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Evaluation of Hydroxyprogesterone Acetate in the Prevention of Canine Estrus

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EVALUATION OF HYDROXYPROGESTERONE ACETATE IN THE PREVENTION OF CANINE ESTRUS

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FOREWORD

Thirty years ago, endocrinology was a laboratory curiosity appreciated by few veterinarians. In those rare instances when the new science escaped from the laboratory and reached the clinic, the endocrine preparations used were extremely crude and often ineffective. In the growth of endocrinology as a science, availability of pure crystalline hormones has played an essential role, and to chemical synthesis must be credited the preparations which make specific endocrine therapy possible today.

In the last two decades endocrinology has come into its own in veterinary medicine, especially in regard to sex endocrinology. But only in the last ten years has particular attention been paid the hormone of the corpus luteum and its synthetic derivatives, which have countless implications in animal production and reproduction. Hydroxyprogesterone acetate is one of these derivatives.

It is the purpose of the author to examine hydroxyprogesterone acetate as an estrual-delaying agent in bitches. Because it is the first oral agent to be evaluated for this use, the drug deserves this attention.

INTRODUCTION

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Although the corpus luteum was recognized as an ovarian structure almost 400 years ago, evidence for existence of a corpus luteum hormone dates back only 50 years. Isolation and synthesis of progesterone date back only 30 years, while preparation of synthetic progesterone derivatives dates back only 20 years. In the last several years, progestogens with high oral activity have been prepared, and there has been remarkable renewal of interest in this class of drugs. Figure 1 presents a chronological brief of the era of the corpus luteum, its hormone, and its derivatives.

Figure 1. Chronological Brief of the Era of the Corpus Luteum, Its Hormone, and Its Derivatives.*

1573 - "Yellow body" noted in mammalian ovaries.

1672 - DeGraaf described corpus luteum, depicted corpus on cow ovary, recognized that number of corpora corresponds to number of fetuses in uterus, that corpora disappear after parturition.

1897 - Beard postulated corpus luteum served necessary function during pregnancy.

*Detailed accounts will be found in two books by George W. Corner, Sr., The Hormones in Human Reproduction, Princeton University Press, 1942, ed. 2, 1946; and Anatomist at Large, Basic Books, New York, 1958.

1898 - Prenant noted glandular appearance of luteal cells.

1900 - Born noted corpus luteum absent in nonplacental mammals: Required for placental development.

1902 - Fraenkel noted rabbits aborted when corpus luteum was removed early in pregnancy.

1907 - Loeb noted corpus luteum prepared uterus of guinea pig for implantation.

1910 - Bouin and Ancel noted corpus luteum produced progestational endometrium.

1911 - Loeb noted corpus luteum ablation to hasten appearance of next estrus.

1913 - Fellner demonstrated existence of corpus luteum hormone: Produced progestational changes with corpus luteum extracts.

1928 - Corner and Allen prevented abortion in rabbits with corpus luteum extracts.

1929 - Corner and Allen described bioassay of corpus luteum extracts in rabbits.

1930-1932 - Potent crystalline preparations of corpus luteum hormone prepared (Hisaw, 1930; Fels and Slotta, 1931; Fevold, 1932; Allen, 1932). Haverlandt delayed estrus in animals with corpus luteum extracts.

1934 - Butenandt, and Wintersteiner and Allen isolated pure crystalline corpus luteum hormone. Slotta defined structure. Butenandt and Fernholz accomplished synthesis. Hormone named "progesterone."

1937 - Makepeace noted progesterone inhibited ovulation in rabbits,[1] Dempsey in guinea pigs.[2]

1938 - Inhoffen synthesized anhydrohydroxyprogesterone and demonstrated oral activity. Robson noted progesterone inhibited ovulation in mice.[3]

1939 - Astwood noted progesterone inhibited ovulation in rats.[4]

1940 - Pfiffner isolated hydroxyprogesterone from adrenals.

1943 - Anhydrohydroxyprogesterone noted clinically effective progestogen.

1948 - Dutt noted progesterone inhibited ovulation in sheep,[5] Christian in cows.[6]

1952 - Progesterone isolated from adrenals. Murray noted injected progesterone delayed estrus in the bitch.[7]

1956 - Hydroxyprogesterone caproate introduced.

It is now fairly well established that development of an antrum in immature ovarian follicles, their secretion of estrogen, and their growth to preovulatory status is stimulated by an anterior pituitary follicle-stimulating hormone (FSH). Rapid swelling of follicles, ending in rupture, requires the combined action of FSH, luteinizing hormone (LH), and progesterone. When the ruptured follicles hea and form corpora lutea, secretion of progesterone becomes accelerated. Progesterone has triple function: (1) inhibition of further FSH discharge, thereby preventing growth of follicles, secretion of estrogen, and physical and psychic signs of estrus during pregnancy; (2) inhibition of further LH discharge, thereby preventing maturation of follicles and ovulation during pregnancy; and (3) production of a progestational endometrium.

Although the estrual-delaying and ovulation-inhibiting actions of progesterone were recognized over 20 years ago,[1-4] the demonstration did not excite practical application. In recent years, several efforts have been made to evaluate the drug in these respects and it has been found that the pituitary-ovarian axis of the bitch was one that could be easily disrupted.[7] When progesterone was administered in sufficient doses, resulting inhibition of pituitary gonadotropin secretion delayed estrus and ovulation. But administration of sufficient doses of progesterone had an inherent limitation; the most practical route of administration was oral, but progesterone had weak oral activity.

Because of difficulties associated with providing potent oral progestogens, considerable chemical and pharmacologic investigation was gauged to solve the problem through synthesis of progesterone derivatives. One of the most potent oral progesterone derivatives, and certainly the most widely evaluated from both safety and estrual-delaying standpoints in bitches, is hydroxyprogesterone acetate.

When hydroxyprogesterone acetate became available, it was early used in bitches.[8] A two-phase 16-month project concerned with safety and estrual-delaying efficacy of the drug was initiated in bitches housed under laboratory conditions. In order to define estrual-delaying effect of the drug, such indices as gross observation, effect

on test studs, vaginal cytology, transillumination examination of ovaries at laparotomy, examination of uteri at laparotomy, necropsy findings, and histology of ovaries and uteri were employed. Despite limitations of the indices, these studies demonstrated that hydroxyprogesterone acetate, administered orally at 1.8 mg. per pound or above, daily, delayed estrus for as long as the drug was administered, apparently suppressed ovulation, and consistently produced progestational endometria. Additional studies included hematology, blood chemistry, liver function tests, hepatic biopsy, renal function tests, urinalyses, and body-weight studies. No pathological variances were observed.

One of the important requirements for any method of delaying estrus is that bitches should be able to cycle normally, conceive, and whelp normal pups after discontinuation of medication. Therefore it became important to determine if hydroxyprogesterone acetate disturbed succeeding estrus. It was demonstrated that medicated bitches exhibited a lag phase, then returned to normal cycling. This signified that the changes in pituitary-ovarian function persisted after the drug was eliminated from the body, followed by gradual resumption of pituitary dominance. Carefully controlled work indicated that subsequent conception, whelping, and litter size and viability were not adversely affected.

ANATOMY OF THE REPRODUCTIVE ORGANS OF THE BITCH

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This consideration of the anatomy of the reproductive organs of the bitch is an orientation to the sections on physiology, endocrinology, and rationale of hydroxyprogesterone acetate prophylaxis which follow.

1. Macroscopic Anatomy[9-12]

Ovaries. The ovaries of the bitch are small, elongated-oval glands less than an inch in length (ca. 2 cm.) on the average. Each is situated a short distance (ca. 1-2 cm.) behind the posterior pole of the corresponding kidney; each is concealed in a peritoneal pouch, the ovarian bursa. The surface of the ovary in the young prepubertal bitch is smooth; in pubertal bitches, uneven because of projecting follicles; in postovulatory bitches, uneven because of both projecting follicles and corpora lutea.

Uterine Tubes (Fallopian tubes, oviducts). The uterine tubes are small and average two or three inches (ca. 5-8 cm.) in length. Each extends from the ovarian bursa to the respective uterine horn.

Uterus. The uterus has extremely long, narrow horns averaging five or six inches (ca. 12-15 cm.) in length and a very short body approximately an inch (2-3 cm.) long. In the nulliparous bitch the uterus lies in the abdomen and pelvis; in the parous bitch it lies

entirely in the abdomen. The cervix is a constricting muscular ring communicating by the cervical canal with the vagina posteriorly.

Vagina. The vagina is relatively long. The anterior portion is narrow; the posterior portion is widened to form the vestibule, which accomodates the bulbus of the male. The hymen is the anterior boundary of the vestibule and posterior to the hymen, on the ventral floor, is the urethral orifice. The ventrally located fossa of the clitoris is the posterior boundary of the vestibule and on the floor of the fossa is the clitoris.

Vulva. The vulva is the common passage of the urinary and genital tracts.

Figures 2 and 3 present orientative views of the gross structure of the bitch's reproductive system.

2. Microscopic Anatomy[10,12]

The ovaries, uterus, and vagina of the bitch undergo continuous, cyclic, complex changes during postpubertal life. While the changes are considered from estrual and endocrinologic aspects in a following section, histologic aspects will be briefly considered here.

Ovaries. The ovary consists of an outer cortex and inner medulla. The cortex is thick and embedded in its interstitial connective tissue are numerous follicles, in which the female sex cells,

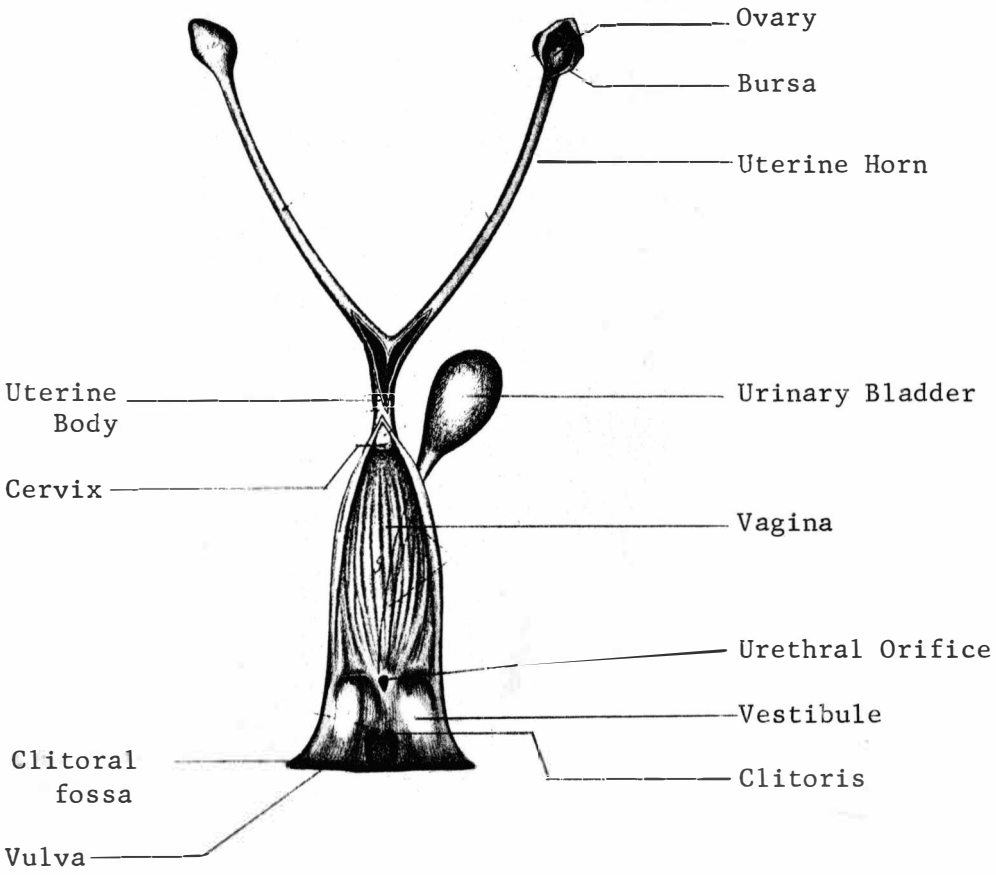


Figure 2. Genital Organs of Bitch (Dorsal View).

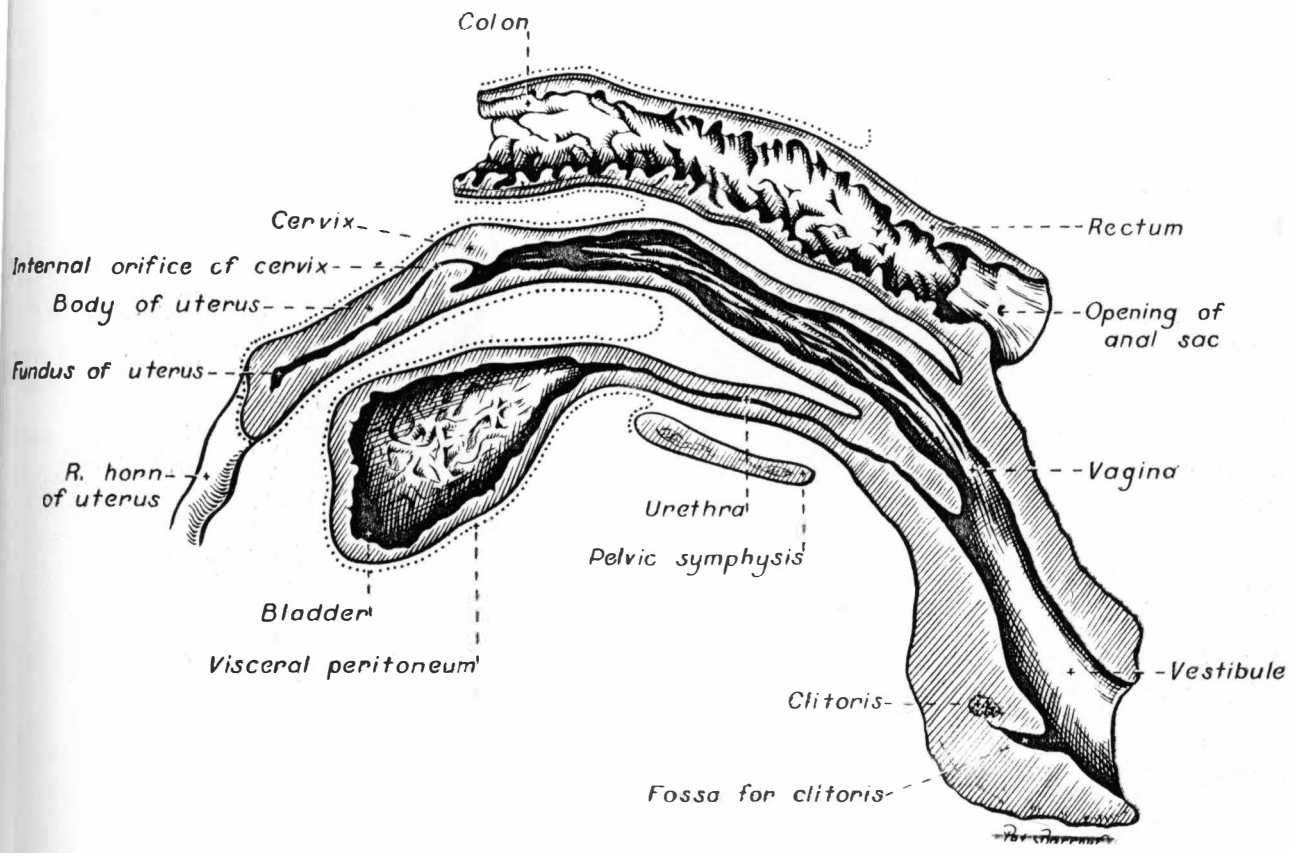


Figure 3. Genital Organs of Bitch (Lateral View).

the eggs, develop.* The medulla consists of connective tissue and blood vessels.

The epithelium investing the free ovarian surface is called germinal epithelium because in the bitch embryo the eggs appear to arise from it. Beneath the germinal epithelium lie the follicles -- the younger the bitch, the more numerous the follicles. They have been estimated to number 350,000 in each bitch ovary at birth, 175,000 at puberty, 34,500 at five years, 500 at ten years.[13] The number of follicles decreases throughout life, chiefly due to atresia.

The vast majority of the follicles are primary follicles. They are chiefly in the cortical periphery and in young bitches form a thick layer. They are spheroidal, about 2-4 mm. in diameter,[14] their center occupied by a large round oogonium.

Primary follicles grow and change into secondary follicles, about 0.2 mm. in diameter, their center occupied by the primary oocyte about 60-80 μ. in diameter. At this stage, several irregular spaces develop

*Some workers indicate that the egg of the bitch is never an ovum. Since fertilization takes place before the second polar body is shed, it is a primary oocyte prior to fertilization. After fertilization it is, of course, a zygote.[10] The term "egg" will be used throughout, rather than ovum. For euphonic and practical reasons, however, it has not been possible to carry this purism to its ultimate conclusion and refer to ovulation as "eggulation."

within the follicle and fill with liquor folliculi. The liquor further increases the size of the follicle. The spaces usually become confluent, forming a single vesicle lined with follicular cells.

The lining of follicular cells is thickened on one side to form the cumulus oophorus, which surrounds the egg. Meanwhile, the connective tissue surrounding the follicular mass has differentiated into a capsule, the theca folliculi, which later differentiates into two layers: the theca interna and the theca externa.

After developing through primary and secondary stages, the follicle becomes mature and is termed a mature follicle, Graafian follicle, or vesicular follicle. It bulges prominently on the free ovarian surface; the liquor is under considerable pressure and the free wall becomes very thin. The contained primary oocyte reaches 80-110μ in diameter.[15] Upon reaching maturity, the follicle either ruptures or involutes, but it can involute at any stage of development; i.e, it can involute as a primary follicle or a secondary follicle.[10]

Since the follicular fluid accumulates faster than the follicle can expand, the wall becomes thinner and thinner. A small spot at the apex of the bulge opens and the fluid escapes; the primary oocyte is discharged with the fluid. The immediate cause of rupture is not known.

After ovulation, the wall of the follicle does not immediately collapse,[14] but bleeding occurs, followed by coagulation and resolution of the clot.[10] The follicle eventually collapses and its wall is thrown into folds. The follicle now has an irregular stellate shape and incorporates a serous fluid. The cells of the theca hypertrophy, attain considerable size and are called lutein cells. The former follicle is now called the corpus luteum. The comprising lutein cells contain a characteristic yellow lipoid pigment, lutein.

If fertilization occurs, the corpus luteum (because of multiple ovulation, often corpora lutea in bitches) grows for a time, and attains a greater size than it does if fertilization does not occur. Maximum size is reached approximately ten days postestrus.[10-16] The cropus luteum begins to regress halfway through pregnancy (about the 30th day),[17,18,10] but has also been described as persisting through term.[16] If fertilization does not occur, the corpus luteum also grows for a time, then begins to regress at about the tenth day of metestrus. In pseudopregnancy, the corpus luteum does not begin to regress untill about the 20th day of metestrus.[10,20] After regression, the atretic corpus luteum can be detected grossly at the time of the next estrus (seven months later)[12] and even two years later on microscopic examination. After this, a scar persists for life and through shrinking causes a distinct retraction of the ovarian surface.

Figures 4, 5, and 6 present orientative views of the microscopic structure of the ovary of the bitch.

Oviducts. The oviduct wall consists of (1) an internal mucous membrane, (2) a middle muscular layer, and (3) an external serosal membrane. The mucous membrane bears cilia that beat in the direction of the uterus. Contraction waves pass from the ovarian end to the uterine end of the oviduct at the time of ovulation.

Uterus. The uterine wall also consists of three layers: (1) a mucous membrane or endometrium, (2) a muscular layer or myometrium, and (3) a serosal layer. The endometrium consists of simple columnar epithelium bearing uterine glands and crypts.

Vagina. The vaginal wall consists of three layers: (1) mucous membrane, (2) muscular layer, and (3) connective tissue layer.[10] The vaginal mucous membrane and fluid reflect cyclic changes in the anterior pituitary, ovaries, and endometrium[10,21-24] During proestrus, the vagina increases in thickness from a few cells to a layer twenty to thirty cells thick. At the end of estrus the stratified layers slough, leaving again an epithelium two cell layers thick during late metestrus and throughout anestrus.[21-24] Vaginal smears rather than biopsy are utilized in clinical determinations of the cyclic status. During proestrus there are numerous erythrocytes, a few leukocytes, a few noncornified epithelial cells, numerous cornified

epithelial cells, and considerable debris. During estrus, there are numerous erythrocytes, no leukocytes, no noncornified epithelial cells, numerous cornified epithelial cells, and no debris. During metestrus, there are few erythrocytes, numerous leukocytes, some cornified epithelial cells, considerable debris. During anestrus, there are few erythrocytes, numerous leukocytes, numerous noncornified epithelial cells, and decreased debris.[10,21-24]

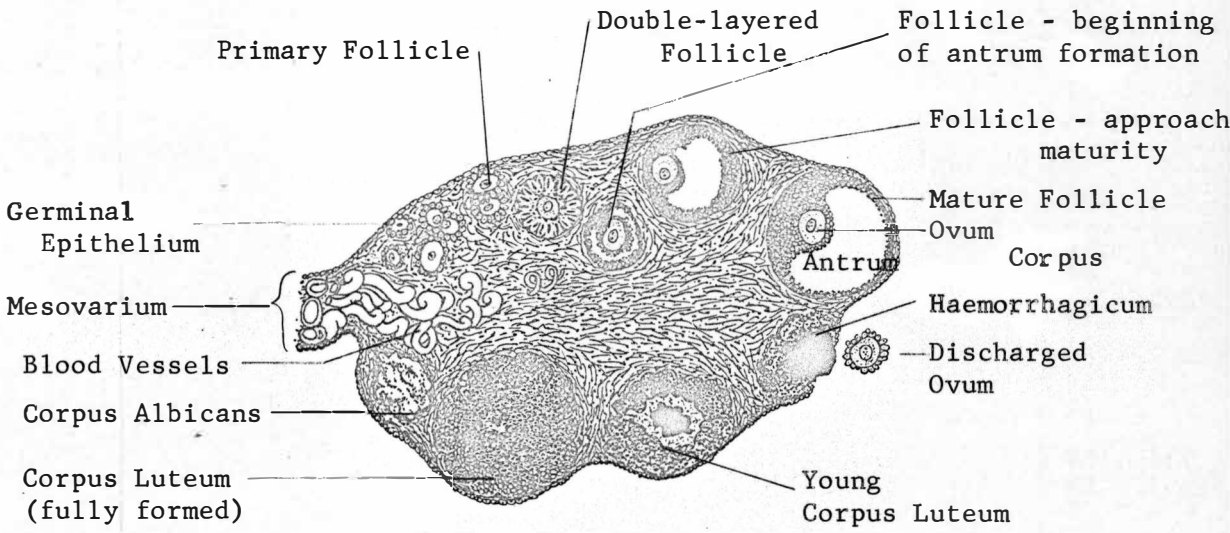


Figure 4. Ovary of Bitch. Sequence of events in origin, growth, and rupture of the follicle, with formation and regression of the corpus luteum (schematic).

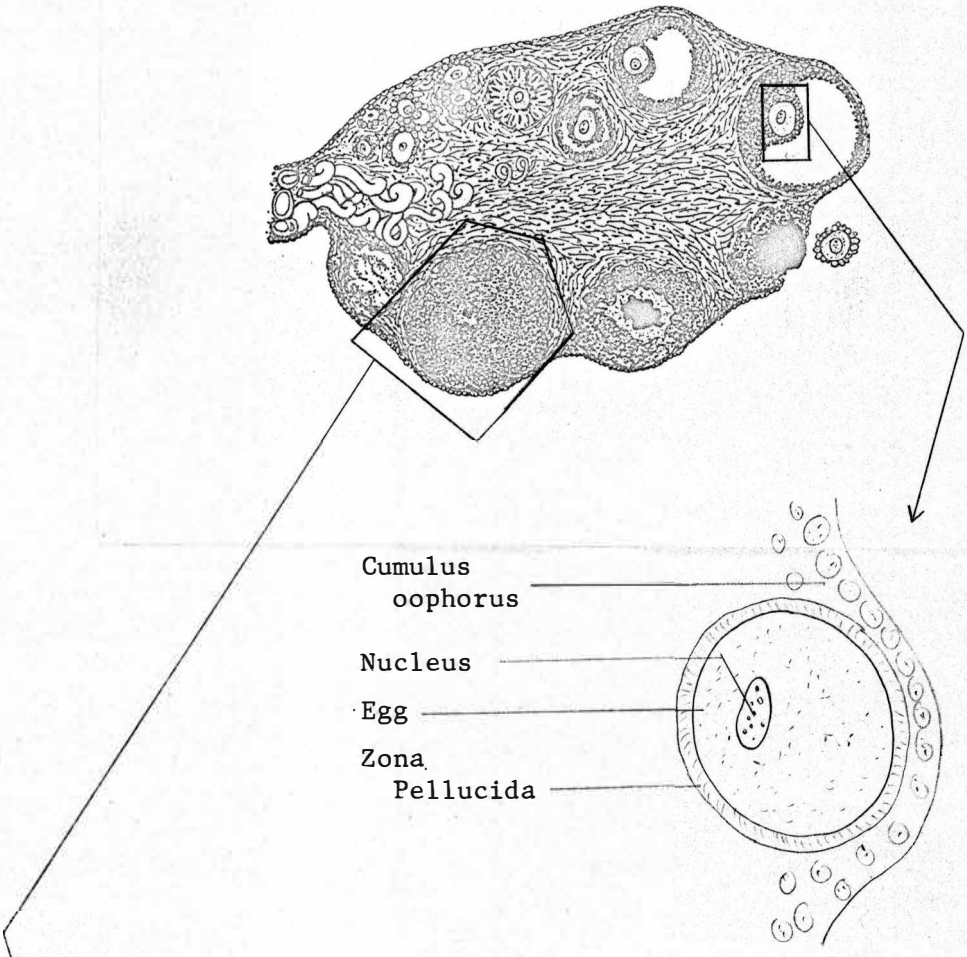


Figure 5. Egg of Bitch.

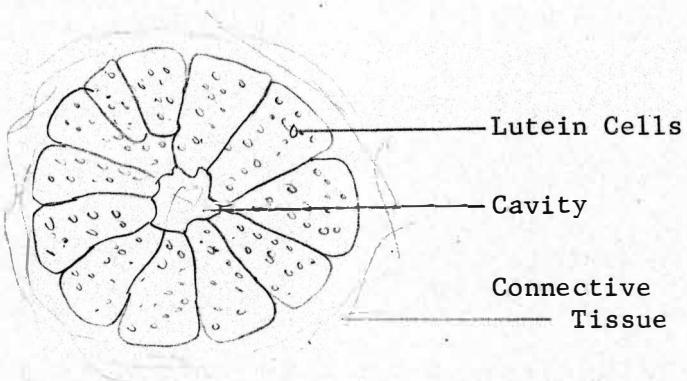


Figure 6. Corpus Luteum of Bitch.

ESTRUAL CYCLE OF THE BITCH

ESTRUAL CYCLE OF THE BITCH

The ovaries, uterus, and vagina of the bitch are the scenes of regularly recurring growth and shrinkage.

1. Tissue Changes

Ovarian Changes. From birth until puberty, the ovaries contain immature eggs, each surrounded by a follicle. At the beginning of the first estrual cycle, and at every one thereafter, some follicles mature. Usually, several reach maturity in each cycle; the others become atretic. Maturation of follicles consists of rapid growth and accumulation of fluid within their cavities. Mature follicles bulge from the surface of the ovary and ten days after the beginning of development they burst, and eggs are extruded into the oviducts. Cells of the ruptured follicles now undergo modification and form solid, yellow masses -- the corpora lutea.

If fertilization does not occur, the corpora lutea grow for the next several weeks, then degenerate. If sperm cells are introduced into the vagina at about the time of ovulation, they travel through the uterus into the oviduct. This is usually the site of fertilization. Once fertilization has occurred, the corpora lutea remain and grow during pregnancy.

Uterine Changes. Proliferation, differentiation, and involution

of the endometrium accompany follicular and luteal growth and degeneration. During follicular growth, the endometrium proliferates. After ovulation, during follicle degeneration and luteal growth, the endometrium differentiates, becomes even thicker; the uterine glands and crypts and supplying blood vessels grow even more. If fertilization has occurred, the endometrium remains in a differentiated progestational (secretory) stage during pregnancy. If fertilization has not occurred, involutionary changes are seen. By the end of this time, the endometrium has returned to its initial resting stage and is ready to repeat the cycle.

Vaginal Changes. Proliferative and involutionary stages corresponding to follicular and luteal growth and degeneration also take place in the lining of the vagina during the estrual cycle. By making a smear of some surface cells and observing it under the microscope, it is possible to determine the stage of the animal's cycle.

The aforementioned ovarian, uterine, and vaginal changes occur during specific phases of the estrual cycle.

2. Estrual Phases

Puberty. The prepubertal bitch does not exhibit an estrual cycle; at puberty, the first cycle is exhibited. This may occur at six months to 24 months. The four phases of the bitch's estrual cycle are proestrus, estrus, metestrus, and anestrus.[20,21,25]

Proestrus. Proestrus is the phase of "coming into heat." It lasts an average of nine days, but may range anywhere from two to 27 days.[14,19,20,22,24-26] There is predominance of FSH, resultant growth of ovarian follicles, and increased estrogen and possibly progesterone secretion. The endometrium is in the proliferative stage, vulvar swelling and hemorrhage occur -- the most objective phenomena of the whole estrual cycle, and quite distinct from the postovulatory hemorrhage (menstruation) of women, monkeys, and cows (associated with cessation of corpus luteum activity). Proestrual hemorrhage is preovulatory and is associated with increased estrogen secretion. The bitch is attractive to male dogs, but will not stand for breeding. The proestrual phase merges imperceptibly into the estrual phase, or heat.

Estrus. Estrus is the period of "sexual desire" during which the bitch accepts breeding. It also lasts an average of nine days, but may range anywhere from four to 13 days.[14,19,20-22,24-26] Just prior to ovulation, so much estrogen has been secreted by the follicle that there is inhibition of FSH and stimulation of LH. Increased LH causes ovulation on the second or third day of estrus, the uterus ultimately receiving the eggs.[14,19,20,22] LH is considered to be the dominant hormone necessary for corpus luteum appearance and growth. From the ruptured follicle, a temporary endocrine

Figure 7. Normal Estrual Cycle of Bitch.

FSH causes follicular growth and estrogen levels rise during proestrual phase; LH causes follicular rupture during estrual phase and progesterone levels rise. Corpus luteum secretes progesterone and during early metestrus, levels peak, with gradual decrease during late metestrus. In later anestrual phase, FSH is again elaborated, causing follicular growth and secretion of estrogen.

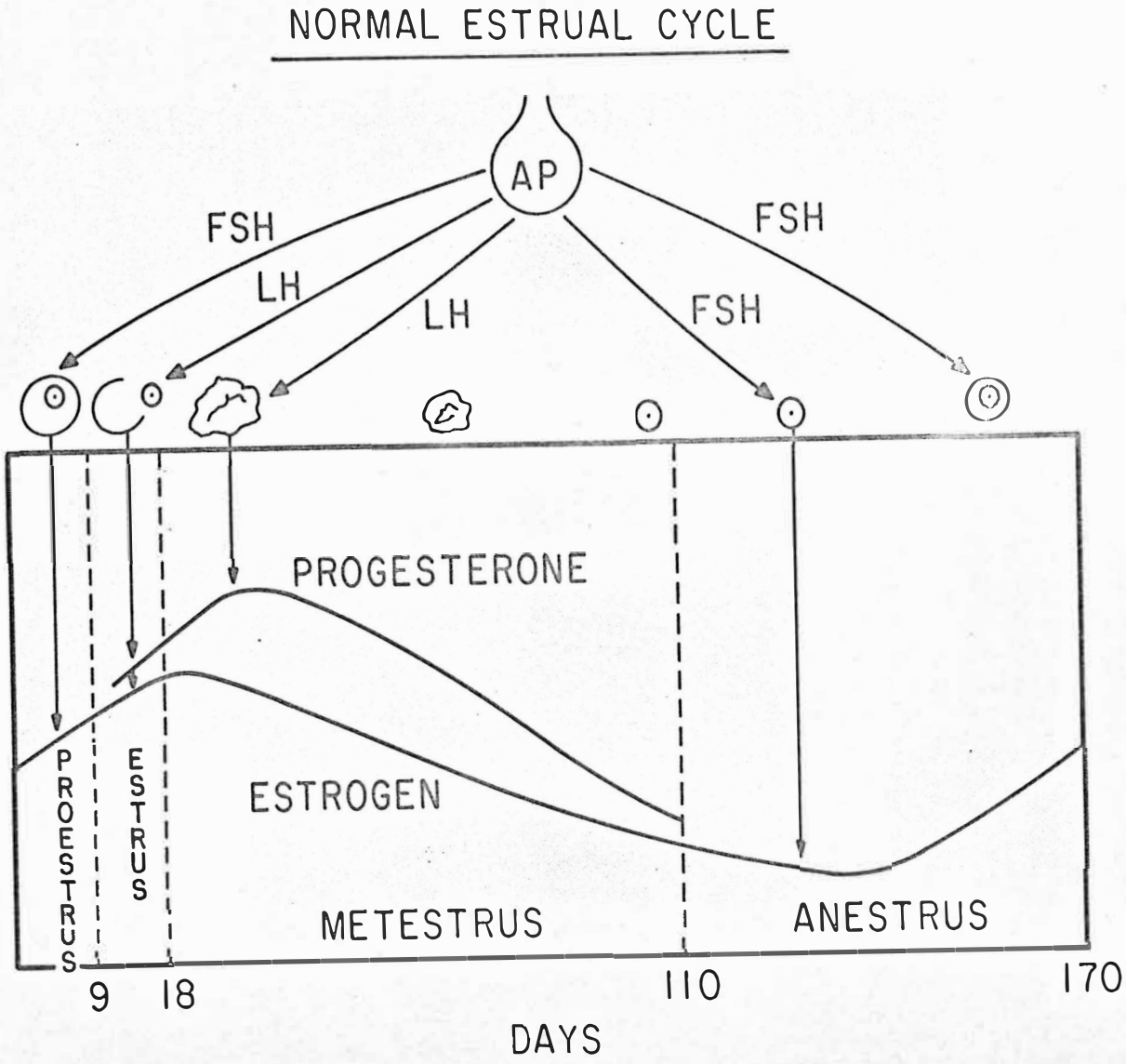

NORMAL ESTRUAL CYCLE
AP
FSH
LH
LH
FSH
FSH
PROGESTERONE
ESTROGEN
PROESTRUS
ESTRUS
METESTRUS
ANESTRUS
9
18
110
170
DAYS

Figure 7.

gland is formed -- the corpus luteum. This secretes progesterone and estrogen. Estrogen secretion gradually diminishes and progesterone secretion gradually increases. The endometrium is transformed from the proliferative stage to the progestational (secretory) stage. The estrual phase imperceptibly merges into the metestrual phase.

Metestrus. Metestrus is the phase of "gradual subsiding. It lasts an average of 80 to 90 days.[14,19,20,22,24-26] There is decreased estrogen output from the remains of the follicle, increased progesterone secretion from the corpus luteum, and inhibition of LH. There is involution of the endometrium. There is decreased progesterone secretion as the corpus luteum becomes smaller.

Anestrus. Anestrus is the phase of "relative quiescence." It lasts approximately 60 days.[14,19,20,22,24-26] There is decreased progesterone secretion as the corpus luteum gradually decreases in size. There is increasing predominance of FSH during the later part of the anestrual phase.

Figure 7 illustrates the various phases of the canine estrual cycle, with prorating of pituitary-ovarian hormones throughout the cycle.

Duration and Interval. Variations in the estrual cycle occur, and this has been observed not only between different breeds but even in the same bitch.[14,19,22,24-26] Some bitches may exhibit three or

four cycles a year; some only two. Differences in cycle length are governed by the length of the anestrual phase -- in rapid-cycling bitches the anestrual phase is shorter than in slow-cycling bitches.[12] In unbred bitches the cycles are longer than in bred bitches. There is progressive increase of cycle length in bitches up to four years of age.[12] If the bitch does not become pregnant, the estrual cycle continues with more-or-less regularity throughout adult life. There are usually two cycles a year and although these are popularly thought to occur in the spring and again in the fall, this is not true.[12,23] Seasonal differences in incidence of estrus are not striking.

3. Pregnancy and Pseudopregnancy

There are some alternatives to the estrual cycle: pregnancy and pseudopregnancy. The bitch may thus take any one of the following "routes":

Pregnancy. After passing through proestrus into estrus, the bitch may become impregnated. Rather than entering metestrus, she then enters an alternate phase -- pregnancy. The corpus luteum persists and there is hence predominance of progesterone. This, in turn, leads to suppression of estrus and ovulation. The uterus remains in progestation.

Variations in pregnancy occur. Although usually ranging from 58 to 63 days, pregnancy may be as short as 53 days or as long as 79

days. Following whelping, the bitch enters anestrus, the uterus involuting over a three-week postpartum period.

Figure 8 illustrates pregnancy in the bitch, with prorating of pituitary-ovarian hormones.

Pseudopregnancy. Another alternative is pseudopregnancy. In this the bitch passes through proestrus and estrus, may be bred, but is not impregnated. Breeding apparently enhances incidence of the condition. Pseudopregnancy is a 90-day phase during which the corpus luteum persists as it does in pregnancy, progesterone is secreted, and the uterus remains progestational. The bitch deposits added abdominal fat, the mammary glands develop, and the bitch may build a nest. A more severe form of pseudopregnancy has been referred to as pseudocyesis. In addition to added abdominal fat, mammary development, and nesting, there may be extreme nervousness, inappetence, adoption of the young of another species or of an inanimate object such as a doll or slipper, and if allowed to nurse there may be milk secretion (50 to 70 days after the end of estrus).[27,28] Pseudocyesis may terminate in relaxation of the vulva and pelvic structures, subnormal temperature, and uterine contractions with expulsion of a small amount of fluid and blood, in what has been termed a "phantom parturition." Following pseudopregnancy, the bitch passes into anestrus.

Figure 8. Pregnancy in the Bitch.

Pituitary-ovarian relationships and hormone levels during proestrual and estrual phases are identical to those of the normal estrual cycle (Figure 7). Pregnancy is substituted for metestrus and, because of corpus luteum persistence, progesterone levels remain elevated until near term. Anestrus follows parturition.

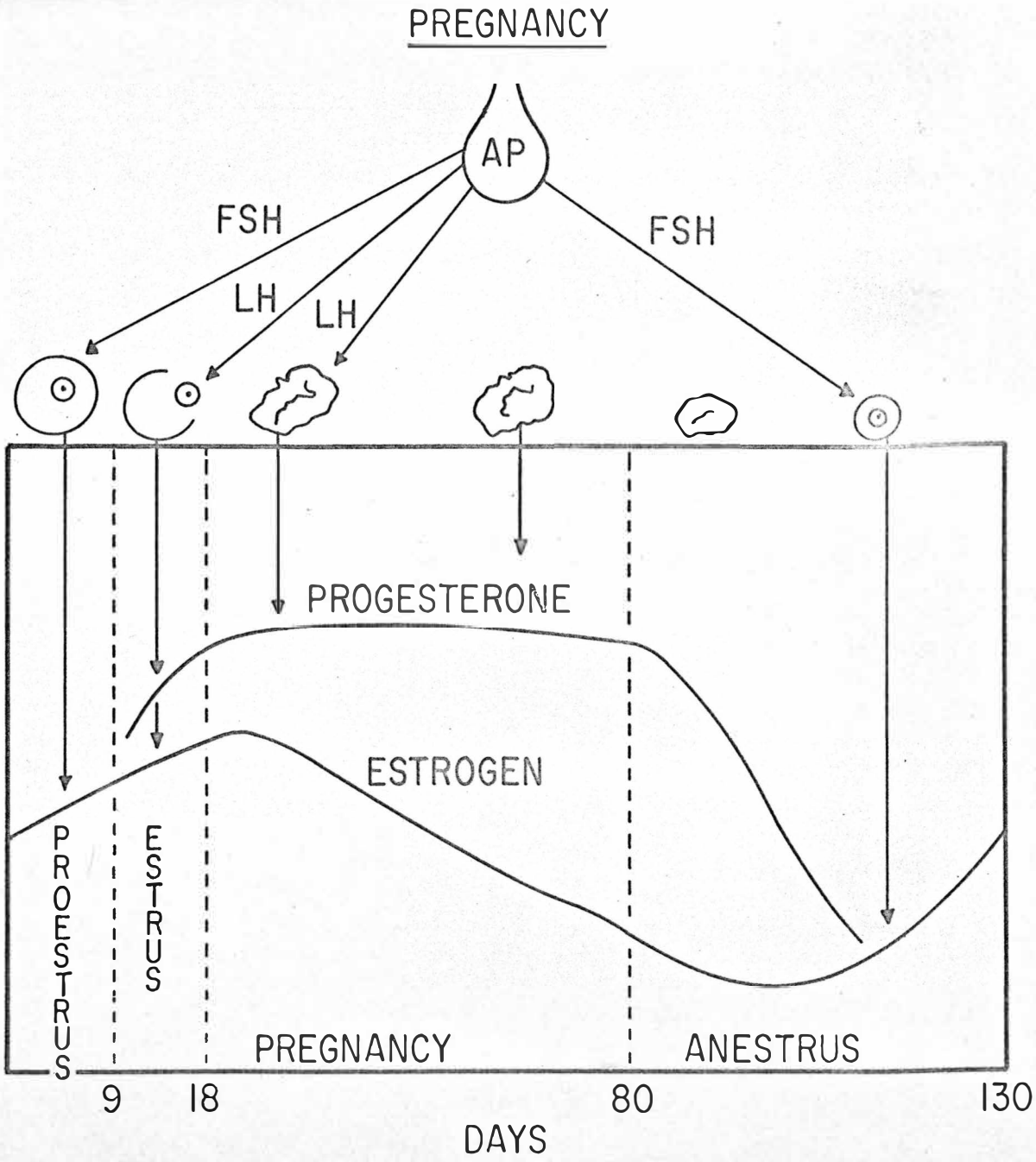

PREGNANCY
AP
FSH
LH
LH
FSH
PROGESTERONE
ESTROGEN
PROESTRUS
ESTRUS
PREGNANCY
ANESTRUS
9
18
80
130
DAYS

Figure 8.

PHYSIOLOGICAL BASIS FOR HYDROXYPROGESTERONE ACETATE ESTRUAL-DELAYING EFFECT IN BITCHES

PHYSIOLOGICAL BASIS FOR HYDROXYPROGESTERONE ACETATE ESTRUAL-DELAYING EFFECT IN BITCHES

To pinpoint the estrual-delaying effect of "oral contraceptives" in bitches, an understanding of the interrelationships of endocrine glands and their target organs is essential.

1. Endocrine Interrelationships

In general, a hormone acts directly on a specific target organ. As the result of responses produced on a specific target organ, the same hormone may be said to act indirectly on other organs (Figure 9). The anterior lobe of the pituitary gland is considered the master coordinator of the endocrine system, although it is itself controlled by the brain and other endocrine glands. It is now thought that certain neurosecretions are released from the hypothalamus into the pituitary portal system to aid in regulating the secretory activity of anterior lobe cells.

Anterior Pituitary Gonadotropins. Anterior pituitary hormones that possess predominantly female sex influences are the gonadotropic hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), and luteotropic hormone (LTH, lactogenic hormone, prolactin) (Figure 10). The anterior pituitary, through its gonadotropic hormones, acts directly on the ovary, its target organ, and only

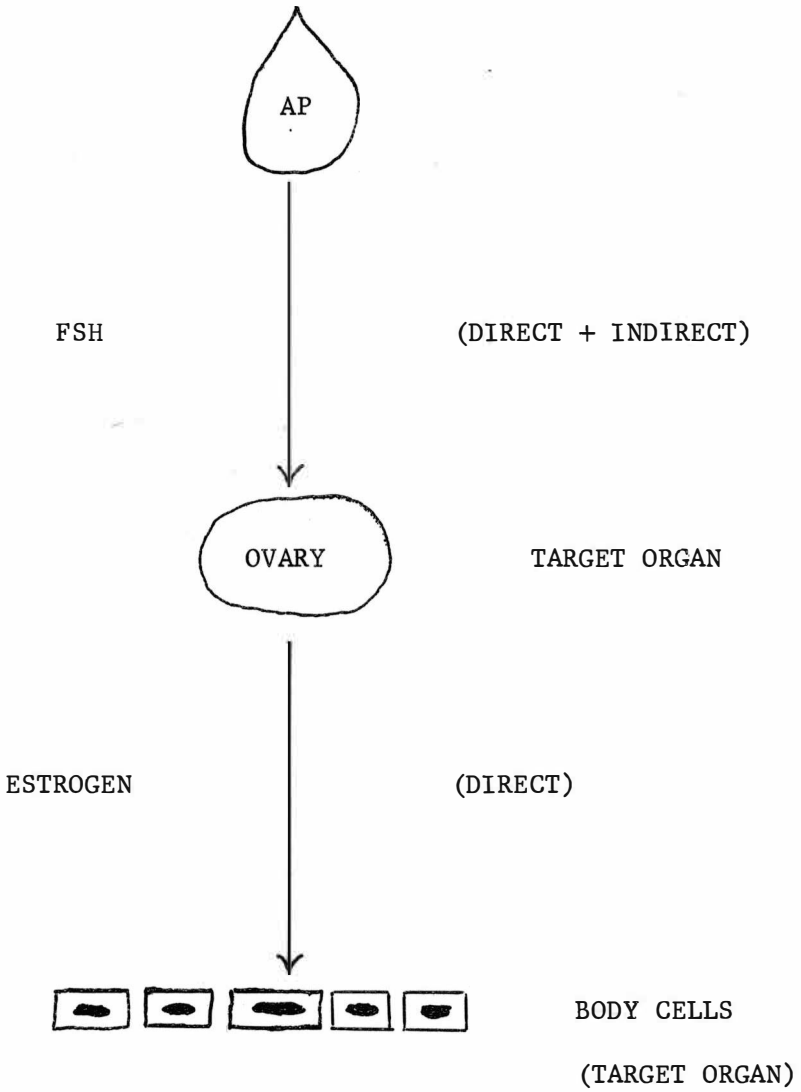

AP
FSH
(DIRECT + INDIRECT)
OVARY
TARGET ORGAN
ESTROGEN
(DIRECT)
BODY CELLS
(TARGET ORGAN)

Figure 9.

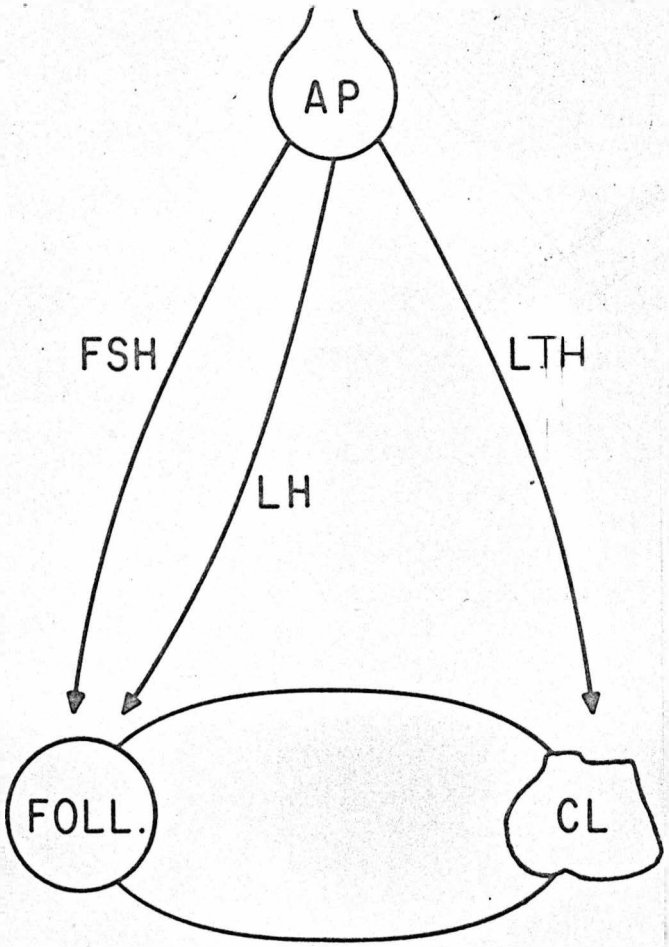


Figure 10.

indirectly on other organs. When the ovary is absent, the pituitary gonadotropic hormones exert no effect.

Cycle Control. FSH and LH are more-or-less continuously secreted, but one or the other alternately predominates. It is this rhythmic domination that produces the estrual cycle of the bitch. In the follicular stage of the cycle, the anterior pituitary elaborates FSH, which causes marked growth of the ovarian follicle and proliferation of the granulosa cells. Estrogen and progesterone are secreted. Low levels of hepatically-inactivated estrogen degradation products, which possess no estrogenic activity in themselves, have a central effect on the anterior pituitary gland in that they stimulate further elaboration of FSH (Figure 11). This causes further estrogen secretion, until high levels of estrogen degradation products inhibit FSH secretion. Meanwhile, the noninactivated estrogen has had peripheral effect on other organs. High levels of estrogen degradation products, in addition to inhibiting FSH secretion, stimulate LH and LTH secretion. Low levels of progesterone also stimulate LH and in synergism with FSH produce follicular rupture and ovulation (Figure 12). After ovulation, in the luteal stage of the cycle, the ovary responds to LH with formation of the corpus luteum and secretion of progesterone, but maintenance of secretion is the function of LTH. Higher levels of progesterone have a central effect on the anterior

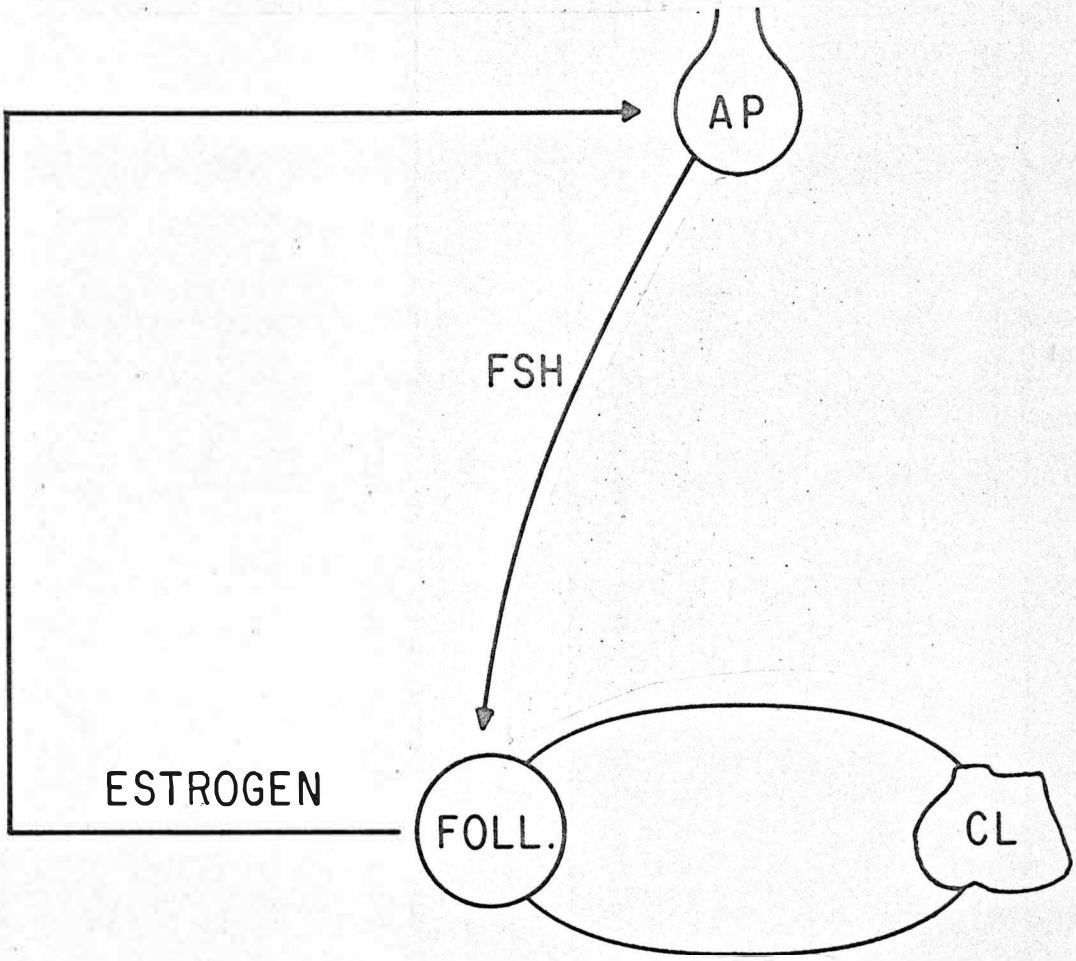


Figure 11.

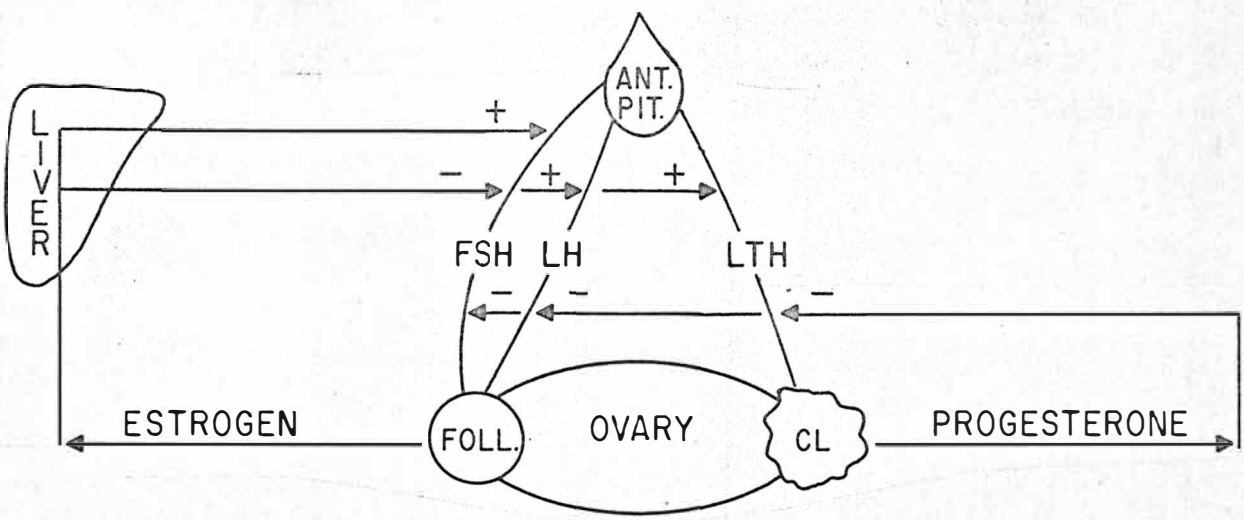

ANT.
PIT.
LIVER
+
−
+
+
FSH
LH
LTH
−
−
−
ESTROGEN
FOLL.
OVARY
CL
PROGESTERONE

Figure 12.

pituitary gland in that they inhibit LH and LTH secretions, thereby preventing maturation of a new follicle. Higher levels of progesterone also inhibit FSH secretion, thereby preventing development of a new follicle. Meanwhile, progesterone has had peripheral effect on other organs. Ultimately, with LH and LTH inhibited, the corpus luteum recedes, progesterone secretion decreases, and the anterior pituitary is released to secrete FSH and a new follicle begins maturation.

As indicated, hormones can act conditionally: they may act only if priming has occurred. Progesterone can act only after estrogen priming; therefore, it is impossible to produce a progestational endomentrium by administering progesterone unless the endometrium has already been proliferated by endogenous or exogenous estrogen. Similarly, LTH acts on the lactogenic structure of the mammary glands only if estrogen and progesterone have primed the ductal and alveolar tissue for functional activity.

2. Estrogen-Progesterone Effect

The central effects of estrogen and progesterone have already been described. Briefly, estrogen degradation products stimulate FSH at low levels, inhibit FSH and stimulate LH and LTH at high levels. Progesterone stimulates LH at low levels, inhibits LH and LTH at high levels, and inhibits FSH at higher levels. Central effects, then may be progonadotropic or antigonadotropic.

The peripheral effects of estrogen (Figure 13) may be genital or extragenital. Genital effects include those on the tubular genitalia and mammary glands; extragenital effects include those on the body in general, on metabolism, and on behavior. Specifically, estrogen produces the following changes in the tubular genitalia: endometrial proliferation, myometrial growth and increased tone, uterine connective tissue growth, vaginal proliferation (demonstrated by an increase in cornification by vaginal smear techniques), vaginal and vulvar relaxation, vulvar swelling, hemorrhage, and production of an attractive estrual odor. Estrogen also causes mammary ductal proliferation, produces female secondary sex characteristics, causes nitrogen retention (anabolic effect) and retention of water and electrolytes (including sodium, potassium, chloride, calcium, and phosphorus). Estrogen also produces typical estrual behavior.

The peripheral effects of progesterone (Figure 14), generally dependent on estrogen priming, also may be genital or extragenital. Progesterone produces endometrial progestational changes, myometrial atony, and vaginal progestational changes (demonstrated by vaginal smear techniques), mammary alveolar proliferation, and secondary sex characteristics. Progesterone also produces increased excretion (or decreased retention) of nitrogen (catabolic effect), sodium, chloride, and phosphorus. Progesterone is also responsible for maternal behavior.

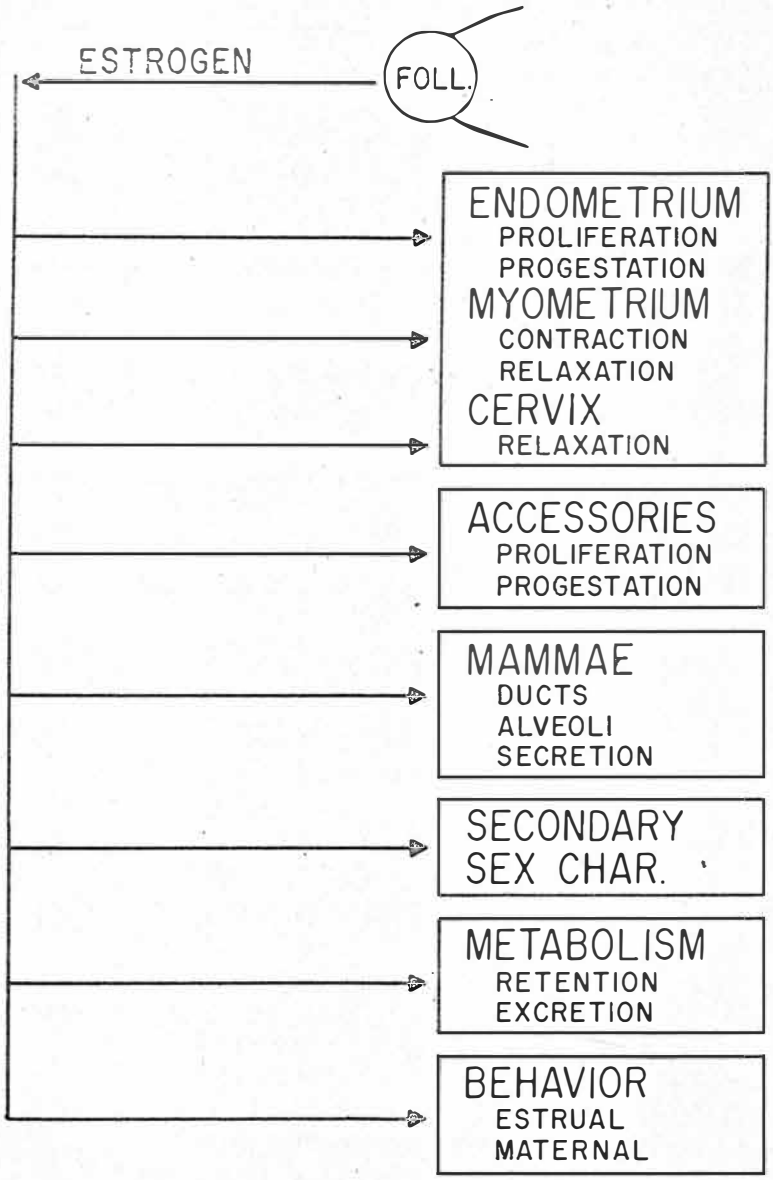
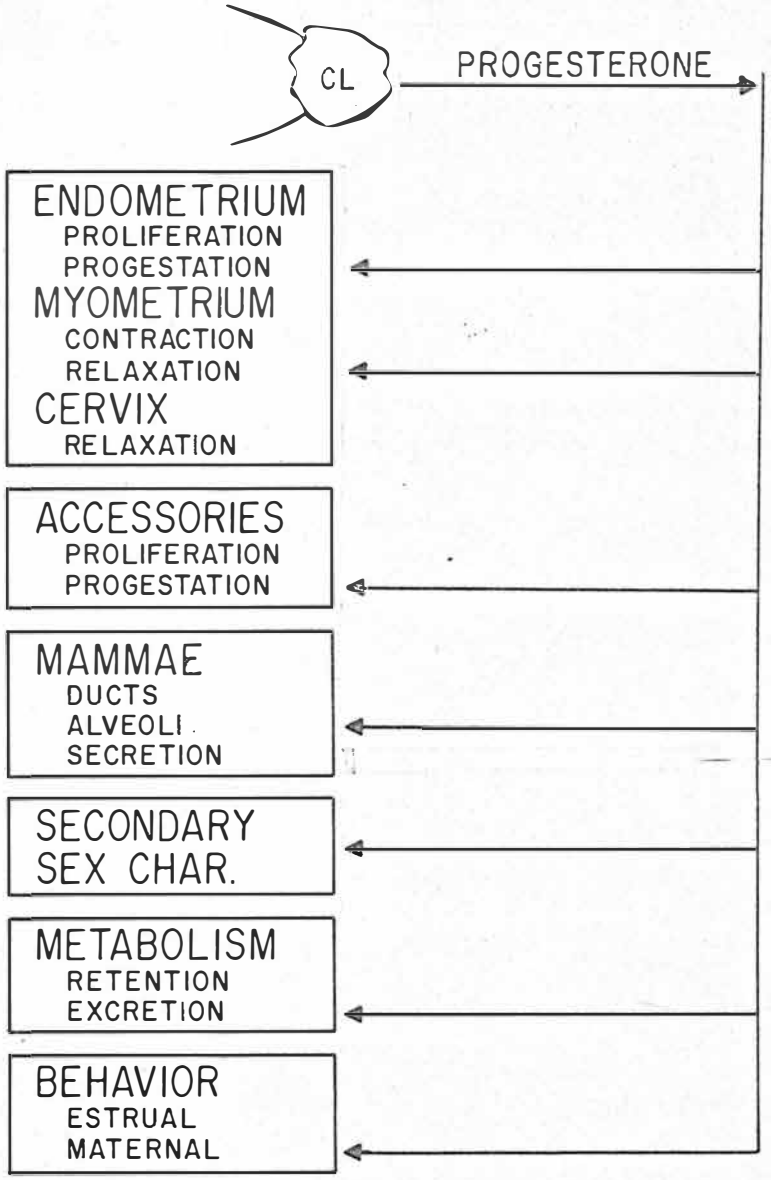

ESTROGEN
FOLL.
ENDOMETRIUM
PROLIFERATION
PROGESTATION
MYOMETRIUM
CONTRACTION
RELAXATION
CERVIX
RELAXATION
ACCESSORIES
PROLIFERATION
PROGESTATION
MAMMAE
DUCTS
ALVEOLI
SECRETION
SECONDARY
SEX CHAR.
METABOLISM
RETENTION
EXCRETION
BEHAVIOR
ESTRUAL
MATERNAL

Figure 13.


CL
PROGESTERONE
ENDOMETRIUM
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PROLIFERATION
PROGESTATION
MAMMAE
DUCTS
ALVEOLI
SECRETION
SECONDARY
SEX CHAR.
METABOLISM
RETENTION
EXCRETION
BEHAVIOR
ESTRUAL
MATERNAL

Figure 14.

Incorporation of an acetoxy group at position 17 of progesterone produces hydroxyprogesterone acetate and apparently changes the route of metabolism of the drug so that it is effective when administered orally. The physiological basis for hydroxyprogesterone acetate-induced delay of estrus in bitches is identical to that for progesterone in that the drug acts (1) centrally to inhibit FSH, LH, and LTH secretion, preventing follicular growth and maturation, estrual signs, and ovulation, (2) peripherally to produce the endometrial and vaginal changes characteristic of progestation (Figures 15-17).

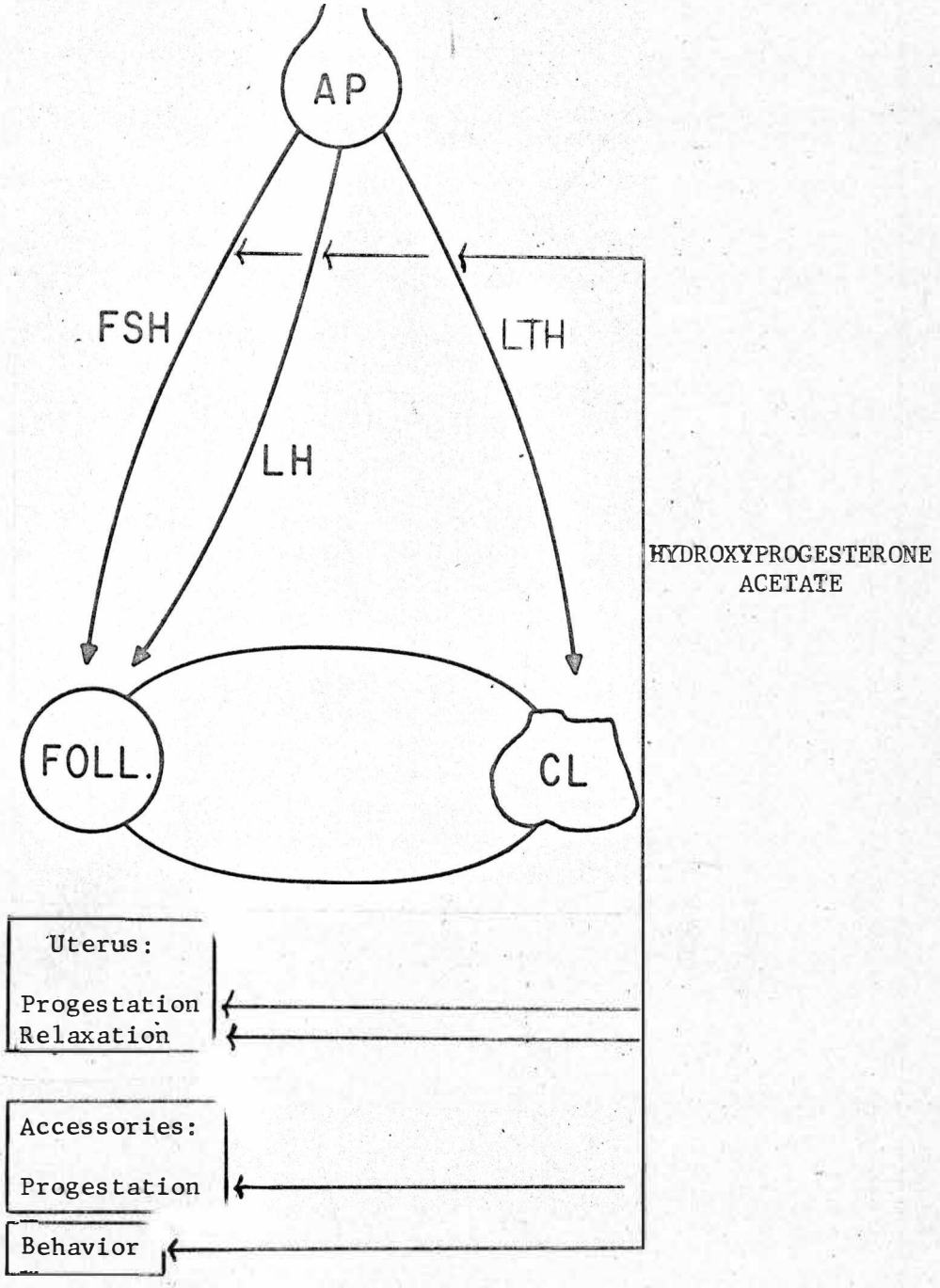

AP
FSH
LH
LTH
HYDROXYPROGESTERONE
ACETATE
FOLL.
CL
Uterus:
Progestation
Relaxation
Accessories:
Progestation
Behavior

Figure 15

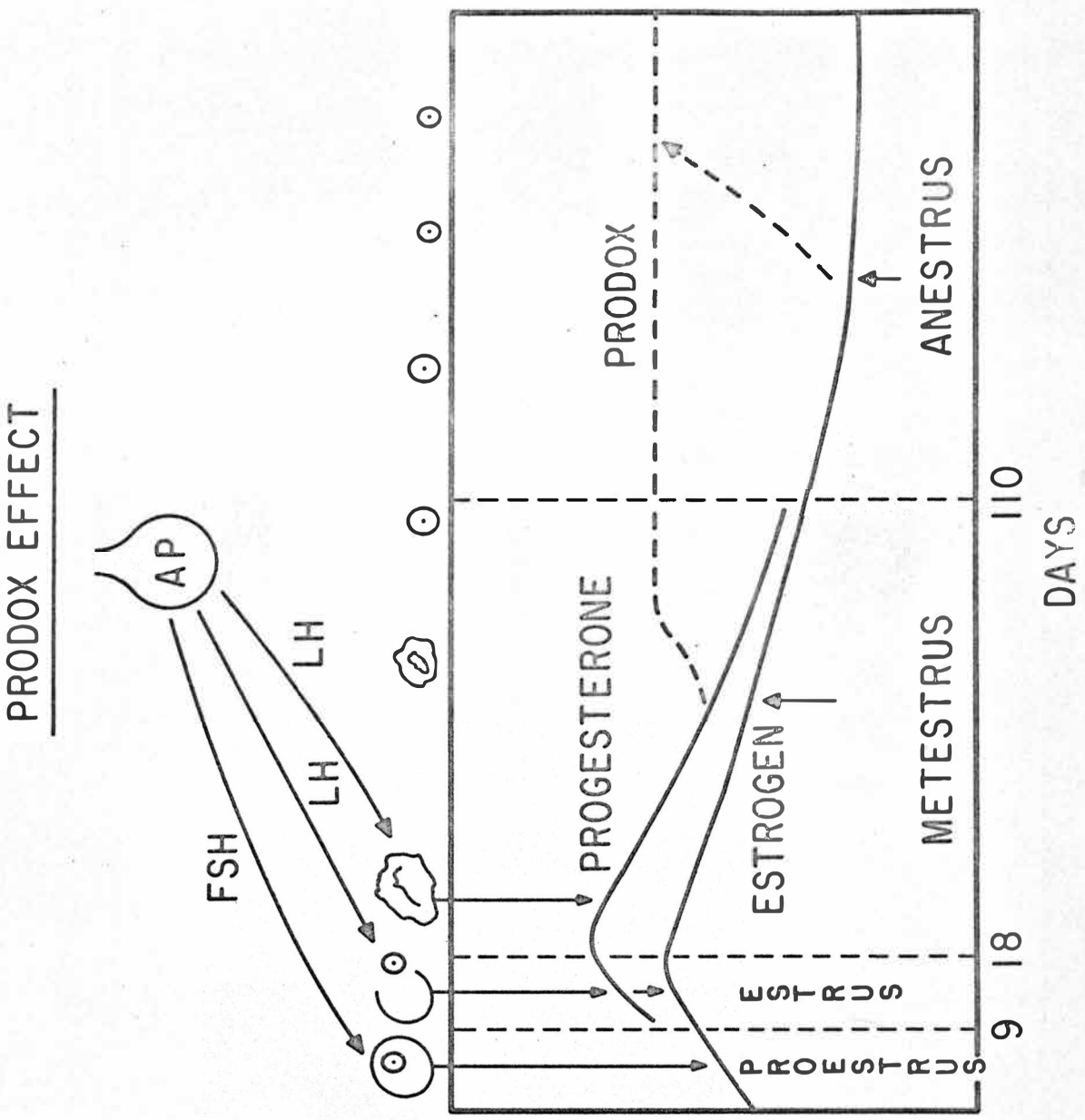


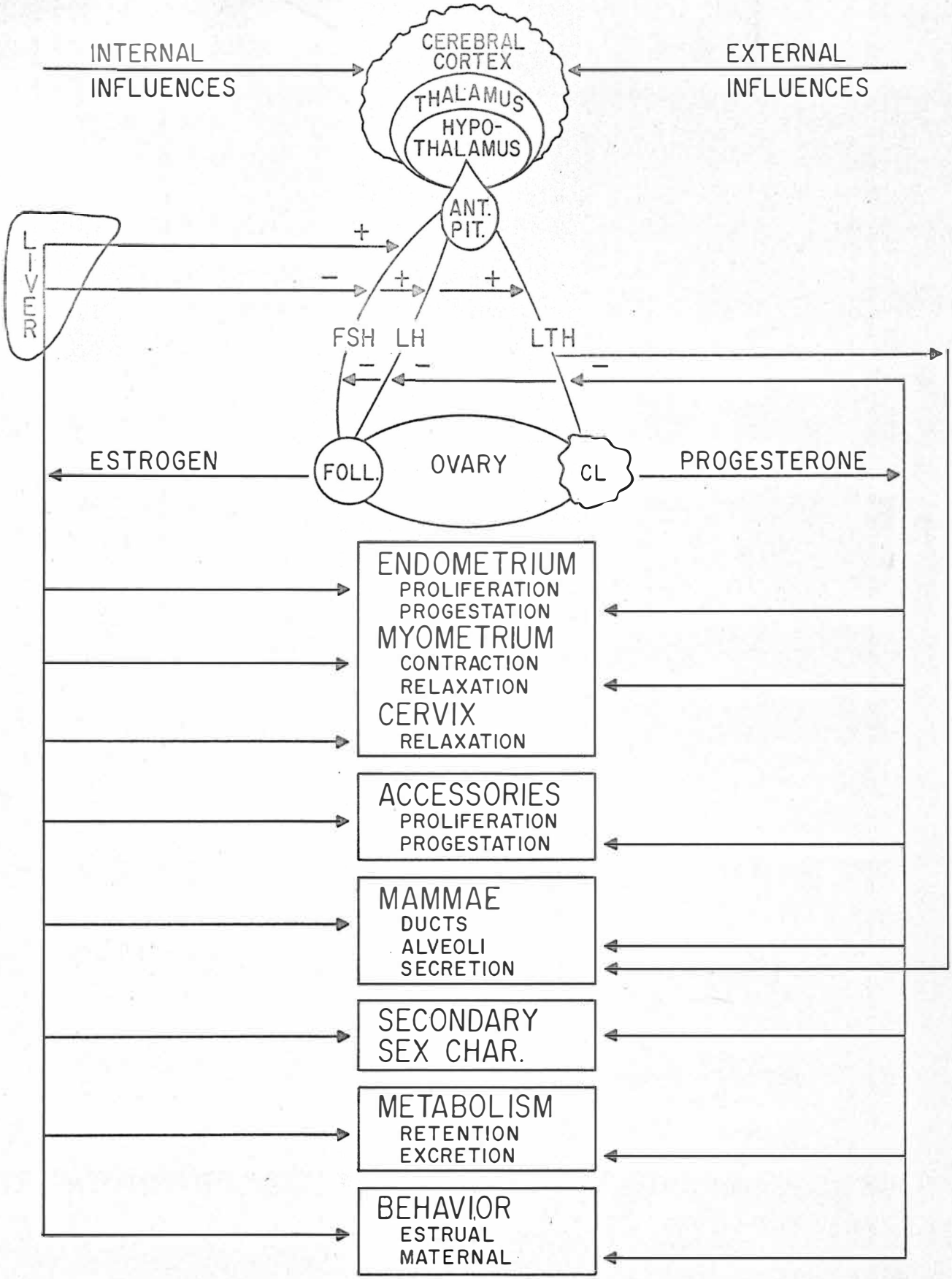
Figure 16. Hydroxyprogesterone (Prodox) Effect.

Administered in metestrual or anestrual phase, the drug maintains progestogen levels.

Figure 17.

At puberty in the bitch, certain internal influences (hereditary background, nutritional level) and external influences (illumination, temperature) act on the cerebral cortex. Nerve impulses are sent from the cerebral cortex to the thalamus and hypothalamus. Neurohumoral secretions from the hypothalamus stimulate the anterior pituitary gland. FSH is released and stimulates the ovarian follicle, which secretes estrogen. This circulates through the liver and, as estrogen degradation products, stimulates the pituitary to release added amounts of FSH (+). This causes further release of estrogen and higher levels of estrogen and higher levels of estrogen degradation products inhibit release of FSH (-) and stimulate release of LH (+) and LTH (+). The central effects of estrogen, then, are at first progonadotropic and later antigonadotropic. The peripheral effects of estrogen are on genital and extragenital tissues, as indicated in the figure.

Release of LH causes further follicular maturation and ovulation, organization of the corpus luteum, and release of progesterone. LTH maintains progesterone release. Progesterone inhibits release of FSH (-), LH (-), and LTH (-). The central effects of progesterone are at first progonadotropic (not indicated in figure, stimulating release of LH) and later antigonadotropic. The peripheral effects of progesterone are on genital and extragenital tissues, as indicated in the figure.


INTERNAL
INFLUENCES
CEREBRAL
CORTEX
THALAMUS
HYPO-
THALAMUS
EXTERNAL
INFLUENCES
ANT.
PIT.
LIVER
+
−
+
+
FSH
LH
LTH
−
−
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ESTROGEN
FOLL.
OVARY
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PROGESTERONE
ENDOMETRIUM
PROLIFERATION
PROGESTATION
MYOMETRIUM
CONTRACTION
RELAXATION
CERVIX
RELAXATION
ACCESSORIES
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MAMMAE
DUCTS
ALVEOLI
SECRETION
SECONDARY
SEX CHAR.
METABOLISM
RETENTION
EXCRETION
BEHAVIOR
ESTRUAL
MATERNAL

Figure 17.

CHEMICAL, PHYSICAL, AND BIOLOGICAL PROPERTIES OF HYDROXYPROGESTERONE ACETATE

CHEMICAL, PHYSICAL, AND BIOLOGICAL PROPERTIES OF HYDROXYPROGESTERONE ACETATE

1. Chemical and Physical Properties

17α-hydroxyprogesterone acetate is an orally active derivative of progesterone. It is a steroid closely related in structure to the natural luteal product, to certain of the androgens, and to the adrenocortical steroids. It is practically insoluble in water, only slightly soluble in sesame oil, methanol, acetone, and ether, and relatively soluble in ethyl acetate, methylene chloride, and chloroform. It is stable under ordinary conditions and temperatures. Its structural formula, compared with that of progesterone, may be represented as in Figure 18.

2. Biological Properties

Incorporation of an acetoxy group at position 17 of progesterone produces hydroxyprogesterone acetate and apparently changes the route of metabolism so that the drug is effective when administered orally. Its physiological basis for delay of estrus is identical to that for progesterone in that it acts centrally to inhibit FSH, LH, and LTH release, preventing follicular growth and maturation, estrual signs, and ovulation, and acts peripherally to produce endometrial and vaginal changes characteristic of progestation.

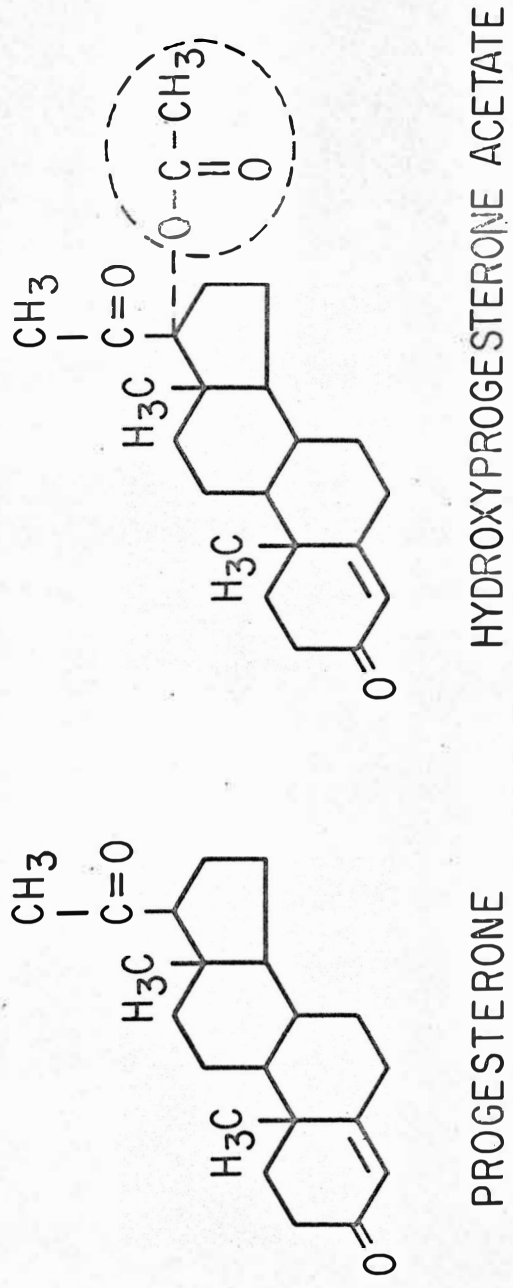

Figure 18. Structural Formulas. Progesterone and hydroxyprogesterone acetate.

Endocrine Bioassays. Oral progesterogenic activity of hydroxyprogesterone acetate is at least twice that of ethisterone, as measured by the endometrial response of young ovariectomized rabbits.[29] Oral estrogenic activity is much less than that of ethisterone, as measured by the uterotrophic effect on ovariectomized rats. Oral androgenic activity is not demonstrable with hydroxyprogesterone acetate, although it is significant for both ethisterone and progesterone, as measured by the seminal vesicle and prostate response of castrate rats.[30]

Safety. In acute oral safety studies in mice, the LD_{50} of hydroxyprogesterone acetate is greater than 10,000 mg. per kg. In acute oral safety studies in rats, 10 and 30 mg. per kg. for 22 days produce no significant deleterious changes in clinical signs, hemograms, or necropsy findings. In chronic oral safety studies in rats, hydroxyprogesterone acetate mixed in the diet at levels of 0.02% and 0.06% (12.5 and 38.5 mg. per kg. average) and fed ad lib. for six months produces no significant deleterious changes in clinical signs or necropsy findings.

LABORATORY AND CLINICAL EXPERIENCE WITH HYDROXYPROGESTERONE ACETATE IN THE BITCH

LABORATORY AND CLINICAL EXPERIENCE WITH HYDROXYPROGESTERONE ACETATE IN THE BITCH

1. Laboratory Experience

When hydroxyprogesterone acetate became available, it was first used in bitches in a two-phase laboratory study concerned with safety and efficacy.[8]

In chronic oral safety studies in bitches, 10 mg. per kg. for nine months and 2.5 mg. per kg. for sixteen months produced no significant deleterious changes in clinical signs, body weight, hemograms, blood chemistry, renal function tests, urinalysis values, hepatic morphology, and liver function tests.

In long-term efficacy studies in bitches, hydroxyprogesterone acetate was administered orally for sixteen months. In order to define the estrual-delaying effect of the drug, indices such as gross observation, effect on studs, vaginal cytology, transillumination examination of ovaries at laparotomy, examination of uteri at laparotomy, necropsy findings, and histological examination of ovaries and uteri were employed. These studies produced the conclusion that hydroxyprogesterone acetate, administered orally at 1.8 mg. per pound (4 mg. per kg.) or above, daily, would inhibit the physical and behavioral signs of estrus, prevent bitches from attracting studs, produce vaginal cytological changes characteristic of progestation,

inhibit Graafian follicle maturation, suppress ovulation, and consistently produce progestational endometria for as long as the drug is administered.

One of the important requirements for any method of delaying estrus is that bitches should be able to cycle normally, conceive, and whelp normal-sized litters of viable pups after discontinuing medication. Therefore, it became important to determine if hydroxyprogesterone acetate disturbed succeeding cycles. It was found that changes induced in the ovaries and uteri did not interfere with return of the bitches to normal breeding. Bitches previously medicated returned to normal cycling, accepted breeding, conceived normally, and whelped normal-sized litters of healthy pups. It appeared, however, that breeding results were better when bitches were bred no sooner than six months after cessation of treatment.

2. Clinical Experience

When safety and antiestrual efficacy of hydroxyprogesterone acetate had been satisfactorily demonstrated in laboratory bitches, the evaluation was expanded to include dogs housed under natural conditions. Accordingly, the drug was made available to selected veterinarians throughout the country.

The drug was administered as drops containing 25 mg. of hydroxyprogesterone acetate per cc. It was given either directly or on the

feed to a diverse group of dogs ranging from prepubertal bitches less than a year of age through mature nulliparas and multiparas to senile surgical risks twelve years of age. Their weights ranged from 2.5 pounds to 90 pounds, with a preponderance of bitches in the 51- to 60-pound category (26% of the total). The majority in this category were racing Greyhounds. In all, thirty breeds were represented.

When the drug was administered daily to metestrual or anestrual bitches at 1.8 mg. per pound of body weight, or above, and begun at least thirty days prior to an expected proestrus, estrus was postponed in 100% of the bitches for as long as the drug was administered. Reports indicated five broad areas in which postponement of estrus is useful:

1. When short-term delay of estrus is desired in hunting, show, derby trial, traveling, companion, or house bitches.

2. When long-term delay of estrus is desired in house bitches or kennelled bitches.

3. When delay of estrus is desirable or required in house bitches or kennelled bitches, to allow litter-spacing.

4. When delay of estrus is medically indicated, as in bitches with tendencies toward prolonged estrus, polyestrus, or excessive hemorrhage during estrus; in ovariectomized bitches demonstrating attractiveness, estrus, or both; and in bitches with estrogen-dependent mammary tumors, in which ovariectomy is contraindicated.

5. When delay of estrus is medically indicated because pregnancy, parturition, or both are contraindicated, as in bitches with abdominal, inguinal, or diaphragmatic herniae, pelvic obstruction, uterine atony, or debility.

Two hundred thirty bitches received the drug prophylactically, approximately 30% for periods up to thirty days and approximately 70% for periods from thirty days to sixteen months. None became estrual while on medication, and many of the bitches are still receiving the drug. Possible side effects observed in thirteen bitches were urinary incontinence (1), enlarged nipples (1), lactation (1), lethargy (2), weight gain (3), and swollen mammae (5).

Only bitches in metestrus or anestrus were candidates for prophylactic effect. The drug prevented estrus in some already in proestrus, and abbreviated the phase in some already estrual, but such use must be considered therapeutic rather than prophylactic. The bitches in which the drug was used therapeutically are not included in the prophylactic total of 230.

Upon cessation of administration, bitches returned to normal cycling within one week to several months. No breeding difficulties were encountered. If another period of delayed estrus was desired, administration was reinstituted when the bitch was again metestrual or anestrual. Hunting bitches have been maintained on hydroxyprogesterone acetate for two consecutive hunting seasons, with cessation of medication, occurrence of estrus, breeding, and conception during the off season.

DISCUSSION

DISCUSSION

Hydroxyprogesterone acetate is not just one more method of preventing estrus and conception in the bitch. The drug is the first of a family of antiestrual-contraceptive compounds that are vastly and entirely different from any method or drug before available; the drug temporarily prevents estrus and the accompanying reproductive processes. Veterinarians and other scientists have long hoped for and sought to develop such a drug -- one which is safe, effective, and simple to use.

When clinical evaluation was begun, a number of investigators feared that prolonged suppression of reproductive processes might result in permanent sterility. The fear was never realized; once medication was stopped, estrus, ovulation, and conception occurred. Other investigators feared that prolonged use of the drug might be dangerous. This fear was also never realized; regular and thorough physical examinations, exhaustive laboratory tests, and necropsies indicated no rise in the general disease rate or any alteration in bioclinical signs. Still other investigators feared the drug would be ineffective. This,too, was never realized; exhaustive clinical evaluations convinced even the most skeptical.

Thus, hydroxyprogesterone acetate and the concept of oral prevention of estrus passed through the three stages through which any innovation passes:

1. The drug and its concept are not safe and effective.
2. The drug and its concept are often safe and effective.
3. The drug and its concept are not only safe and effective but "I"discovered the whole concept.

In June, 1960, the Division of Veterinary Medicine, Bureau of Medicine, Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C., declared finally effective the new drug application of The Upjohn Company, Kalamazoo, Michigan, said application pertaining to the use of hydroxyprogesterone acetate in preventing estrus in dogs. It was a routine action because the law requires the Food and Drug Administration to permit the marketing of any drug as soon as adequate clinical evaluation demonstrates the drug to be safe for use as recommended by the applicant. Oral control of estrus in the bitch is now an accomplished fact. It has opened completely new and unimagined horizons to the veterinarian and dog owner, and these horizons are constantly widening.

It must be admitted that all the answers are not yet known. The need for constant administration disturbs many veterinarians, and the possibility of long-term side effects will not be settled for many

years. A number of these are theoretically possible. Naturally, the longer detailed observations continue, the less apprehension there will be concerning possible long-range effects on the pituitary, adrenals, liver, ovaries, uterus, and pups born of medicated bitches.

Partial and preliminary answers to certain aspects of these questions have been provided by the present studies. For example, one important consideration has been the possibility of harmful long-range effects on the genital tract. A fairly substantial amount of biopsy, vaginal cytological, and necropsy data relates to this specific phase of the problem. In short, within the limits of the techniques, there was no evidence that hydroxyprogesterone acetate produced harmful effects in genital tracts for the period of administration reported.

SUMMARY

SUMMARY

Field evaluation of hydroxyprogesterone acetate for postponing estrus in bitches was initiated after laboratory studies had indicated safety and efficacy of the compound.

A total of 230 metestrual or anestrual bitches were successfully medicated for periods up to sixteen months. An oral dose of 1.8 mg. per pound, daily, effectively postponed estrus for as long as continued in 100% of the cases. Possible side effects were minimal. Bitches returned to cycle upon cessation of medication and whelped normal litters. No breeding difficulties were encountered.

PROJECTION

PROJECTION

The ultimate means of preventing conception will probably never be found. There are many possibilities:

Progestogens, more potent and with long durability, have already been discovered. Medroxyprogesterone acetate is one of these. Parenteral administration of depot-type preparations is a definite possibility. Some of these may have selective central or peripheral effects -- either antigonadotropic or endometrotropic effect.

Antiprogestogens, which may interrupt luteal-phase phenomena.

Estrogens, with central antigonadotropic effect, but without peripheral effect.

Antiestrogens, which interrupt follicular-phase phenomena.

Specific ovistatic or ovicidal compounds, which may be without effect on the endometrium. Dr. Warren O. Nelson and Dr. Sheldon V. Segal, and other scientists, have conducted investigations with a nonsteroidal compound which, when fed to laboratory rats as late as four days postmating, has halted development of eggs and provided 100% prevention of conception.

Specific antinidatory compounds, which may be without effect on the egg. In Israel and India scients have conducted investigations with compounds that prevent adherence of an egg to the endometrium, halting further development.

Specific spermistatic or spermicidal compounds. These would be administered to males and would act by decreasing sperm production, decreasing sperm motility, decreasing percent of normal sperm, or by killing sperm. Members of the bis (dichloroacetyl) diamines have been shown to possess such properties.

Immunizing biologics, which temporarily, semipermanently, or permanently immunize against sperm or egg, and can be used in either males or females. The sperm and egg would act against each other in an antigen-antibody reaction. Conception has been prevented in sea urchin, mice, rabbits, and cattle by this method.

Whether these or still other lines of research will produce a contraceptive method as good as, or better than, the present hormone technique, only time will tell.

But how will these agents be used? We have been considering different lines of pharmacologic and biologic research geared to promoting infertility. What are possible (even probable) areas of utility?

1. Contraceptive use in people, in which the agent prevents conception, hence pregnancy and childbearing.
2. Antiestrual use in small animals, in which the agent prevents estrus, hence ovulation, conception, pregnancy, and litter-bearing. In addition to the current antiestrual use in dogs and cats, is there utility in zoo animals (many are

removed from display when estrual)? In wild animals, as in the fox, to break the rabies cycle? (Wild animals are known to serve as a permanent rabies reservoir and to infect domestic animals and man. Foxes are not the only reservoir host and almost certainly they are not uppermost in the hierarchy -- skunks, raccoons, and rodents are probably higher -- but foxes are more accessible. Feeding an oral antiestrual agent for one cycle would break the rabies cycle; at least veterinarians in public health roles and other scientific roles think this may be so.) What are other possibilities in zoonoses?

3. Estrus synchronization, in which the agent interrupts the estrual cycle for a predetermined time; upon withdrawal, animals come into heat within a reasonably short interval. At first glance, one unacquainted with the economics of animal production would think that simultaneous estrus in a large herd of dairy cattle or a large drove of swine would be chaos on a mass scale. But this assumption is naive. Estrus synchronization would simplify artificial insemination, preparturient care, parturient care, postparturient care of the dam and young, weaning, breaking to solid feed, immunization, husbandry, and marketing. And this could apply

to all producing animals -- dairy and beef cattle, swine, sheep, poultry (where uniformity of egg production would be sought), and perhaps even to fur producers, where there would be estrus synchronization, simplification of husbandry techniques, and grouping of pelting activities.

And why restrict our thoughts to promoting infertility? If we are on the brink of finding numerous methods for inhibiting and disrupting various stages of reproduction, it means that we are also armed with means for correcting reproductive defects -- in short, for promoting fertility. Fertility and infertility are two sides of the same coin.

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APPENDIX

Age	Wt. (lb.)	Cc.*	Mg./ lb.	Total Days	Results**	Side Effects***	Comments
3	21	1	1.1	48		Yes	Listless and sleepy.
3	54	2.5	1.15	60			Slight vaginal swelling 10 days after start.
4	56	2	0.8	49			
7	22.5	1.25	1.4	72			Mammary adenoma--size reduced (good)
2	50	4	2.0	15			Endometritis (good)
1	5	0.5	2.4	16			Endometritis with cystic ovaries. (excellent) nymphomania
8	40	3	1.8	10			Mammary adenocarcinoma with liver metastases (?)
1.5	18	1.5	2.0	20			Abortion with endometritis (?)
6 mo.	5	0.5	2.4	15			Undescended testicles (poor)
10	15	1.25	2.0	24			Mammary adenocarcinoma (good) decreased in size.
2	35	2.75	1.9	10			Abortion with endometritis (fair)
2	60	6	2.5	30			Heat 30 days after stopping. Bred N/litter.
6 mo.	15	2	3.3	30			Heat 30 days after stopping.
1	25	2	2.0	75			Heat 45 days after stopping.
2	60	6	2.5	30			No heat 90 days after stopping.
1	30	3	2.5				Still on drug. Heat 45 days after stopping.
10 mo.	7	1	3.5	30			Still on.

* 25 mg./cc.

** Remained anestrual unless otherwise designated.

*** None, unless otherwise designated.

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
6 mo.	15	1.25	2.0	180+			Still on drug.
7 mo.	72	5	1.7	150		Yes	Urinary incontinence. Reduced to 3 cc--recovered.
9 mo.	40	3	1.8	100			Estrual 1 month after cessation.
1	25	2	2.0	30	Heat		Proestrual (late) when begun
2	60	4.5	1.8	60	Poor		Nymphomania. No response.
1.5	20	1.5	1.5	180+			Still on drug.
4	30	2.25	1.8	100	Poor		Mast cell tumor.
5	60	4.5	1.8	40+			Still on drug.
1	15	1.25	2.0	60+			Still on drug.
3.5	24	2	2.0	100+			Killed by car.
5	32	2.25	1.7	50+			
1.5	50	4	2.0	60			Died (myiasis).
7	15	1.25	2.0	60	Doubtful		Attracts males periodically about every three months. (anestrual) Does not have regular periods of estrus.
5	28	2	1.8	30			Due to be in heat. Preparatory for mammary tumor removal (not identified.) Tumor regressed, more circumscribed.
5	18	1.25	1.7	60			To prevent estrus.
12	47	4	2.1	90			To prevent estrus in bitch that has had dystocia every delivery.
1	2.5	0.5	4.8	30			Post estrus, to prevent continuous heat.
5	5	0.5	2.4	30			To prevent postpartum hemorrhage.
3	40	3	1.8	45			To break habits after spaying. Stopped riding.
11 mo.	13	1	1.9	45			To prevent heat.

Age	Wt. (lb.)	Cc.	Mg. / lb.	Total Days	Results	Side Effects	Comments
2	22	2	2.2	30			To suppress male activity. Bites people when bitch is in estrus. Stopped biting.
15	45	4	2.2	38			For repressing tumor (not identified)*
3	18	1.5	2.0	90			Postestrus
1.5	6	0.5	2.0	15			False pregnancy.
1	3	0.5	4.0	12			Dog in heat 10 weeks.
8	7	1	3.5	?	No results		Used to prevent heat. Probably irregularly administered.
3	4	0.5	3.0	14			Uterus hemorrhage.
5	35	3	2.1	30			Estrus for 6 weeks.
2	45	5	2.7				Good
6	3+	3	1.0	90+			
3	5	0.5	2.5	30			False pregnancy.
11	33	2.75	2.0	70			To study repression of tumor.
9 mo.	22	1.5	1.6	21			To prevent estrus.
11	12	1	2.0	21			False pregnancy.
3.5	40	7	4.3	10			Stilbestrol overdose.
	40	7	4.3	21	Poor		Tumor--no regression.
6	25	5	5.0	10			Mismating.
9	20	3	3.7	10			False pregnancy.
5	25	2.5	2.4		Fair		Stilbestrol overdose.
2	25	5	5.0				Postestrual hemorrhage.
14	50	2.5--2 weeks 1.2 3.7--15 weeks 1.8					Tumor--regression.
16 mo.	28	1	0.89	240		Yes	Loss of pep. Regained on stopping and no heat for 30 days.
						Yes	Spayed: Organs grossly normal. Wt. gain (2.5--3 lb.) lost in 12--14 days after stopping.
				60			Mammary tumor--no further growth.
				60			Postestrual endometritis.

* Used when tumor grows for 1 week.

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
				14			Heat 10 days after stopping.
2	12	1	2.0	45			Heat 7 days after stopping.
4	25	2	2.0	30			Heat 15 days after stopping.
7	50	2	1.0	30			Heat 30 days after stopping but not attractive.
3	35	2	1.4				Still on drug.
2	5	0.25	1.24	120			Proestrual when begun. No heat for 1 period.
5.5	4	0.25	1.55	270			No heat for 2 periods.
4	15	0.5	0.83	210			No heat for 1 period.
3	25	1	1.0	240			No heat for 1--2 period.
3	45	1	0.55	60			In feed.
1	50	2--7 days 1	1.0 0.5				In feed. Still on drug.
5	45	2	1.1	7	Heat		In feed. Began drug on 3rd day of bleeding.
1	15	1	1.6				In feed. Still on drug.
2	60	4	1.6	195			
6	55	4	2.0	313		Yes	Enlarged nipples? Still on drug.
1	50	4	2.0	193			No heat 125 days after stopping.
10.5	25	1	1.0	455			
4	25	1	1.0	545			Still on drug.
9	13	1	1.9	47			Still on drug.
1	10	2	5.0	90			Still on drug.
3.5	13	2	3.8	90			Still on drug.
2	25	0.5	0.5	120			

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
4	22	2--5 da 1--1	2.2	60			
2	18	2--5 da 1--1	2.7		Heat		Already in heat when begun.
2	5	0.5	2.5	67			Missed 7 days: vulvar swelling.
6	12	1	2.0	30			Killed by car.
				60			
				60			
				60			
				60			
				135			
				135			
				21	Heat		
2	45	4	2.2	105			
2.5	65	4	1.53	7			
3	70--80	4	1.4--1.25	7			
1	65	4	1.5	60			
4	75	2	0.66	47			Habitual aborter. Begun 2 weeks after breeding until 1 week before term. 5 N/pups 61 days.
8 mo.	50	4	2.0	390			
3	50	4	2.0	150			
9 mo.	18	1	1.3	21	Heat		In estrus at time.
9 mo.	55	2	0.9	21	Heat		In estrus at time.

	Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
	2	55--60	3	1.2--1.36	90	No--9 Heat--3		Began testosterone.
	10	22	2	2.2	60			
	8	47	3	1.0	40			
	0.5	25	2	2.0	240			
	1	20	2	2.5	240			
	3	20	2	2.5	240			
	5	50	4	2.0	30	Heat		Heat after less than 30 days administration.
	7	40	4	2.5	240			Estrus medically contraindicated.
	5	25	1	1.0	30			
	7 mo.	35	2	1.4	45			Proestrual when begun. Spay--developed and vascular uterine horns.
	12	40	2	1.25				Estrus medically contraindicated: multiple mammary tumors.
	10	55	2	0.9	15			Estrual at time: 14 day heat.
	5	40	2	1.2	60			No heat for 11 months.
(3)	2--4	35--60	2	1.4-0.99	15--30			For field trial (all due).
	3	35	2	1.4	30			Hunted; estrual when begun. Bred 7 months, 6 pups.
	2	40	2	1.2	15			Estrual 2 months after cessation.
	7	30	1	0.8	150			
	5.5	16	1	1.5	261		Yes	Weight gain. Heat medically contraindicated--hernia, endometritis.
	13 mo.	13	1	1.9	60			
	4	65	2	0.7	1	Heat		Started on 2nd day of heat.
	3	55	2	0.9	60			

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
4	35	4	2.8	75			Heat 3rd week after stopping.
6	25	3	3.0	20			Started on 2nd day of heat. Suppressed Heat after stopping (1)
2	27	1	0.92	21			
6	22	1	1.1	21			
4	10	0.5	1.2	73			
4	18	1	1.3	28			
6	47	1	0.5	35			Estrus 36 days after cessation. Spayed. In feed.
2	55	2	0.9	1	Poor		Stilbestrol overdosage.
10	60	0.6	0.25				Pseudocyesis treated.
3	20	1	1.2	32			Persistent estrus treated.
7	60	2	0.83	7	Exc.		Pseudocyesis treated (3 days duration)
5	30	1.5	1.25	7	Exc.		Pseudocyesis treated (12 days duration)
11	26	2	1.9	1	Exc.		Pseudocyesis treated.
2.5		3			Exc.		
12		1			Exc.		To control pregnancy.
7	13	1	1.9	1	Exc.		To control pseudocyesis.
2	90	4	1.1	270			Began 3rd week before heat: no swelling or attractiveness.

	Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
	3	40	4	2.5	450			Spayed: ovaries atypical for anestru
	6 mo.	25	2	2.0	120			Died--food poisoning.
	1.5	18	2	2.7	180			Killed by car.
	2	62	4	1.6	21			
	3	74	4	1.3	2	Heat		Heat after 2 days.
	3	22	2	2.2	180			
	2	19	2	2.6	180			
	5	35	2.5	1.78	77			Started when in heat: subsided in 2 days.
	2	5	0.5	2.5	21			Difficulty in administering (Chi.)
	1	4	0.5	3.1	21			Difficulty in administering (Chi.)
	3	55	4	1.8	77			
	3	55	4	1.8	84			
	3	60	4	1.6	180			
	8	20	1	1.2	180		Yes	Weight gain. Dystocia.
	4	6	1	4.0	90			
	9	30	2	1.6	90			Excess bleeding.
	4	15	1	1.6	30			Cystic ovaries.
	2	65	2	0.7	30			Cystic ovaries.
	5	40	2	1.25	45	Heat		Heat and attractive 45 days after starting.
	4	20	1	1.2	30			Long estrus.
	10 mo.	23	1	1.0				
	4	15	1	1.6				
	2	61	2.5	1.0				
	8 mo.	20	1	1.2				
	14 mo.	42	2	1.1				
(15)						13--no 2--Heat		2 in heat when begun.

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
1.5	3	0.5	4.0	10	Exc.		Nymphomania 90 days. N in 10 days.
3	20			15	Exc.		Feminized, being ridden.
3	20			7			Nymphomania 35 days. N in 7.
		1		60			In heat 2 days after cessation.
2.5	52	2	0.9	170			Increase to 1 teaspoon 30 days later.
2	51	1 tsp.	1.9	170			
18 mo.	62.5	2	0.8	60		Yes	Lactated (owner) attractive
5	62	2	0.8	60		Yes	Swollen Breasts.
5	50	2	1.0	60			
15 mo.	50	1 tsp.	2.0	121			
15 mo.	50	1 tsp.	2.0	121			
15 mo.	50	1 tsp.	2.0	121			
15 mo.	58	1 tsp.	1.7		Heat		In heat after 4 days on.
15 mo.	54	1 tsp.	1.8	121			
15 mo.	54	1 tsp.	1.8	121		Yes	Swollen breasts.
15 mo.	56	1 tsp.	1.7	121		Yes	Swollen breasts.
1	51.5	1 tsp.	1.9	76			
1	53	1 tsp.	1.8	76			
17 mo.	52	1 tsp.	1.9	90			
17 mo.	52	1 tsp.	1.9	90			
19 mo.	50	1 tsp.	2.0	53		Yes	Mammary swelling | Decreased when taken off.
19 mo.	54	1 tsp.	1.8	53		Yes	Mammary swelling
23 mo.	54	1 tsp.	1.8	53			
3	54	1 tsp.	1.8	147			
3	54	1 tsp.	1.8	147			
3	54	1 tsp.	1.8	147			
3	54	1 tsp.	1.8	147			
3	54	1 tsp.	1.8	147			
18 mo.	54	1 tsp.	1.8	147			

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
4	30	3	2.5	22			
5	10	1	2.5	30			
1	50	5	2.5	30			
2	5	1	5.0	40			
3	30	2.5	2.0	35			
6	55	4	1.8	26			Male breeding behavior for 2 days.
1.5	50	4	2.0	26			
3	40	4	2.5	25			Almost estrual when begun; suppressed in 5 days.
6	2.5	2	20.0	21			
4	30	4	3.3	15			
9 mo.	30	4	3.3	15			
4	40	4	2.5	15			
8	50	4	2.0	15			
2	4	2	12.5	21			
4	50	4	2.0	14	Heat		Heat 2 days after beginning.
5	22	2	2.2				Heat 60 days after cessation.
3.5	23	3	3.2				
11	28	4	3.5	14			
9	65	4	1.5	21			
2.5	12	1	2.0	547			
5.5	40	1	0.62-1.2	365			On 1 cc for 7 months, then 2 cc. for 5 months ≥1 year.
3	65	2	0.76	210			Begun 1 month before expected estr
3	15	1	1.6	365			
4	40	1	0.62	30			

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
18 mo.	54	1 tsp.	1.8	147			
3	53	1 tsp.	1.8	147			Hemorrhage: doubled dose: controlled.
2	55	1 tsp.	1.8	147			
1.5	77	1 tsp.	1.2	147			
1.5	60	1 tsp.	1.6	147			
15		1 tsp.		147			
15		1 tsp.		147			
15		1 tsp.		147			
15		1 tsp.		147			
15		1 tsp.		147			
15		1 tsp.		147			
15		1 tsp.		147			
4	53	1 tsp.	1.8	55			
4	53	1 tsp.	1.8	55			
4	54	1 tsp.	1.8	55			
2	56	1 tsp.	1.7	139			
2	60	1 tsp.	1.6	139			
2	56	1 tsp.	1.7	139			
2	58	1 tsp.	1.7	139			
3	58	1 tsp.	1.7	139			
1	46	1 tsp.	1.0	139			
1	46	1 tsp.	1.0	139			
1	46	1 tsp.	1.0	139			
2.5	56.5	1 tsp.	1.7	103			
19 mo.	55	1 tsp.	1.8	30			
19 mo.	56	1 tsp.	1.7	30			
27 mo.	54	1 tsp.	1.8	30			
27 mo.	54	1 tsp.	1.8	30			
23 mo.	53	1 tsp.	1.8	30			
23 mo.	50	1 tsp.	2.0	30			
18 mo.	53	1 tsp.	1.8	30			
23 mo.	54	1 tsp.	1.8	30			

Age	Wt. (lb.)	Cc.	Mg./ lb.	Total Days	Results	Side Effects	Comments
8 mo.	19	1.5	1.9	45	Exc.		Profuse bleeding.
10	20	1.5	1.9	14			In heat after 2 months.
4	50			7	Exc.		Estrus in ovariectomized bitch.
1	45	3	1.6				
2	70	4	1.4				
1	60	3	1.2				
1.5	50	3	1.5				Heat 30 days after stopping.
6	58	3	1.2				
1	20	1	1.2				
1.5	46	1	0.5		Heat		Misunderstanding on dose.
3	50	2	1.0	300			Still on drug and racing.
4	60	2.5	1.0	330			Still on drug and racing.
3.5	65	2.5	0.96	300			Still on drug and racing.
2	25	1	1.0	120			Heat 60 days after stopping.
6	40	2	1.25	120			(Second course begun for second
2	50	2	1.0	120			hunting season.)
3	45	2	1.11	70	Heat		Began 1 month prior (excess bleeder). Repeated--heat again.
14 mo.	85	2	0.58				Began 3 weeks prior to heat. Slight bleeding, not [illegible] Ortho.

	Age	Wt.	Cc.*	Mg./ Lb.	Total Days Admin.	Results**	Side Effects***	Comments
	4½	40	1½	0.9	90			
	4	35	1½	1.0				False pregnancy
	10	65	4½	1.7	5			Mammary carcinoma. Died in 5 days.
	8	65	4½	1.7	38		Yes	Lethargy, dry coat, mucus vag. disc. after stopping. Lasted 10-12 days; dog then became peppy, coat improved.
								Pyometra before. 66 day lapse.
			4½	1.7	17			Signs returned. 14 day lapse.
			½	0.18	9			Signs returned. Stopped drug; pep returned.
	11	6	½	2.0	93			Mammary tumor. No inc. in size.
					Cats			
		7-10	½	1.7-1.2				

*25 mg./cc.
**Remained anestrual unless otherwise designated.
***None, unless otherwise designated.