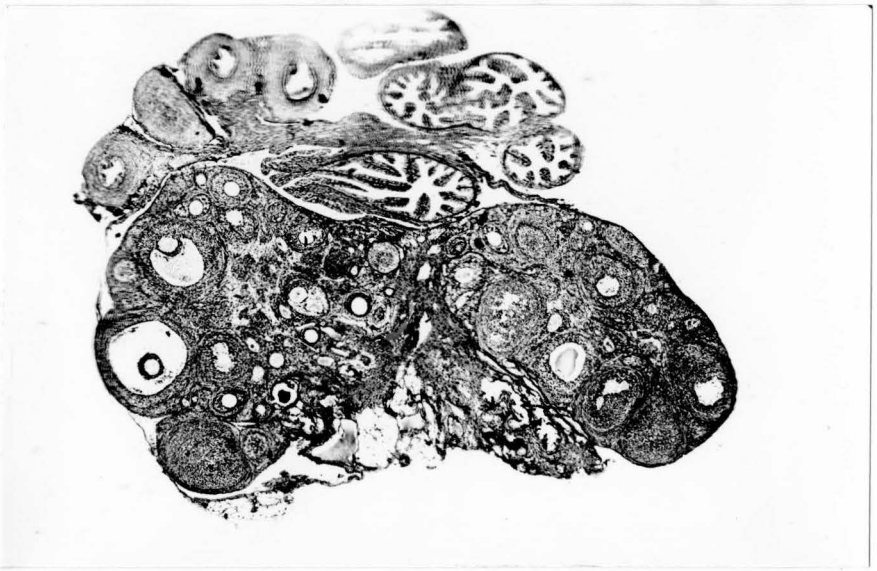


Figure 17

PLATE VII

Uterus of immature rat, 33 days of age, of intermediate uterine weight range for this class (class II).

EXPLANATION OF FIGURES

Fig. 18. Uterus, x25. Uterine enlargement is obvious, and inpocketings of uterine luminal epithelium are more pronounced than previously seen.

Fig. 19. Higher power of uterus shown in figure 18, x250. Mitotic figures obvious in luminal epithelium in area dipping into stroma.

PLATE VII

Figure 18

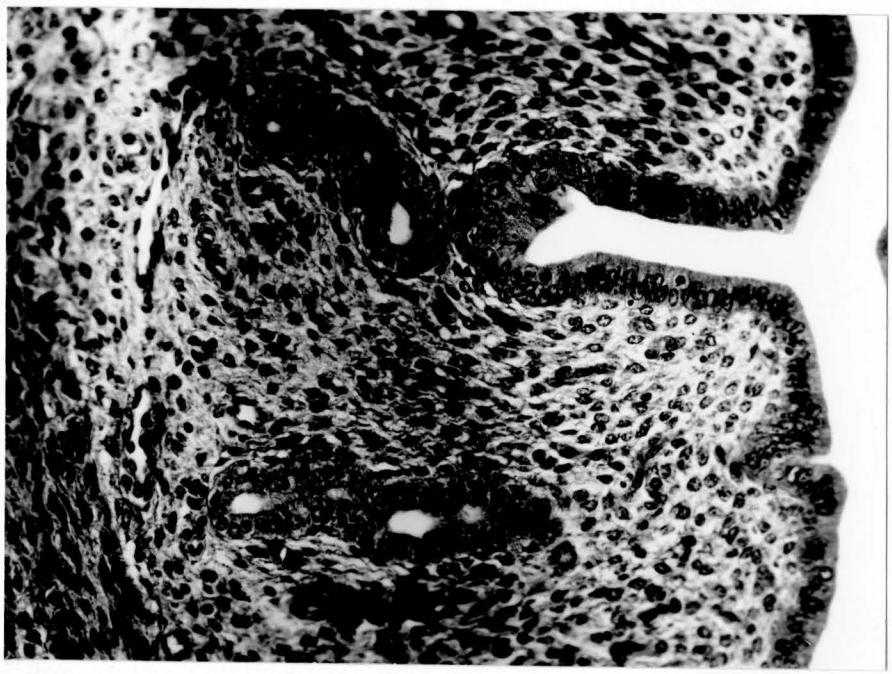


Figure 19

PLATE VIII

Higher magnifications of uterus of immature, 33 day old rat, shown in figure 18 (class II).

EXPLANATION OF FIGURES

Fig. 20. Low columnar uterine luminal epithelium, x1000. Note mitotic figure.

Fig. 21. Higher power detail of inner circular muscle, x400. Note mitotic figure at center of photo.

Fig. 22. Mitotic figure shown in figure 21 at higher magnification, x1000.

PLATE VIII

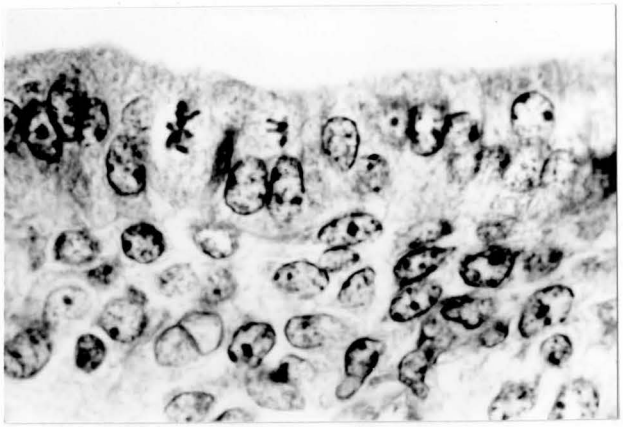


Figure 20

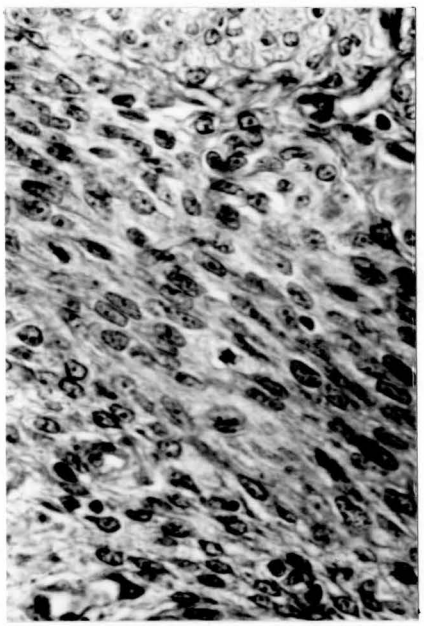


Figure 21

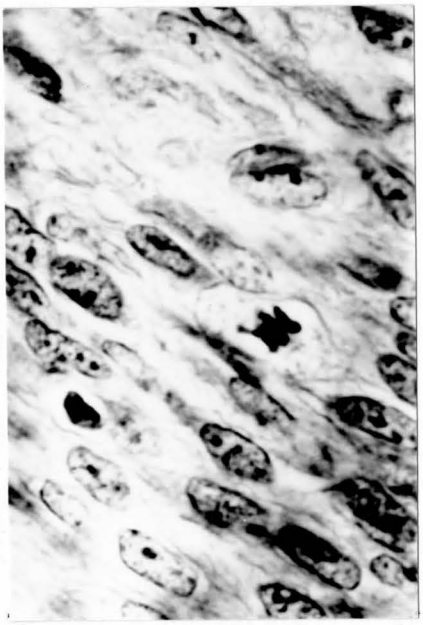


Figure 22

PLATE IX

Ovary of immature rat, 33 days of age, whose uterus is shown in figure 18 (class II uterus).

EXPLANATION OF FIGURE

Fig. 23. Ovary, x25. Large vesicular follicles are obvious. There is a decided increase in follicular population over that previously seen in younger animals.

PLATE IX

Figure 23

PLATE IX

Ovary of immature rat, 33 days of age, whose uterus is shown in figure 18 (class II uterus).

EXPLANATION OF FIGURE

Fig. 23. Ovary, x25. Large vesicular follicles are obvious. There is a decided increase in follicular population over that previously seen in younger animals.

PLATE X

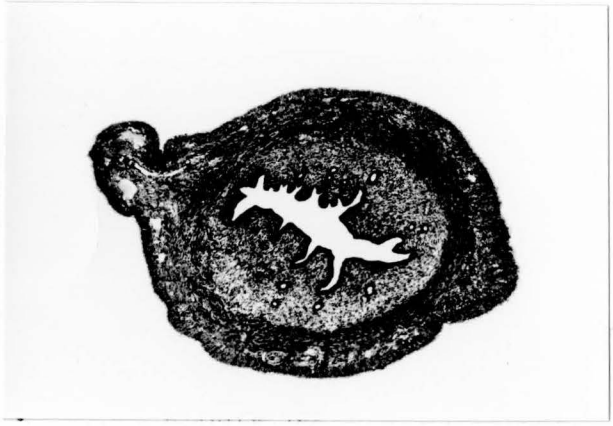


Figure 24

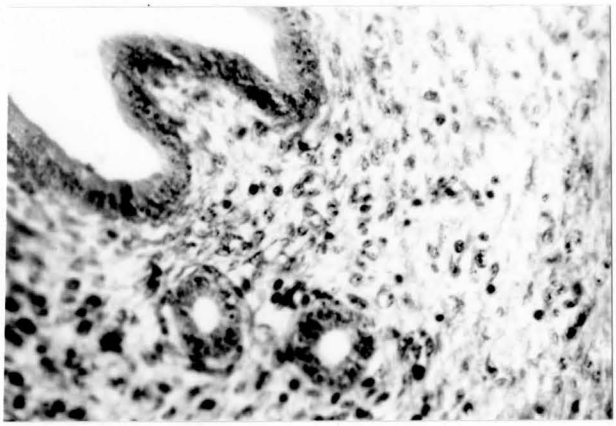


Figure 25

PLATE XI

Ovary of immature rat, 34 days of age, whose uterus is depicted in figure 24.

EXPLANATION OF FIGURES

Fig. 26. Ovary, x25. Note near uniform appearance of vesicular follicles.

Fig. 27. Higher magnification of follicle shown at 8:00 o'clock in figure 26, x400. Note condensing chromosomes.

Fig. 28. Higher magnification of egg cell of a follicle of ovary shown in figure 26, x600. Note process of meiosis.

PLATE XI

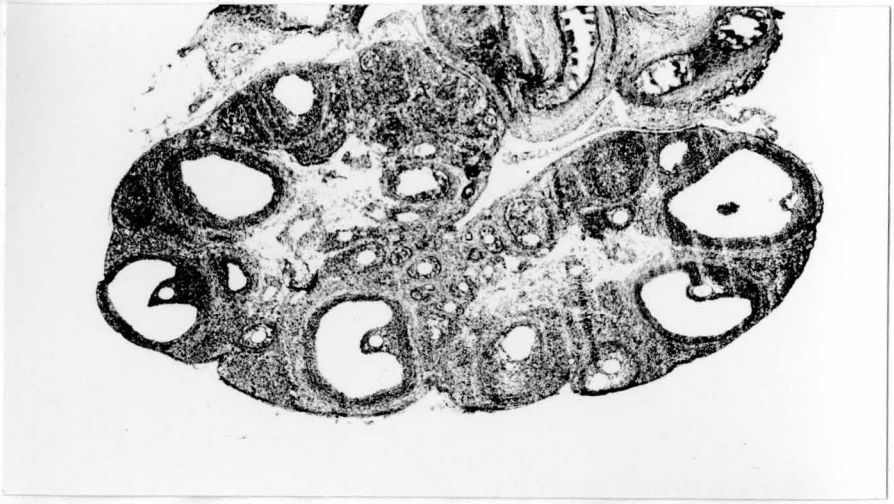


Figure 26

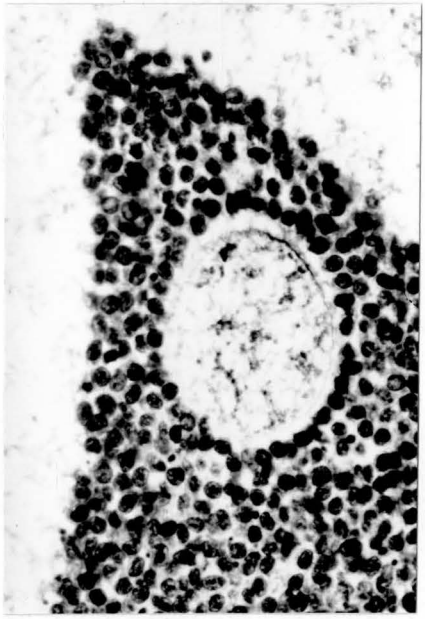


Figure 27

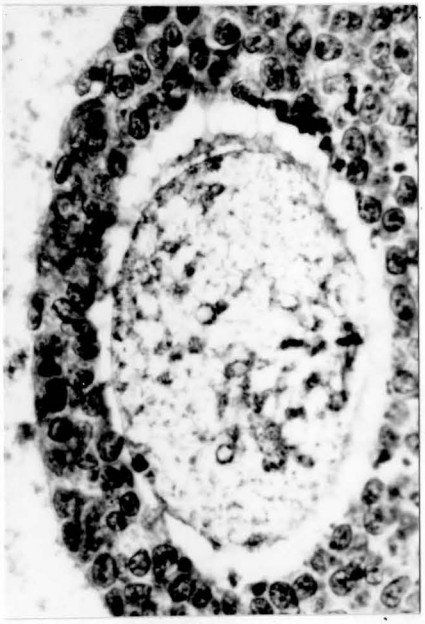


Figure 28

PLATE XII

Uterus of immature rat, 37 days of age, of lower uterine weight range for this class (class III: height of uterine luminal epithelium increased to 20 to 30 micra)

EXPLANATION OF FIGURES

Fig. 29. High power view of uterus emanating from uterine luminal area, x100. Note increased height of luminal epithelium and marked stromal and muscular cellularity.

Fig. 30. Higher power magnification of uterus shown in figure 29, x250. Note distinctive appearance of luminal epithelium.

Fig. 31. Increased magnification of figure 29, x1000. Note highly active uterine luminal epithelium.

PLATE XII

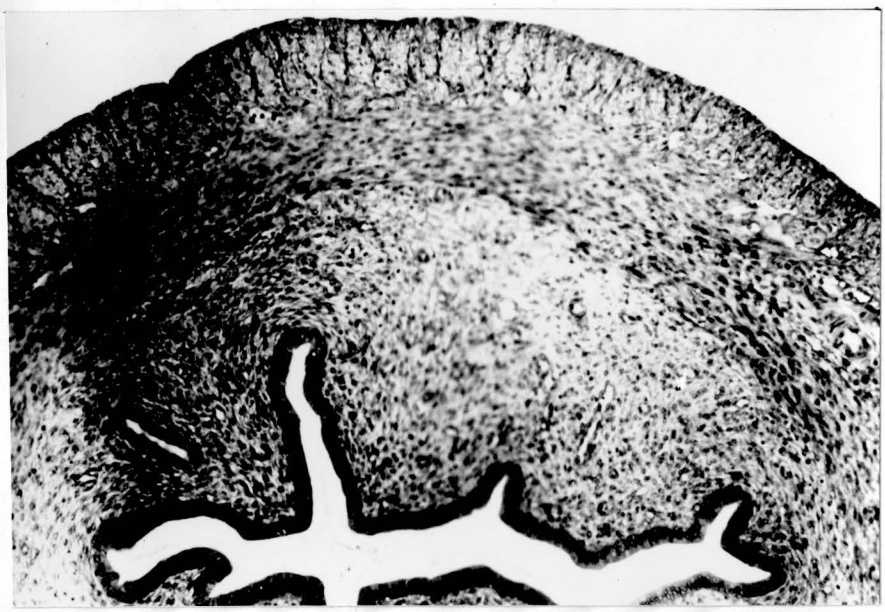


Figure 29

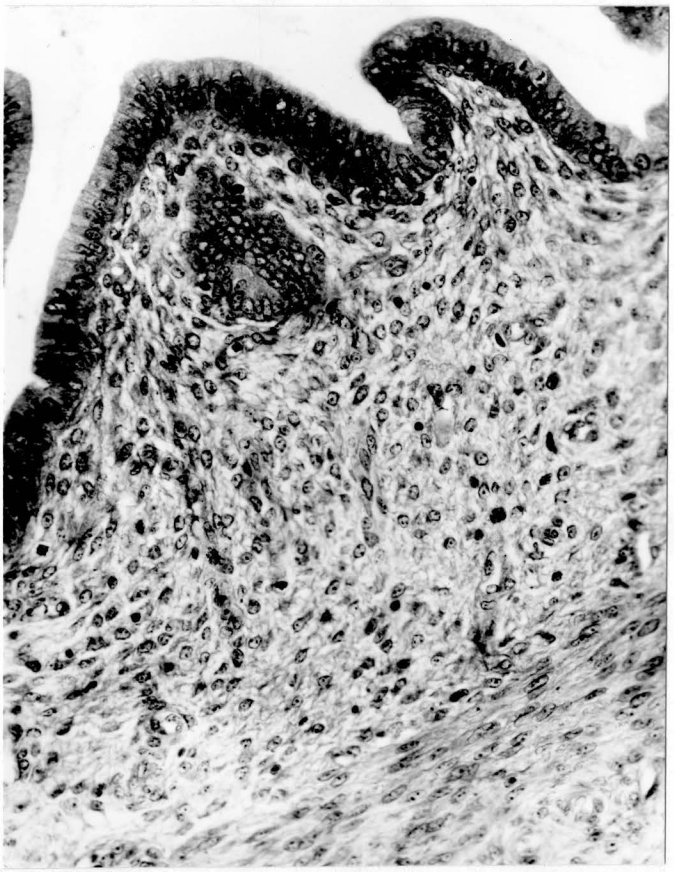


Figure 30, at left

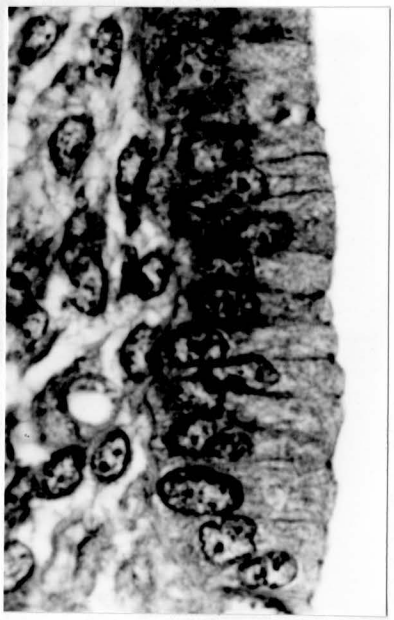


Figure 31

PLATE XIII

Ovary of immature rat, 37 days of age, whose uterus was depicted in figure 29.

EXPLANATION OF FIGURES

Fig. 32. Ovary, x25. Note enlarged vesicular follicles with near- and pre-ovulatory swelling.

Fig. 33. Ovary, x250. Higher power view of follicle shown at lower aspect of figure 32, with meiotic figure.

PLATE XIII

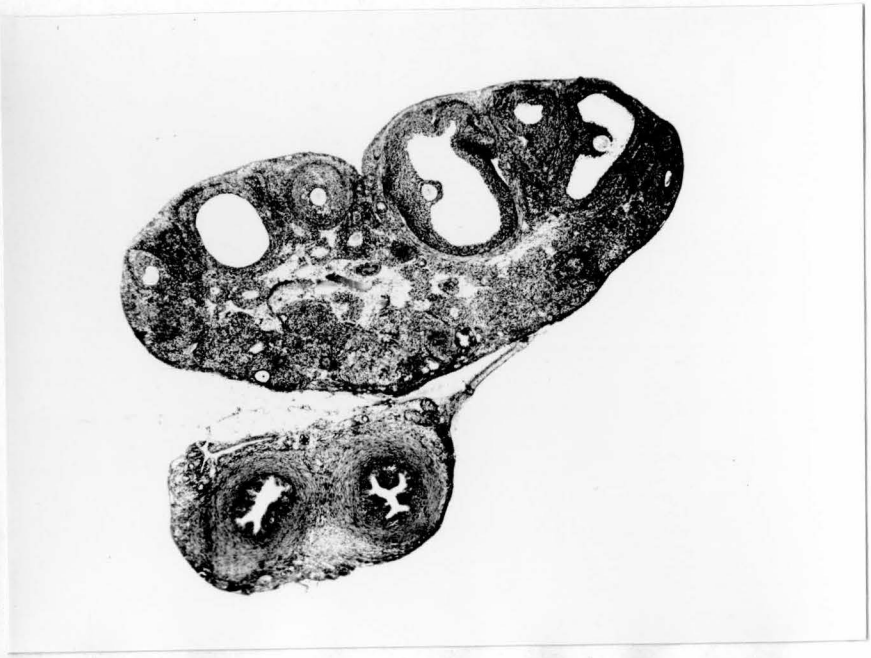


Figure 32

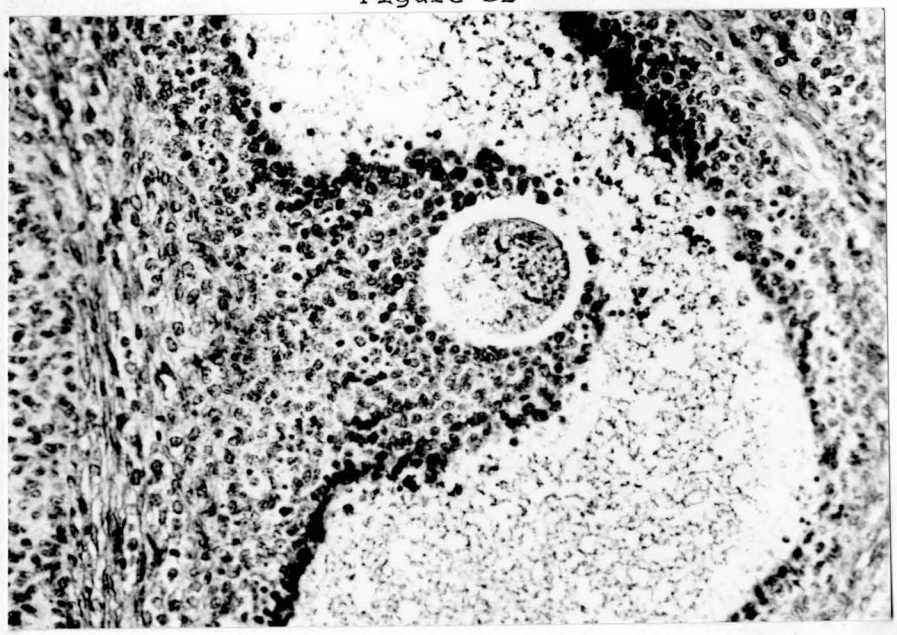


Figure 33

PLATE XIV

Uterus of immature rat, 37 days of age, of higher uterine weight range for this class (class III).

EXPLANATION OF FIGURES

Fig. 34. Uterus, x250, with special attention given to the well-developed, highly active uterine luminal epithelium.

Fig. 35. Higher power of uterus shown in figure 34, x1000. Note mitotic figure and distinct cellular compartmentalization.

PLATE XIV

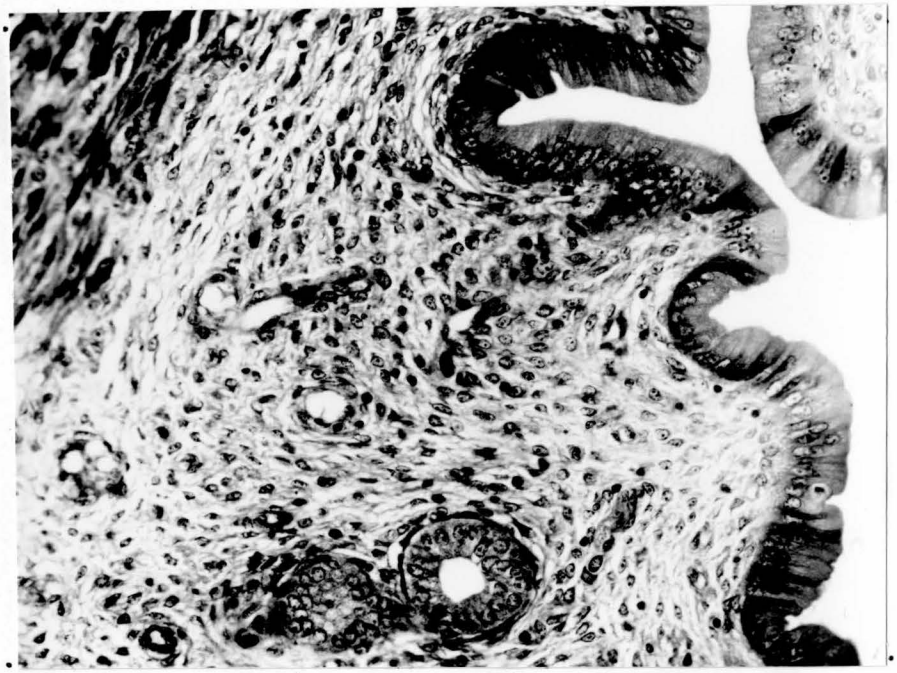


Figure 34

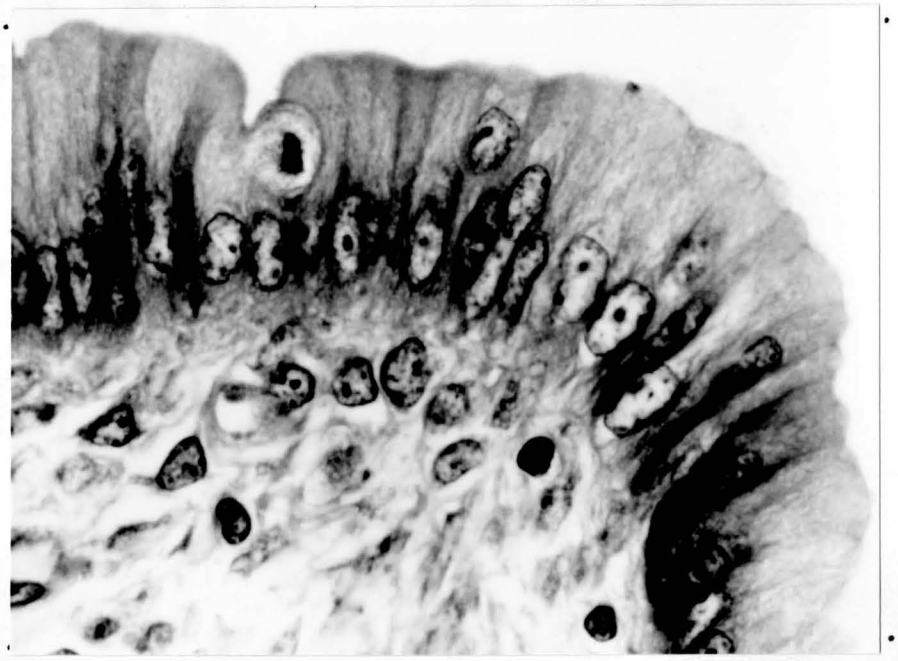


Figure 35

PLATE XV

Uterus of immature rat, 41 days of age (class IV, i.e. uterine epithelium over 30 micra and without vacuolar degeneration - typical of proestrus or early estrus).

EXPLANATION OF FIGURES

Fig. 36. Uterus x25. Note well-developed uterine luminal epithelium and inpocketings.

Fig. 37. Higher magnification of uterus shown in figure 36, x250, showing high columnar uterine luminal epithelium, which, however, does not have the typical tufted appearance of the luminal epithelium of mature animals in estrus.

Fig. 38. Intermediate magnification of uterus shown in figure 36, x100. Note uniformly basal position of nuclei of luminal epithelium and lack of stromal edema.

PLATE XV

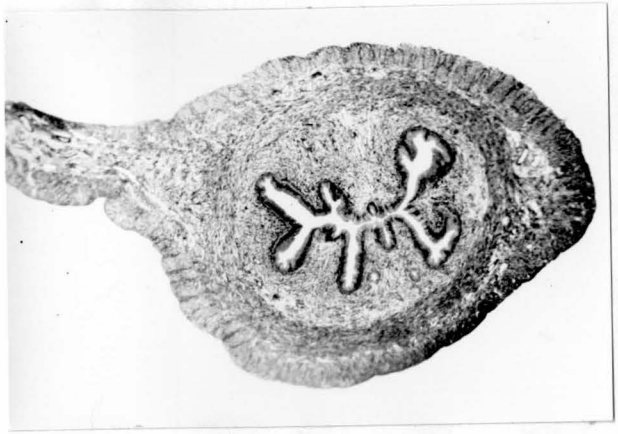


Figure 36

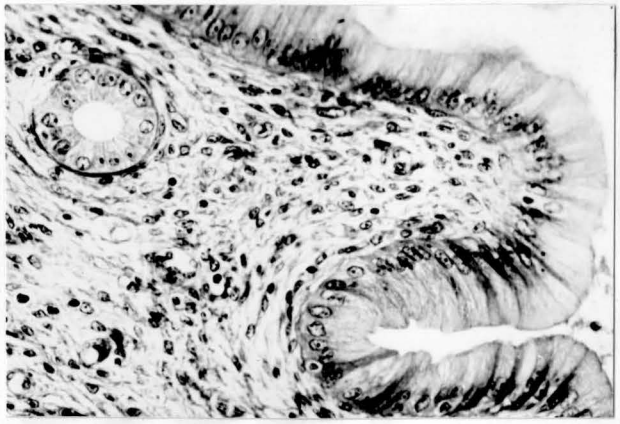


Figure 37

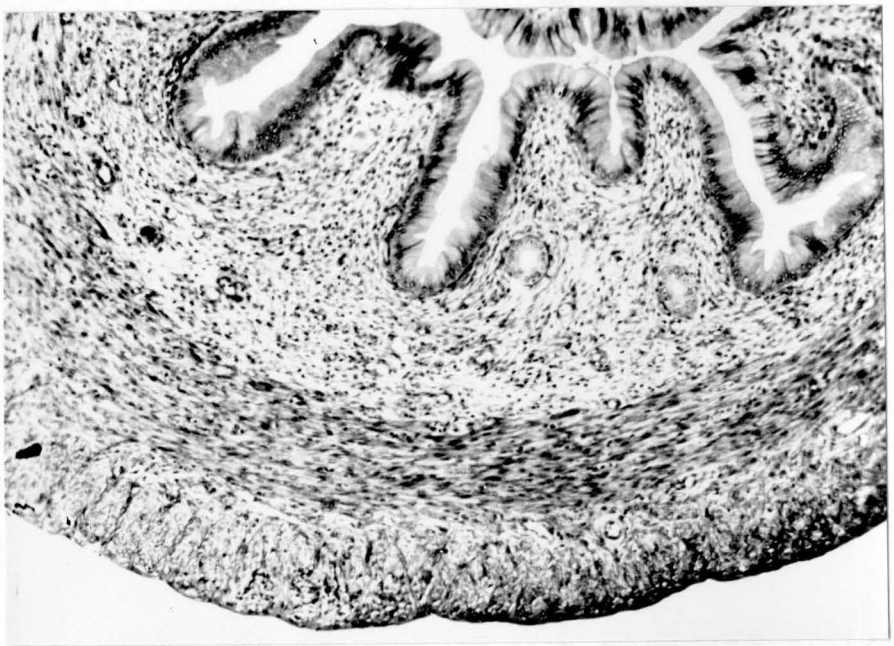


Figure 38

PLATE XVI

Ovary and uterus of immature rat, 41 days of age (immature rat whose uterus is of class IV); same rat as shown on plate XV.

EXPLANATION OF FIGURES

Fig. 39. Higher power magnification of uterus, x1000, showing uterine luminal epithelium. Note the high, columnar epithelium, distinct cellular compartmentalization and basally oriented nuclei.

Fig. 40. Ovary, x25. Note marked follicular development at periphery.

Fig. 41. Higher magnification of largest follicle shown in figure 40, x400. Note condensing chromosomes in egg cell.

PLATE XVI

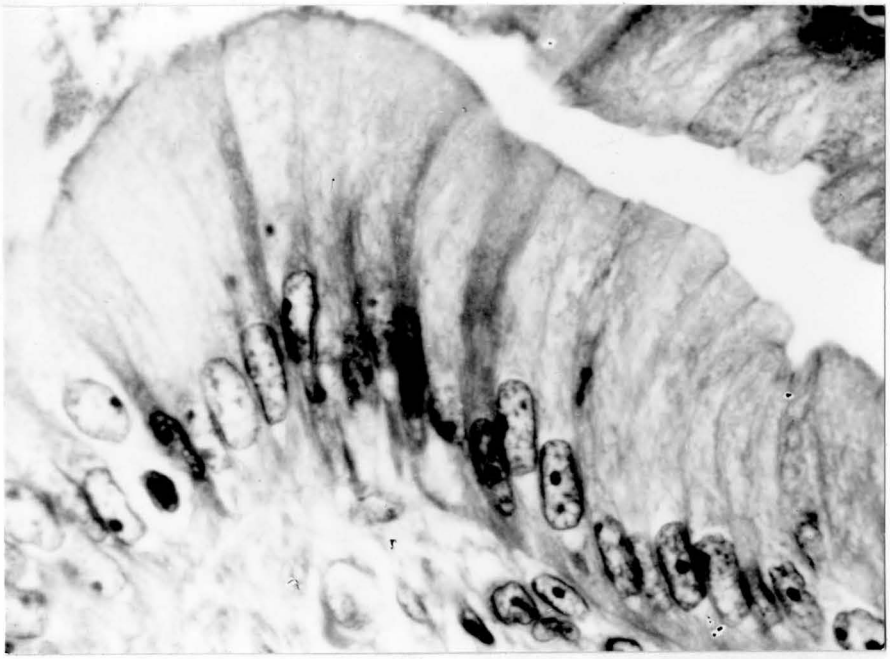


Figure 39

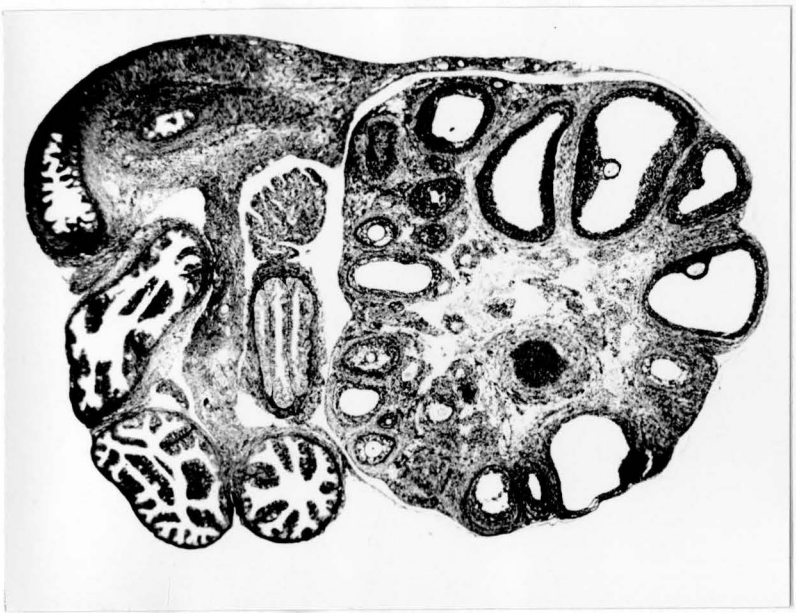


Figure 40

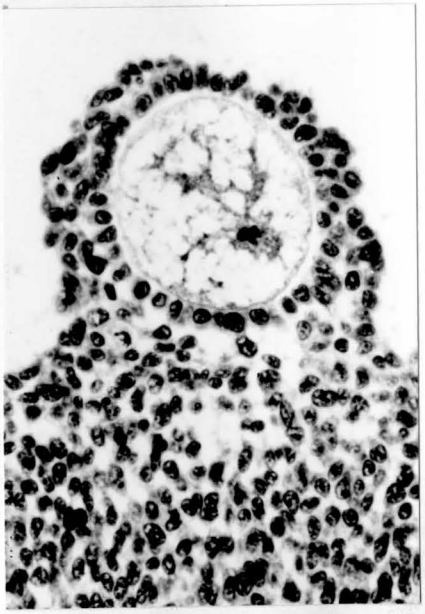


Figure 41

PLATE XVII

Uterus of immature rat, 34 days of age (class V, i.e. vacuolar degeneration of luminal epithelium typical of late estrus to metestrus in the cycling, adult animal).

EXPLANATION OF FIGURES

Fig. 42. Uterus, x25. Note extensive inpocketing of uterine luminal epithelium.

Fig. 43. Uterus, showing region from lumen through myometrium, x100. Note tufts of vacuolated epithelium and marked cellularity of endometrium and myometrium.

Fig. 44. Higher power view of myometrium, x1000. Inner, circular muscle with prophase mitotic figure is depicted.

PLATE XVII

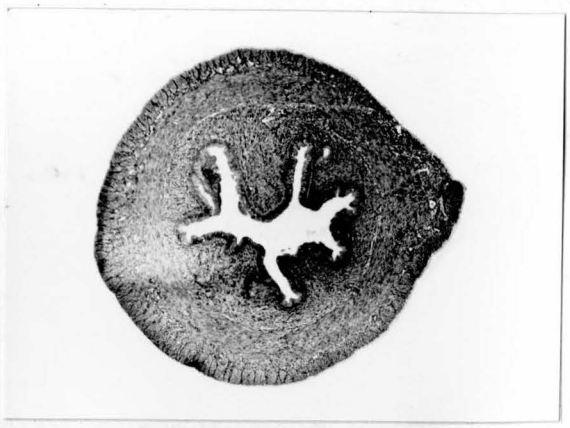


Figure 42

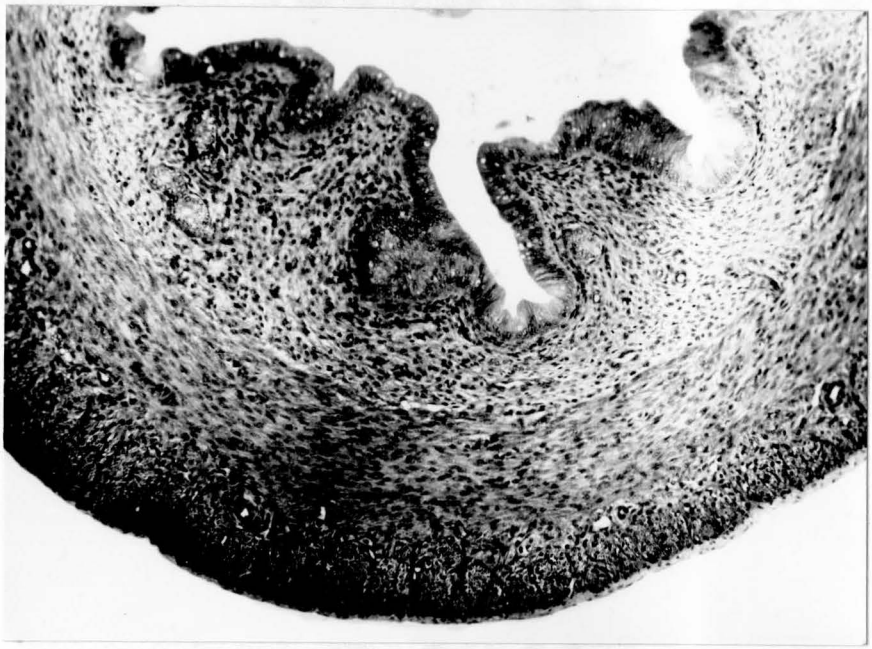


Figure 43

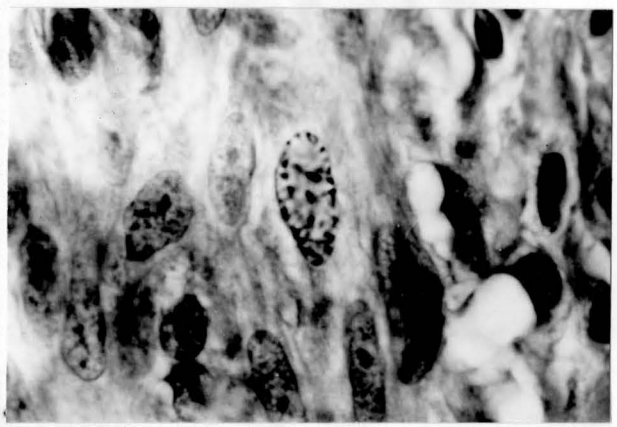


Figure 44

PLATE XVIII

Uterus of immature rat, 34 days of age (class V). Higher magnification of uterus shown in figure 42, plate XVII.

EXPLANATION OF FIGURES

Fig. 45. Detailed view of uterine luminal epithelium and surrounding stroma, x250.

Fig. 46. Detailed view, continued, extended through myometrium, x250.

PLATE XVIII

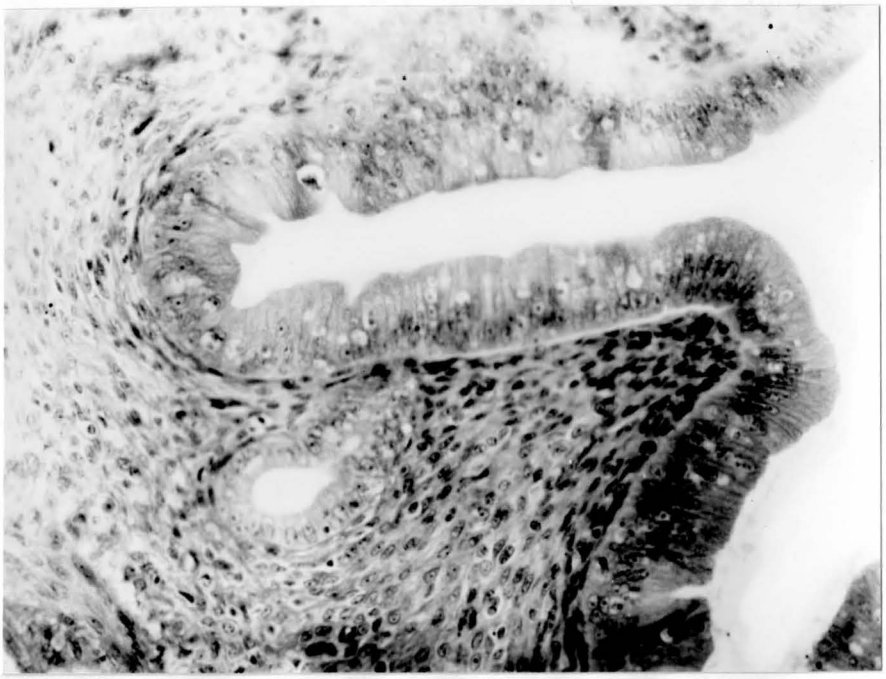


Figure 45

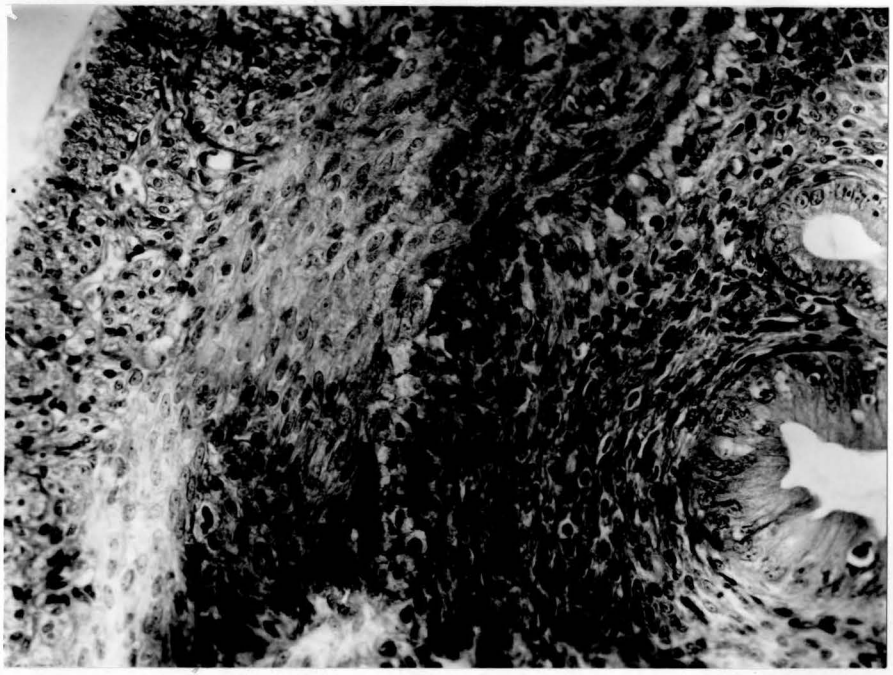


Figure 46

PLATE XIX

Uterus and ovary of immature rat, 34 days of age (class V). Tissues of the same animal whose uterus is shown on plates XVII and XVIII.

EXPLANATION OF FIGURES

Fig. 47. Uterine luminal epithelium, x1000. Note the tall, columnar epithelium, cells with vacuoles and material contained in vacuoles.

Fig. 48. Ovary, x50. Note follicle with dehisced coronal cells surrounding their vital cargo, the ovum.

PLATE XIX

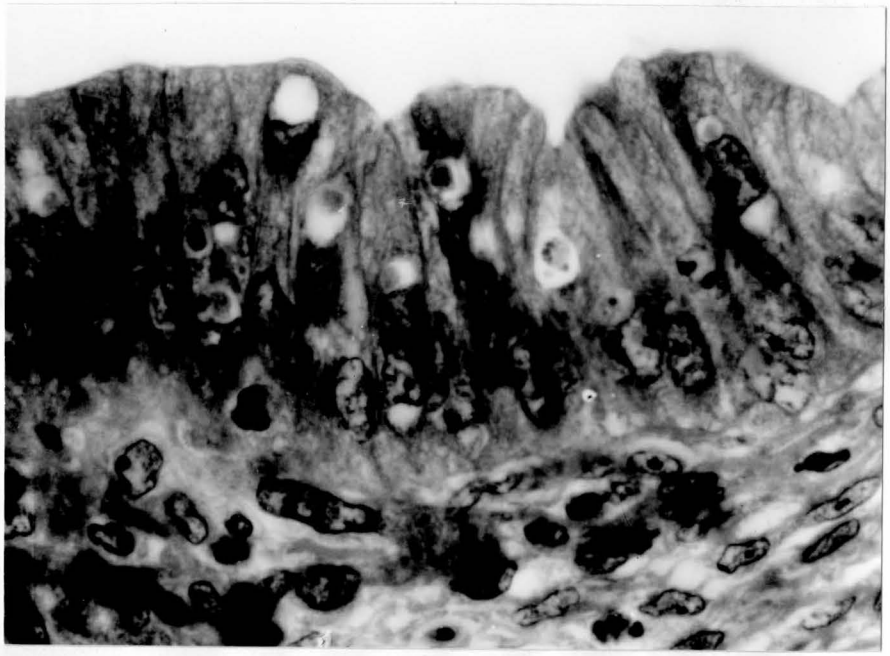


Figure 47

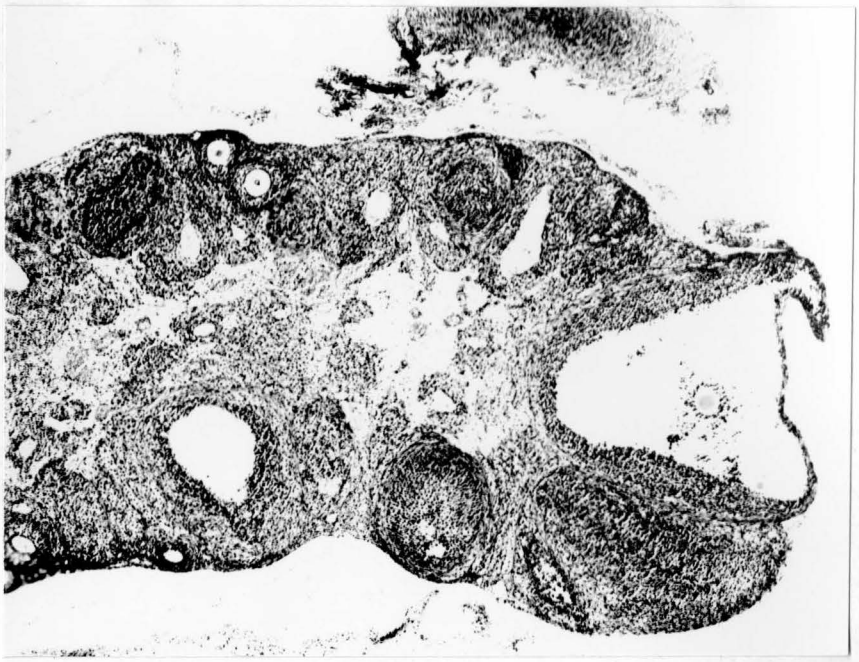


Figure 48

PLATE XX

Uterus and ovary of pubertal rat, 35 days of age, necropsied on first day of finding of vaginal opening.

EXPLANATION OF FIGURES

Fig. 49. Uterus, x25. Note typical preproestrous characteristics: widened uterine lumen with small uterine luminal epithelial inpocketings, and low columnar apithelium.

Fig. 50. Higher power view of uterus shown in figure 49, x250. Note low columnar epithelium.

Fig. 51. Ovary, x25. Note corpora lutea, indicating the previous occurance of ovulation, prior to the time this animal exhibited vaginal opening.

PLATE XX

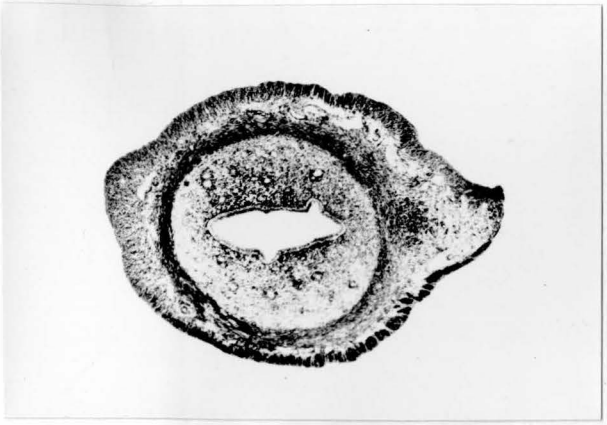


Figure 49

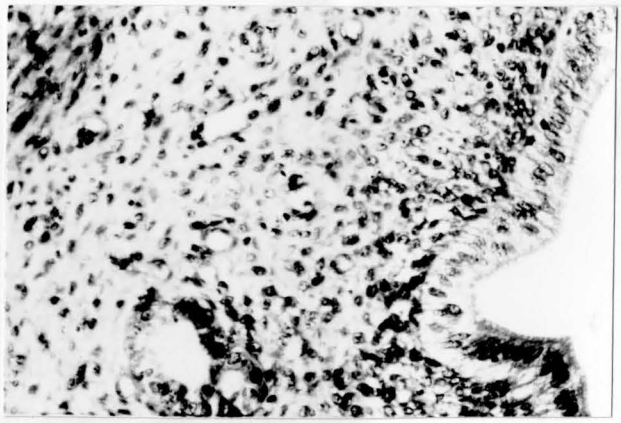


Figure 50

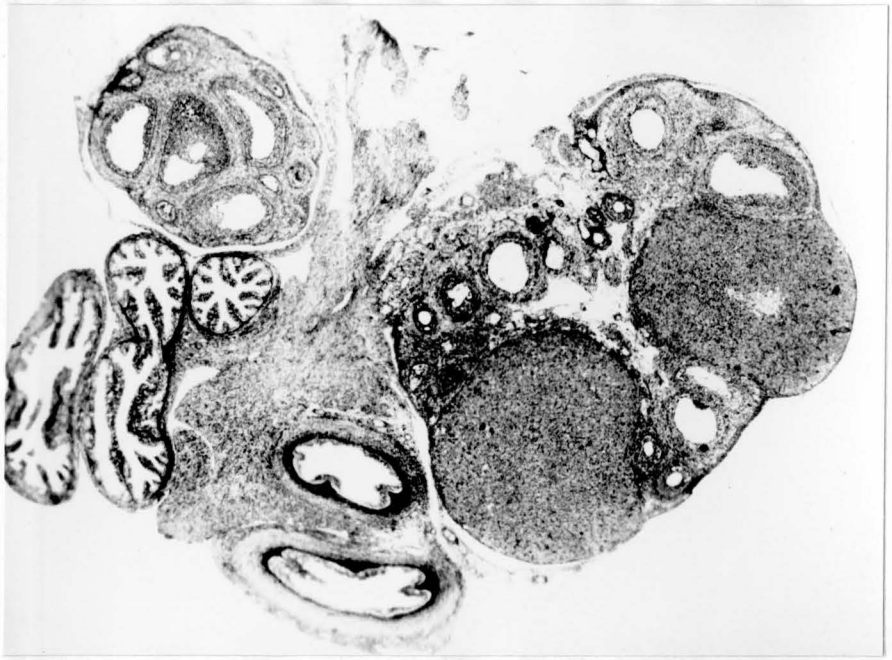


Figure 51

PLATE XXI

Ovary of a pubertal rat, 35 days of age, necropsied at 35 days of age, i.e. on first day of vaginal opening.

EXPLANATION OF FIGURES

Fig. 52. Ovary x25. Note both large vesicular follicles and corpora lutea.

Fig. 53. Higher power of ovary shown in figure 52, x50, detailing a corpus luteum.

Fig. 54. Higher power view of corpus luteum shown in figure 53, x250. The pie-shaped area of the corpus luteum emanates from the central coagulum.

PLATE XXI

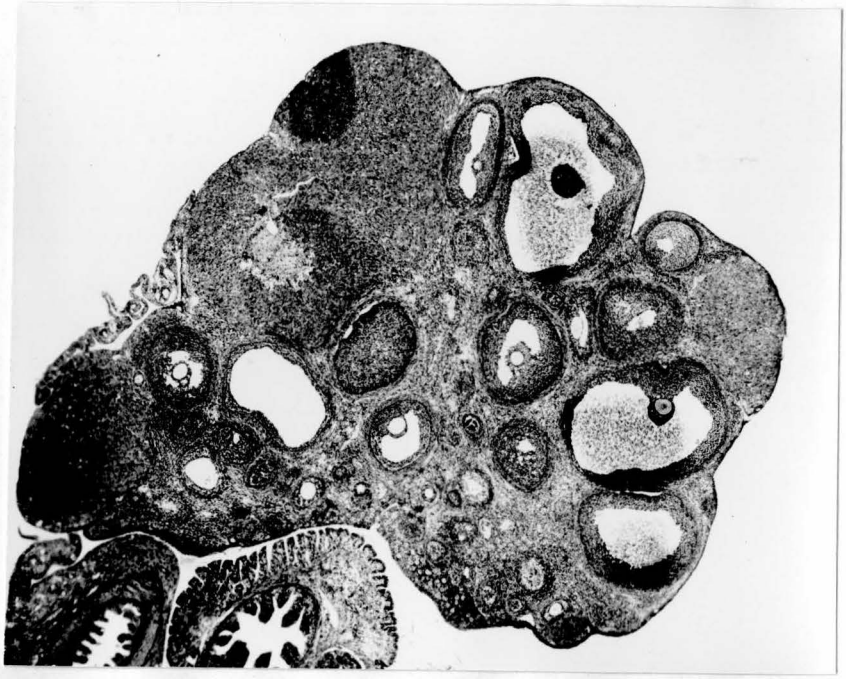


Figure 52

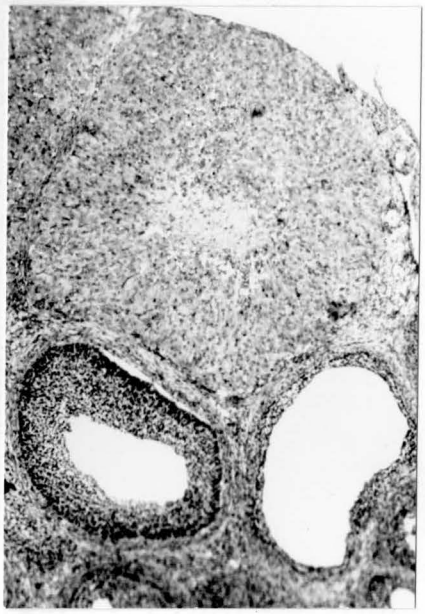


Figure 53

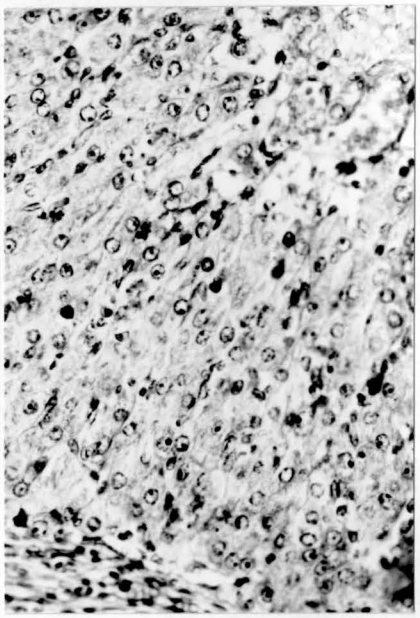


Figure 54

PLATE XXII

Uterus of pubertal rat, 35 days of age, at late proestrus preceding first estrus.

EXPLANATION OF FIGURES

Fig. 55. Uterus, x25. Note the numerous and deep inpocketings of the uterine luminal epithelium typical of proestrus.

Fig. 56. Higher power view of uterus seen in figure 55, x250. Note the high columnar uterine luminal epithelium with basally oriented nuclei seen for most of its cells.

Fig. 57. Intermediate power of uterus shown in figure 55, x100. Note highly cellular luminal epithelial surface lining and the slight stromal edema, compare with figure 38, plate XV for this similar stage in the prepubertal rat.

PLATE XXII

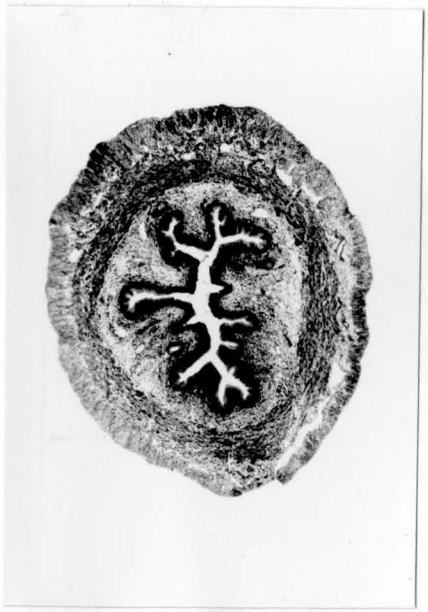


Figure 55

Figure 56

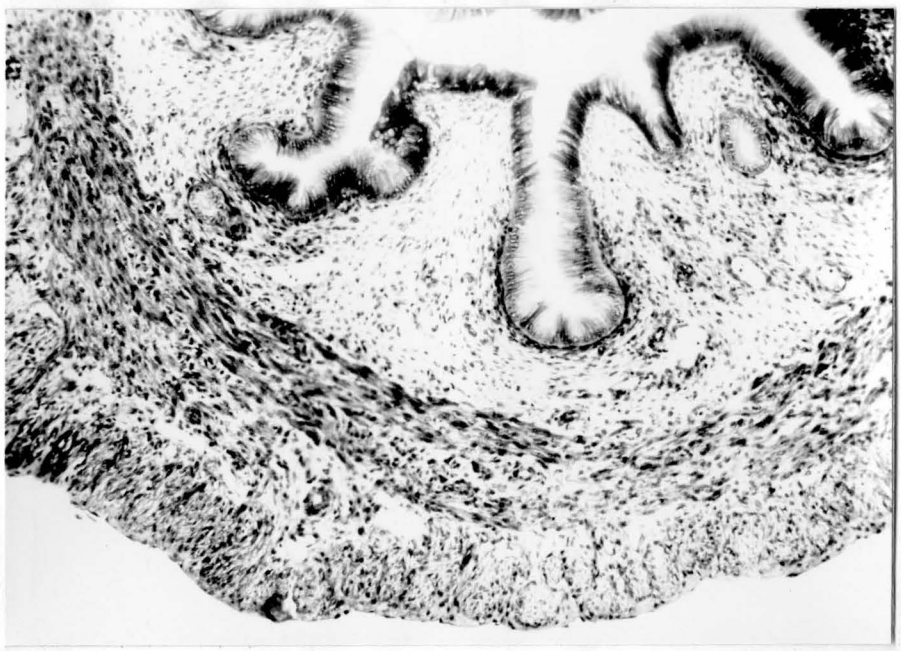


Figure 57

PLATE XXIII

Ovary of pubertal rat, 35 days of age, at proestrus preceding first estrus. Same animal whose uterus is depicted on plate XXII.

EXPLANATION OF FIGURE

Fig. 58. Ovary, x25. Note the many large vesicular follicles, several of which show preovulatory swelling.

PLATE XXIII

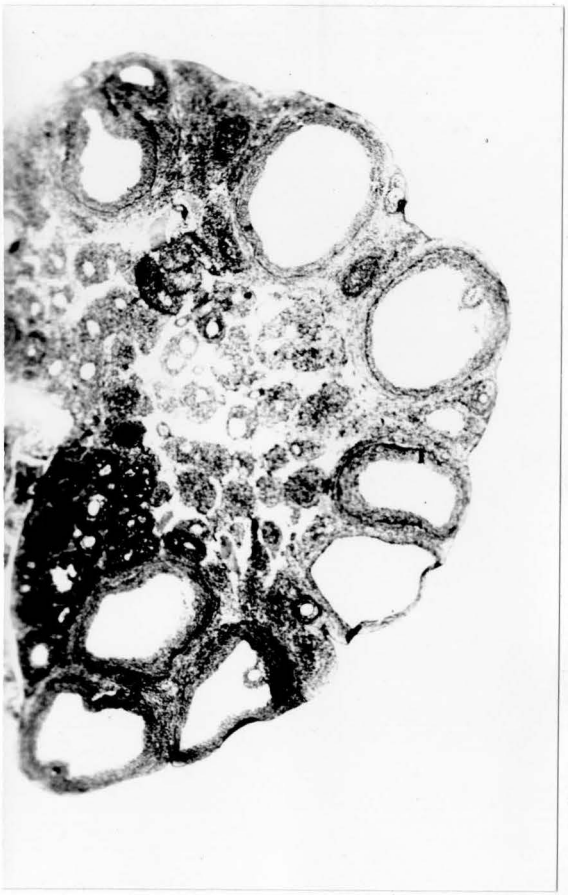


Figure 58

PLATE XXIV

Uterus and ovary of pubertal rat, 38 days of age, on first day of vaginal opening and in first estrus.

EXPLANATION OF FIGURES

Fig. 59. Uterus, x25. Note the typical estrous pattern in the uterus, which, however, has not yet reached typical size of this stage.

Fig. 60. Higher power of region of uterus shown in figure 59, x250. Note high columnar epithelium containing large number of small circular vacuoles with entrapped material.

Fig. 61. Ovary, x25 (inset x 400). Note vesicular follicles of varying size. The ovum of the largest follicle, however, shows a meiotic spindle, cf. inset.

PLATE XXIV

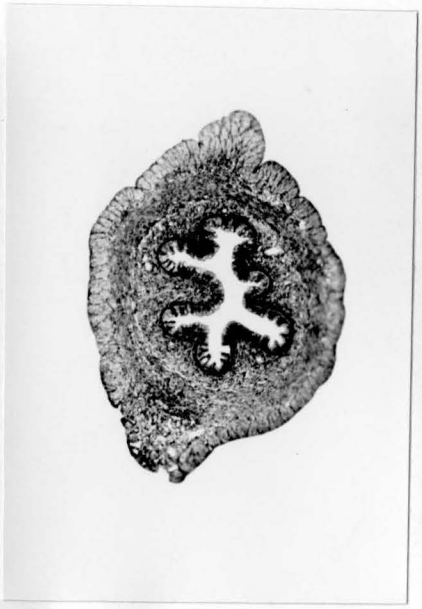
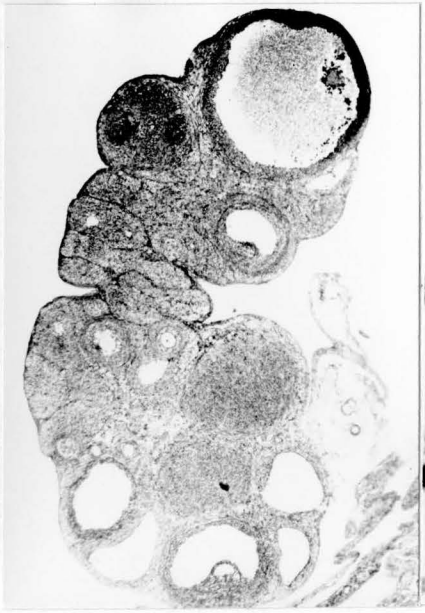


Figure 59

Figure 60



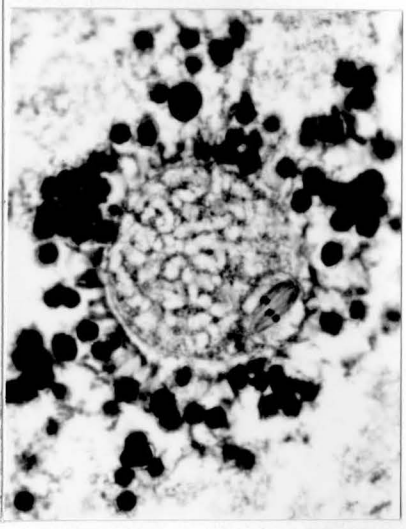


Figure 61

PLATE XXV

Uterus and ovary of pubertal rat, 41 days of age, at first estrus.

EXPLANATION OF FIGURES

Fig. 62. Uterus, x25. Note typical appearance of uterine luminal epithelium during estrus. Also obvious is the typical increase in uterine size characteristic of estrus, cf. also figure 59.

Fig. 63. Ovary of the same rat whose uterus is shown in figure 62, x25. Vesicular follicle with stigma of ovulation is seen at the far right, and an ovum is seen clearly in the oviduct.

PLATE XXV

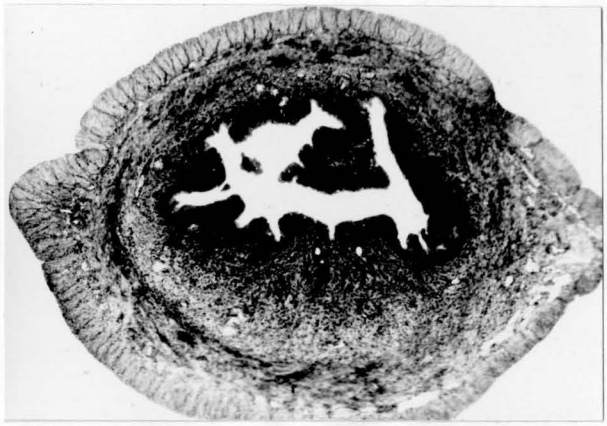


Figure 62

Figure 63

PLATE XXVI

Uterus and ovary of pubertal rat, 42 days of age, at first diestrus.

EXPLANATION OF FIGURES

Fig. 64. Uterus, x25. Note reduced size of uterus, typical for this stage of the estrous cycle, and straight, barely inpocketed uterine lumen.

Fig. 65. Higher power view of uterus observed in figure 64, x250. Note cuboidal-like appearing uterine luminal epithelium.

Fig. 66. Ovary, x25. Note prominent corpora lutea. Vesicular follicles are also present.

PLATE XXVI

Figure 64

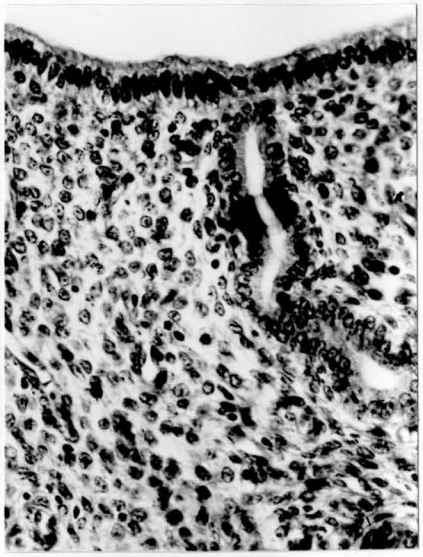


Figure 65

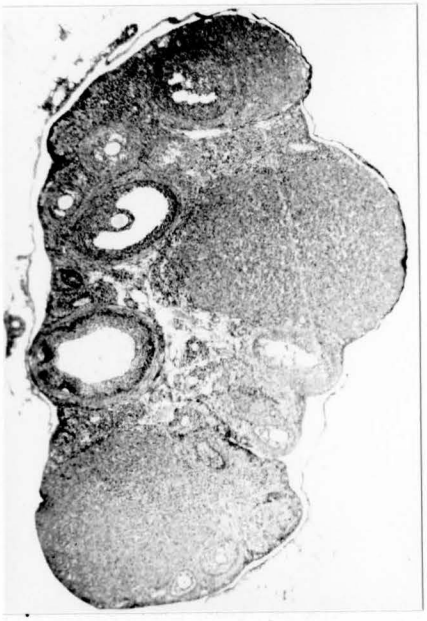


Figure 66

PLATE XXVII

Uterus and ovary of pubertal rat, 42 days of age, necropsied three days after vaginal opening while in preproestrus preceding second estrus.

EXPLANATION OF FIGURES

Fig. 67. Uterus, x25. Note typical appearance of uterus of preproestrous rat.

Fig. 68. Higher power view of uterus observed in figure 67, x400. Note mitotic figure in anaphase in circular muscle.

Fig. 69. Ovary, x25. Note corpora lutea, all of the same generation.

PLATE XXVII

Figure 67

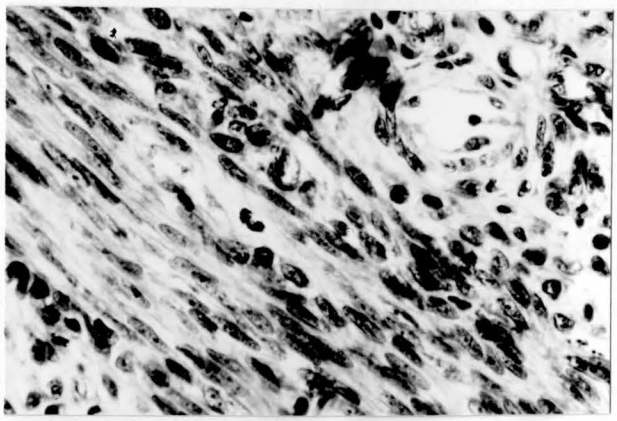


Figure 68

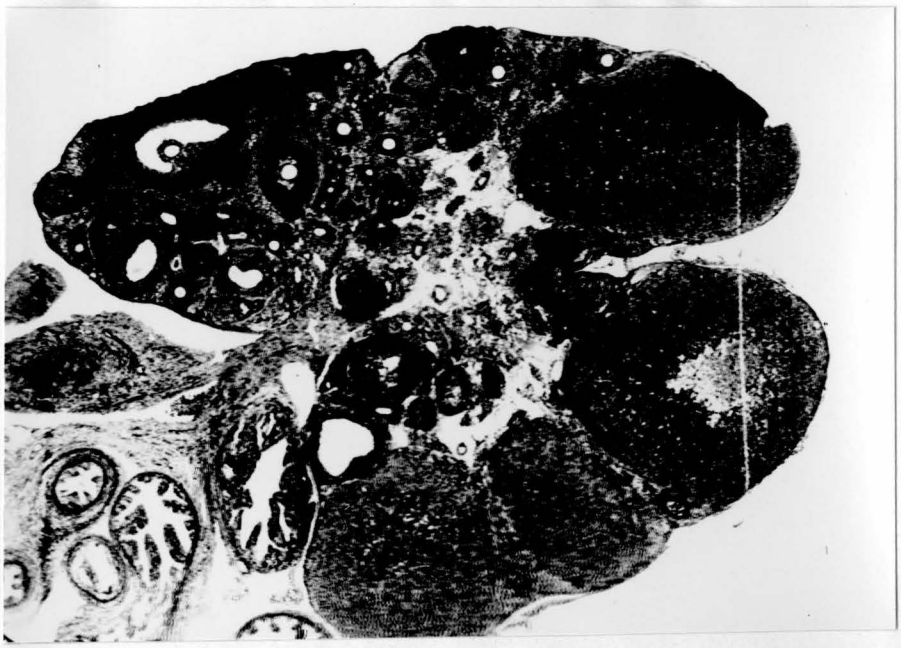


Figure 69

PLATE XXVIII

Uterus of pubertal rat, 41 days of age, and uterus and ovary of pubertal rat, 43 days of age, in second estrous period.

EXPLANATION OF FIGURES

Fig. 70. Uterus of rat 41 days of age, x25. Note typical estrous endometrial pattern.

Fig. 71. Uterus of rat 43 days of age, x25. This animal was necropsied later in the estrous period than the animal whose uterus is shown in figure 70.

Fig. 72. Higher power view of uterus shown in figure 71, x250. Note moderately high columnar epithelium containing numerous, small vacuoles. Nuclei have migrated centrally.

Fig. 73. Ovary of rat 43 days of age, x25, showing dehiscing germ hill and enlarged corpora lutea of the last generation.

PLATE XXVIII

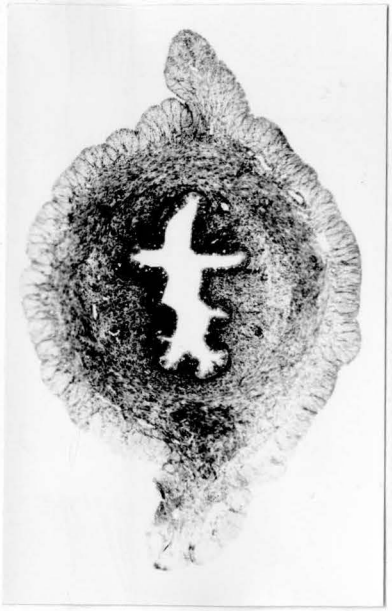


Figure 70

Figure 71

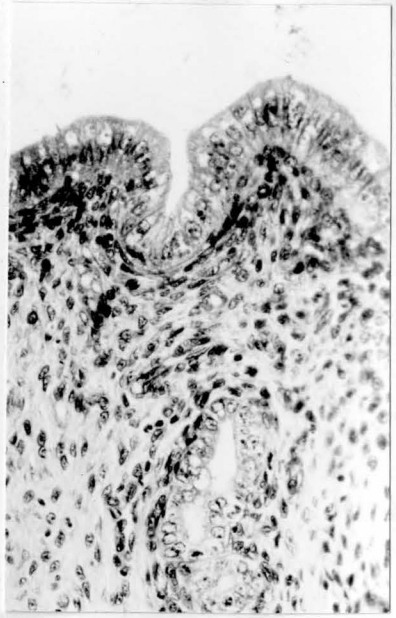


Figure 72

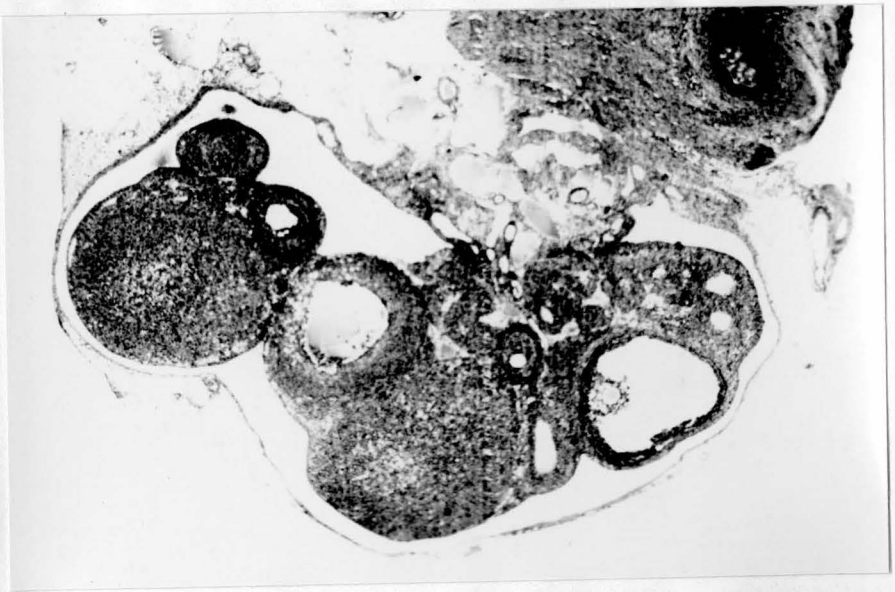


Figure 73

PLATE XXIX

Uterus of cycling, young adult rat, 65 days of age, in preproestrus.

EXPLANATION OF FIGURES

Fig. 74. Uterus, x25, showing typical uterine pattern of rat in preproestrus. Note enlarged size when compared with uteri depicted previously of prepubertal and pubertal animals.

Fig. 75. Higher power view of uterus shown in figure 74, x250. Note columnar luminal epithelium, mitotic figure in anaphase in luminal epithelium close to the inpocketing, stromal edema and developing glands in the stroma.

Fig. 76. Higher power of section shown in figure 75, x1000. Note moderate height of luminal epithelial cells and mitotic figure in luminal epithelium.

PLATE XXIX

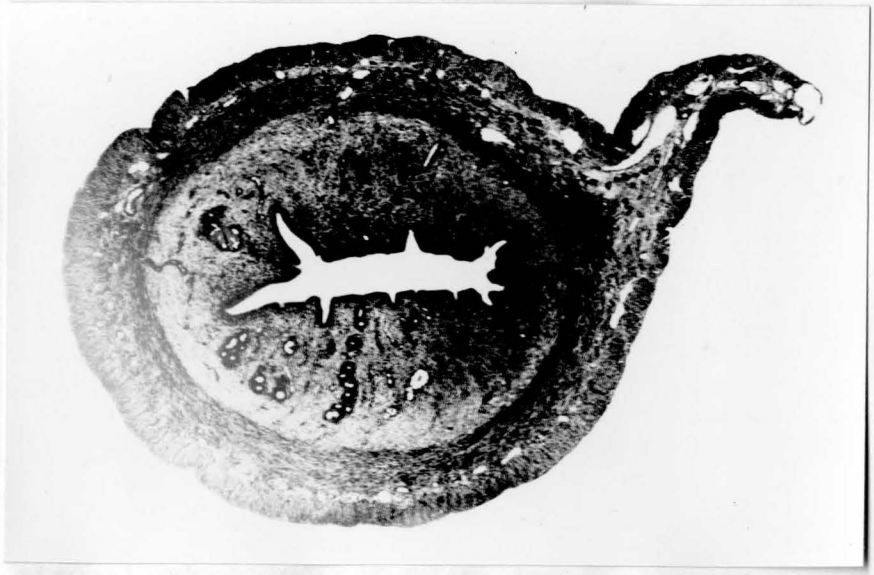


Figure 74

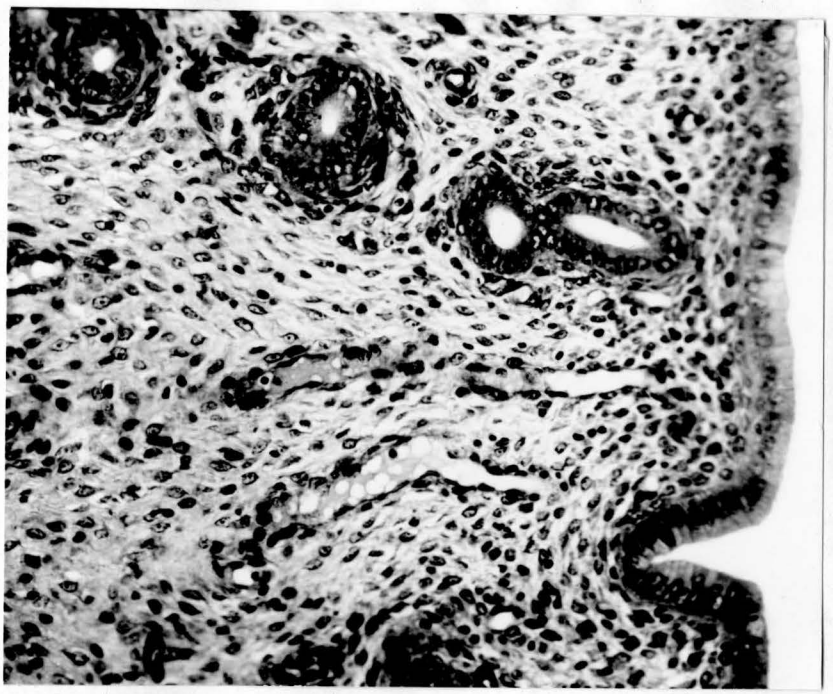


Figure 75

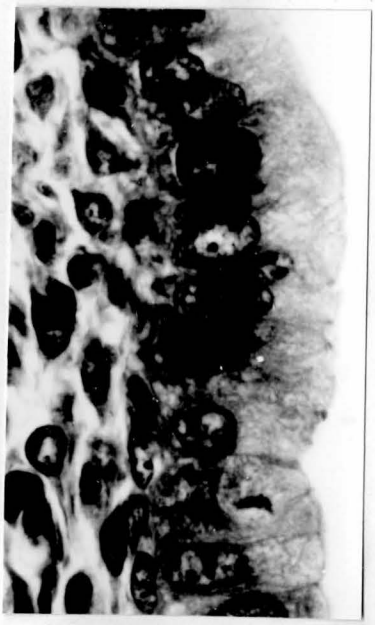


Figure 76

PLATE XXX

Uterus of cycling, young adult rat, 55 days of age, in early estrus.

EXPLANATION OF FIGURES

Fig. 77. Uterus, x25, typical of early estrus. Markedly indented and stellate-appearing luminal epithelium in the antimesometrial aspect and numerous glands throughout the stroma suggest high metabolic activity.

Fig. 78. Higher power view of uterus shown in figure 77, x1000, showing tall columnar uterine luminal epithelium and stromal edema typical of estrus in the adult rat.

PLATE XXX

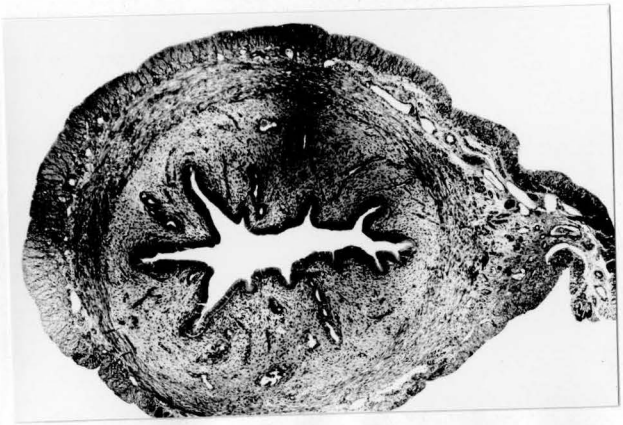


Figure 77

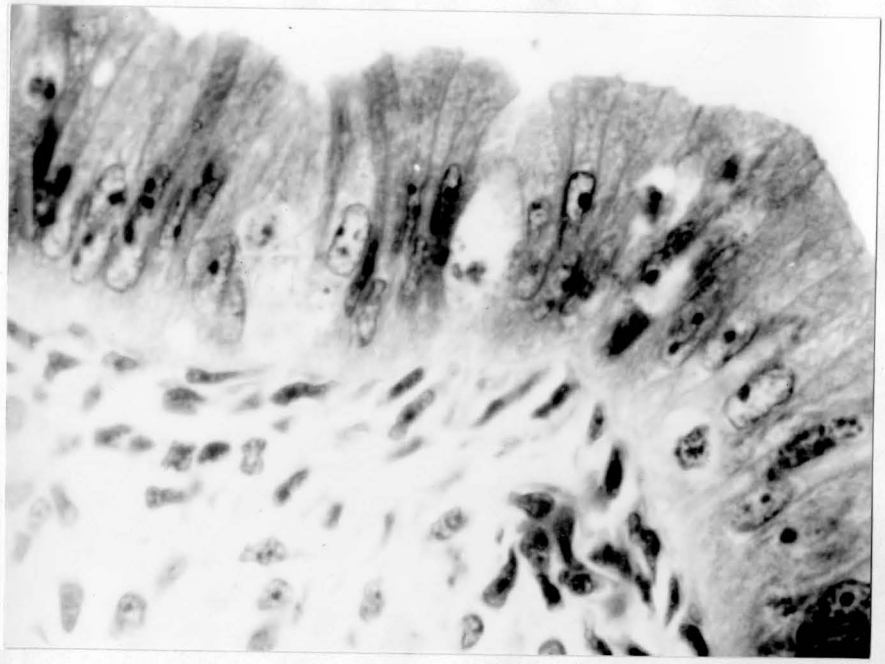


PLATE XXXI

Uterus of cycling, young adult rat, 55 days of age, in early metestrus.

EXPLANATION OF FIGURES

Fig. 79. Uterus, x25. Note enlarged uterine lumen, and general pattern of metestrus, an intermediate pattern between estrus and diestrus.

Fig. 80. High power view of uterine luminal epithelium of uterus shown in figure 79, x1000. Note typically ragged appearance of luminal epithelium with cells of varying heights and shapes.

PLATE XXXI

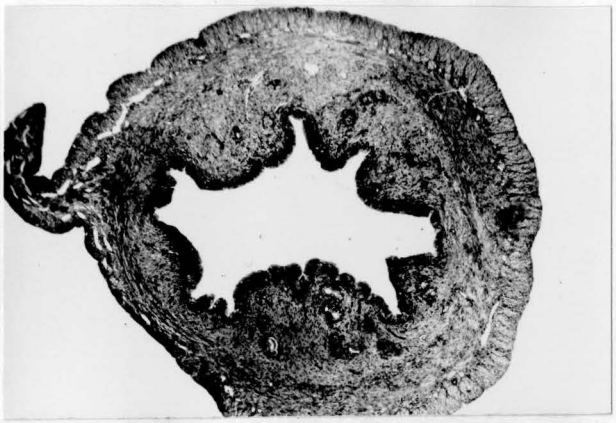


Figure 79

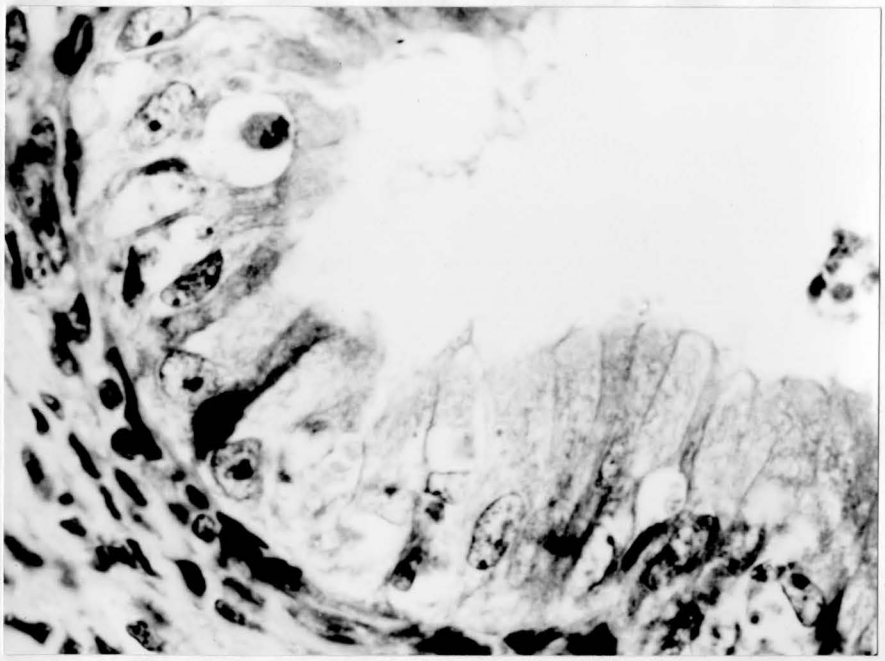


Figure 80

PLATE XXXII

Uteri of cycling, young adult rats, 55 days of age, in early diestrus.

EXPLANATION OF FIGURES

Fig. 81. Uterus, x25, showing a typical diestrous pattern: cuboidal uterine luminal epithelium, narrowed uterine lumen without inpocketings, and reduced uterine size.

Fig. 82. Uterus of another 55 day old rat in early diestrus, x1000. The uterine luminal epithelium has not yet reached cuboidal shape. Note also large basally oriented vacuoles containing material.

Fig. 83. Uterus of a third rat, x250. The inner circular muscle occupies most of the area of the photograph. Note mitotic figure in the circular muscle.

Fig. 84. High power view of uterine field shown in figure 83, x1000, highlighting mitotic figure.

PLATE XXXII

Figure 81

Figure 82

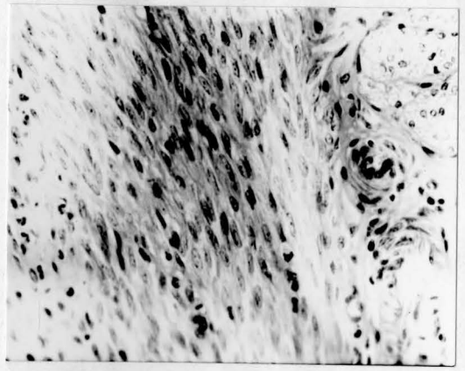


Figure 83

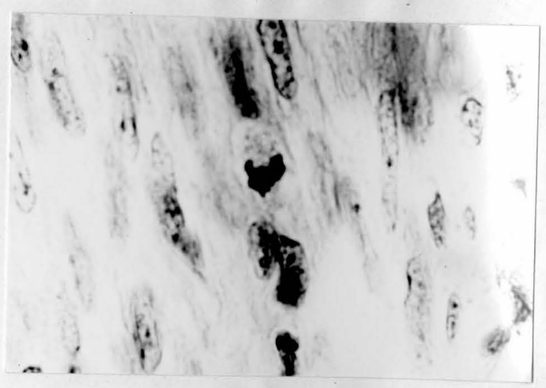


Figure 84

PLATE XXXIII

Uteri of bilaterally ovariectomized, young adult rats, 57 days of age, given hormonal vehicle, sesame oil one hour prior to necropsy.

EXPLANATION OF FIGURES

Fig. 85. Uterus, x25. Note reduction in size and minimal inpocketing of uterine luminal epithelium.

Fig. 86. Uterus of a second rat, x25. Reduction in size of uterus is more marked than in that shown in figure 85; also, note straight, narrow uterine lumen.

Fig. 87. Higher power view of uterus shown in figure 86, x250, illustrating reduced height of uterine luminal epithelium. The one gland seen in the whole cross section is included in this field.

Fig. 88. Higher power view of uterus shown in figure 86, x1000. Note decreased height of uterine luminal epithelium and small, basally-oriented vacuoles in the epithelial cells, typical of non-stimulated uteri.

PLATE XXXIII

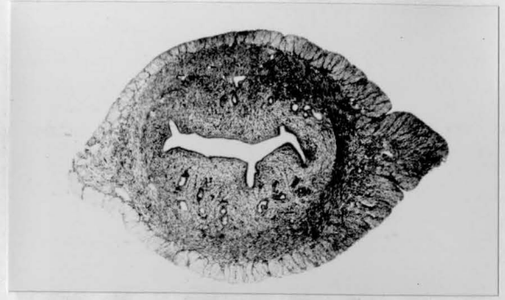


Figure 85

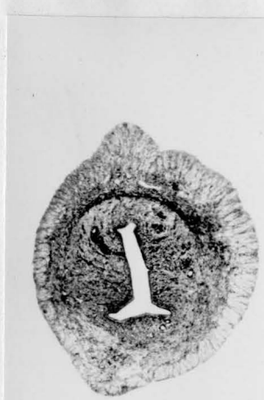


Figure 86

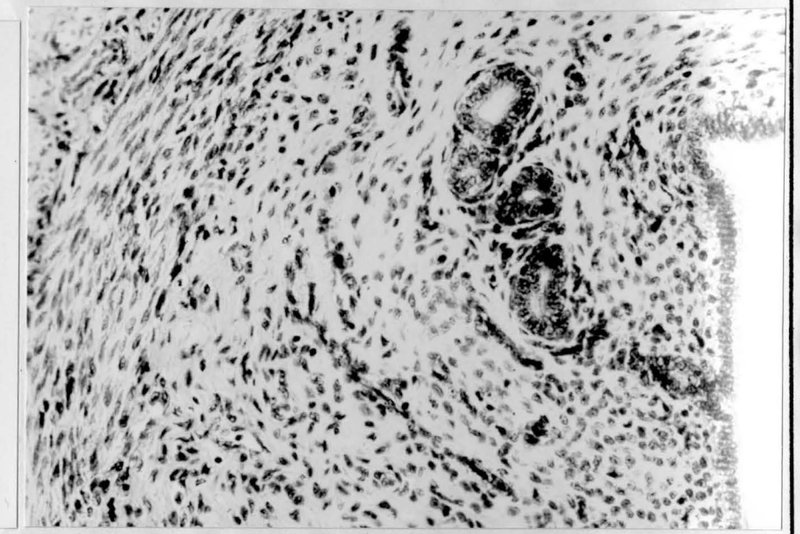


Figure 87

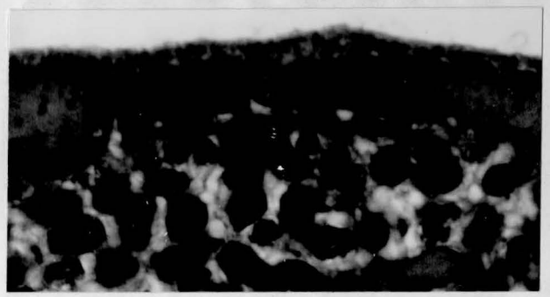


Figure 88

PLATE XXXIV

Uteri of bilaterally ovariectomized, young adult rats, 57 days of age, given ovarian sex steroid hormones or hormonal vehicle three hours prior to necropsy.

EXPLANATION OF FIGURES

Fig. 89. Uterus, x250, of rat given 0.2 cc sesame oil as control. Note marked atrophy of uterus.

Fig. 90. Uterus, x250, of rat given 0.1μg estradiol-17β, showing stromal edema, a feature of early uterine 'awakening.'

Fig. 91. Uterus, x250, of rat given 1.5 mg progesterone, showing lack of stimulation.

Fig. 92. Uterus, x250, of rat given 0.1μg estradiol-17β and 1.5 mg progesterone, showing early 'awakening' pattern, especially stromal edema.

PLATE XXXIV

Figure 89

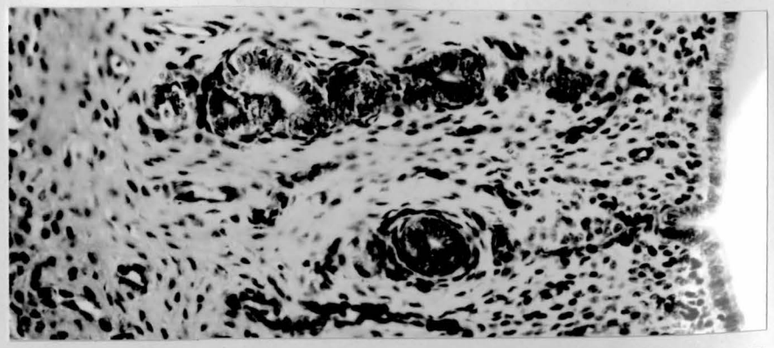


Figure 90

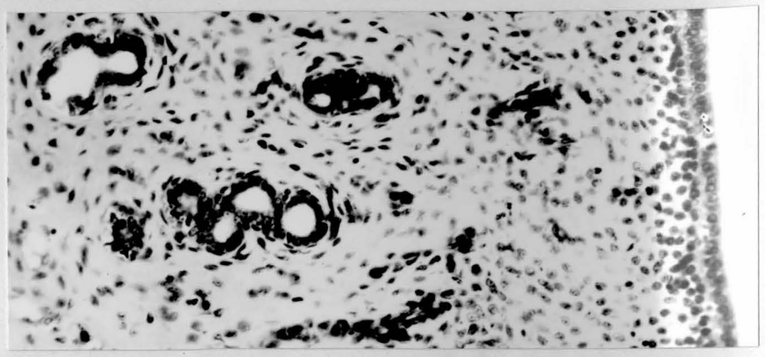


Figure 91

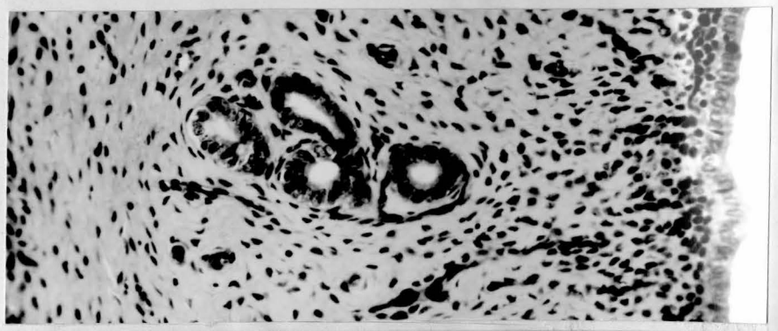


Figure 92

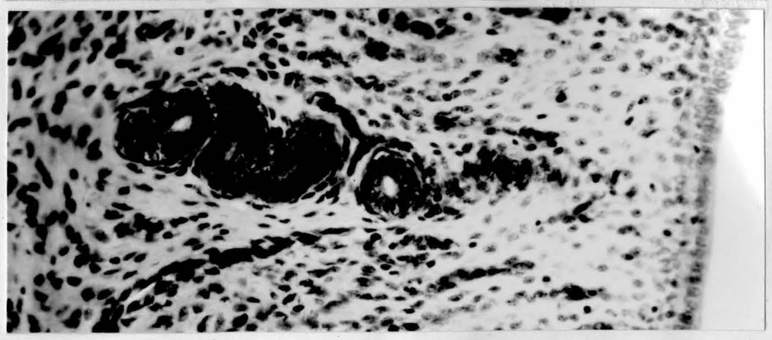


PLATE XXXV

Uteri of bilaterally ovariectomized, young adult rats, 74 days of age, given ovarian sex steroid hormones or hormonal vehicle in 13 dosages over a 17 day period.

EXPLANATION OF FIGURES

Fig. 93. Uterus, x250, of rat given 0.2 cc sesame oil as control. Note marked atrophy of uterus.

Fig. 94. Uterus, x250, of rat given 0.1μg estradiol-17β daily for 13 days of a 17 day treatment period. Note marked uterine endometrial growth with tall columnar epithelium, compare with figure 56, plate XXII.

Fig. 95. Uterus, x250, of rat given 1.5 mg progesterone daily for 13 days of a 17 day treatment period. Note actively appearing endometrial stroma.

Fig. 96. Uterus, x250, of rat given 0.1μg estradiol-17β and 1.5 mg progesterone daily for 13 days of a 17 day treatment period. Note marked uterine stromal activity and moderately tall uterine columnar epithelium, containing numerous mitotic figures. These characteristics reveal that the early uterine horizons II and III observed in prepubertal and pubertal rats can be duplicated in large measure with combinations of estradiol-17β and progesterone.

PLATE XXXV

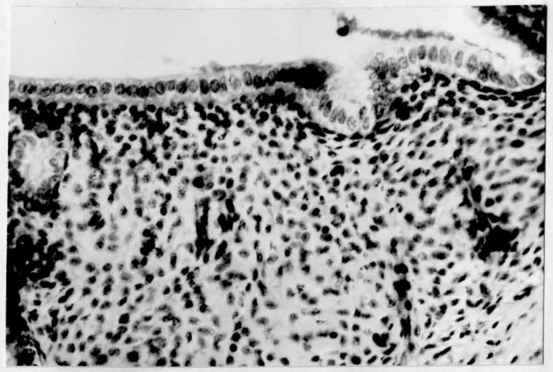


Figure 93

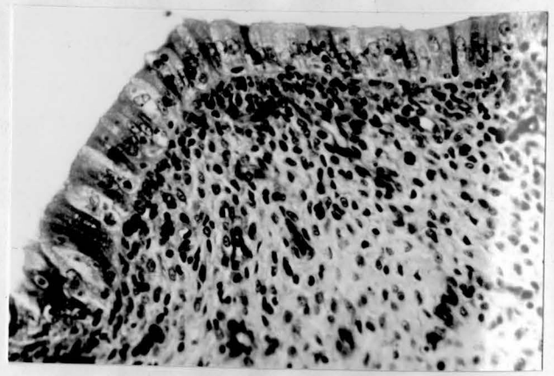


Figure 94

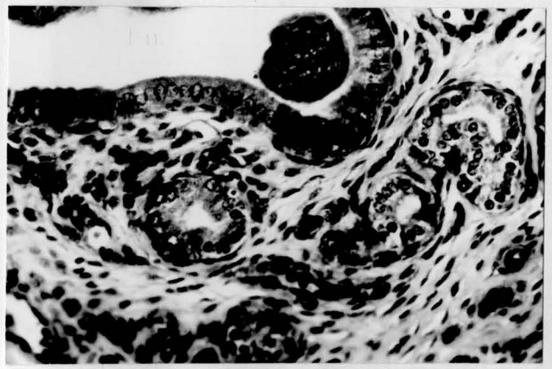


Figure 95

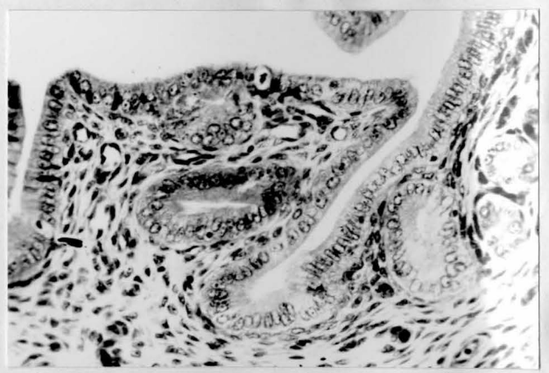


Figure 96

ABSTRACT

Utilizing intact rats in a graded series of ages from immaturity through adulthood, and bilaterally ovariectomized young adult rats, given physiological dosages of sex steroid hormones for short periods of time, it was determined that distinctive patterns of uterine growth and functional development could be followed sequentially. In intact rats of ages 23 to 133 days of age, it was possible to observe the transitional stages from immaturity through maturity gravimetrically and cyto- and histologically. Five distinctively different cyto- and histomorphological types of uteri were observed in prepubertal animals, which have been designated as classes. Also, it has been possible to establish four major early horizons in uterine growth and development.

Early uterine horizon I is typical of all investigated criteria of uteri of 33 day old prepubertal rats, and was highlighted by a 100 per cent weight increase over immature uteri of 23 day old rats.

Early uterine horizon II is typical of the studied characteristics of uteri of 37 day old prepubertal rats, which exhibited emerging patterns of maturity.

Early uterine horizon III is typical of the observed uterine changes associated with the opening of the vagina in the pubertal rat.

Early uterine horizon IV is typified by all of the characteristics of the uterus of the 55 day old rat in estrus, which now exhibits the

true criteria of the uterus of the mature rat in estrus.

Utilizing a large series of bilaterally ovariectomized young, adult rats given subcutaneous injections of estradiol-17β, progesterone, or a combination of the two hormones, it has been possible to quantify the time-span and hormonal dosages required to duplicate early uterine horizons I, II and III. These data conclusively reveal that 0.1μg estradiol-17β when injected once daily for three days elicits the first early uterine horizon; furthermore, when 0.1μg estradiol-17β and 1.5mg progesterone were injected according to the experimental model system over a 17 day treatment period, the pattern of early uterine horizon II was induced, as well as early aspects of horizon III.

These data firmly establish the guidelines for further researches especially for ultrastructural and biochemical studies.

APPROVAL SHEET

The dissertation submitted by Barbara A. Kasprow has been read and approved by five members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

DATE May 23, 1969

Prof. Joseph Lomas Vilardo

Signature of Advisor