UTERO-OVARIAN RELATIONSHIPS IN THE GUINEA PIG

by

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INTRODUCTION

For nearly 40 years it has been known that there exists a functional relationship between the uterus and the corpus luteum in some species. The effect of hysterectomy has been studied in at least 15 species. Complete hysterectomy has been observed to cause persistence of the corpora lutea for a period of time approaching or exceeding the length of gestation in the guinea pig, cow, pig, pseudopregnant rat and pseudopregnant rabbit. Hysterectomy of the ferret, opossum, monkey, estrous rabbit and cycling rat has been reported to have no effect on ovarian function or estrous behavior. Corpora lutea are maintained for a period which approaches or exceeds that of pregnancy or pseudopregnancy following hysterectomy of pregnant or pseudo-pregnant rats and rabbits. The mechanism by which the uterus regulates the life span of the corpus luteum remains to be determined.

This investigation deals with the relation of quantity of uterus to the length of time the corpora lutea persist in the partially hysterectomized guinea pig. The effect of unilateral ovariectomy at the time of partial hysterectomy, transplantation of the uterus, and chemical destruction of the endometrium were also studied. In addition, this study includes some preliminary observations on the effects of various reproductive hormones on the estrous cycle and on the maintenance of the corpora lutea in the guinea pig.

REVIEW OF LITERATURE

Reproductive Biology of the Guinea Pig

The guinea pig under laboratory conditions is polyestrous the entire year. Estrus lasts less than half a day and the estrous cycle is about 16.5 days. Ovulation is spontaneous and the corpora lutea of the estrous cycle are functional. Gestation is about 67 to 68 days, with a litter size of about 3 to 4 (Asdell, 1946).

Estrous cycle and gestation

Ishii (1920) reported the age of puberty as 55 to 70 days. Young et al. (1939) stated the average age of puberty is 67.8 days with a range of 33 to 134 days. The vaginal opening is closed by a membrane except for a period of about 4 days at each estrus. Kelly and Papanicolaou (1927) described this structure as a solid epithelial vulvar cord extending 1 to 2 mm. in depth. The opening of the membrane occurs at the inflammatory stage of the sexual cycle. Opening is caused by the cornification and subsequent desquamation of the central part of the vulvar cord.

Young et al. (1933) and Young et al. (1935) reported the average length of the estrous period as about 8 hours with a range of 1 to 18 hours. The former authors report ovulation occurs within an hour after the termination of estrus. Young et al. (1935) observed about 64 percent of all heats begin be-

tween 6 p.m. and 6 a.m. with the high point of estrus between 11:52 p.m. and 1:32 a.m. Observation by Dempsey et al. (1934) showed that light is not necessary for the maintenance of the reproductive rhythm. Confinement to complete darkness causes a loss of the tendency to come in heat at night, but the cycle length is not affected. Young et al. (1933) found no relationship between the length of estrus and the number of ovulations, while Young et al. (1938) reported those with the greatest numberof ovulation have the most intense heat, as judged by the frequency with which they are mounted.

Boling et al. (1939) observed estrus immediately after parturition in about 64 percent of the females. Estrus usually begins within 2 hours after parturition, but it lasts only about 3.5 hours instead of the normal average of 8.6 hours. This estrus is always accompanied by ovulation, while ovulation also occurs in about half of those animals not exhibiting heat. Ibsen (1928) observed little or no difference in the number of ova shed from the 2 ovaries, as 51 percent of the embryos was found in the left horn. Kinney (1923) and Kelly (1928) have reported that internal migration of ova is impossible on anatomical grounds, since the internal bifurcation is in the cervix. According to Blandau and Young (1939) the maximum life of the ova, as shown by insemination at various times, is about 30 hours. A marked drop in fertilization was observed about 8 hours after ovulation. Ishii (1920) reported pregnancy is rare following copulation in the first 3 or 4 hours of estrus.

Nicol (1932-33), Young et al. (1935) and Young et al. (1933) reported average estrous cycle lengths of 17.7 days, 16 days 6 hours and 16 days 7 hours, respectively. The latter authors observed a range of 13 days to 21 days 12 hours, based on constant observation during 321 estrous cycles. Young et al. (1935) found that neither sterile copulation nor unsuccessful artificial insemination have any effect on cycle length. Loeb (1923b), Papanicolaou (1926) and Dempsey (1937) stated that removal of all the corpora lutea soon after ovulation shortens the estrous cycle to about 11 days. Young et al. (1938) observed that unilateral ovariectomy doubles the number of ovulations in the remaining ovary.

The average litter size of 11,945 litters as reported by Haines (1931) was 2.58 with a range of 1 to 8. Blandau and Young (1939) determined the average length of 395 gestations to be 68 days with a range of 58 to 72 days. Herrick (1928) found ovariectomy before day 26 of gestation always resulted in abortion or resorption of the embryos. Ovarian transplants at the time of ovariectomy prevented abortion in 9 of 14 cases.

Histology of the ovary

Loeb (1911) reported the presence of follicles which contained cavities at 4 to 7 days of age. The theca interna of these follicles is not well differentiated at this age. By 18 days of age some follicles are undergoing atresia. During

the estrous cycle Myers et al. (1936) found that a new wave of follicular growth begins about 2 or 3 days after ovulation. These follicles grow at a fairly constant rate until the beginning of estrus. During the first part of estrus the growth rate increases to about 9 times that during diestrus. Asdell (1946) determined that about 40 to 50 follicles are produced during each estrous cycle, of which only about 2 will ovulate. Those not destined to ovulate can be detected about 2 days before estrus by degenerative changes in the granulosa layer.

Loeb (1906) reported that the corpora lutea of the guinea pig are formed from both the granulosa and the theca interna cells, which after a time can not be distinguished from each other. Loeb (1923b) found that the corpora lutea are well formed by 4 days following ovulation. These corpora lutea begin to regress about 13 to 15 days following ovulation as shown by the vacuolization around their periphery. Rowlands (1956) reported the corpora lutea reach their maximum size about the twelfth day of the estrous cycle. The corpora lutea of gestation grow at a constant rate for 18 to 20 days and maintain this size until parturition.

Schmidt (1936a) and Young et al. (1938) observed rete ovarii were present in the ovaries of nearly all guinea pigs. The latter authors found that the cystic condition of rete ovarii increases with the age of the animal. However, they found no relationship between the presence of rete cysts and the length

of estrus.

Histology of the uterus

During the estrous cycle the uterus undergoes characteristic histological changes. At estrus the cells of the uterine epithelium are tall and pseudostratified, and the surface is somewhat irregular. These cells tend to become vacuolated during estrus. The mucous layer becomes very edematous and leucocytes invade the stroma. At estrus the uterine glands are not well developed and are inactive. Shortly after the time of ovulation patches of the epithelium are shed. At 1 to 2 days after estrus the edema has subsided and repair of the epithelium is in progress. During this period the stroma is very vascular and the glands are swollen with secretion. Three days postestrus the glands are more tortuous and the glandular epithelial cells are swollen so as to obliterate the lumen. The surface epithelium is smooth and the cells are tall with the nuclei at their base. At 7 days postestrus the glandular cells have shrunk in size, and the uterine epithelium is cuboidal to columnar. The glands are retrogressing and less coiled at day 10. By day 14 the glandular retrogression is more apparent (Asdell, 1946).

Histology of the vagina

The vagina also undergoes marked changes during the estrous cycle. Following estrus the vaginal epithelium becomes a low

stratified squamous type. About 8 to 10 days after ovulation the lower layer of the epithelium is cuboidal with long flat cells overlying it. An intense proliferation then takes place; the epithelium becomes thicker, with the superficial layer flattened. At estrus the cells become cornified and desquamate. In the latter part of estrus the vaginal wall becomes edematous and is invaded by large numbers of leucocytes which pass into the lumen. These changes during heat produce the typical vaginal smear. At the first part of estrus the leucocytes disappear and squamous epithelial cells are present in the smear. This is followed by cornified cells, then small epithelial cells and finally by an invasion of leucocytes (Stockard and Papanicolaou, 1917 and Kelly, 1929).

Deciduomata formation

Traumatization of the uterine mucosa between the third and ninth day of the estrous cycle causes the production of deciduomata. These deciduomata persist about 15 days, at which time they become necrotic. The estrous cycle is prolonged to about 20 to 30 days when deciduomata are produced. It is the uterine mucosa which is specific for decidual formation. The presence of corpora lutea or the injection of progesterone is necessary for this reaction. Removal of the ovaries or corpora lutea soon after ovulation prevents the formation of deciduomata in the traumatized uterus. Administration of progesterone to the ovariectomized guinea pig with a traumatized uterus produces

deciduomata. Prolonged progesterone injections before trauma-
tization cause the uterus to become refractory to deciduomata
formation. Estrogen does not intensify the effect of proges-
terone in production of deciduomata. Also, deciduomata are
formed in pieces of uterus transplanted 5 to 7 days after ovu-
lation (Loeb, 1908, 1909, 1910, 1914, 1923b and 1932, and
Blumenthal and Loeb, 1942).

Hormone concentrations

Schmidt (1936b) reported no estrogen in the urine of guinea
pigs during diestrus or early pregnancy. Four-fifths of the
extract of a three-day collection of urine taken at estrus was
necessary to produce estrus in the immature rat. Urine at 20
days of gestation contains about the same quantity of estrogen
as urine at estrus. By 40 to 63 days of gestation this quantity
of estrogen is doubled. Rowlands and Short (1959) reported the
progesterone content of the guinea pig corpora lutea to be 16.2,
7.7, 14.9, 15.5, 25.2, 26.6, 23.5 and 37.6 μg. per gm. of luteal
tissue at day 6 and 11 to 13 of the cycle, day 6, 11 to 13, 21
to 23, 41 to 43 and 62 to 64 of gestation and in hysterectomized
animals at 62 to 64 days after the preoperative estrus, re-
spectively.

The gonadotrophic level of the pituitary was reported by
Nelson (1935) to be only about one quarter that of the rat.
Ovariectomy increases the level of gonadotrophin, whereas es-
trogens depress it. Schmidt (1937) observed follicle stimu-

lating hormone to be low at estrus with its maximum between days 11 and 15 of the estrous cycle. Leuteinizing hormone was reported by Lipschütz (1931) to be less than one tenth that in the rat pituitary. Reece and Turner (1937) found a low pituitary content of prolactin. The content per gram of anterior pituitary was 217 bird units in the immature guinea pig and 520 bird units in the sexually mature female. Estrogen was found to increase the pituitary concentration of prolactin.

Hormonal induction of estrus

Induction of estrus in spayed guinea pigs is difficult with estrogen injections. Boling et al. (1938) reported estrus in spayed guinea pigs occurred in only 14 of 138 cases following injections of 200 international units of theelin. If 0.2 international units of progesterone were given 24 to 48 hours following the injection of 200 international units of theelin, 80 percent of these animals exhibited estrus. Sexual receptivity was observed by Dempsey et al. (1936) in only 14 percent of normal females and 31 percent of spayed females following injections of 1000 international units of theelin. However, 40 international units of estrin followed in 36 to 48 hours with 0.2 international units of progesterone produced estrus in all of 24 spayed animals. Injection of 0.2 gram equivalents of sheep luteinizing hormone produced heat and ovulation in 19 of 24 guinea pigs, (latent period 5.8 hours) when injected on the twelfth to fifteenth day of the estrous cycle. No effect

was obtained when injected earlier in the cycle. Asdell (1946) reported that the ovaries of the guinea pig are very refractory to injections of gonadotrophic hormones or to pituitary implants, due to the ease with which the theca interna becomes luteinized. Implantation of 4 guinea pig pituitaries daily is required to grow normal follicles. Injection of pregnant mare's serum was found by Rowlands (1938) to cause little increase in the ovarian weight of the immature guinea pig, but the uterus was greatly enlarged.

Hysterectomy

There has been a great interest in the role of the uterus on the life span of the corpora lutea since Loeb (1923a) discovered a functional relationship between the uterus and the corpora lutea. Effects of hysterectomy on the corpora lutea have been studied in at least 15 species.

Hysterectomy of the mouse

Westman (1929) hysterectomized infantile mice and observed normal cycles when the animals reached puberty. Hall (1934) states that the endometrium of the mouse has a definite relation to endocrine function. Prolan was given 10 to 21 days following transplantation of the uterus in completely hysterectomized mice. Regressive changes found in the ovaries of hysterectomized mice were prevented by uterine transplants and prolan injections.

Bradbury (1932) reported hysterectomy as early as the eleventh day of gestation in the mouse is followed by milk secretion within 48 hours. He states that hysterectomy before the appearance of interlobular canals inhibits mammary development even though corpora lutea are present. Pituitary extracts induce alveolar tissue in hysterectomized mice, if some interlobular tissue is present.

Hysterectomy of the rat

Durrant (1926), Loeb (1927), Kok (1930), Durrant (1931) and Ball (1934) observed normal estrous cycles following hysterectomy of cycling rats. Bradbury (1937) and Bradbury et al. (1950) also found no effect of hysterectomy in cycling rats, but the hysterectomy of pseudopregnant rats extended the life span of the corpora lutea of pseudopregnancy from 12 to 18 days. They reported injections of endometrial suspensions hastened the involution of the corpora lutea following hysterectomy of pseudopregnant rats. Hysterectomy between the sixth and sixteenth day of pregnancy tended to cause the corpora lutea to persist for a time approaching what would have been term for that pregnancy.

Long and Evans (1922) observed normal estrous cycles in hysterectomized rats unless the cervix was stimulated to produce pseudopregnancy. Typical estrous cycles were observed following transplantation of the ovaries. They also reported the production of pseudopregnancy in rats with transplanted

ovaries and found that transplanted ovaries from 21-day-old rats into mature rats hastened maturity of the ovaries by 2 months. Perry (1961) found pseudopregnancy was extended about 4 days by hysterectomy of the rat, but that the corpora lutea do not grow larger than those of normal pseudopregnant rats. Perry and Rowlands (1961) observed that normal pseudopregnancy was 12.9 days, and that pseudopregnancy was 19.4 days following hysterectomy on the fifth day of normal pseudopregnancy. Three subsequent pseudopregnancies following hysterectomy averaged 18.2 days with cycles between pseudopregnancies being 4 to 5 days.

Kross (1922) reported hysterectomy produced ovarian degeneration in the rat; while Ranney et al. (1947) found no microscopic differences between the ovaries of rats hysterectomized for 16 weeks and those from intact animals. Shelesnyak and Schwarz (1944) observed marked luteinization and depressed follicular development in 23 rats hysterectomized before puberty and autopsied 6 to 21 weeks later. Murphy (1934) found no effect of hysterectomy on ovarian weight. Equal weights of both ovaries 10 days or more following unilateral hysterectomy were reported by Mandl and Zuckerman (1951).

Hechter et al. (1940) observed a shortening of the time to postoperative estrus in rats hysterectomized 4 to 6 days after copulation and implanted with estrous rat uteri in the peritoneal cavity. The time to postoperative estrus was about 11

days as compared to 21 days for nonimplanted rats and 21 days
for rats implanted with diestrous rat uteri, frozen estrous rat
uteri or estrous rabbit uteri. Haupstein and Buhler (1936)
stated that the pituitary of hysterectomized rats is low in
luteinizing hormone. Silbiger and Rothchild (1961) observed
that pseudopregnancy was prolonged in 85.1 percent of 87 rats
hysterectomized before day 9 of pseudopregnancy and 50 percent
of 58 rats hysterectomized later in the diestrus. Ovarian
hypertrophy and increased pituitary folliculotrophic potency
were not seen until the fifteenth day of pseudopregnancy.
Forty-nine and three-tenths percent of 49 hysterectomized rats
bearing homotransplanted pituitaries had pseudopregnancy longer
than 16 days, as compared to only 6 percent in hysterectomized
controls.

Hysterectomy of the rabbit

Studies of the effect of hysterectomy on the ovaries were
reported in the rabbit in 1889 by Grammatikati. He found no
regressive changes in the ovaries of rabbits 6 months following
hysterectomy. Bond (1906), Carmichael and Marshal (1907) and
Unterberger (1930) reported that the ovaries of hysterectomized
rabbits appeared normal at the time of sacrifice 3.5 to 5 months
after the operation. According to Mandl and Buerger (1904)
many follicles were present in ovaries of most rabbits 8 months
to 3 years after hysterectomy, with some cases of cystic de-
generation attributed to injury of the ovarian blood supply.

Tenney et al. (1955) reported total hysterectomy or tubal ligation in the rabbit first caused follicular stimulation followed in a few weeks by follicular degeneration and development of the interstitial body. They concluded that hysterectomy caused a premature aging of the rabbit ovary. Tenney et al. (1958) prevented ovarian degeneration in the rabbit following removal of the uterus by administering aqueous extract from the human uterus.

Asdell and Hammond (1933) reported the life span of corpora lutea in the mated hysterectomized rabbit is between 24 and 29 days. This is about 10 days longer than the life span of the corpora lutea of normal pseudopregnancy and approximately 6 days shorter than during gestation. Gillard (1937) observed that the eighteen-day pseudopregnancy of rabbits was extended to 25 days by hysterectomy. He reported that there was no difference between the mammary development of pseudopregnant and pseudopregnant-hysterectomized rabbits. Siegmund (1934) and Loeb and Smith (1936) also found an increase in the life span of the corpora lutea of pseudopregnancy following hysterectomy.

Pincus (1937) and Pincus and Zahl (1937) reported that normal rabbits excrete estriol following injections of estrone; but they found no estriol in the urine of either hysterectomized rabbits or of rabbits which had been ovariectomized for a long time. Also, it was found that estradiol is converted to estrone in the estrous and hysterectomized rabbit, to both estrone and

estriol in the pregnant and pseudopregnant animal and is not converted in ovariectomized females. It was concluded that estradiol is converted into estrone in animals with ovaries, and that estrone is converted to estriol in animals with a functional uterus. Fish and Dorfman (1942) were unable to isolate any estriol from the urine of normal rabbits. Also, they were not able to isolate any estriol from urine of ovariectomized-hysterectomized rabbits during or in the 3 days following the injection of 100 mg. of alpha-estradiol daily for 5 days. However, they isolated both estrone and beta-estradiol. It was concluded that estrone is an intermediate product in the conversion of alpha-estradiol to beta-estradiol, and that this conversion takes place in the absence of both the ovaries and the uterus. Heckel (1942) was able to maintain the corpora lutea of pseudopregnant rabbits with intact uteri for more than twice the usual life span by injecting estradiol benzoate. Removal of part of the uterus prolonged pseudopregnancy in proportion to the quantity of uterus removed. The amount of estrogen (20 to 40 rat units), insufficient to increase the life span of the corpora lutea of intact animals, prolonged pseudopregnancy of partially hysterectomized animals passed that of similarly hysterectomized controls. Greep (1941) reported that hysterectomy in the second half of pregnancy causes a decrease in the size of the corpora lutea, but that estrogen injections prevented this involution. Hysterectomy and hypophysectomy showed that the estrogen effect was not through the pituitary.

Chu et al. (1946) decreased the life span of the corpora
lutea of hysterectomized-pseudopregnant rabbits to that of nor-
mal pseudopregnancy by implantation of uterine tissue. They
also increased the life of the corpora lutea of rabbits hys-
terectomized during pregnancy with implants of placenta.
Sessums and Murphy (1933) compared the ovaries of intact rab-
bits with the ovaries of rabbits 6 to 9 months following hys-
terectomy or following hysterectomy with autotransplants of
pieces of the endometrium. They concluded that hysterectomy
in the rabbit brings about inhibitory and degenerative changes
in the ovaries. Autotransplants of the endometrium tended to
limit the inhibitory and degenerative changes. Mishell and
Motyloff (1941) stated that endometrial extracts from nonpreg-
nant cows regulate follicular proliferation, protect the func-
tional elements of the ovary and retard the process of atrophy
when administered to hysterectomized rabbits. Micale (1940)
reported either vitamin E or folliculin prevented involution of
the corpora lutea of rabbits hysterectomized during early preg-
nancy, but that gonadotrophin or vitamin C had no effect on
corpora lutea.

Hysterectomy of the guinea pig

Loeb (1923a) described a functional relationship between
the uterus and the corpora lutea. He found that the removal of
the entire uterus in the guinea pig caused the maintenance of
the corpora lutea for at least 60 days and in some cases as

long as 80 days following the preoperative estrus. If one-fourth to one-third the length of the uterus remained intact, the full effect was not observed, as estrus occurred 21 to 32 days following the preoperative ovulation. The completely hysterectomized animals showed mammary development with mitotic proliferation as late as 74 days after the preoperative estrus. Later Loeb (1927) reported ovulation does not usually occur in guinea pigs within 3 months following hysterectomy. Absence of the uterus as such does not prevent ovulation, since ovulation occurred following removal of the corpora lutea after hysterectomy and at puberty following hysterectomy of sexually immature guinea pigs. He found no shortening of the life span of the corpora lutea by transplanting the uterus at either the time of hysterectomy or 7 to 10 weeks after the operation. Loeb and Kountz (1927) observed that injections of follicular extract, which caused opening of the vagina and proliferation of the vaginal epithelium in immature, castrated and cycling guinea pigs, did not produce these effects in hysterectomized guinea pigs. Desclin and Brouha (1931) completely hysterectomized 8 guinea pigs and found estrus did not occur before the time of sacrifice, 52 to 58 days following the preoperative estrus. They stated that the pituitaries were larger than normal and were comparable to those during late pregnancy or from animals in which deciduomata were produced. Hysterectomy during gestation was reported by Desclin (1932) to cause corpora lutea to

persist about 3 months. Herlant (1933) found no estrus for 5 months following hysterectomy either a few days after estrus or during gestation. Following hysterectomy the pituitary gland enlarged and the eosinophilic cells showed increased amounts of chromatin.

Rowlands and Short (1959) demonstrated that the corpora lutea of hysterectomized guinea pigs reached a size as great as during pregnancy. The progesterone content of these corpora lutea 62 to 64 days after the preoperative estrus was greater than corpora lutea from animals 21 to 64 days of pregnancy. Rowlands (1961a) stated that the corpora lutea of hysterectomized guinea pigs regressed slowly with an almost permanent inhibition of the estrous cycles and ovulation. Rowlands (1961b) found that the vaginal membrane did not open for 8 months in 4 of 5 animals hysterectomized on the fifth day of the luteal phase. Corpora lutea of animals hysterectomized on day 10 of the estrous cycle enlarged and maintained their size for about 50 days. Hysterectomy on the fifteenth day of the estrous cycle lengthened the cycle only an average of 2.5 days over that of the normal estrous cycle.

Nelson (1934) demonstrated that removal of the uterus and ovaries from guinea pigs at 35 to 60 days of gestation caused lactation within 40 hours. Hysterectomy or ovariectomy alone had no effect on lactation. When only the ovaries and fetuses were removed, lactation did not occur; but lactation did occur

when the placentae were also expelled. Turner and Gomez
(1934) reported milk secretion in guinea pigs hysterectomized
at 25 to 30 days of gestation, if the ovarian blood supply was
ligated. However, when the ovarian blood supply was not li-
gated, milk secretion did not occur. They also found lacta-
tion occurred in non-pregnant females within 48 hours following
the injection of lactogenic hormone, if the animals were in
estrus. However, they could not produce lactation with this
hormone at other times during the estrous cycle.

Hysterectomy of miscellaneous animals

Unterberger (1930) observed no effect of hysterectomy on
ovarian function in the dog. Cheval (1934) reported most folli-
cles in ovarian grafts were in stages of atresia after complete
hysterectomy of the dog. This follicular atresia was prevented
with the uterus in situ or with the transplantation of pieces
of uterus in hysterectomized animals.

Baidin (1939) found that the ovaries of 3 cats had not
atrophied 3 to 4 years following hysterectomy. The follicles
continued to mature and normal corpora lutea were present.

Carmichael and Marshal (1907) reported the ovaries of the
ferret are normal 3 months after hysterectomy. Deanesley and
Parkes (1933) observed estrus, copulation and ovulation in the
ferret following hysterectomy toward the end of anestrus, at
6 days after coitus or at 3 weeks of gestation.

Hartman (1925) concluded hysterectomy of the opossum is

without effect on ovarian function.

Hysterectomy of the thirteen-lined ground squirrel, either pregnant or nonpregnant, has no effect on the normal cycle of development and regression in the ovary as reported by Drips (1919).

Klein (1938) reported the corpora lutea were undergoing regression 78 hours following complete removal of the uterus at 8 to 13 days of gestation in the golden hamster. By 92 hours after hysterectomy the corpora lutea had regressed to a non-functional state and new ovulations had occurred. Neither estrone nor progesterone maintained the corpora lutea following hysterectomy. However, the corpora lutea persisted following the removal of only the fetuses with the placentae retained.

Hysterectomy of the ewe, cow and sow

Wiltbank and Casida (1956) found the corpora lutea persisted in 10 ewes following hysterectomy on day 3 to 8 of the estrous cycle. Corpora lutea marked during the operation were found at the time of sacrifice 20 to 107 days after the pre-operative estrus. They also reported marked corpora lutea present in 4 completely hysterectomized heifers at the time of slaughter, 27 to 154 days after the preoperative estrus.

Anderson (1962) found that the corpora lutea in 4 heifers, completely hysterectomized on day 8 to 11 of the estrous cycle, persisted for 270 or more days following the preoperative estrus. The life span of corpora lutea in 12 partially hysterec-

tomized heifers were prolonged to some degree depending on the quantity of tissue removed. Armstrong and Hansel (1959) were unable to cause corpora lutea regression in hysterectomized heifers with injections of oxytocin which produced corpora lutea regression in intact heifers.

Spies et al. (1960) reported the corpora lutea of 4 gilts hysterectomized 7 days after the beginning of estrus persisted to the time of slaughter, 31 to 119 days following the preoperative estrus. Injections of progesterone, administered from 10 days to 25 days following breeding in pregnant gilts or gilts hysterectomized on the seventh day of gestation, reduced the corpora lutea size and their progesterone concentration to about half that of similar untreated controls. Corpora lutea persisted to the time of slaughter, 24 and 30 days after the preoperative estrus, in 4 hysterectomized gilts with uterine transplants. Du Mesnil Du Buisson and Dauzier (1959) hysterectomized 8 nonpregnant sows on day 8 of the estrous cycle and the corpora lutea persisted 117 days following the preoperative estrus. Ten sows hysterectomized at 30 days of gestation had persisting corpora lutea followed by an intense ovulation about 200 days following the preoperative estrus. Du Mesnil Du Buisson (1961) reported conservation of the anterior 26 cm. of one horn prolonged the estrous cycle more than 25 days in 5 of 12 cases. When the length of conserved anterior horn was less than 26 cm. heat did not occur to the time of sacrifice, 40 and 50 days

postestrus. In these gilts the corpora lutea had regressed on the side with the conserved uterine segment and persisted in the opposite ovary. Perry (1961) also reported a long delay in recurrence of estrus in hysterectomized gilts. Anderson et al. (1961) found persisting corpora lutea at the time of slaughter, 119 to 121 days after the preoperative estrus, in 5 gilts completely hysterectomized on day 8 to 11 of the estrous cycle. Partial hysterectomy of 25 gilts, in which various portions of the uterus remained, prolonged the estrous cycle to some degree with a relationship between the quantity of tissue removed and the estrous cycle length. About one-fourth of one horn was found to be the minimum amount of tissue needed to cause the corpora lutea to regress. The progesterone concentration of corpora lutea from hysterectomized gilts, 25 to 50 days following the preoperative estrus, was found to be comparable to those of the luteal phase of the estrous cycle or to those of early pregnancy.

Hysterectomy of the monkey

Burford and Diddle (1936) completely hysterectomized 5 monkeys and found a normal cyclic pattern as determined by vaginal washings and changes in the sex skin. During their 121 to 217 day postoperative observations, laparotomies showed that ovulation, corpora lutea formation and corpora lutea regression occurred. They concluded hysterectomy in the monkey had no influence on the ovary and that there was no evidence of

the uterus contributing any endocrine influence. Van Wagenen
and Catchpole (1941) and Speert (1942) concluded hysterectomy
in the monkey had no effect on ovarian activity. Te Linde and
Wharton (1960) performed laparotomies and ovarian biopsies 2
months to 2 years following the hysterectomy of 6 monkeys.
Ovulation occurred and the size, function and histology of
ovaries and adrenal glands remained normal for at least 2 years.

Hysterectomy of the human

Abel (1899) reported complete removal of the uterus in
women resulted in complete atrophy of the ovaries and menopausal
symptoms similar to those occurring after ovariectomy. Leaving
a portion of the uterine mucous membrane allowed continued men-
struation and no menopausal symptoms. Doran (1905) agreed that
leaving the cervix during hysterectomy allows menstruation to
continue. Grammatikati (1889) stated that ovaries 6 months to
3 years after hysterectomy undergo degenerative changes with
atresia of the Graafian follicles. Holzback (1906) reported
ovaries of hysterectomized women do not atrophy, and that ovari-
an degeneration is probably due to damage to neural connections
during the operation. Hawks (1921) reported onset of meno-
pause was delayed when one ovary was left and further delayed
when both ovaries remained. Tamis (1934) reported that the
duration of ovarian function is not related to the quantity of
the ovary conserved, and that ovarian activity persisted in
women hysterectomized under 35 years of age. However, there

was some tendency toward premature onset of menopausal symptoms with both ovaries conserved.

Kretzschmar and Gardiner (1935) reviewed 2042 cases of hysterectomy and concluded that the greater delay in onset of hot flashes following subtotal hysterectomy than after complete hysterectomy would suggest an endocrine function of the uterus. Sessums and Murphy (1932) studied 143 cases and concluded that conservation of ovaries in hysterectomized women reduced the severity and delayed the onset of menopause. Te Linde and Darner (1928) also recommended conserving ovaries where possible. Vineberg (1915) reported cases of ovarian degeneration following hysterectomy, although it was not known if nerve or blood supply had been damaged. Graves (1917) states that vasomotor disturbances result as often following hysterectomy with retained ovaries as when the ovaries are removed or transplanted. He further states that after hysterectomy the ovaries are of little or no importance and may even produce trouble due to formation of adhesions or cysts. Sache (1959) reported a case of complete hysterectomy and ovariectomy in which 2 weeks after the operation lactation started with abundant secretion 10 or more days later. Jones and Te Linde (1941) injected 40 to 60 mg. of progesterone per day for 3 days into normal, hysterectomized and hysterectomized-ovariectomized women. Pregnanediol was recovered in all cases in about equal quantities, indicating the uterus is not necessary for conversion of progesterone to preganediol in women.

MATERIALS AND METHODS

Hysterectomy

Fifty-six virgin sexually mature guinea pigs (7 groups of 8 each) were totally or partially hysterectomized. Total hysterectomy (Group I) consisted of the complete removal of the uterus from os uteri to the tubo-uterine junction. In Groups II through VII partial hysterectomies were performed with the following portions of the uterus left in situ: Group II, cervix; Group III, anterior one-fourth of one horn; Group IV, uterus posterior of the external bifurcation; Group V, uterus posterior of the external bifurcation plus the posterior one-fourth of one horn; Group VI, anterior one-half of one horn; and Group VII, one horn.

The animals were maintained on a commercial guinea pig ration (Purina Guinea Pig Chow) supplemented with lettuce. All animals had completed one or more normal estrous cycles (range 15 to 18 days) before the operation. The occurrence of estrus was determined by the vaginal smear technique described by Stockard and Papanicolaou (1917). Smears were taken twice daily when the vaginal closure membrane was open. As a control procedure, sham operations were performed on 4 guinea pigs. They were subjected to the same operation as the experimental animals except for the actual removal of the uterus.

Operations were performed during the early luteal phase of the estrous cycle (day 5 or 6). The animals were anesthe-

tized with an intraperitoneal injection of 30 mg. pentobarbital sodium (Abbott Laboratories) per kg. of body weight. The uterus was removed through a mid-ventral incision about one inch in length. Only the vaginal or cervical stub was ligated as the bleeding could be controlled by clamping off the uterine blood supply with hemostats. Care was taken not to damage the blood supply to the ovaries or remaining uterine segment. The wound was closed with a continuous suture of 00 catgut in the muscle layers and broken sutures in the skin. A small amount of p-aminobenzenesulfonamide was applied to the incision just prior to suturing the skin.

Following the operation the guinea pigs were placed with males and vaginal smears taken twice daily when the vaginal closure membrane was open. Either the presence of sperm or a normal estrous smear was taken as an indication of estrus. The animals were sacrificed the day following their third postoperative estrus or 130 days after their preoperative estrus if a third estrus had not yet occurred. At the time of sacrifice, data were obtained on the size and weight of the ovaries and remaining portions of the uterus, as well as any gross structures or abnormalities of the ovaries and uterine segments.

The ovaries and remaining portions of the uterus were fixed in Bouin's solution and stored in 70 percent ethanol. Paraffin sections of the ovaries were cut serially at 7 micra and stained with hematoxylin and eosin. Samples of remaining

uterine tissue were also cut at 7 micra and stained with hema-
toxylin and eosin. Histological comparisons of these tissues
were made with tissues taken during various stages of the
estrous cycle and pregnancy.

Chemical Destruction of the Endometrium

In a group of 16 guinea pigs an irritant or corrosive was
injected into the lumen of the uterus to study the effect of
endometrial destruction on the estrous cycle length. The cervix
was removed from 3 animals, and the uterine horns injected with
either Bouin's fixative, 10 percent phenol or saturated picric
acid. After 10 minutes the horns were flushed with physiologi-
cal saline. In the other 13 animals the following substances
were injected into the lumen of the intact uterus: 4 animals,
0.6 cc of 10 percent phenol; 4 animals, 0.6 cc of Bouin's fixative
(diluted 2:1 with distilled water); 4 animals, 0.6 cc of 10 per-
cent silver nitrate; and 1 animal, 1 cc of 10 percent silver ni-
trate. Animals were sacrificed following their third estrus or
after 50 days if estrus had not occurred. One animal from each
of the groups treated with 0.6 cc of phenol or Bouin's fixative
was sacrificed after 3 days to determine the degree of endometrial
destruction. Uteri from all animals were studied histologically
to determine the amount of remaining endometrium.

Uterine Transplantation

In order to determine if the effect of hysterectomy on the
life span of corpora lutea is mediated by neural or chemical

means, autotransplants of the entire uterus or endometrium were made in 45 animals. In the case of 24 animals, the autotransplants were made as follows: cross-sectional slices (2 mm.) were placed in the abdominal cavity (1 animal), under the neck skin (3 animals) or abdominal skin (1 animal); whole uterus was placed in the abdominal cavity (2 animals) or under the abdominal oblique muscles (8 animals); endometrial scrapings were placed under the skin over the ribs (4 animals); and finely chopped uterus was placed under the rib skin (4 animals) or the kidney capsule (1 animal).

The most successful autotransplants were achieved in a two-stage operation on 21 animals. The first stage consisted of separating the anterior portions of the uterine horns from the broad ligament as well as the oviducts and placing them under the abdominal oblique muscles. The posterior ends of the horns remained attached to the cervix and posterior broad ligament. In the second stage of the operation the posterior portion of the uterus was transplanted under the abdominal oblique muscles without separation from the transplanted anterior horns. This operation was carried out after the animal had recovered from the first stage of the transplantation. In 13 of these 21 guinea pigs the puberal estrus occurred during the time between the 2 operations. The other 8 guinea pigs consisted of 4 sexually mature and 4 prepuberal animals. All experimental animals were kept with intact males following the operation. Vaginal

smears were taken and estrus determined as previously described. Puberal estrus was used in evaluating cycle length when transplantations were completed before puberty. Tissue for histological evaluation of the transplants was placed in Bouin's fixative and stored in 70 percent ethanol.

Unilateral Ovariectomy and Partial Hysterectomy

In order to study the effect of the ovaries on the estrous cycle length following partial hysterectomy, another series of operations was performed. The following tissues were removed in three groups of animals: Group A (8 animals), left ovary; Group B (16 animals), left ovary, left uterine horn and anterior three-fourths of the right uterine horn; and Group C (16 animals), left uterine horn and anterior three-fourths of the right uterine horn. These operations were performed on day 5 of the estrous cycle through a lateral incision. The number of corpora lutea was noted in the removed ovary at the time of the operation. These animals were sacrificed 2 days after their first postoperative estrus. The number of corpora lutea present at the time of operation in the remaining ovaries was determined by a count of the failing corpora lutea in histological preparation of these ovaries taken at the time of sacrifice.

Miscellaneous Observations on the Effects of Reproductive Hormones

A group of 33 sexually mature female guinea pigs was injected subcutaneously with oxytocin or various gonadotrophic

hormones to study their effects on the estrous cycle of the intact animal. Six animals were injected with 5, 10 or 40 international units of oxytocin (Armour's Purified Oxytocic Principle, Lot No. T15503) from day 1, 3 or 16 through day 5 to 7 of the estrous cycle. The gonadotrophin injections were started on either the day of estrus or the ninth day of the estrous cycle and continued through the twentieth postestrous day or next estrus, depending on which occurred first. The gonadotrophins used and the amounts injected daily were: ovine luteinizing hormone (Armour's Pituitary Gonadotropin, Lot No. R377279), 1 to 8 mg.; ovine prolactin (Armour's AVL-0210, Lot No. R041155), 25 to 200 international units; and human chorionic gonadotrophin (Squibb's Follutein, Control: 9A43562), 25 or 50 international units. The animals were kept with intact males and estrus determined as previously described. Animals were sacrificed 1 to 5 days following their first estrus after started on treatment. In addition, 1 animal received 8 mg. of luteinizing hormone, one 16 mg. of luteinizing hormone and another 75 international units of chorionic gonadotrophin on day 12 of the estrous cycle and was sacrificed the following day. Histological studies were made of the ovaries and uterine tissue taken at the time of sacrifice.
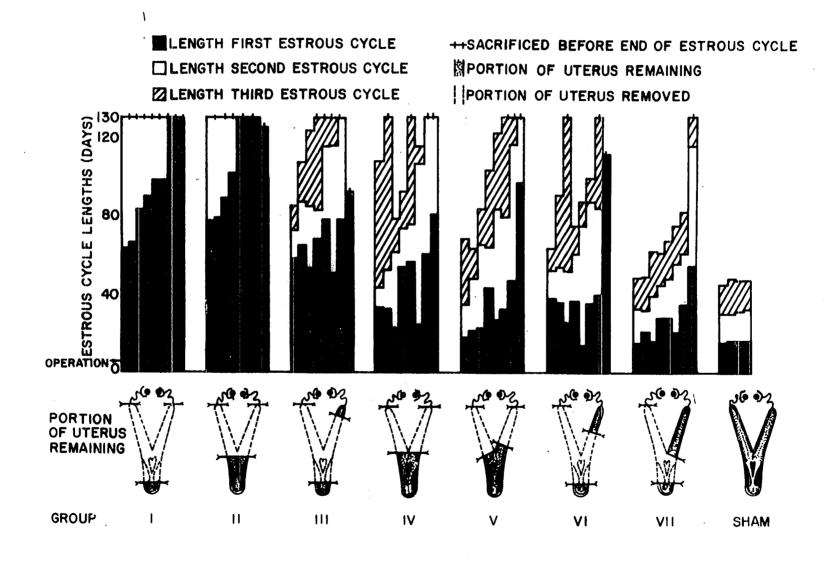
RESULTS AND DISCUSSION

Hysterectomy

Figure 1 presents the estrous cycle lengths of the 7 groups of hysterectomized animals and of the 4 animals on which sham operations were performed. The average length of estrous cycle, the standard error and number of animals cycling for each group are shown in Table 1. These data indicate that the quantity of uterine tissue removed had a direct relationship to the length of time the corpora lutea persisted. The preserved cervical tissue appeared to have no effect on the life span of the corpora lutea, while a smaller or equal quantity of uterine horn (one-fourth or one-half horn) caused a marked shortening of the time to postoperative estrus over that of completely hysterectomized animals. Although the second and third estrous cycles remained quite variable in length, there was a trend toward a shortening of these cycle lengths over that of the first postoperative cycle. This shortening of the second and third estrous cycle lengths might be due to a hypertrophy or an increased functional activity of the remaining portion of the uterus.

One or more animals in each group of subtotally hysterectomized guinea pigs failed to cycle comparable to the others in the group. Some of these animals ran a first or a first and second cycle comparable to the group and then stopped

Figure 1. Effect of quantity of uterine tissue on estrous cycle lengths in the guinea pig

LENGTH FIRST ESTROUS CYCLE ++SACRIFICED BEFORE END OF ESTROUS CYCLE
LENGTH SECOND ESTROUS CYCLE PORTION OF UTERUS REMAINING
LENGTH THIRD ESTROUS CYCLE PORTION OF UTERUS REMOVED

ESTROUS CYCLE LENGTHS (DAYS)

OPERATION

PORTION OF UTERUS REMAINING

GROUP   I   II   III   IV   V   VI   VII   SHAM

Table 1. Estrous cycle lengths following hysterectomy in the guinea pig

Group	No. of animals	Lengths of estrous cycles		
		1st	2nd	3rd
I	8	83 ± 6[a] (6)[b]	---	---
II	8	87 ± 6 (4)	---	---
III	8	64 ± 4 (7)	31 ± 8 (6)	23 ± 8 (3)
IV	8	45 ± 7 (8)	32 ± 11 (6)	27 ± 12 (4)
V	8	39 ± 9 (8)	39 ± 7 (7)	29 ± 5 (5)
VI	8	32 ± 3 (7)	35 ± 7 (7)	17 ± 5 (5)
VII	8	27 ± 4 (8)	26 ± 6 (8)	19 ± 1 (7)
Sham	4	16 ± 0 (4)	15 ± 0 (4)	16 ± 1 (4)

[a]Mean $\pm$ standard error.

[b]Numbers in parenthesis indicate number of animals cycling.

cycling, while others behaved more like completely hysterectomized animals. This may have been due to varying degrees of necrosis at different times following the operation, to differences in functional activity of uterine tissue or to differences in threshold response to uterine tissue.

Table 2 shows the level of significance between group means of the first estrous cycle following hysterectomy, as determined by the t-test. Complete hysterectomy was not significantly different from leaving only the cervix. However, cycle lengths of both completely hysterectomized animals and animals with only the cervix remaining were significantly longer than those of all other groups.

Guinea pigs in which the entire uterus was removed or in which only the cervix remained did not exhibit estrus before 63 days after the preoperative estrus. Six of these 16 animals had not been in estrus at the time of sacrifice, 124 to 130 days after their preoperative estrus. The inhibition of estrus was accompanied by the maintenance of the corpora lutea for a period of time equal to or longer than the length of gestation (58 to 72 days, Blandau and Young, 1939). Although the corpora lutea were not marked for later identification, the absence of corpora albicantia and the thickness of the blood vessel walls in the corpora lutea of animals in Groups I and II indicated that these corpora lutea were not from recent ovulations.

The inhibition of estrus and the persistence of the

Table 2. Level of significance between group means of first estrous cycle length following hysterectomy as determined by t-test

Group	II	III	IV	V	VI	VII	Sham
I	<.400	<.025	<.001	<.001	<.001	<.001	<.001
II		<.005	<.001	<.001	<.001	<.001	<.001
III			<.025	<.025	<.001	<.001	<.001
IV				>.500	<.200	=.050	<.025
V					<.500	<.400	<.100
VI						<.500	<.010
VII							<.200

corpora lutea are in agreement with the work of Loeb (1923a),
who reported that the corpora lutea may be preserved 60 to
perhaps as long as 80 days after preoperative estrus in com-
pletely or almost completely hysterectomized guinea pigs.
Loeb (1927) reported estrus usually does not occur within 3
months following complete hysterectomy of guinea pigs, and
that removing only the cervix and about one-fifth to one-sixth
of the two uterine horns results in the occurrence of estrus
21 to 30 days after the preoperative estrus. Rowlands (1961b)
reported that vaginal opening did not occur for 8 months in 4
of 5 guinea pigs hysterectomized on the fifth day of the lu-
teal phase. If hysterectomy was performed on day 10 of the
cycle, the corpora lutea persisted for about the length of
pregnancy; while following hysterectomy at day 15 of the cycle,
he found only a 2.5 day extension of the estrous cycle. Row-
lands (1961a) states that the corpora lutea of the guinea pig
regress at an extremely slow rate following hysterectomy, with
an almost permanent inhibition of the estrous cycle and ovula-
tion.

Due to the common occurrence of ovarian rete cysts in
both operated and control animals and to the difference in
time from last estrus to sacrifice, a direct and valid compari-
son of the effect of hysterectomy on ovarian weight could not
be made. However, ovarian weights following complete hys-
terectomy appeared to compare closely with those of pregnancy.

The occurrence or size of rete cysts showed no relation to estrous cycle lengths. This is in agreement with the finding of Young et al. (1938), that there is no relation between the presence of rete cysts and the occurrence of estrus.

An estimate of the amount of follicular development (based on histological observation), the diameter of the largest follicle and an estimate of the percent of follicles undergoing atresia are given in Table 3 for control and completely hysterectomized animals. These data indicate a similar follicular structure in ovaries of completely hysterectomized animals and the ovaries from guinea pigs near the middle of gestation.

Data representing the product of the 3 diameters of corpora lutea, in mm^3, are presented in Table 4, as an expression of the size of these organs. These values are in agreement with those reported by Rowlands (1956) for cycling and pregnant guinea pigs. These data show that the corpora lutea following complete hysterectomy attain a greater size than during the normal estrous cycle. Rowlands and Short (1959) reported the weights of the corpora lutea in hysterectomized guinea pigs 62 to 64 days following the preoperative estrus to be equal to those from 21 to 64 days of gestation. They also reported progesterone content of these corpora lutea to be greater than during pregnancy. Table 4 also presents the estimated corpora lutea activity (based on histological appearance) and the luteal and total cell counts per mm^2 (based on 12 counts per

Table 3. Follicular development in cycling, pregnant and hysterectomized guinea pigs

	No. of animals	Estimated follicular development	Average diameter of largest follicle	Estimated follicular degeneration
			μ	%
Cycling				
Day 1	3	1[a]	317	90
Day 5	3	2	540	30
Day 10	3	3	893	30
Day 14	3	4	1040	80
Pregnant				
Day 16	2	5	995	45
Day 25	2	5	912	35
Day 40	2	4	1050	40
Day 55	2	2	1200	50
Complete hysterectomy	8	5	1034	42

[a]Rated histologically on increasing follicular development from 1 to a maximum of 5.

Table 4. Comparisons of luteal development in cycling, pregnant and hysterectomized guinea pigs

	No. of animals	Product of 3 diameters of corpora lutea	Estimate of corpora lutea activity	Luteal cells/ mm^2	Total cells/ mm^2
		mm^3		$x10^3$	$x10^3$
Cycling					
Day 1	3	1.7	5[a]	2.8	5.4
Day 5	3	2.6	5	1.7	6.2
Day 10	3	3.2	5	1.4	9.2
Day 14	3	1.4	1	0.8	10.1
Pregnant					
Day 16	2	5.6	5	1.3	7.6
Day 25	2	5.5	5	1.3	6.3
Day 40	2	6.3	5	1.0	5.3
Day 55	2	5.1	3	0.9	4.3
Complete hysterectomy	8	3.5 (1.1 to 5.2)	4 (2 to 5)	1.2 (0.5 to 1.7)	7.2 (5.9 to 8.5)

[a]Rated histologically on increasing luteal development from 1 to a maximum of 5.

animal in a microscopic field of 0.00774 mm^2) in sections cut

at 7 micra. Using both these cell counts the corpora lutea

following hysterectomy compare more closely with those of preg-

nancy than with those of the estrous cycle.

When an anterior portion of a uterine horn was left in

situ, the segment of horn became filled with fluid and greatly

distended. The weight of this segment and its fluid content

averaged about 100 times (range about 5 to 1300) the weight of

tissue which had been left at the time of operation. Even after

removing the fluid, the remaining tissue averaged about 30

times (range about 3 to 340) the original weight. This fluid

accumulation did not appear to influence cycling, as there was

no apparent relation between the amount of fluid accumulation

and the estrous cycle length. Loeb (1927) also noted this fluid

collection in the remaining anterior horns. Uterine segments

connected to the cervix and vagina did not accumulate fluid.

The muscularis layer of the uterine segment containing

fluid was thickened and the endometrium was thinner than normal.

The endometrium was edematous and the cytoplasm of the endo-

metrial cells stained lighter than in normal cycling animals.

The luminal epithelium was lower in the uterine horns which were

distended with fluid than in the nondistended posterior segments.

This lower epithelium was probably due to the expansion of the

lumen of the uterus by the fluid collection. In animals in

which either an anterior half or quarter horn had remained,

uterine glands were usually absent and the endometrium was very thin. Animals in which a whole horn remained did not show as marked a histological change in this remaining uterine segment. The endometrium was thicker and uterine glands were present in all cases. However, there was no apparent relation between the presence of uterine glands and the length of estrous cycle. Uterine sections from animals in which the posterior portion of the uterus remained appeared normal histologically. The endometrium remained thick and the uterine glands were abundant. Epithelial cells of both the lumina and glands were of a height comparable to those present in normal cycling animals.

Sperm were present in only 41 of 82 cases of estrus in which the hysterectomized animals were in the presence of a male. There was no apparent relationship between the occurrence of mating and the length of the preceeding estrous cycle. Furthermore, no relationship was observed between male acceptance and the location of the remaining uterine segment. This low incidence of male acceptance may be due to an imbalance of estrogen and progesterone. Boling et al. (1938) reported a low incidence (10.1%) of heat following injection of 200 international units of theelin in spayed guinea pigs. When the theelin was followed by 0.2 international units of progesterone, 80 percent of these same animals showed heat. Vaginal smears were not as distinct in most cases following hysterectomy as in normal cycling animals. In some cases the cornification was

not complete, and several smears were never completely free of leucocytes. This, also, may indicate an imbalance of ovarian hormones. Ford and Young (1951) reported a synergistic action of estrogen and progesterone in producing vaginal cornification in ovariectomized guinea pigs.

Chemical Destruction of the Endometrium

Table 5 shows the effect of chemical destruction of the endometrium on the estrous cycle length. Eight of the 10 animals had the life span of the corpora lutea extended by these treatments. The time from the estrus prior to treatment until the next estrus occurred ranged from 16 to 53 days (average 30 days), with 2 animals failing to exhibit estrus by the time of sacrifice (50 days after the preoperative estrus). Histological study of the uterus following sacrifice indicated that those animals with the greatest endometrial destruction had the greatest prolongation of the life span of the corpora lutea. Two animals sacrificed 3 days following treatment had necrosis of about three-fourths of the endometrium and some smaller areas of the muscularis layer. Three animals had normal second and third estrous cycles following a prolonged first cycle. This was probably due to a regeneration of endometrium, as near normal amounts of endometrium were present at the time of sacrifice. These data indicate the endometrium may be the tissue responsible for the increased life span of the corpora lutea since the destruction was mostly limited to the endometrium.

Table 5. Effect of chemical destruction of the endometrium on estrous cycle length

	Estrous cycle length			Amount of endometrium remaining
	1st	2nd	3rd	
10 minute treatment				
Bouin's fixative	53	-[a]	-	little
Saturated picric acid	16	11	12	moderate
10% phenol	17	16	15	near normal
Injected into lumen				
.6cc 33% Bouin's fixative	-[a]	-	-	little
.6cc 10% phenol	35	16	14	moderate
" " "	27	15	18	near normal
" " "	-[a]	-	-	little
.6cc 10% silver nitrate	26	-[a]	-	moderate
" " " "	38	sacrificed		moderate
1cc " " "	31	15	10[b]	moderate

[a]Sacrificed 50 days following the prior estrus.

[b]Had not ovulated.

Uterine Transplantation

In the 45 guinea pigs, in which autotransplants of the uterus were made, transplanted tissue was recovered from 42 animals at the time of sacrifice. On histological examination 24 of these transplants consisted of myometrium, dense connective tissue, necrotic tissue and little or no endometrium. Four of these animals exhibited estrus following uterine autotransplantation. The first estrus occurred in these animals 50, 51, 69 and 72 days following the preoperative or puberal estrus. In 18 animals the transplant showed more than a trace of normal appearing endometrium. Two of these had not exhibited estrus at the time of sacrifice 75 and 80 days after puberal estrus. The amount of functional endometrium in these animals may have been below the threshold amount necessary for cycling. Four animals had cycle lengths of 45, 53, 57 and 65 days. Table 6 summarizes data on the occurrence of estrus in the remaining 12 animals. These animals had first postoperative estrous cycle lengths ranging from 15 to 32 days (average 22 days). Estrus, as evaluated by the opening of the vagina and the characteristic smear, was observed in 25 instances. However, male acceptance, as indicated by the presence of sperm in these smears, was noted only 11 times. In 5 of these animals estrous cycles ceased after the first or second estrus. Reduced endometrial function in the transplants may have been the cause of cessation of

Table 6. Estrous cycle lengths following uterine autotransplants in guinea pigs[a]

Animal no.	Cycle lengths			Days from last estrus to sacrifice
	1st	2nd	3rd	
149[b]	24	12	46[c]	1
103[b]	16	—	—	60
123[b]	31	48	—	49
216[b]	22	20	20	2
133	15	17	20	1
135	21	28	19	2
137	32	—	—	59
138	24	—	—	60
187	20	Sacrificed		5
256	19	13	Sacrificed	2
264	22	18	22	2
273	17	17	—	50

[a]Endometrial autotransplant in animal No. 149. Complete uterine autotransplant in all other animals.

[b]Mature guinea pigs (all others immature at time of transplantation).

[c]Underlined indicates male acceptance.

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estrous cycles in these animals. In animal number 273, sacrificed 50 days after the second postoperative estrus, ovulation had occurred the day prior to sacrifice without opening of the vaginal membrane. This animal had also shown libido the day previous to sacrifice.

In 6 sexually mature females, totally hysterectomized at day 5 or 6 of the cycle, the time interval from preoperative to postoperative estrus ranged from 63 to 98 days (average 83 days). Estrus had not occurred in 2 totally hysterectomized animals at the time of sacrifice (130 days). In 2 guinea pigs hysterectomized at 4 or 5 days of age, estrus had not occurred at the time of sacrifice, 75 days following their puberal estrus.

These data show a marked shortening of the life span of the corpora lutea in animals with a uterine transplant containing endometrium, over those that were totally hysterectomized or that had little or no surviving endometrium in the transplant. These findings suggest a functional relationship between the endometrium and the life span of the corpora lutea. The endometrium may produce a luteolytic substance which causes involution of the corpora lutea in the normal cycle, or it may metabolize a substance necessary for the maintenance of the corpora lutea and thereby cause regression.

Results of other workers also indicate a chemical relationship between the endometrium and the life span of the corpora lutea. Bradbury et al. (1950) injecting endometrial

suspensions and Hechter et al. (1940) using intraperitoneal implants of the uterus have reported shortening of the life span of the corpora lutea in hysterectomized-pseudopregnant rats. Chu et al. (1946) found the life span of the corpora lutea of hysterectomized-pseudopregnant rabbits was shortened following intraperitoneal implants of the uterus. Duncan et al. (1961) observed in pigs that endometrial filtrates from day 12 and 13 of the estrous cycle increased in vitro progesterone synthesis by luteal tissue, whereas filtrates from days 16 and 18 showed definite inhibitory effect on hormone production. It is of interest that Loeb (1927) using guinea pigs and Spies et al. (1960) using pigs found no effect of uterine transplants on the occurrence of postoperative estrus in these animals.

Unilateral Ovariectomy and Partial Hysterectomy

Table 7 shows the average and range of estrous cycle lengths of guinea pigs following unilateral ovariectomy, unilateral ovariectomy at the time of partial hysterectomy and partial hysterectomy alone. Partial hysterectomy consisted of the removal of the left uterine horn and the anterior three-fourths of the right uterine horn. Estrous cycle lengths following removal of only the left ovary averaged 14.9 days (range 13 to 16 days) as compared to an average of 15.5 days (range 14 to 17 days) for the preoperative cycles. This dif-

Table 7. Influence of unilateral ovariectomy on life span of corpora lutea of partially hysterectomized guinea pigs

Group	Operation	Number of animals	Average estrous cycle length[a] (days)	Range in cycle length (days)
A	Unilateral ovariectomy	8	14.9	13 to 16
B	Unilateral ovariectomy	15	18.1	15 to 23
	and partial hysterectomy	1	$<40^b$	—
C	Partial hysterectomy	13	22.2	17 to 36
		1	$>24^c$	—
		2	$>40^d$	—

[a]Significance of t-test: Group A vs Group B, P < .005; Group A vs Group C, P < .05; and Group B vs Group C, P = .01 (4 non cycling animals excluded).

[b]Ovulated without opening of vaginal membrane.

[c]Killed before postoperative estrus.

[d]Degenerated endometrium.

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ference was not statistically significant at the 5 percent level. The removal of the left ovary at the time of partial hysterectomy shortened the time to first postoperative estrus from an average of 22.2 days (range 17 to 36 days) following partial hysterectomy alone to an average of 18.1 days (range 15 to 23 days). This difference was statistically significant at the 1 percent level, but data from 4 animals were excluded. Histological examination of the remaining ovaries and uterine tissue of these 4 animals excluded from the analysis showed deviations from others in the groups. One animal in group B was sacrificed 40 days following the preoperative estrus without exhibiting a subsequent estrus. Histological study of the remaining ovary showed this animal had ovulated without opening of the vaginal membrane. One animal in group C had only a partially cornified smear 22 days after the preoperative estrus. When sacrificed 2 days later, ovulation had not occurred and histologically the ovaries were like those from animals about the twelfth day of the normal estrous cycle. Two other animals of group C were sacrificed 40 days after the preoperative estrus without showing a subsequent estrus. Histological study revealed degeneration of much of the endometrium of these 2 guinea pigs. One of these animals also had a large active deciduoma at the point where the uterus had been severed. All other animals had only traces of active tissue in otherwise necrotic deciduomata.

Histological studies of the ovaries removed at the time of sacrifice, 2 days following the first postoperative estrus, showed that the number of corpora lutea remaining at the time of the operation varied from 1 to 3 in groups A and B and 2 to 5 in group C. No relationship was noted between the time to postoperative estrus and the number of remaining corpora lutea, weight of remaining uterine tissue or the presence or weight of degenerating deciduomata.

Nelson (1935) reported ovariectomy increased the pituitary gonadotrophins of guinea pigs, while estrogen injections decreased gonadotrophin content. Heckel (1942) was able to double the life span of the corpora lutea in the pseudopregnant rabbit by the injection of estrogen. Greep (1941) prevented the decrease in size of corpora lutea, which occurs in rabbits hysterectomized during the second half of pregnancy, by estrogen injection regardless of whether or not the pituitary was removed. Pincus (1937) reported that estriol was found in the urine of normal rabbits following injection of estrone, but was not found in the urine of injected hysterectomized rabbits. However, Fish and Dorfman (1942) were unable to isolate estriol from the urine of normal rabbits or the urine of ovariectomized-hysterectomized rabbits injected with alpha-estradiol.

In the completely hysterectomized animal there might be
an accumulation of estrogenic compounds or metabolites of
estrogens which act directly or through the hypothalamus or
pituitary to maintain the corpora lutea. According to this
theory animals with larger portions of the uterus remaining
following partial hysterectomy would be able to metabolize
estrogenic compounds at a greater rate. This could account
for the inverse relationship found between the quantity of
uterus remaining and the time to first postoperative estrus.
However, estrogen might be inhibiting the production of a
substance by the uterus which causes corpora lutea regression
in the normal cycling animal. This would also be in agreement
with the inverse relationship between the life span of the
corpora lutea and the quantity of uterus remaining in partially
hysterectomized animals. Therefore, the uterus may be utiliz-
ing a substance necessary for maintenance of the corpora lutea
or producing a substance responsible for corpora lutea re-
gression. This substance could act directly on the corpora
lutea or through the hypothalamus or pituitary.

Miscellaneous Observations on the Effects
of Reproductive Hormones

Six guinea pigs were injected subcutaneously with 5, 10
or 40 international units of oxytocin daily from day 1, 3 or
16 of the estrous cycle through day 5, 6 or 7 of the cycle.
All 6 animals had normal estrous cycle lengths and ovulations.

Seven animals were injected daily with 25, 50, 100 or 200 international units of prolactin from the day of estrus or day 9 of the estrous cycle to the time of next estrus. These animals also exhibited normal estrous cycle lengths and ovulations.

Table 8 presents the effects of luteinizing hormone on the estrous cycle length and ovulation. Estrous cycle lengths, as determined by opening of the vaginal membrane and positive vaginal smears, were increased with all but the 1 mg. daily dose. However, ovulation occurred in only 5 of 12 cases. The only animal in which sperm was found in the smear did not ovulate. Histological study of the ovaries showed luteinization in all ovaries, but the corpora lutea which were present at the start of injections had regressed. One animal on the 1 mg. dose had 7 new ovulations and 8 luteinized follicles, while the other had 4 luteinized and many partially luteinized follicles. All other animals had extensive luteinization. This varied from luteinization of a large number of follicles to what appeared to be luteinization of almost all of the ovary. The ovarian weights of these animals were 2 to 4 times those of untreated animals and appeared to be due to the luteinization. In some cases only a few small follicles and a small amount of connective tissue were not luteinized. Some blood-filled follicles were present in most ovaries. Also, some cystic uterine glands were found in most animals. Two animals

Table 8. Effect of subcutaneous injections of luteinizing hormone in the intact guinea pig

| Daily dose (mg.) | Day of estrous cycle | | Estrous cycle length | No. of ovulations |
	First injection	Last injection		
2	Estrus	16	16	0
2	Estrus	20	33	4
8	Estrus	20	24[a]	0
8	Estrus	20	25	0
1	9	14	14	7
1	9	14	14	0
2	9	19	17	0
2	9	20	41	3
4	9	20	21	0
4	9	20	18	0
8	9	20	41	6
8	9	20	24	many

[a] Only animal showing male acceptance.

were injected with either 8 or 16 mg. of luteinizing hormone on the twelfth day of the estrous cycle and sacrificed the following day. Histological study showed no effect of the 8 mg. injection. However, the 16 mg. injection induced 2 ovulations with active appearing corpora lutea still present.

Table 9 shows the effect of chorionic gonadotrophin on the intact guinea pig. These results are quite similar to the luteinizing hormone results, both from estrous cycle length, ovulation, size of ovaries, and histological examination. Both blood-filled follicles and cystic uterine glands were present in most animals. Animals on chorionic gonadotrophin had a larger number of normal appearing follicles in the ovaries than those on luteinizing hormone. Also animals receiving chorionic gonadotrophin had a greatly enlarged cervix. One animal received a single injection of 75 international units of chorionic gonadotrophin on day 12 of the estrous cycle and was sacrificed the following day. The ovaries of this animal contained a large number of luteinized follicles. These appeared very similar to corpora lutea one day following ovulation except they contained ova.

Table 9. Effect of subcutaneous injections of chorionic gonadotrophin in the intact guinea pig

Daily dose (I.U.)	Day of estrous cycle		Estrous cycle length	No. of ovulations
	First injection	Last injection		
25	Estrus	17	17	4
25	Estrus	16	16	0
50	Estrus	13	11	0
50	Estrus	18	18	4
25	9	20	29	4
25	9	20	22	0
50	9	20	26	0
50	9	20	19	0

SUMMARY

This investigation deals with the effect of the uterus on the life span of the corpora lutea in the guinea pig. In 7 groups of 8 animals per group the following portions of uterus were left in situ: Group I, none; Group II, cervix; Group III, anterior one-fourth of one horn; Group IV, uterus posterior to the external bifurcation; Group V, uterus posterior to the external bifurcation plus the posterior one-fourth of one horn; Group VI, anterior one-half of one horn; and Group VII, one horn. Sham operations were performed on 4 animals. These operations were performed at day 5 or 6 of the estrous cycle. In 6 of 8 completely hysterectomized animals the corpora lutea persisted for 63 to 98 days, whereas the other 2 animals had not shown estrus by the time of sacrifice at 130 days. The time to the first postoperative estrus following complete hysterectomy was not significantly different from that of leaving only the cervix, but both these groups were significantly different from all other groups. In the partially hysterectomized animals an inverse relationship was found between the quantity of uterus remaining and the life span of the corpora lutea. Sham operations had no effect on estrous cycle length. Development of the corpora lutea and follicles in completely hysterectomized animals, as determined by histological preparations, was similar to those found about the

middle of pregnancy. Chemical destruction of the endometrium gave effects similar to those of hysterectomy.

Transplanted tissue was recovered from 42 of 45 guinea pigs in which autotransplants of the entire uterus or endometrium were made at various locations in the animals. Eighteen of these 42 transplants contained some normal appearing endometrium. Estrous cycle lengths of 15 to 32 days (average 22 days) were observed in 12 of the 18 animals with surviving endometrium in the transplants. In the other 6 animals in this group the amount of functional endometrium may have been below the threshold amount necessary for cycling. Animals with no surviving endometrium behaved similarly to those that were completely hysterectomized.

A study was made of the effect of unilateral ovariectomy on estrous cycle length of partially hysterectomized guinea pigs. The following tissues were removed at day 5 or 6 of the estrous cycle: Group A (8 animals), left ovary; Group B (16 animals), left ovary, left uterine horn and anterior three-fourths of right uterine horn; and Group C (16 animals), left uterine horn and anterior three-fourths of right uterine horn. The removal of the left ovary at the time of partial hysterectomy shortened the time to first postoperative estrus from an average of 22.2 days following partial hysterectomy alone to an average of 18.1 days. This difference was significant at the 1 percent level. Removal of only the left ovary had no

effect on estrous cycle length.

Six intact guinea pigs were injected with 5, 10 or 40 international units of oxytocin daily from day 1, 3 or 16 of the estrous cycle through day 5, 6 or 7 of the cycle. Seven intact animals were injected daily with 25, 50, 100 or 200 international units of prolactin from estrus or day 9 of the estrous cycle to the time of next estrus. Neither oxytocinnor prolactin had any effect on estrous cycle length or ovulation. Twelve intact guinea pigs were injected daily with 1, 2, 4 or 8 mg. of luteinizing hormone and 8 with 25 or 50 international units of chorionic gonadotrophin. Injections were started at estrus or day 9 of the estrous cycle and continued to the next estrus or to 20 days after the estrus prior to treatment. Estrous cycles were prolonged with both hormones. However, estrus was not usually accompanied by ovulation. Both these hormones cause massive luteinization of the follicles, but did not maintain the corpora lutea which were present at the start of injections.

Results of these investigations indicate a functional relationship between the uterus and the life span of the corpora lutea. The data suggest the endometrium is the tissue involved in the regulation of the life span of the corpora lutea. Furthermore, this regulation appears to be independent of neural connections to the uterus. The data also indicate that the quantity of ovarian tissue plays some role in the control of

the life span of corpora lutea. The uterus may be utilizing a substance necessary for maintenance of the corpora lutea or producing a substance responsible for corpora lutea regression. This substance could act directly on the corpora lutea or indirectly by way of the hypothalamus and pituitary.

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