THE EFFECTS OF A BOVINE UTERINE POWDER ON THE CORPORA LUTEA OF PSEUDOPREGNANT RABBITS, RATS AND HYSTERECTOMIZED GUINEA PIGS

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INTRODUCTION

One of the central problems of reproductive physiology at the present time is the control of the secretory activity and life span of the corpus luteum. The nongravid uterus appears to be concerned in determining the life span of the corpus luteum by initiating or facilitating involution or regression of the luteal tissue. Much evidence exists to suggest the production of a uterine luteolysin; however, isolation of a specific luteolytic substance in the uterus has not been shown. It has been reported that removal of the uterus during the estrous cycle prolongs the duration of luteal function in guinea pigs (Butcher et al., 1962), swine (Anderson et al., 1961), sheep (Wiltbank and Casida, 1956) and cattle (Anderson et al., 1962). Hysterectomy also prolongs the life span of corpora lutea in pseudopregnant rats (Bradbury et al., 1950), rabbit (Asdell and Hammond, 1933) and hamsters (Caldwell et al., 1967).

The transplantation of uterine tissue into hysterectomized animals has provided positive evidence concerning the existence of a uterine luteolytic factor. Luteal regression and estrous cycles occurred in hysterectomized, pseudopregnant rabbits (Sessums and Murphy, 1933; Chu et al., 1946; Flerko and Bardos, 1961), rats (Hechter et al., 1940), guinea pigs (see Melampy and Anderson, 1968), swine (Anderson et al., 1963; du Mesnil du Buisson and Rombauts, 1963) and sheep (Rowson, Hay and Moor,

unpublished data; see Melampy and Anderson, 1968) with transplanted uterine tissue.

Uterine extracts have been utilized to demonstrate the existence of a uterine luteolytic substance in swine (Schomberg, 1967), rats (Bradbury et al., 1950), hamsters (Mazer and Wright, 1968) and cows (Williams et al., 1967).

As a result of the data reported by Williams et al., (1967), indicating the presence of an extractable luteolytic substance in the bovine uterus, the present study was designed to investigate the effects of acetone-dried powder preparations of early luteal bovine uterus on the morphology and the histology of corpora lutea of pseudopregnant rats, rabbits and hysterectomized guinea pigs, in an attempt to demonstrate and/or further isolate a luteolysin in the bovine uterus.

REVIEW OF LITERATURE

The primary function of the corpus luteum in unmated animals is the control of the length of the diestrus phase of the cycle. In general, the corpus luteum is functional for a period of time ranging from just a few days to about two weeks, depending upon the species (Melampy and Anderson, 1968). In many species of mammals the secretory life span of the corpus luteum is significantly prolonged during pregnancy and has an inherent life span approximately equivalent to the length of gestation (Moor, 1968).

It has long been known that the ovary exerts an influence upon the uterus (Loeb, 1908) and that the uterus exerts an influence upon the ovary (Loeb, 1923). In the past, experimental attempts relative to the control of ovarian and luteal functions were primarily in terms of the central nervous system, pituitary gonadotrophins and gonadal steroids (Melampy and Anderson, 1968). The failure to expect a protein factor to be involved was primarily due to the fact that the endometrium is embryologically of mesodermal origin and thus should secrete steroids. Also, prior to 1923, it tended to be a common belief that the nongravid uterus exerted no regulatory influence on ovarian activity.

HYSTERECTOMY

According to Melampy and Anderson (1968) the ovarian and luteal function following total hysterectomy has been investigated in at least 17 species. The mechanism by which hysterectomy prolongs the functional life of the corpus luteum in the

cow, pig, sheep and guinea pig is relatively unknown. It could act either by direct means on the ovary or by an indirect route through the hypothalamus and thus modify the function of the pituitary. Eckstein (1965) proposed several likely factors and routes involved:

a) A pituitary luteotrophin, a mixture of FSH and LH or some unknown factor.

b) A pituitary luteolysin

c) A uterine luteolysin which brings about the periodic destruction of the corpus luteum of the nonfertile cycle. This uterine factor may be carried by blood or lymph or it may diffuse through the tissue to its site of action. It may act not only locally but it may also be mediated by systemic pathways.

It was in the guinea pig that the luteotrophic effect of hysterectomy during the luteal phase was first demonstrated by Loeb (1923). If the hysterectomy was performed two to three days post-ovulation then ovulation had not yet occurred three months following the operation due to the presence of the corpus luteum. But if the hysterectomy was performed on immature guinea pigs, ovulation occurred as soon as the animal had recovered from the effects of the operation and follicles had reached maturity (Loeb, 1927).

Loeb and Kountz (1928) observed that the ovary, 40 to 46 days post-hysterectomy, closely resembled that of mid-pregnancy (corpora lutea large, vascular and well preserved) and that these

effects on the sexual system were produced, in a large measure, through their action on the corpora lutea. In another experiment, Loeb and Smith (1936) observed that in the hysterectomized guinea pig there was a constant development of large mature follicles, whereas during the cycle, mature follicles appeared only during the latter part of the cycle. Rowland and Short (1959) found that corpora lutea increase in size and weight following hysterectomy. As a result, they concluded that the stimulus which maintains luteal function in nonpregnant guinea pigs not only persists for a period of time as a result of hysterectomy but also becomes more intense. On Day 11 to 13 and Days 21 and 23 they observed a three-fold increase in progesterone content of the corpus luteum of pregnant guinea pigs, which they assumed coincided with the production of a luteotrophin. Throughout the pregnancy, corpus luteum progesterone content was maintained at a high level and was similar to the amount found in corpora lutea of hysterectomized guinea pigs.

Butcher et al. (1962) reported an inverse relationship between the quantity of uterus and the time to the first postoperative estrus in partially hysterectomized guinea pigs. They found that if the cervix was retained there was no affect on the luteal life span but if one-fourth to one-half of a uterine horn remained, there was a marked shortening of the

luteal life span when compared to completely hysterectomized guinea pigs. Corpora lutea were maintained in the hysterectomized controls for a period equal to or longer than the length of gestation. Also, if they injected an irritant or a corrosive into the lumen of the uterine horns the corpus luteum life was extended. The greatest prolongation was observed in animals with the most endometrial destruction. Therefore, it was suggested that there is a functional relationship between the endometrium and the life span of the corpus luteum. Furthermore, the uterus may be producing a substance responsible for luteal regression. In the same study, they observed that unilateral ovariectomy, at the time of the partial hysterectomy, shortened the corpus luteum life span to 18 days as compared to 22 days if partially hysterectomized only. Also, the estrous cycle was not affected by unilateral ovariectomy in animals with an intact uterus. As a conclusion, they felt there may be an uterine substance involved in regulation of the luteal life span. Mishell and Motyloff (1941) and Loeb (1923) had previously suggested the presence or at least the possible existence of such a factor.

Heap et al. (1965) on the basis of partial hysterectomy suggested that the uterus may somehow utilize estrogen. Fischer (1965) observed that unilateral hysterectomy plus ipsilateral ovariectomy resulted in normal 16 day cycles, whereas if ovariectomy was on the contralateral side, the cycle was 30 to 33 days in length. Furthermore, corpora lutea on the ovaries, on

the operated side of the uterus, were maintained but on the non-operated side the corpora lutea regressed as usual. He concluded that uterine inhibition is a direct effect on the corpus luteum mediated locally.

Bland and Donovan (1965, 1966) also investigated the local effect of the luteolytic action of the guinea pig uterus. One horn was distended with glass beads and the volume of the corpora lutea was used as an index of the rate and degree of regression. The corpora lutea on the distended side regressed while those on the ovary adjacent to the non-distended horn were maintained. It was concluded that in the guinea pig, one horn, if suitably stimulated by beads, can exert a local effect to induce regression of corpora lutea on that side but not in the contralateral ovary. Donovan and Traczyk (1962) found that beads in both horns, early in the cycle, shortened the cycle from 15 or 16 days to 12 days.

If the concept of a local luteolytic effect is accepted, then removal of a uterine horn should result in maintenance of corpora lutea on the corresponding side and normal regression on the opposite side. Bland and Donovan (1966) found that if one horn was removed on Day 3 or 4 and the ovaries were examined on Day 14, they observed the corpora lutea on the unoperated side regressed normally and on the operated side the corpora lutea were maintained and much larger.

Butcher et al. (1962) and Rowlands (1961) reported that the corpora lutea were maintained for a period of time equal to or

longer than gestation. Furthermore, due to the fact that they performed the hysterectomies at various times post-ovulation, it was reported that a critical stage appears to be attained in the luteal-uterine relationship prior to Day 16 of the estrous cycle and that if hysterectomy is done prior to Day 16 a functional corpus luteum will persist. Spies et al. (1964) stated that Day 15 of the cycle was a stage at which luteal regression is already well advanced.

Heap et al. (1967) reported that if hysterectomy or hypophysectomy is performed before Day 9 after ovulation, the luteolytic stimulus is eliminated and if removed after Day 10 the effect of the stimulus is greatly reduced. He reported that their observations could best be interpreted in terms of a positive luteolytic stimulus involving the pituitary and the uterus. Contrastingly, Moor (1968) reported that normal luteal function in the guinea pig is independent of hypophyseal control from a very early stage after ovulation.

Ovarian activity and estrous behavior remain unaltered after hysterectomy in the cycling rabbit (Loeb and Smith, 1936; Pincus, 1937), cycling rat (Perry and Rowlands, 1961; Long and Evans, 1922; Durrant, 1926; Loeb, 1927; Bradbury et al., 1950), opossum (Hartman, 1925) and rhesus monkey (Burford and Biddle, 1936). However, continued luteal maintenance and an absence of estrus are observed after uterine removal in the cycling ewe (Wiltbank and Casida, 1956; Kiracofe and Spies, 1963; Denamur and Mauleon, 1963; Rowson and Moor, 1964), cycling sow (Spies

et al., 1958, 1960; du Mesnil du Buisson and Dauzier, 1959; Anderson et al., 1961, 1963; du Mesnil du Buisson and Leglise, 1963), cycling cow (Wiltbank and Casida, 1956; Anderson and Neal, 1961; Anderson et al., 1962; Kaltenbach et al., 1964; Hansel and Seifart, 1968), pregnant and pseudopregnant rabbit (Loeb and Smith, 1936; Gillard, 1937; Greep, 1941; Chu et al., 1946; Coon, 1964; Hunter and Casida, unpublished data), pseudopregnant rat (Bradbury, 1937; Hechter et al., 1940; Perry and Rowlands, 1961; Melampy et al., 1964).

Bradbury (1937) suggested that because the uterus plays an important role in estrogen metabolism, hysterectomy may lead to an accumulation of nonmetabolized steroids or end-products that may influence luteotrophic release by the pituitary or it may act directly upon the corpus luteum. Silbiger and Rothschild (1963) suggested that the prolongation resulting from hysterectomy is mediated through the hypophsis. DeJongh and Wolthius (1964) concluded that the intact rat uterus may consume progesterone or possibly inactivate it by reduction, whereas in the formation of decidua or hysterectomy, progesterone is spared. Contrastingly, Heckel (1942) postulated an estrogen-sparing effect of hysterectomy in the rat.

AUTOGRAFTS

The autotransplanted uterus is as effective in shortening the luteal life span as the uterus in situ (Richardson, 1967; Barley et al., 1964). Butcher et al. (1962) and Anderson et al.

(1963) reported that autotransplants of the entire uterus in guinea pigs resulted in a marked shortening of the time to postoperative estrus over that of completely hysterectomized controls, 15.5 days and 21.5 days, respectively. Evidence that the uterus does affect lysis of the corpus luteum and is depenent upon the quantity of viable endometrium in pseudopregnant rabbits was reported by Chu et al. (1946) and Sessums and Murphy (1933). This utero-ovarian relationship was also reported in hysterectomized rats (Hechter et al., 1940; Bradbury et al., 1950; Gitsch, 1958; Flerko and Bardos, 1961; Barley et al., 1964; Anderson, 1968). Caldwell et al., (1967) reported that transplants of myometrium did not reduce the duration of pseudopregnancy in rabbits but homotransplants of uterine segments to the cheek pouch of the hysterectomized hamster did reduce the duration of pseudopregnancy.

Rowson et al. (unpublished data) reported that endometrial autografts placed between the flank muscles resulted in the termination of luteal function in 7 of 14 hysterectomized ewes. The length of the estrous cycles that occurred in the animals with successful endometrial grafts were all extended and ranged from 20 to 45 days.

Du Mesnil du Buisson and Rombauts (1963) observed that in the hysterectomized pig, continued estrous cycles resulted from uterine autografts containing viable endometrium. Furthermore, in 1966, they reported continued estrous cycles if the uterine horn transplants were made on the body wall. However, an absence

of cycles in sows following similar autografts to the small intestine. These data suggest that a uterine luteolytic factor (ULF) is carried directly by the portal circulation from the site of the autograft on the small intestine to the liver where it is inactivated.

Normal reproductive performance including conception, pregnancy, parturition and subsequent lactation has been reported in the ewe, rabbit and dog by Zhordania and Gotsiridze (1963) following autotransplantation of the female reproductive tract (uterus, oviducts, ovaries, mesosalpinges and mesovarium). Therefore, they suggested that direct afferent neural pathways from the uterus are not essential in luteolytic action in the unmated female or in the uterine function during pregnancy.

Anderson (1968) demonstrated that transplantation of rat uterine segments to hysterectomized females reversed the luteal prolongation brought about in the hysterectomized controls. Relying also on data obtained from parabiotic rats, Anderson concluded that the uterine luteolytic factor was blood borne and local control was secondary in importance.

NEURAL OR HORMONAL CONTROL

Uterine control of luteal regression may be mediated through the nervous system (Hill and Alpert, 1961) or it may be by way of a luteolytic hormone secreted by the endometrium which acts directly upon the corpus luteum (Williams *et al.*, 1967). Evidence suggesting both neural and hormonal utero-

ovarian relationships have been reported. Beads implanted in the guinea pig uterus, on Day 3 following estrus, shortened the cycle, while Day 8 implants lengthened it. Denervation of the implanted areas destroyed these effects (Moore, 1961).

Nalbandov and St. Clair (1958) suggested that the physical presence of an embryo within the uterus might act via nervous pathways and stimulate luteal maintenance through the central nervous system. Contrastingly, Rowson and Moor (1967) have shown that the physical presence of an embryo, which had been killed by heating, was ineffective in prolonging the cycle but homogenates did prolong it (22.4 days vs. normal 16 days); therefore, they concluded it is not a CNS involved prolongation due to the physical presence of an embryo in the sheep uterus. Apparently the embryo counteracts the luteolytic effect of the endometrium by continual secretion of an anti-luteolytic substance.

UTERINE DISTENSION

Evidence for the existence of a local uterine luteolytic activity in sheep comes from investigations of Moore and Nalbandov (1953) and Nalbandov et al. (1955). The cycle was shortened from 16 or 17 days to about 11 days by inserting a plastic bead in one horn on Day 3. The effect was abolished by denervating the segment around the bead. As a result, the duration of the corpora lutea reverted to the normal length, which suggested a neurohumoral arc, wherein the afferent

stimuli arising from the distended uterine fragment served as the neural component of the arc. Anderson (1962) observed that distension of the uterine horns with beads failed to modify the length of the estrous cycle in the sow.

Hansel and Wagner (1960) found alteration of bovine estrous cycles and luteal function following infusion of seminal and preputial fluids as well as seminal sediment. Also, they found that if they inserted a rubber catheter through the cervix and into the uterus during estrus or on Day 1, and filled a balloon on the end of the catheter with water, they were able to shorten the cycle length from 20.8 to 13.2 days. They concluded that they had only modified uterine function.

Yamauchi et al. (1967) observed that estrous cycle lengths of heifers were shortened when a rubber tube was inserted into the uterine horn during the first half of the cycle. Also, if they injected a viscous gel-like substance intrauterine at early luteal phase they were able to accelerate luteal regression and on the contrary, the treatment at late luteal phase delayed regression. Ovulation occurred 7 to 12 days after intrauterine injection of the gel-like material when administered in early, functional or late luteal phases of the cycle. From the results, they supposed that the inflammatory changes of the endometrium may be related to the life span of the corpus luteum.

Barley et al. (1966) utilized unilateral and bilateral hysterectomy and ovariectomy in several combinations to investigate local or systemic utero-ovarian relationships in pseudo-

pregnant rats. They observed that even though the proximity of the uterus and ovary was necessary for a pseudopregnancy of normal duration, an intact uterine horn contralateral to the retained ovary was able to appreciably affect that ovary. They concluded that the results occurred by way of the vascular system.

Investigations in the rat (Clemens et al., 1968), guinea pig (Oxenreider and Day, 1967) and sheep (Kiracofe et al., 1963) indicate that interruption of uterine venous drainage or a separation of the uterine and ovarian blood systems is sufficient to result in maintenance of luteal activity and thus indicating that the luteolytic factor is blood-borne.

INTRAUTERINE DEVICES

Experimental evidence has accumulated within recent years indicating that foreign objects placed in the uterine lumen affect the development and maintenance of corpora lutea. According to a review by Hawk (1968) intrauterine devices (IUD) have been reported to interrupt pregnancy in the rat (Marston and Chang, 1964), prolong pseudopregnancy (Ershoff and Deuel, 1943; Peckham and Greene, 1948; Velardo et al., 1953), suppress estrus in rats in which the products of conception were replaced with paraffin (Selye, 1933), interrupt pregnancy in rabbits (Marston and Chang, 1964; Brown and Foote, 1966) and shorten estrous cycles in the cow (Yamauchi and Nakahara, 1958; Hansel and Wagner, 1960; Chatterjee and Luktuke, 1961; Hawk et al., 1964;

Anderson et al., 1965; Ginther et al., 1966), ewe (Ginther et al., 1965), guinea pig (Ginther et al., 1966) and woman (Vorys et al., 1964). Furthermore, IUD's had no apparent effect in pseudopregnant mice (Kamell and Atkinson, 1948), cycling and pseudopregnant hamsters (Orsini, 1965; Kent and Atkins, 1959), cycling sows (Anderson, 1962; Gerrits and Hawk, 1966) or monkies (Kar et al., 1965).

Presumably the triggering of the IUD-activated luteolytic mechanism occurs in the uterus. Whether IUD's affect the pituitary and whether the altered pituitary function is involved in IUD effects on corpora lutea is not known.

EXOGENOUS HORMONES

Results of experimental work suggests a relationship between estrogen and luteal function in some species. Estrogen has been described as luteotrophic in rabbits (Keyes and Nalbandov, 1968) and rat (Ramirez et al., 1964) but yet prevent the formation and/or maintenance of the corpus luteum in the cow. Estrone is luteotrophic in hypophysectomized, pseudopregnant rabbits (Robson, 1937; Greep, 1941; Spies et al., 1967) as well as the intact doe (Greep, 1941; Chu et al., 1946; Hammond and Robson, 1951). Keyes and Nalbandov (1967) reported estradiol - 17B was capable of maintaining pregnancy in rabbits with X-irradiated ovary containing corpora lutea.

Maintenance of corpora lutea in hypophysectomized rabbits administered exogenous estrone or a combination of estrone and

bovine LH support the theory that estrogen has a direct luteotrophic effect in rabbits (Spies et al., 1967). Hechter et al. (1940) postulated that hysterectomy leads to an accumulation of estrone and thus maintenance of the luteal tissue in the rabbit. Estrone is not converted to estriol in the hysterectomized rabbit as it is in the intact animal (Pincus and Zahl, 1937). Everett (1961) suggested a release of gonadotrophic activity by the pituitary which is responsible for the production of ovarian estrogen, which acts locally and thereby causes luteal maintenance.

Greenwald (1965) reported that estrogen exerts a luteolytic and an antifollicular effect in pregnant hamsters, if injected before Day 4 of pregnancy. The site of action of the hormone is considered to be at the pituitary level (Choudray and Greenwald, 1968). Luteal regression was observed in the pseudopregnant rabbit following HCG or LH by Spies et al. (1966). It was suggested that this action may have resulted from an interference with endogenous estrogen through a direct effect on the ovary.

In the rat, estrogen acts at one time as a stimulus to LH secretion for ovulation (Bradbury et al.; 1950) and later as an inhibitor of LH secretion when LH might cause luteolysis (Rothchild and Schwartz, 1965) as an essential for deciduomata maintenance but destructive of it when it is present in excess (Rennels and Guillet, 1963) and finally as a stimulus to LTH

production (Everett, 1956) and/or LTH synergist (Alloiteau and Bouhours, 1965).

Bogdanove (1966) reported estrogen is capable of not only initiating but also maintaining pseudopregnancy in the rat. It appears that the anterior pituitary is involved in the luteotrophic activity of estrogen (Melampy and Anderson, 1968) as hypophysectomy prevents this action (Desclin, 1949). The exogenous estrogen increases the pituitary level as well as the secretion of prolactin (Reece and Turner, 1937).

A synergistic action of estrogen and progesterone on male acceptance and vaginal cornification in the hysterectomized guinea pig has been reported by Boling et al. (1938); Ford and Young (1951). According to Rowland (1961), in the guinea pig, estrogen provided by follicles, that continue to develop following removal of the uterus, may contribute to luteal maintenance.

Choudray and Greenwald (1968) reported that estrogen (ECP) administered on Day 3 causes complete luteolysis by Day 10 in the cyclic guinea pig. Spies et al. (1964) observed that neither estrogen, relaxin nor progesterone, administered from Day 6 of the estrous cycle, was able to induce luteolysis in the cyclic guinea pig. Choudray and Greenwald (1968) reported that the estrogen must be administered before Day 6. The ECP resulted in complete luteolysis and precocious ovulation which is due to the complete luteal regression and therefore removes the block to the ovulatory surge or release of LH. Exogenous estrogen administered in early pregnancy causes luteal regression and the

termination of pregnancy (Deanesly, 1963; Greenwald, 1967). Deanesly (1963) suggested that the luteal regression in the pregnant guinea pig was induced by estrogens affecting the pituitary gonadotrophic secretions.

Deanesly (1966) set forth that it was unlikely that estrogen-induced luteolysis involved a stimulation or inhibition of LH secretion, since LH has neither luteotrophic or luteolytic effects in the cyclic guinea pig. Choudray and Greenwald (1968) suggested that ECP blocked the release of pituitary hormones essential for luteal maintenance as well as graffian follicles. The simultaneous arrest of follicular development suggests that FSH is involved in luteal maintenance in the guinea pig. Luteal maintenance in the guinea pig may be dependent upon a hormonal complex, rather than a single hormonal factor (Greenwald, 1967; Choudray and Greenwald, 1968).

Results of experimental work indicate that estrogen is not luteotrophic in the ferret (Donovan, 1965), woman (Fluhmann and Hoffman, 1935; Brown et al., 1948; Vande Wield and Turksoy, 1965) or cow (Kaltenbach et al., 1964). Administration of estrogen before Day 10 but not after Day 15 will cause luteolysis in the cycling, pregnant or hysterectomized heifer. The site of action is considered to be at the pituitary level (Wiltbank, 1966).

According to Choudray and Greenwald (1968) neither exogenous progesterone nor testosterone proprionate exerted any adverse

influence on corpora lutea when administered on Days 1 to 9 of the estrous cycle. Spies et al. (1964) found progesterone not luteolytic in the hysterectomized guinea pig when administered on Day 6 post hysterectomy. Contrastingly, Aldred et al. (1961) reported that progesterone administered to pregnant and non-pregnant guinea pigs causes partial destruction of the corpus luteum. The progesterone injections caused luteal regression if given prior to Day 4 of the cycle or following Day 20 of pregnancy.

Progesterone does not regress the corpus luteum in cycling gilts or prevent the formation of the cyclic corpus luteum but does prevent the luteal tissue from lasting beyond the usual cyclic span (Sammelwitz et al., 1961). Continual administration of progesterone causes lysis of the corpus luteum in the pregnant (Sammelwitz and Nalbandov, 1958; Sammelwitz et al., 1956; Spies et al., 1958, 1959, 1960; Sammelwitz et al., 1961) and hysterectomized gilts (Spies et al., 1958, 1960). The luteolytic effect on the luteal tissue of pregnancy is presumably due to a blockage of a second release of luteotrophin (Sammelwitz et al., 1961).

Progesterone administered on the day before proestrus causes ovulation to occur 24 hours early in the rat (Everett and Sawyer, 1949) and prevents ovulation in the rabbit (Hiroi et al., 1965). According to Spies et al. (1964), progesterone causes early regression in pregnant mice (Burdick, 1942) but has no effect in pregnant rabbits (Ulberg, 1952) or rats

(Sammelwitz et al., 1961).

Moor et al. (1966) reported that the exogenous administration of progesterone maintains the uterus in a lytic condition well beyond the length of the normal cycle in artifically ovulated, progesterone-treated ewes and thus causes luteal regression in pregnant ewes (Zimbelman et al., 1959). Woody et al. (1966) have reported that exogenous progesterone exerts an inhibiting action on the corpus luteum of the ewe. These investigators concluded part of the action of the progesterone on the corpus luteum may be mediated through the uterus.

Luteinizing hormone (LH) has been reported to be luteolytic when administered to the intact pseudopregnant rabbit (Stormshak and Casida, 1964; Coon and Spies, 1964; Spies et al., 1966; Stormshak and Casida, 1965; Keyes and Nalbandov, 1968) or if given 5 to 7 hours after hypophysectomy (Kilpatrick et al., 1964), whereas a luteotrophic effect is exhibited in the hypophysectomized, pseudopregnant rabbit (Kilpatrick et al., 1964; Keyes and Nalbandov, 1968). Spies et al. (1967) reported no luteotrophic properties from bovine LH, administered subcutaneously, to hypophysectomized, pseudopregnant does.

The luteolytic effect of LH may be due to a block of estrogen since estrone given concurrently with LH prevents regression of the corpora lutea (Coon and Spies, 1964). Kilpatrick et al. (1964) ascribed the luteolytic effect of LH, administered within 5 to 7 hours after hypophysectomy, to the fact that the ovarian follicles ovulate in response to the LH.

It has been suggested (Stormshak and Casida, 1965; Spies, 1964) that LH may cause luteal regression in combination with a luteolytic substance possibly of pituitary origin or that it may interfere with the secretion of estrogen by the ovarian interstitial tissue.

Maintenance of the weight of the luteal tissue in hypophysectomized does given exogenous estrone alone or in combination with bovine LH supports the hypothesis that estrogen has a direct luteotrophic effect in rabbits. Furthermore, since estrogen can prevent the luteolytic effect of exogenous LH in pituitary intact rabbits (Spies et al., 1966; Stormshak and Casida, 1965) and can support pregnancy by maintenance of luteal function in antigonadotropin-treated (Spies and Quadri, 1967; Quadri et al., 1966) or ovarian X-irradiated (Keyes and Nalbandov, 1967) animals, the evidence strongly suggests that the effects of LH are exerted via regulation of ovarian estrogen synthesis (Spies et al., 1967).

LH appears to be the principal luteotrophic factor, while a prolactin-like luteotrophin may become important later, as in the pregnant and hysterectomy-induced pseudopregnant gilt (du Mesnil du Buisson, 1966). Bunde and Greep (1936) found LH to be luteolytic in the rat prior to hypophysectomy. Hansel and Seifart (1968) concluded from the sparse data available that pituitary LH content increases during the time of luteal degeneration in the cow.

According to Armstrong (1966), evidence indicates that LH

stimulates steroidogenesis in the bovine luteal tissue primarily by increased conversion of cholesterol to progesterone and that it acts preferentially upon newly formed cholesterol. Inavailability of this recently synthesized cholesterol may play a role in initiating the luteolytic process (Hansel and Seifart, 1968). Armstrong and Black (1966) found that at the 18th day, luteal tissue loses its ability to produce increased progesterone in response to LH added in vitro. This adds support to the theory of an active luteolytic process in the presence of high LH (Hansel and Seifart, 1968).

Bovine LH-antisera and estrogen caused partial regression of the luteal tissue in hysterectomized heifers, whereas bovine LH, HCG and crude anterior pituitary extracts were luteotrophic. The luteotrophic effects of the LH-containing preparations were abolished when incubated with urea (Hansel and Seifart, 1968).

Prolactin has been reported to not stimulate progesterone synthesis by luteal tissue in women, monkeys, sows, ewes, rabbits and guinea pigs (Turner, 1967). Smith et al. (1957) and Hansel (1966) reported that prolactin is ineffective in maintaining the corpus luteum after hysterectomy in heifers. According to Richardson (1967), prolactin is equal to hysterectomy in prolonging the luteal life span, despite the presence of a non-traumatized uterus in the pseudopregnant rat, which implicates prolactin having a role in the maintenance of the rat corpora lutea. Everett (1956) suggested that the same neurohumoral factors from the hypothalamus that simulates LH discharge

inhibits prolactin release in the rat. Shelesnyak (1957) reported that ergocornine, which is thought to block prolactin release from the pituitary, will interrupt luteal metabolism if given early in the life of the corpus luteum, Days 1 to 6, but becomes ineffective at later periods.

Exogenous vasopressin shortened the pseudopregnant period in hysterectomized rats but not in intact rats (Malven and Hansel, 1965), and since it stimulates pituitary LH release (McCann et al., 1962; Giuliani et al., 1961) and LH is luteolytic even in the presence of sustained LTH (Rothchild and Schwartz, 1965) a possible mechanism for luteolysis mediated through the hypophysis is suggested (Malven and Hansel, 1965).

Luteal function is not altered when oxytocin is injected into the hysterectomized heifer (Donaldson and Takken, 1968; Malven and Hansel, 1964) whereas in the intact cow, the luteal tissue function is markedly inhibited (Armstrong and Hansel, 1959; Hansel and Wagner, 1960; Hansel and Seifart, 1968). Donaldson and Takken (1968) suggested that exogenous oxytocin may act through a hypothalamo-pituitary pathway shared commonly with the uterus. Donaldson et al. (1965) hypothesized that the luteolytic effect of oxytocin in heifers may not be due to a release of a luteolytic factor but since it is associated with decreased levels of plasma LH, the stored supply of pituitary LH may have been exhausted by the stimulus.

Oxytocin treatment effectively reduced the length of the estrous cycle in partially hysterectomized heifers or in heifers

with the uterus opened in situ (Anderson et al.,1965). Furthermore, in heifers with hypophysial stalk transection at estrus, oxytocin injections induced luteal regression by Day 12 (Henricks et al.,1967). These results suggest oxytocin initiates local uterine luteolytic action.

The mode of action of oxytocin on initiating inhibition or regression of the bovine corpus luteum is not known; although, it is not improbable that the modification of the bovine luteal tissue may be the result of the production of a luteolytic factor(s) by the oxytocin stimulated uterus (Melampy and Anderson, 1968). It was further suggested that it may be afferent nerve impulses causing an indirect inhibition of secretion of the anterior pituitary luteotrophins although a direct action of the polypeptide hormone on the corpus luteum could not be denied. Ginther (1967) demonstrated with unicornal heifers not only that oxytocin affects luteal regression but also emphasized that the uterine luteolytic effects acts in a local manner in the cow.

Exogenous oxytocin does not exert a definite luteolytic effect in hysterectomized pseudopregnant rats as it does in rats with renal autografts of pituitary tissue (Fauklner and Hansel, 1962) and cattle (Armstrong and Hansel, 1959).

Wiltbank et al. (1961) reported prolongation of the functional life of the bovine corpus luteum as a result of HCG administration. Lynn et al. (1965) observed that bovine luteal tissue responded to in vitro HCG with an increased conversion of pregnenolone to progesterone.

HYPOPHYSECTOMY

Rowlands (1962) reported that hypophysectomy performed approximately 50 days following hysterectomy resulted in maintenance of the corpora lutea in guinea pigs. Dempsey (1937); Rosenbusch-Weihs and Ponse (1957) observed that if hypophysectomy was performed early in the normal cycle, the luteal tissue persists. Heap et al. (1967) reported implantation occurred, after hypophysectomy on Day 2, suggesting that the surviving luteal tissue continued to secrete progesterone over the critical period. Their observations were most readily interpreted in terms of a positive luteolytic stimulus involving both the pituitary and the uterus. The stimulus is removed if the pituitary or the uterus is removed after Day 9 after ovulation and is reduced if removed after Day 10. Perry and Rowlands (1962) found hypophysectomy on either Day 2 or 5 of the cycle resulted in luteal maintenance well beyond the normal cycle length. If hypophysectomy was performed on Day 10, luteal regression proceeded as in the intact guinea pig. The authors suggested the presence of a pituitary luteolysin which was being released between Days 5 and 10 in the guinea pig.

If the rat pituitary is separated from the central nervous system influence by a hypothalamic lesion (Taleisnk and McCann, 1961) or an autotransplant, it ceases FSH and LH secretion and secretes LTH continuously (Everett, 1964) as evidenced by the maintenance of the preformed corpora lutea for indefinite periods

of time. MacDonald and Armstrong (1965) reported prompt implantation and the maintenance of pregnancy in rats which were both hypophysectomized and castrated soon after ovulation and insemination.

Hypophysectomy in sheep (Denamur and Mauleon, 1963) and swine (du Mesnil du Buisson and Leglise, 1963) on the day of ovulation did not affect the development or the maintenance of the luteal tissue for the duration of the cycle and regression occurred at a time corresponding to the end of the estrous cycles in each species. According to Short (1964), immediate regression of the corpora lutea occurred when Denamur and Martinet (1961) performed hypophysectomy on or after Day 50 in pregnant ewes.

Rennie et al. (1964) reported that artifically induced corpora lutea or of pregnancy regress promptly if hypophysectomy is performed on the rabbit. Exogenous estrogen will maintain these corpora lutea and allow pregnancy to go term.

DECIDUAL CELL RESPONSE

Loeb (1907) noted that injury to the uterus of the guinea-pig resulted in a massive proliferation of the decidual cells, a deciduoma, similar to that observed during pregnancy. This report stimulated investigations of the many aspects of the phenomena. Shelesnyak and Kraicer (1961) and De Feo (1963) demonstrated that on the fourth day of leukocytic vaginal smears during pseudopregnancy or pregnancy, the uterus is most sensitive to traumatization by mechanical and chemical means. Shelesnyak

(1960) reported two essential requirements for successful induction and maintenance of decidualization. These are: (1) a responsive endometrium achieved by the action of ovarian hormones, estrogens and progestogens in proper sequence and proportion; and (2) stimulation of the process achieved by histamine, directly or by histamine-releasing agents, or actions such as trauma.

According to Finn (1966) there are three main hormonal stages in the sensitization of the uterus for the decidual cell response (DCR); the stimulation of the endometrium by estrogen at estrus, progesterone and the 'nidatory surge' of estrogen. Yochim and De Feo (1963) reported that maximum sensitivity to trauma was on Day 4 of pseudopregnancy and the deciduomata was associated with an increase in both uterine weight and water content.

The magnitude of the DCR is progesterone-dependent but yet progesterone alone is incapable of producing the maximum response of 2,000 mg., which was observed in intact pseudopregnant and in ovariectomized rats receiving an estrone: progesterone ratio of 1:2,000 or 1:4,000 (Yochim and De Feo, 1962). Rothchild et al. (1940) reported that placentomata in rats, ovariectomized and traumatized on Day 4 of pseudopregnancy, were larger with increasing doses of progesterone.

Richardson (1967) concluded in his review that an FSH-LH interaction is important in secretion of estrogen to produce a maximum uterine stimulation in intact rats. Nghiem (1969) observed a 500 mg increase in weight of the traumatized horn,

in rats that received LH-antisera and injected with 3 mg progesterone daily. Major et al. (1967) demonstrated that LH stimulates progestin production from luteal tissue in the rat and increases deciduoma weight (Yochim and De Feo, 1962). According to Nghiem (1969) pituitary LH may be capable of promoting progesterone secretion in the rat and LH antisera interferes with estrogen secretion.

EXTRACTS

The observation, in certain mammals, that hysterectomy was followed by failure of the corpus luteum to regress with cessation of ovarian cyclic activity has suggested the uterine production of a luteolytic substance. As a result, many attempts have been made to determine if various types of extracts were able to reverse the effects of hysterectomy on the corpus luteum.

Parkes and Bellerby (1926) observed that follicular extracts terminated the pregnancy in mice and that larger amounts were needed in the later stages of pregnancy to cause lysis of the luteal tissue. Leob and Kountz (1928) observed that follicular extracts administered for a six day period, beginning on Days 5, 6, 7, 10 or 12, had no luteolytic effect in pregnant, hysterectomized and cycling guinea pigs. As a result of the lack of ovulations during the injection period they concluded that the extract acted in a manner similar to a corpus luteum. However, when the extract was administered to undernourished females, the ovaries lacked corpora lutea and the follicles were larger than

the controls but yet ovulation did not occur.

Mishell and Motyloff (1941) administered daily injections of bovine endometrial extracts for 90 days to hysterectomized rabbits and observed that the extract increased the longevity of the luteal tissue. They concluded that the extract regulated the process of follicular proliferation and protected the functional elements of the ovary and retarded the process of atrophy. Contrastingly, Greep (1941) reported that acetone and saline extracted placental tissue, from does 15 to 25 days pregnant, failed to maintain the corpora lutea when administered to does that were hysterectomized on Day 15 of gestation. Furthermore, he concluded that the placenta does not produce sufficient estrogen to maintain the corpus luteum of pregnancy. Allen and Corner (1930) reported that crude extracts of corpora lutea maintained the pregnancy until term in rabbits that were ovariectomized soon after mating.

Acetone-dried bovine uterine powder, administered intraperitoneally to pseudopregnant rabbits was reported to cause lysis of luteal tissue, follicular development and depression of acetate incorporation into progesterone (Williams et al., 1967). They concluded that the mechanism which brought about the observed luteal regression and follicular development was the direct luteolytic effect of a protein hormone on the ovary. It was suggested but rejected that the effect was due to one or more of the proteins present in the preparations was absorbed

into the circulation and acted hormonally at the pituitary-hypothalamic level to initiate FSH or LH release. They also excluded the possibility that the uterine powder possessed FSH or LH activities and thus result in the luteal regression and follicular development.

Hansel (unpublished data) observed decreased weights and progesterone content of luteal tissue in hamsters treated with bovine endometrial suspensions and acqueous extracts. The most luteolytic activity of the acqueous extracts was found to be present in the 55% ammonium sulfate fraction. Skeletal muscle and myometrium were ineffective.

Astwood and Greep (1938) described a luteotrophic effect of a rat placental extract administered to pseudopregnant rats but could find no FSH or LH properties. Endometrial suspensions from estrual rats shortened the life span of the luteal tissue in hysterectomized pseudopregnant rats (Bradbury et al., 1950). Contrastingly, Kiracofe and Spies (1966) reported that injections of ether soluble extracts and lyophilized uterine homogenates of estrus rats gave no indication of decreasing the pseudopregnancy period of hysterectomized pseudopregnant rats. They did report corpora lutea regression was hastened in hysterectomized pseudopregnant rats that were in parabiotic union with ovariectomized partners. Malven and Hansel (1965) failed to demonstrate a luteolysin in pseudopregnant rats administered acqueous and ether extracts of late luteal bovine endometrium.

The extracts tended to prolong the hysterectomized-sustained rat corpora lutea.

Duncan et al. (1961) observed that swine endometrial filtrates from Days 12 and 13 of the estrous cycle increased in vitro progesterone synthesis by luteal tissue, whereas filtrates from Days 16 and 18 indicated a definite inhibitory affect on progesterone production. These results suggest that a critical alteration occurs in the utero-ovarian functional relationship prior to Day 16 of the cycle. Similarly, Schomberg (1967) reported that sow uterine flushings on Days 14 to 18 had a definite luteolytic effect on pig granulosa cell growth, in vitro, whereas flushings from Day 12 indicated a slight lytic effect and Day 1 to 10 and Day 20 flushings had no detrimental effect on the growth of the granulosa cells. The appearance of the luteolytic activity of the uterine flushings coincided with the decline in progesterone production (Gomes et al., 1965; Masuda et al., 1967). The lytic activity persisted during the anatomical regression of the luteal tissue and was absent by the time of the next estrus. Duncan et al. (1961) suggested that the active principle(s) was a relatively thermostable, dialysable compound but the preliminary studies of Schomberg (1967) indicated a thermolabile, nondialysable substance.

Contrastingly, Anderson and Melampy (1962) reported that infusion of endometrial extracts obtained from proestrous sows into the uterus of luteal phase pigs did not hasten luteal regression or subsequent ovulations.

Short (1964) reported that the levels of progesterone secreted by the ovine ovary varies with the number of corpora lutea present but was not responsive to ovine prolactin, FSH, LH, HCG, PMS, STH, TSH or endometrial extracts.

Kiracofe et al. (1966) reported that injections of ether soluble extracts or homogenates of lyophilized uterine tissue, taken from ewes at various stages of the estrous cycle did not affect corpora lutea weight or histology of hysterectomized or pregnant ewes.

Rowson and Moor (1967) observed that daily intrauterine infusions of 14 or 15 day sheep embryo homogenates prevented corpora lutea regression of nonpregnant ewes although intramuscular injections of the homogenates did not prolong luteal function. They suggested that the embryonic substance probably acts on the endometrium in an "anti-luteolytic" manner and thus counteracts the action of the endometrium which would otherwise result in luteal regression. In order that the infusion could exert its normal effect, a continuous secretion of the anti-luteolytic substance is reguired from the embryo.

Mazer and Wright (1968) reported further evidence for the production of a thermolabile, nondialyzable uterine luteolytic factor on the 6th and 7th days of pseudopregnancy in the hamster. The uterine homogenate was successful in shortening the duration of pseudopregnancy but was incapable of interfering with established pregnancies, which they felt indicates that embryos or embryonic membranes prevent synthesis of a uterine luteolytic

factor. It was concluded that the primary relationship between the uterus and ovary is local (via lymphatics) with systemic control (blood-vascular) as a somewhat less important secondary.

Pharriss and Wyngarden (1969) reported that Prostaglandin $F_{2\alpha}$, which is a potent and selective venoconstrictor and the major prostaglandin associated with the endometrium, either infused constantly for 48 hours into pseudopregnant rats or subcutaneous injections on Days 1 to 7, resulted in average pseudopregnancies of 8 days. The controls remained pseudopregnant for an average of 17 days. They postulated that it is an indirect luteolytic mechanism involving either the hypothalamo-pituitary-gonadal relationship or it may be causing a chemical hypophysectomy or it may be toxic to luteal tissue or it may be that ovarian blood flow is restricted and the venous drainage common to the ovary and the uterus. As a result, the corpus luteum succombs to such conditions as ischemia, limited substrate availability or accumulation of metabolites. The latter mechanism is indicated to be involved but further studies are necessary to resolve the validity of this hypothesis. In the same study, Pharriss reported that prostaglandin, administered to pseudopregnant rabbits on Days 4 to 8 of pseudopregnancy and the ovaries collected on Day 11, was 100% effective in 12 rabbits tested while 11 of the 12 controls had normal appearing luteinized ovaries.

METHODS AND MATERIALS

UTERINE POWDERS:

Preparation I.

Uteri of five early luteal (Days 3 to 5) cows, exhibiting normal reproductive histories, were removed immediately after slaughter, trimmed of extraneous tissue and the ovaries examined for reproductive state. Each uterus was then cut into small pieces, coarsely ground in a meat grinder, the ground tissue placed into individual glass containers of acetone, equal to 94-97% (v/w) and held for 24 to 48 hours at 4 degrees Centrigrade.

The acetone was removed with a Buchner funnel and the residue was washed with anhydrous ether repeatedly until no further discoloration of the ether could be detected. The residue was then placed on a porcelainized tray and allowed to air-dry in a refrigerated hood. The dried material was then Wiley-milled to a 60-mesh-screen size and stored frozen.

Preparation II.

The uterus of one early luteal cow (Day 4), exhibiting a normal reproductive history, was prepared as in the above manner. This acetone dried powder was then concentrated by ammonium sulfate fractionation. The uterine powder was placed in 0.9% saline and extracted for one hour. Recovery of the soluble material was facilitated by using a Buchner funnel and a vacuum flask. The

residue was discarded after having been washed several times with 0.9% saline. The soluble material was then taken to 50% saturation with ammonium sulfate and the residue recovered with acetone and the soluble material was discarded. The acetone was then removed by evaporation and the residue was freeze-dried and stored frozen.

Preparation III.

The uterus of an early luteal cow (Day 3), exhibiting a normal reproductive history, was prepared by the same procedure as the second preparation except the soluble material was taken to 100% saturation with ammonium sulfate. The subsequent residue was recovered with acetone then freeze-dried and stored frozen.

Preparation IV.

An aliquot 50 g sample of the acetone extracted uterine powder was subjected to 107.2°C (225°F) for a period of one hour and stored under refrigeration.

LAPAROTOMIES:

Laparotomies were performed on Day 4 to evaluate the reproductive state of the rabbit ovary. The does alloted to Trial I were subjected to bilateral laparotomy whereas those in Trial II were subjected to unilateral laparotomy. The does were prepared for surgery by injection of Sodium Pentabarbitol (Haver-Lockhart Laboratories, Kansas City, Missouri), which

had been diluted to one part Sodium Pentabarbitol to two parts sterile saline, into the marginal ear vein. The degree of anesthesia was attained when the animal failed to exhibit the retina reflex.

The hair was clipped from the entire area bounded by the vertebrate dorsally, the mammary glands ventrally, the last rib anteriorally and the hip posteriorally. The clipped area was then antiseptisized with alcoholic roccal and a surgical shroud was applied.

A two inch lateral incision was made through the abdominal wall approximately two and one-half inches posterior to the last rib and one and one-half inches ventral of the longissimus dorsi muscle. The ovary was then withdrawn from the abdominal cavity to the exterior, placed upon the surgical shroud and visually examined. The number and location of corpora lutea, corpora albicantia, blood-filled follicles and the size, number and location of follicles were recorded. In Trial I, one corpus luteum on each ovary was marked by injection of india ink into the body proper of the corpus luteum, whereas in Trial II no attempt was made to mark a corpus luteum.

After the appropriate data was recorded, the ovary was replaced, the incision closed with sutures and the animals were returned to their cages and allowed to recover.

HYSTERECTOMY

Twenty eight mature, virgin female guinea pigs, weighing

400-600 g and having estrous cycles 16 to 18 days long, were randomly assigned to one of two experimental groups. Estrous cycles were determined by daily examination of the vaginal plate for at least two cycles. Day 0 was designated as the last day that the vaginal plate was open. Each pig was anesthesized with anhydrous ether on Day 10, the hair was clipped and the skin was antiseptisized with alcoholic roccal in the surgical region before applying a surgical shroud. Ovaries were exposed, through one-inch para-lumbar incisions and the corpora lutea were visually examined. The number and location of corpora lutea, corpora albicantia, blood-filled follicles and the size, number and location of follicles were recorded. The uteri were removed via a mid-ventral incision, transection being made anterior to the tubo-uterine junction and in the mid-cervical region. Blood vessels were cauterized to prevent bleeding and caution was taken to not interrupt the ovarian blood supply. The ovary was replaced after the appropriate data were recorded. The incision was then sutured and the animals were placed in cages to recover.

The guinea pigs were maintained on a 14-hour light regime in a temperature controlled laboratory and fed pellets and water ad lib.

Experiment I.

Trial I.

Five mature New Zealand White does were injected intrave-

nously, via the marginal ear vein, with 250 I.U. of HCG (Chorionic Gonadotropin, The Upjohn Company, Kalamazoo, Michigan) to induce ovulation and pseudopregnancy. Laparotomies were performed the morning of Day 4 with the day of HCG injection designated as Day 0. Four received subcutaneous injections of 500 mg of uterine powder (Preparation I), suspended in 6 ml of sterile saline on the afternoon of Days 5, 6, 7 and 8 of pseudopregnancy. The remaining doe received 6 ml of sterile saline and served as a control.

On Day 9 of pseudopregnancy, the does were sacrificed and the physiological state of the ovaries were recorded. The corpora lutea were counted and described grossly as to color or degree of apparent vascularity. All follicles were counted and measured.

Each corpus luteum was then exsected, weighed and placed in individual vials of 10% formalin and prepared for histological study.

Trial II.

Pseudopregnancy was induced in twenty-seven mature New Zealand White does as in Trial I except they received 100 I.U. of HCG. Sixteen does were randomly selected to serve as treated animals and the remaining five does served as controls. The treated does were then randomly alloted so that the group receiving uterine preparation I was composed of four does; the group receiving uterine preparation II was composed of six does;

and six does in the group receiving uterine preparation III.

The does received intraperitoneal injections of 1.0 g of the respective uterine powder suspended in 10 ml of sterile saline on the afternoon of Days 5, 6, 7, 8 and 9 of pseudopregnancy. The controls received only saline.

On Day 10 of pseudopregnancy, the does were sacrificed and the data was collected as in Trial I.

The formalinized corpora lutea were dehydrated in a series of isopropyl alcohol baths and infiltrated with fresh paraffin. They were then sectioned at five to seven microns and mounted with Mayer's albumin on glass slides. The tissues were stained with Mallory's Triple Stain and PAS for histological examination.

Photomicrographs were taken at 100x to aid in the evaluation of the effect of the treatments on the corpora lutea.

The does were maintained on a 14-hour light regime in a temperature controlled laboratory and fed pellets and water ad lib.

EXPERIMENT II.

Group I, consisting of 13 guinea pigs, served as hysterectomized controls. These females were sacrificed by ether euthanization on Day 16 post-hysterectomy and the corpora lutea were exsected, weighed, fixed in Susa fixative; washed with water; dehydrated in an alcohol series; cleared with xylol; embedded in paraffin; sectioned at 5-7 microns; mounted on 1" X 3" glass slides using Mayer's albumen fixative; and stained with Mallory's

Triple Stain and PAS.

Group II, consisting of 15 guinea pigs, was hysterectomized on Day 10, injected with early luteal acetone extracted bovine uterine powder suspended in sterile saline on the afternoon of Days 12, 13, 14, 15 and 16 and sacrificed on the afternoon of Day 17. The corpora lutea were prepared for histological study as in group I.

EXPERIMENT III.

Ten mature, virgin female albino rats of the Holtzman strain, weighing 150-200 g and having at least two, four or five day estrous cycles, were utilized to investigate the effects of early luteal acetone-extracted bovine uterine powder on decidual cell response (DCR). Pseudopregnancy was induced by twenty seconds of cervical stimulation facilitated by the use of an electric tooth brush fitted with a metal rod. The presence of leukocytes in the vaginal lavage indicated the first day of diestus and was designated as Day 1.

At midday ($\pm$ 1 hour) of Day 4 of pseudopregnancy the rats were anesthesized by anhydrous ether inhalation and the left horn of the uterus was traumatized with a needle via mid-ventral incision. The needle was inserted into the lumen of the left horn at the bifurcation and gently passed to the tubo-utero junction. The needle was withdrawn with the point pressed against the antimesometrial endometrium. Unnecessary handling of the uterine horn was avoided and care was taken to inflict

uniform traumatization.

Ten rats received two daily intraperitoneal injections of 0.5 ml each of supernatant from the acetone extracted uterine powder suspended in saline, on Days 5, 6, 7 and 8 of pseudo-pregnancy and the controls received only saline. On Day 9, the rats were weighed and sacrificed. The uterine horns were exsected and individually weighed to the nearest 0.1 mg on a Mettler Gram-O-Matic balance. The horns were then placed in an oven at 150 C for 24 hours and the dry weight recorded. The ovaries were also collected, weighed and fixed in individual vials of 10% formalin for histological examination.

The rats were maintained on a 14-hour light regime in a temperature controlled laboratory and fed pellets and water ad lib.

RESULTS AND DISCUSSION

The data presented in Tables 1 and 2 indicate the affect of early luteal bovine uterine preparations on the weight of corpora lutea of pseudopregnant rabbits. Gross observations of color or degree of apparent vascularity at sacrifice, was indicative of the degree of histological regression of the corpora lutea; however, the variability observed among animals and between ovaries of the same animal causes considerable doubt as to its reliability as a suitable criteria for describing the functional or histological regressive state of corpora lutea. This is not to say that it should not be utilized as a research tool but rather that it should be utilized only as an aid in interpretation of data.

Our data indicates that when compared to histological appearance, the mean corpora lutea weight was not an accurate criteria for determining functional capabilities of the luteal tissue, unless the rabbit died or exhibited anaphylaxis. For instance, only five of the eighty-six corpora lutea exsected in the acetone extracted uterine powder group failed to histologically exhibit signs of regression but yet the mean corpora lutea weight (15.32 mg) of these animals is equal to that of animals treated with the heated uterine powder (15.52 mg) which did not show histological signs of regression. Histologically, the luteal cells, of the heated powder treated animals (Figures 1 and 2), were plump appearing with vesicular nuclei whereas the corpora lutea of the animals receiving the acetone extracted uterine powder (Figures 3 and 4),

TABLE 1. THE EFFECTS OF UTERINE PREPARATIONS ON THE MORPHOLOGICAL CHARACTERISTICS OF RABBIT CORPORA LUTEA

Treatment	Rabbit No.	No. of corpora lutea	Avg. corpora lutea wt. (mg)	Color of corpora lutea
Acetone extracted uterine powder	3ABA	18	12.94	PA-W
	3NB[a]	13	9.62	PA
	5AB	9	17.34	P-PA
	1	6	7.70	W
	7	10	20.42	PA-W
	8	12	18.02	PA-W
	10	15	18.01	PA
	12	16	12.82	PA
Saline	D	7	21.26	R
	2PA[b]	11	7.25	R
	Y	11	18.70	R
	9	19	19.38	VR
	L	9	16.52	VR
	GRB	12	15.32	R
	J[c]	10	*	P
	1L3[d]	9	*	P
50% SAS	L	7	21.17	P-R
	21C	13	17.69	PA
	1DB[d]	*	*	*
	14	15	17.79	PA-R
	2CA[e]	14	6.45	*
	2AR	18	15.39	PA
100% SAS	20E[f]	8	14.28	VR
	4AD	9	21.39	P
	2BD	9	20.82	PA-R
	BMC	10	18.44	PA-P
	X	10	18.89	P-R
	60-Y[g]	10	10.89	P-R
Heated uterine powder	5LU	7	12.87	VR
	RKB-1	13	15.55	VR
	RKB-2	9	17.87	VR
	RKB-3	16	15.38	VR

* Not recorded
[a] Died on Day 5
[b] Died on Day 7
[c] Died on Day 6
[d] Died on Day 6
[e] Died on Day 5
[f] Died on Day 9
[g] Died on Day 10

VR= Very red
R= Red
RP= Reddish pink
P= Pink
PA= Pale
W= White

TABLE 2. THE EFFECT OF UTERINE PREPARATIONS ON THE WEIGHT OF RABBIT CORPORA LUTEA

Treatment	No. of animals	No. of C.L.	Avg. C.L. Wt. (mg)
Acetone extracted uterine powder	7	86	15.32
Saline	5	58	18.24
50% SAS	4	53	18.01
100% SAS	4	38	19.89
Heated uterine powder	4	45	15.42
Others (died)	6	56	9.70

exhibited luteal cells that were vacuolated and shrunken with condensed cytoplasm and picnotic nuclei.

The mean corpora lutea weight of does treated with 50% ammonium sulfate fractionated powder are equal to those of the saline treated controls (18.01 and 18.24 mg., respectively), but histologically are quite different (Figures 5 to 8). The corpora lutea, from the 50% SAS treated does, contained shrunken, vacuolated luteal cells with hyperchromatic, picnotic nuclei which appear to be incapable of producing sufficient quantities of progesterone, whereas the corpora lutea of the controls consisted of large, plump appearing luteal cells with vesicular nuclei. The latter cells appeared to be capable of producing normal quantities of progesterone.

The luteal cells that embodied the corpora lutea of those animals injected with 100% SAS (Figure 9), histologically dis-

played the normal functional appearance of the saline treated controls except in localized areas. The cells immediately surrounding blood vessels and capillaries exhibited lysis while those more removed appeared relatively normal. The mean corpora lutea weights of these does were not significantly different than the saline controls.

A decreased luteolytic activity was observed in the 100% SAS powder. Luteolytic activity was present in the non-fractionated as well as the 50% SAS powder and thus should be present at the same concentration in the 100% SAS powder. The latter statement is based on the assumption that in the ammonium sulfate fractionation process, the large proteins, due to their molecular weight, would be observed in the initial fractions and the smaller molecular weight proteins would be observed in the succeeding fractions as the concentration of ammonium sulfate approached 100% SAS (Johnston et al., 1967). Such factors as heat, acidity and alkalinity are capable of denaturing proteins and thus destroy the physiological activity (Morrison and Boyd, 1965). In this instance, it is unknown to the author which factor(s) resulted in the decreased luteolytic activity of the powder.

The results of an one-way analysis of variance indicates no significant difference in the mean corpora lutea weights of the treatment groups, although, the decreased corpora lutea weights observed in the animals that died during treatment (9.70 mg) when compared to controls (18.24 mg) was highly signi-

ficant ($P < 0.005$).

The observed luteal regression following injection of the uterine powder may have occurred via several possible mechanisms. It is conceivable that one or more of the proteins present in these preparations was absorbed into the circulation and acted hormonally at the pituitary-hypothalamic level to initiate FSH or LH release. Either one or both of these may then act at the ovarian level to bring about luteal regression. If the pituitary were involved then the uterine powder would not be expected to have similar effects in rabbits, rats and guinea pigs, due to the inherent heterogeneity of luteotrophic mechanisms involved. Of course, it is also possible that the injected uterine powders possessed these hormonal activities, acting directly on the ovary to give the present results. Coon and Spies (1964) observed that LH administered intravenously, after Day 4 post-ovulation, in pituitary intact does appeared to block ovarian estrogen synthesis, thus causing the corpora lutea to regress. Estrone administered concurrently with the LH was able to reverse the effects of the LH and prevent regression of the luteal tissue. Hillard et al. (1964) have reported that LH increases progestin output in vivo and in vitro (Armstrong et al., 1966; Kaltenbach et al., 1967).

According to Stormshak and Casida (1964), an intravenous LH dosage of 10 ug was found to be ovulatory on Day 9 of pseudo-pregnancy, whereas 15 ug or more of LH were necessary to bring about luteal regression as measured by luteal weights. Spies

et al. (1966) suggested that HCG or LH in pseudopregnant does causes corpus luteum regression by interfering with endogenous estrogen via a direct effect on the ovary. Keyes and Nalbandov (1968) hypothesised that LH, administered to pituitary intact rabbits, ovulates follicles which presumably serve as the endogenous source of estrogen; therefore, in the absence of the endogenous estrogen, the corpus luteum degenerates.

Williams et al. (1967) reported that intraperitoneal dosages of 100 to 500 IU of HCG and greater than 100 ug of LH, was required to induce ovulation in does. Furthermore, these workers did not observe new ovulations following four days of intraperitoneal injections of uterine powder into pseudopregnant rabbits. Likewise, neither intraperitoneal injections of 1.0 gram of uterine powder into four estrual rabbits nor the intravenous injections of saline extract of uterine powder, known to produce luteal regression and follicular development, were able to induce ovulations. An immature rat ovarian weight assay for FSH at a dosage of 300 mg of uterine powder, was observed by Williams et al. (1967) to be negative.

A third possible mechanism to explain these observations involves the direct luteolytic effect of the protein hormone on the ovary. Williams et al. (1967) concluded that the effect of bovine uterine extracts on corpora lutea of pseudopregnant does was due to a direct uterine luteolytic hormone (ULH) effect on the ovary. His conclusion was based primarily on depression of incorporation of acetate-2-C^{14} label into progesterone by the luteal tissue in only seven of eleven treated does. It is

interesting to note that incorporation of acetate-2-C^{14} label into progesterone was not significantly affected when all eleven of the animals receiving injections of the uterine powder were compared to controls. The data in the present experiment is insufficient to allow speculation as to whether the effect observed was due to a true uterine luteolytic factor (ULF).

Another possible process to explain the observed results involves the question of whether the effect of the uterine powder was directly toxic to the luteal tissue. It is granted that six animals died during the experimental period; however, the author feels that a sickness, not associated with the treatment, was responsible. Four of the six does, 3NB, 2CA, 1DB and 2PA, expired on Days 5, 5, 6 and 7 respectively, while rabbits 20E and 60-Y died on Days 9 and 10 of pseudopregnancy. Several of the above does were diagnosed as having respiratory congestion at the time of laparotomy. Doe 1, in the group receiving the acetone extracted uterine powder exhibited symptoms of anaphylaxis on the second day of injections and at each successive injection throughout the trial. At sacrifice, the corpora lutea exsected from this doe were grossly observed to be very small and white and was assumed to be regressed. Histological examination verified the total regression of the luteal tissue. Only in rabbits that died or exhibited anaphylaxis was complete lysis of the luteal tissue observed (Figures 11 and 12).

Furthermore, the decidual cell response (DCR) data (Table 3) indicates that the uterine powder was not toxic to the uterine

TABLE 3. THE EFFECT OF ACETONE EXTRACTED BOVINE UTERINE POWDER ON THE DECIDUAL CELL RESPONSE IN THE ALBINO RAT

Treatment	Rat No.	Weight of the Traumatized Uterine Horn		Weight of the Nontraumatized Uterine Horn		Wet Ovarian Wt. (mg)
		Wet	Dry	Wet	Dry	
Acetone extracted uterine powder	CJ2	1,677.8	270.7	157.8	37.5	56.6
	CJ3	2,152.4	339.0	153.4	35.5	49.4
	CK2	1,398.5	235.6	158.6	37.3	56.5
	CM2	1,661.7	268.1	157.1	34.1	60.0
	DE3	2,643.2	385.4	161.5	*	54.5
	DG3	2,598.0	403.5	405.6	37.2	53.2
	DA1	2,666.3	403.3	157.3	28.7	*
	DF1	2,641.2	394.4	150.7	29.7	71.5
	DB2	2,234.6	347.6	179.4	31.4	76.4
	DE1	2,116.2	326.3	148.2	26.2	69.4
Total Wt. (mg)		21,789.9	3,373.9	1,829.6	317.6	547.5
Mean Wt. (mg)		2,179.0	337.4	183.0	35.3	60.8
Saline	DB1	2,367.6	371.8	156.8	29.5	62.0
	DC2	1,444.8	232.8	148.0	27.2	55.1
	DF2	2,135.0	329.4	143.5	26.7	63.4
	DG2	1,356.4	211.5	142.2	25.9	61.3
	DD1	2,127.6	349.1	161.7	31.2	55.6
	*	1,796.7	*	165.8	*	56.7
	*	1,837.1	*	210.3	*	54.0
	*	1,590.6	*	200.0	*	54.4
	*	1,621.2	*	156.9	*	56.2
	*	1,771.3	*	195.6	*	72.0
Total Wt. (mg)		18,048.3	1,494.6[a]	1,680.8	140.5[a]	590.7
Mean Wt. (mg)		1,804.8	298.9[a]	168.1	28.1[a]	59.1

* Not recorded

[a] Based on 5 animals

tissue of the rat. The progesterone synthesis was not decreased, as indicated by the increased weights of the traumatized horn. If anything, there was a stimulation to increase progesterone production by the ovary. Also, histologically the corpora lutea were normal appearing and quite capable of functioning at a normal physiological level (Plate 13). Additionally, death loss or expressable symptoms of anaphylaxis were not observed in the guinea pigs and yet the luteal tissue is regressed, as evaluated by "blossom cell" counts (Table 5).

Another possible mechanism by which the acetone extracted uterine powder could affect the corpora lutea and bring about the observed results is the role of a venoconstrictor. According to Pharriss (1968), the uterus is the site of synthesis or storage of a potent venoconstricting substance, Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), whose release or synthesis is under the control of the ovary and stimulated by hormones emanting from the corpus luteum. Reacting to these hormones the uterus releases the venoconstricter into the venous blood and the utero-ovarian vein flow is thereby reduced and as a result the actual perfusion of the ovary is reduced. The ovary at this time has reached peak luteal activity and is making heavy demands on the existing blood flow. Therefore, under this new restriction, luteal metabolism cannot be maintained and the subsequent failure of the luteal tissue would occur due to inavailability of substrate, a buildup of waste or metabolic products and result in either poisoning or biochemical end-product inhibition or local ischemia

due to the high oxidative need of the steroidogenic tissue.

Pharriss (1968) has observed that $PGF_{2\alpha}$ infusion for 48 hours (1 mg/kg body weight/day) caused a dramatic shift in the steroid pattern of the ovaries. Progesterone levels were depressed and 20α-hydroxypregn-4-ene-3-one levels were elevated to such an extent that the ratio of these two steroids was dramatically shifted to the 20α reduced form much in predominance. This shift has been described by Linder and Shelesnyak (1967) as an early indication of luteal degeneration. Similar results were obtained by subcutaneous injections, twice daily, and the data was correlated with vaginal estrus.

Pharriss (personal communication) has observed that the corpora lutea, of pseudopregnant does sacrificed 18 to 36 hours post-injection of $PGF_{2\alpha}$, appear morphologically functional but biochemical analysis indicates that the luteal tissue is in the terminal stages of regression.

Data from a Parlow Ovarian Ascorbic Acid Depletion Assay (Table 4) a bioassay for LH, in which 52 rats were involved, indicates that 1.0 ml of the saline supernatant of the acetone extracted uterine powder had a depleting effect on the ovarian ascorbic acid equivalent to 2.75 ug of LH. Pharriss (personal communication) stated that PGF_2 has an affect on ascorbic acid of the immature rat ovary quite similar to LH, although depletion is approximately 40 to 50% as effective as similar quantities of LH. In our experiment, 0.5 and 1.0 ml of saline supernatant of the acetone extracted uterine powder depleted ovarian

TABLE 4. ASCORBIC ACID LEVELS IN OVARIES OF IMMATURE ALBINO RATS AFTER INJECTION OF LH OR UTERINE PREPARATIONS

Treatment	Mean ug Ascorbic Acid/100 mg Ovary	Mean ug LH
Saline	2.2	0.00
1.0 ug LH[1]	1.8	
2.0 ug LH	1.4	
4.0 ug LH	1.3	
16.0 ug LH	0.9	
0.5 cc U.P.S.[2]	1.9	0.80
1.0 cc U.P.S.	1.3	2.75
0.5 cc H.U.P.S.[3]	2.2	0.00

[1]NIH-LH-S14. NIH, Endocrinology Study Section, Bethesda, Maryland

[2]Uterine Powder Supernatant

[3]Heated Uterine Powder Supernatnat

ascorbic acid 13.6% and 41.0% respectively.

The acetone extracted uterine powder was further tested on mature female rats utilizing the decidual cell response (DCR) as an endpoint. The assumption being that if the uterine powder was capable of exerting a luteolytic influence in the rat, the decreased progesterone production by the corpora lutea would result in a decreased weight of the traumatized horn. The data in Table 3, which involved 20 rats, indicates a 374.16 mg mean increase in the weight of the traumatized horn in those rats

receiving 0.5 cc., twice daily, intraperitoneally of the acetone extracted uterine powder. The increase in cornual weight was found to be significant at the $P < .10$ level and approached significance at the $P < .05$ level as calculated by an one-way analysis of variance.

The increase in the mean weight of the traumatized horn was due either to a decreased estrogen production and/or an increased progesterone production by the rat ovary or an increase in the pituitary gonadotrophin, prolactin. The latter is necessary throughout the life of the corpus luteum for its maintenance (Nikitovitch-Winer and Everett, 1958). In the rat, significant production of estrogen by the follicles requires both FSH and LH (Turner, 1965). Pharriss (1968) has demonstrated in the rat that Days 5 to 7 post-ovulation is the time at which the luteal synthesis and content of progesterone reaches a peak. Mitchell and Yochim (1968) reported that HCG administered to female rats increases the ovarian and uterine weight and caused vaginal cornification by stimulating estrogen secretion.

It has been reported by Pharriss and Wyngarden (1969) that *in vitro* LH stimulates rat ovaries to produce progesterone but yet *in vivo* Rothchild (1965) observed that LH is not luteotrophic in the rat and may even cause luteolysis if given over a five to nine day period during pseudopregnancy. Pharriss (1968) observed that Prostaglandin $F_{2\alpha}$, when incubated *in vitro* with pseudopregnant rat ovaries, did not decrease progesterone synthesis or the progesterone to 20α-reduced progesterone ratios. Furthermore,

these workers felt that there was a stimulation to increase progesterone concentrations in the ovaries.

The increase in the weight of the traumatized horn in the DCR experiment was possibly due to the presence of a prostaglandin in the uterine powder. According to Pharriss (personal communication) exogenous LH causes an initial increase in progesterone production by the pseudopregnant rat ovary which is followed by lysis of the luteal tissue. The latter action is hypothesized by Pharriss to occur because the exogenous LH disrupts the fine balance between the blood supply to the ovary and the vascular demands of the ovary and actually by stimulating the luteal metabolism, hastens the termination of the luteal tissue. Similar results have been obtained with $PGF_{2\alpha}$.

The data in Table 5 presents the effect of the acetone extracted uterine powder on the corpora lutea of the hysterectomized guinea pig. The determination of luteal regression was made by counting the number of "blossom cells" under oil immersion, according to the procedure of Spies et al. (1964). The average of four randomly chosen fields was taken as representative for a given corpus luteum. Progesterone content of corpora lutea and the number of "blossom cells" is highly positively correlated (Spies et al., 1959). The decrease in mean luteal cells per field was found to be highly significant ($P < .005$) by one-way analysis of variance.

Spies et al. (1964) reported that progesterone was not luteolytic in guinea pigs whereas Deanesly and Perry (1965)

TABLE 5. THE EFFECT OF A BOVINE UTERINE EXTRACT ON LUTEAL CELL NUMBER IN HYSTERECTOMIZED GUINEA PIGS

Treatment	Animal No.	Mean Luteal Cells/Field	
Acetone extracted uterine powder	525	6.69	
	527	[a]	
	529	3.25	
	530	3.53	
	531	6.15	
	532	5.75	
	533	3.92	
	534	4.42	
	535	6.94	
	5-3	5.11	
	24-13	5.53	[b] 5.13
Saline	1-1	15.00	
	1-30	21.33	
	6-15	11.66	
	11-24	14.75	
	13-8	12.12	
	15-16	10.56	
	17-10	10.00	
	18-21	[a]	
	25-16	11.93	
	526	11.88	[c] 13.25

[a] CL completely regressed; no histological data

[b] based on 10 animals

[c] based on 9 animals

reported that progesterone was effective in causing luteal regression. Contrastingly, Choudray and Greenwald (1968) have reported estrogen to be luteolytic but not progesterone or testosterone proprionate in the cycling guinea pig.

According to Perry and Rowlands (1962), the uterus appears to limit the size to which the corpus luteum can grow and the

pituitary gland controls its persistence as a morphological entity. Deanesly and Perry (1965) concluded that the uterine factor associated with normal cyclic corpus luteum regression, which is lacking after hysterectomy, presumably acted on the hypophysis rather than directly on the ovary. They also found that pituitary LH suffices to cause ovulation in the hysterectomized guinea pig.

Pharriss (1968) has emphasized that species in which $PGF_{2\alpha}$ has been effective in lysis of luteal tissue have one common factor; the final common venous pathway between the ovary and the anterior portion of the uterus on the same side. Pharriss (personal communication) has stated that PGE and $PGF_{2\alpha}$ both have the biological activity of exerting the constricting effect primarily on the venous side of the vascular system with little or no effect on the arterial side.

The mechanism by which the luteal regression occurred in the guinea pigs in the present experiment is felt to be due to the presence of a prostaglandin in the acetone extracted uterine powder; although, the involvement of the pituitary-hypothalamo axis cannot be rejected.

EXPLANATION OF PLATE I

Fig. 1. Luteal tissue from rabbit treated with heated acetone-extracted uterine powder exhibiting large plump luteal cells with vesicular nuclei (150x).

Fig. 2. Luteal tissue from rabbit treated with heated acetone-extracted uterine powder exhibiting large plump luteal cells with vesicular nuclei (600x).

Fig. 3. Luteal tissue from rabbit treated with acetone extracted uterine powder exhibiting shrunken cells, condensed cytoplasm and pyknotic nuclei (150x).

Fig. 4. Luteal tissue from rabbit treated with acetone extracted uterine powder exhibiting shrunken cells, condensed cytoplasm and pyknotic nuclei (600x).

PLATE I

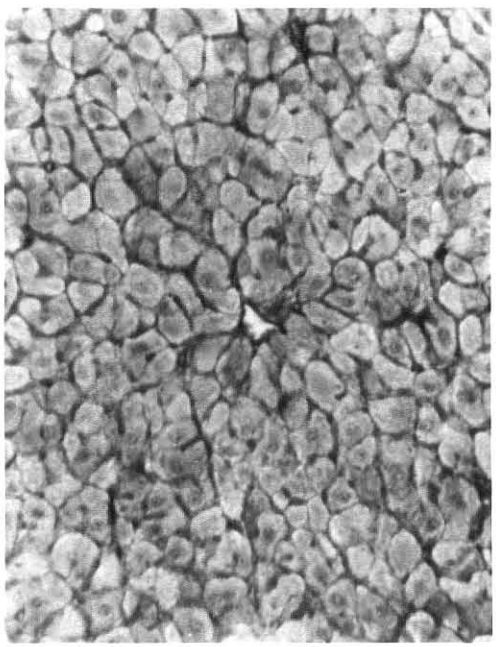


Fig. 1

Fig. 2

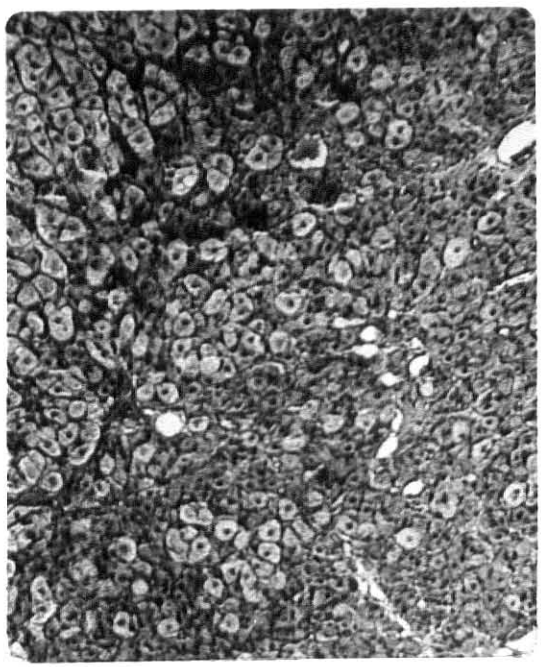


Fig. 3

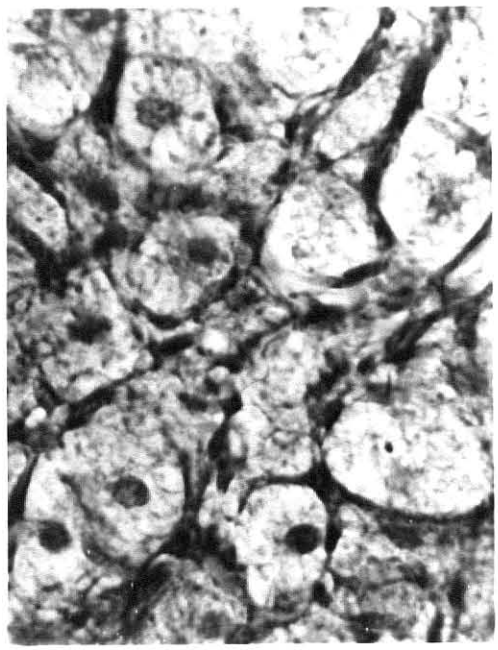


Fig. 4

EXPLANATION OF PLATE II

Fig. 5. Luteal tissue from rabbit administered 50% ammonium sulfate fractionated acetone-extracted uterine powder exhibiting shrunken cells, condensed cytoplasm and pyknotic nuclei (160x).

Fig. 6. Luteal tissue from rabbit administered 50% ammonium sulfate fractionated acetone-extracted uterine powder exhibiting shrunken cells, condensed cytoplasm and pyknotic nuclei (600x).

Fig. 7. Luteal tissue from rabbit administered physiological saline exhibiting large plump cells with vesicular nuclei (150x).

Fig. 8. Luteal tissue from rabbit administered physiological saline exhibiting large plump cells with vesicular nuclei (600x).

PLATE II

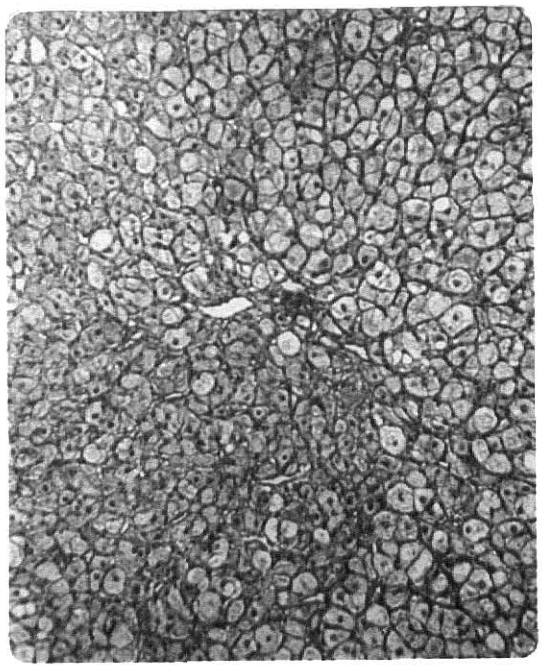


Fig. 5

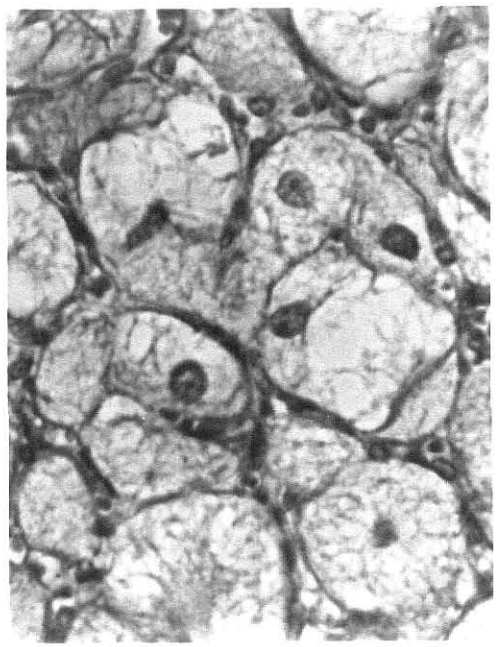


Fig. 6

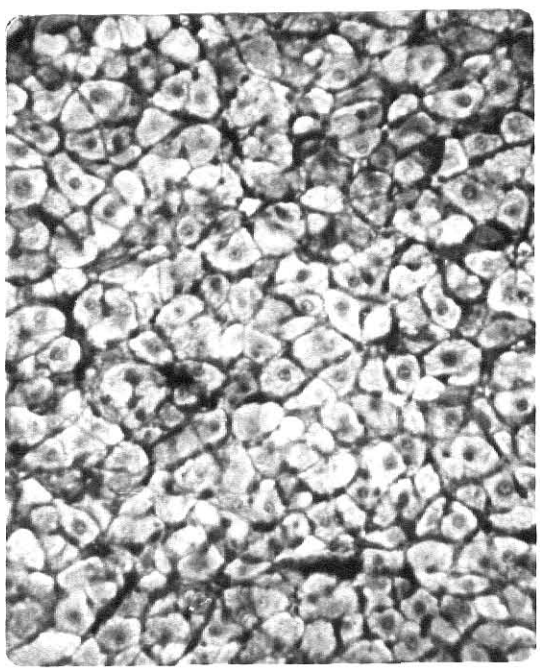


Fig. 7

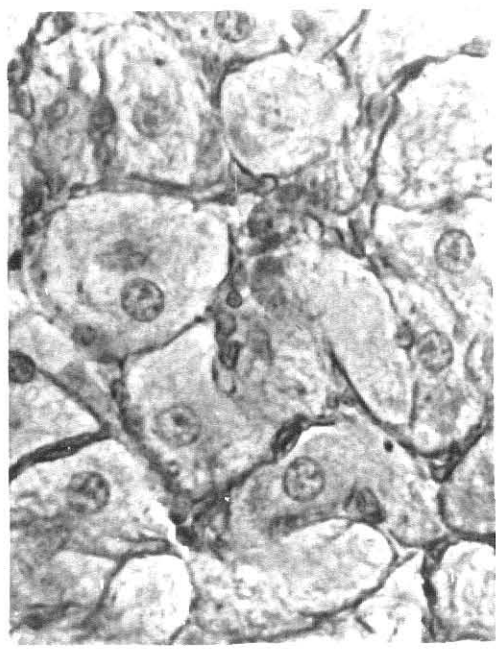


Fig. 8

EXPLANATION OF PLATE III

Fig. 9. Luteal tissue from rabbit administered 100% ammonium sulfate fractionated acetone-extracted uterine powder exhibiting lysis of luteal cells immediately around blood vessels but cells more removed appear quite normal (150x).

Fig. 10. Luteal tissue from rabbit administered 100% ammonium sulfate fractionated acetone-extracted uterine powder exhibiting shrunken cells, condensed cytoplasm and pyknotic nuclei (600x).

Fig. 11. Luteal tissue from rabbit administered acetone-extracted uterine powder but exhibited anaphylaxis (160x).

Fig. 12. Luteal tissue from rabbit administered acetone-extracted uterine powder but exhibited anaphylaxis (600x).

PLATE III

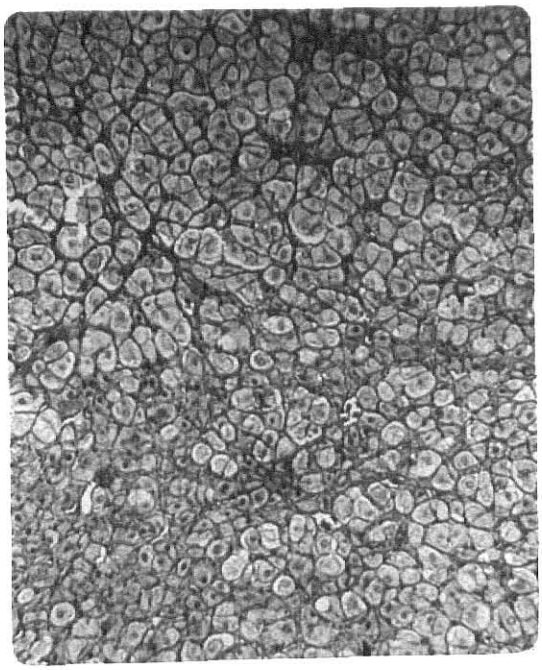


Fig. 9

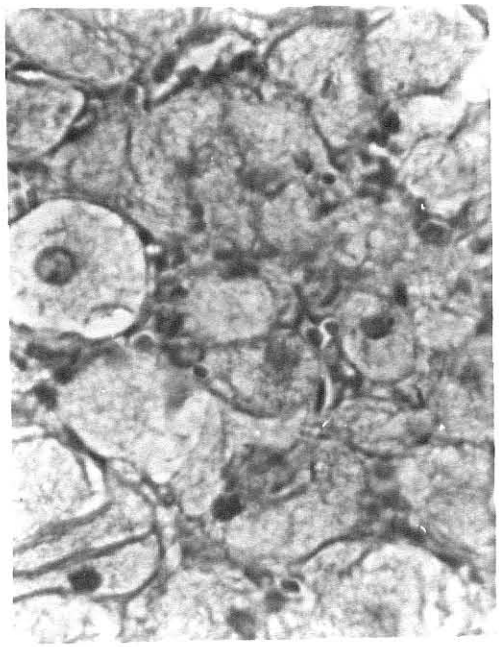


Fig. 10

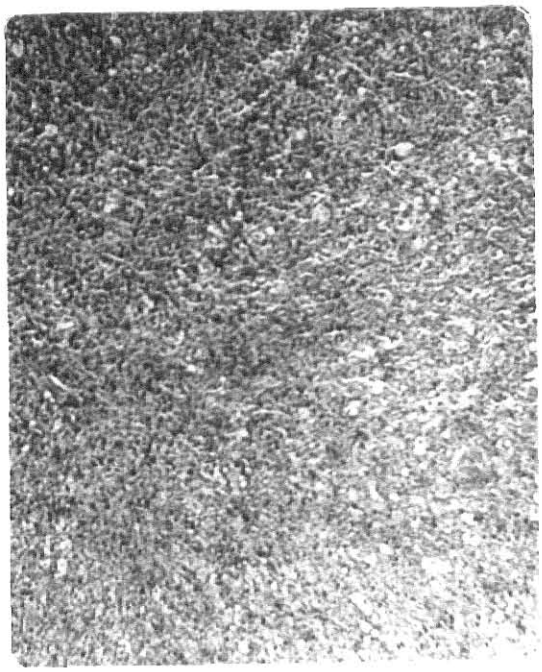


Fig. 11

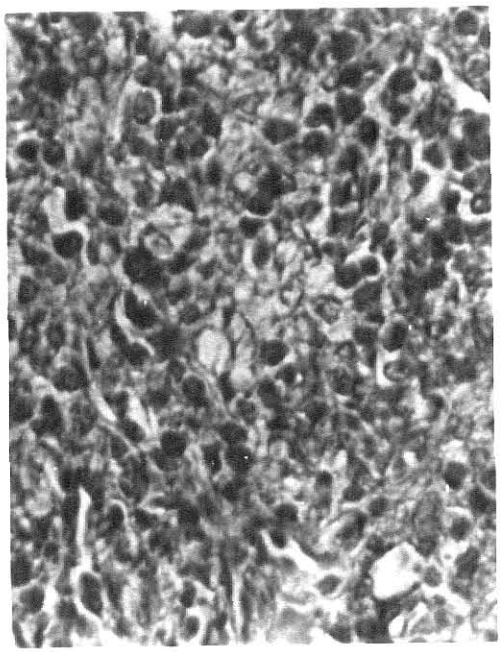


Fig. 12

FIG. 1 ASCORBIC ACID STANDARD CURVE

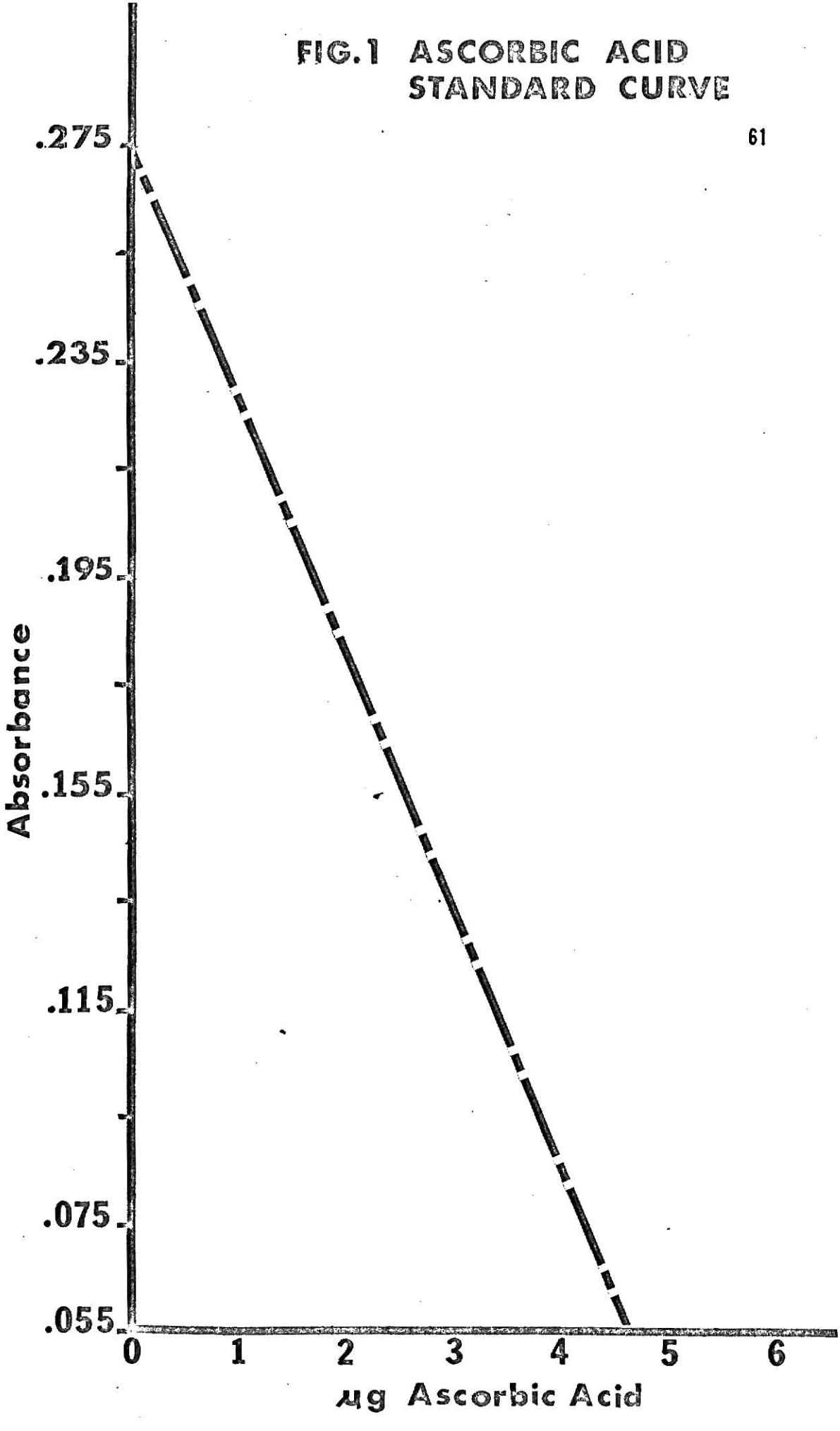
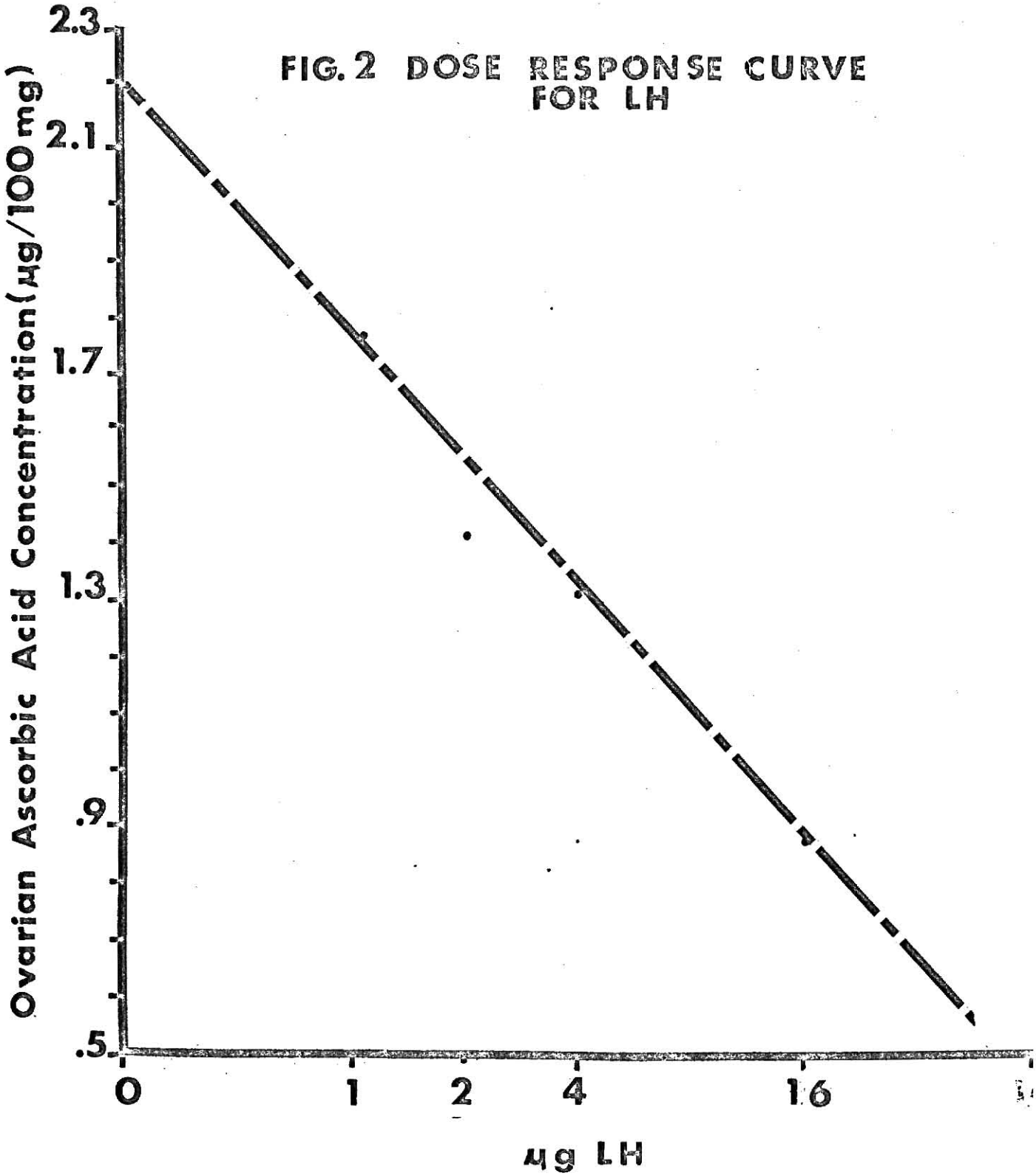


FIG. 2 DOSE RESPONSE CURVE FOR LH



SUMMARY

Acetone extracted and 50% ammonium sulfate fractionated bovine uterine powder when injected intraperitoneally into mature pseudopregnant rabbits caused histological regression of the corpora lutea whereas, the 100% ammonium sulfate fractionated powder exhibited a localized luteolytic effect on those cells immediately surrounding blood vessels and capillaries. Luteal regression, determined by "blossom cell" count, was noted in hysterectomized mature guinea pigs that received intraperitoneal injections of the acetone extracted uterine powder. Contrastingly, the same powder did not exhibit luteolytic properties in the mature pseudopregnant rat, as evaluated by the proliferation of the traumatized horn in the decidual cell response.

A Parlow ovarian ascorbic acid depletion assay revealed that 1.0 ml of saline supernatant of the uterine powder had a depleting effect on the ascorbic acid of the immature rat ovary equivalent to 2.75 ug of LH. These data indicate the presence of LH or a substance with LH-like activity in the acetone extract of the early luteal bovine uterus. It is further indicated that the luteolytic effect of the uterine powder was due to a blood-borne, protein-like, water soluble and heat labile substance.

These data make the proposal that the luteolytic effect of the uterine extracts was due to the presence of a venoconstricing

prostaglandin, most probable, particularly when these data are considered with the reports by Pharriss (1968). It is granted that the involvement of the hypothalamo-pituitary axis cannot be dismissed.

According to Rothchild (1965), "It is wrong to ascribe failure of the corpus luteum solely to the failure of the luteotrophic process, or solely to the activity of the direct luteolytic process, unless one or the other factor can be clearly eliminated".

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ADDENDUM

Subsequent to the preparation of the manuscript, the acetone extracted uterine powder was analyzed for luteinizing hormone (LH) content by Dr. D. J. Bolt (U.S.D.A., Beltsville, Maryland) by double antibody radioimmuno assay.

Five grams of uterine powder was reconstituted in 50 ml of saline and 20 ml of the supernatant was shipped frozen for analysis. The uterine extract supernatant arrived thawed but was immediately frozen upon arrival. At the time of assay, the material was thawed, centrifuged and dispensed into 30 assay tubes. The centrifugation was necessary to clear the extract of some undesirable flocculi. The material was then assayed at 10 dose levels with three tubes per level. Two standard LH curves with 16 levels of NIH-LH-S15 were determined concomitantly.

In the assay, a dilution of 1:80,000, Niswender LH antibody No. 573, No. 1056-C2 LER LH for iodination, Iodine 131 and second antibody No. DJB-19-88 were utilized according to a modified procedure of Niswender et al. (1969).

The data indicates that the activity of the saline supernatant was equivalent to 7.1 millimicrograms LH per ml of uterine extract; however, it is felt that the assay does not have a high degree of reliability above 80%. As a result, only those values obtained from 500 microliters through the 100 microliters of sample (tubes 7 to 21) were confidently accepted. Therefore,

under these limitations it is suggested that the saline supernatant of the acetone extracted uterine powder exhibited a mean activity equivalent to 5.52 millimicrograms LH per ml.

It should be noted that the flocculi was present in the saline supernatant administered to the various test animals but was removed for the assay. It is the belief of the author that a portion of the activity was present in the flocculi and therefore the mean value of 5.52 millimicrograms LH per ml of uterine extract supernatant should be considered as a minimum rather than a maximum value.

Furthermore, the acetone extracted uterine powder was analyzed for follicle stimulating hormone (FSH) content by a modified method of the HCG Augmentation bioassay of Steelman and Pohley (1953).

Eight assay animals received subcutaneous injections twice daily for three days of saline supernatant from two grams of the uterine powder reconstituted in 15 ml of sterile saline. The remaining 12 rats received either one of three dosage levels of FSH (NIH-FSH-S-6) or saline alone. All rats received a total of 30 IU HCG.

The uterine extract was administered at a dosage of 0.50 ml per injection whereas the FSH was administered at levels of 10, 40 and 60 micrograms per injection (total FSH of 0.06, 0.24 and 0.36 milligrams per rat) and the sterile saline at 0.50 ml.

The uterine extracts were assayed concomitantly with sheep

pituitary extracts from another experiment. The ovarian weights of the rats treated with the pituitary extracts exhibited a marked increase due to the FSH content whereas the data in Table 3 indicates that the saline supernatant of the uterine extract was equivalent to the zero FSH controls.

Niswender et al. 1969. Endocr. 84:1166.

Steelman, S. L. and F. M. Pohley. 1953. Assay of the follicle-stimulating hormone based on the augmentation with human chorionic gonadotrophin. Endocr. 53:604.

TABLE 1. DETERMINATION OF LH ACTIVITY IN ACETONE EXTRACTED UTERINE POWDER BY RADIOIMMUNO ASSAY

Tube	Uterine extract (mul)	Egg white albumen (mul)	cpm	cpm corrected $t_{\frac{1}{2}}$ of I^{131}	% of zero LH	mug LH/tube	mug LH/ ml extract	Mean value/ dose level (mug/ml)
1			20,263	48,621	183.3			
2			28,188	67,731	255.3			
3			99,965	60,022	262.3			
4			2,689	1,380	5.2			
5			2,171	1,068	4.0			
6			1,205	1,068	4.0			
7	500	0	5,283	11,326	42.7	3.45	6.90	4.70
8			27,904	15,409	58.1	1.72	3.44	
9			26,982	14,853	56.0	1.88	3.76	
10	300	200	28,252	15,618	58.9	1.69	5.63	4.27
11			33,821	18,976	71.5	0.90	3.00	
12			7,759	17,297	65.2	1.25	4.17	
13	200	300	7,591	16,892	63.7	1.35	6.75	6.30
14			7,655	17,046	64.2	1.30	6.50	
15			7,957	17,774	67.0	1.13	5.65	
16	150	350	8,173	18,295	69.0	1.04	6.93	6.14
17			8,093	18,102	68.2	1.08	7.20	
18			8,848	19,923	75.1	0.64	4.30	
19	100	400	8,230	18,433	69.5	1.01	10.10	6.20
20			8,919	20,094	75.7	0.71	7.10	
21			10,891	24,849	93.7	0.14	1.40	
22	75	425	8,786	19,773	74.5	0.76	10.10	6.59
23			11,930	27,355	103.1	0.04		
24			10,496	23,897	90.1	0.23	3.07	

Continued TABLE 1. DETERMINATION OF LH ACTIVITY IN ACETONE EXTRACTED UTERINE POWDER BY RADIOIMMUNO ASSAY

Tube	Uterine extract (mul)	Egg white albumen (mul)	cpm	cpm corrected $t_{\frac{1}{2}}$ of I^{131}	% of zero LH	mug LH/tube	mug LH/ ml extract	Mean value/ dose level (mug/ml)
25	50	450	10,624	24,205	91.2	0.14	2.80	7.53
26			9,713	22,009	83.0	0.48	9.60	
27			9,473	21,430	80.8	0.51	10.20	
28	25	475	11,985	27,487	103.6	0.04		5.08
29			10,633	24,227	91.3	0.19	7.60	
30			11,210	25,618	96.6	0.06	2.56	
31	10	490	10,927	24,936	94.0	0.12	12.30	10.15
32			13,710	31,647	119.3	0.04		
33			11,120	25,401	95.7	0.08	8.00	
34	5	495	11,285	25,799	97.2	0.05	10.60	14.10
35			11,086	25,319	95.4	0.09	17.60	
36			9,472	21,427	80.8	0.51	102.00	
37	0	500	10,113	22,973				
38			12,040	27,620				
39			11,191	25,573				
40			10,884	24,832				

TABLE 2. STANDARD CURVES FOR LH(NIH-LH-S15)

Tube	mug LH/ml[1]	cpm	cpm corrected for $t_{\frac{1}{2}}$ of I^{131}	% of zero LH	mug LH/ml
1	0	8,587	18,915		
2	0	7,566	16,461		
3	0	8,177	17,930		
4	0.4	6,403	13,667	76.9	
5	0.4	1,794	2,593	14.6*	
6	1.0	4,866	9,974	56.1	
7	1.0	1,003	695	3.9*	
8	4.0	7,136	15,428	86.8	
9	4.0	6,023	12,754	71.8	
10	10.0	5,956	12,593	70.9	
11	10.0	4,287	8,583	48.3	
12	40.0	2,693	4,753	26.7	
13	40.0	1,838	2,699	15.2	
14	100.0	2,229	3,638	20.5	
15	100.0	1,616	2,165	12.2	
16	400.0	1,298	1,401	7.9	
17	400.0	932	522	2.9	
18	S131	8,245	18,093	101.8	
19	S131	8,738	19,277	108.5	
20	S123	4,261	8,521	48.0	25.0
21	S123	2,971	5,421	30.5	29.0
1	0	9,189	21,733		
2	0	8,478	19,917		
3	0	8,684	20,443		
4	0.4.	6,733	15,461	74.7	
5	0.4	1,386	1,807	8.7*	
6	1.0	4,371	9,430	45.6	
7	1.0	1,129	1,151	5.6*	
8	4.0	6,997	16,135	78.0	
9	4.0	5,574	12,502	60.4	
10	10.0	5,915	13,372	64.6	
11	10.0	4,454	9,641	46.6	
12	40.0	2,581	4,859	23.5	
13	40.0	2,078	3,574	18.1	
14	100.0	2,449	4,521	21.8	
15	100.0	1,622	2,410	11.6	
16	400.0	1,317	1,631	7.9	
17	400.0	1,230	1,409	6.8	
18	S131	7,900	18,441	89.1	
19	S131	7,622	17,731	85.7	
20	S123	4,041	8,587	41.5	34.0
21	S123	2,770	5,341	25.8	35.0

[1] NIH-LH-S15

*Values considered not acceptable; therefore, not used in calculations

TABLE 3. DETERMINATION OF FSH ACTIVITY IN ACETONE EXTRACTED UTERINE POWDER BY HCG AUGMENTATION BIOASSAY

Group	Total Ovarian Wt.[a] (mg)	Mean Ovarian Wt. (mg)
Saline	51.9	
	52.8	54.3
	58.1	
0.06 ug FSH	172.6	
	153.7	127.9
	120.9	
	64.2	
0.24 ug FSH	163.3	
	154.0	185.6
	196.4	
	228.6	
0.36 ug FSH	163.1	
	259.0	222.9
	232.1	
	237.2	
Uterine Extract	46.7	
	50.9	
	57.9	
	64.3	52.8
	57.1	
	50.0	
	56.1	
	39.1	

[a]Weight of both ovaries

THE EFFECTS OF A BOVINE UTERINE POWDER ON THE CORPORA LUTEA OF PSEUDOPREGNANT RABBITS, RATS AND HYSTERECTOMIZED GUINEA PIGS

by

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A series of experiments were conducted to determine the effects of an acetone-dried, early luteal bovine uterine powder on the corpora lutea of pseudopregnant rabbits, rats and hysterectomized guinea pigs.

Uteri were collected from seven normal cycling cows 3 to 5 days after estrus. Each uterus was coarsely ground, acetone extracted, air-dried and Wiley-milled to powder form. Ammonium sulfate fractionation (50 and 100% SAS) was performed on powder from two uteri. Furthermore, an aliquot of the acetone dried uterine powder was heated to 225 F for a period of one hour.

Thirty-two pseudopregnant New Zealand White rabbits were randomly alloted to 1 of 5 groups and injected either subcutaneously or intraperitoneally with 0.5 or 1.0 gram of the various uterine preparations in sterile saline or saline alone on Days 5 to 9 or 6 to 10 of pseudopregnancy and sacrificed on Day 10 or 11. Mean corpus luteum weights for those receiving acetone-dried powder, saline, 50% SAS, 100% SAS and heated uterine powder were 15.32, 18.24, 18.01, 19.89 and 15.42 mg, respectively. Six rabbits that died or exhibited anaphylaxis during the treatment had a mean corpora lutea weight of 9.70 mg. Although the bovine uterine powders did not significantly decrease corpora lutea weight in the rabbit, histological evidence of regression was observed. This regression appeared to begin around blood vessels and capillaries and progress outward indicating the presence of a substance in the blood that was detrimental to the life of the luteal cells.

Pseudopregnancy was induced in 20 mature albino rats and designated as Day 0. On Day 4, one uterine horn was traumatized to induce the decidual cell response (DCR). Ten rats were injected intraperitoneally with 0.5 cc, twice daily, of a saline supernatant of the acetone extracted uterine powder, whereas the ten controls received saline alone, on Days 5 to 8 of pseudopregnancy. The acetone extracted uterine powder did not appear to be toxic or luteolytic in the rat. A 374.16 mg mean increase was noted in the weight of the traumatized horn of injected rats. This value approached significance at the $P<0.05$ level. Furthermore, 1.0 ml of the saline supernatant from the acetone extracted uterine powder depleted ovarian ascorbic acid in immature rats equivalent to 2.75 ug LH.

The uterine powder was further tested in 28 guinea pigs hysterectomized on Day 10 post-estrus. Fifteen animals were injected intraperitoneally with 1.0 ml of the saline supernatent of the acetone extracted bovine uterine powder while 13 received saline alone on Days 12 to 16. Treatment regressed corpora lutea in these animals as evaluated morphologically on color and size. The number of luteal cells per microscopic field was decreased from 13.25 in the controls to 5.13 ($P < .005$).

From these data it is proposed that the physiological action of the uterine powder was due to the presence of a blood-borne, protein-like, water soluble and heat labile substance that exhibited LH-like properties. The results of this series of experiments were observed to have many parallelisms with those results obtained by Pharriss (1968) with prostaglandin F_2^{α}, a potent venoconstricting agent.