THE INFLUENCE OF PROGESTINS ON CORPORA LUTEA OF THE RAT

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ABSTRACT

THE INFLUENCE OF PROGESTINS ON CORPORA LUTEA OF THE RAT

by Abubakar A. Shaikh

Normal cycling rats, hysterectomized rats and pregnant rats were treated with various dosage levels of 6-α-methyl-17-acetoxy progesterone (MAP) daily by oral route. Corpora lutea were maintained in all the animals tested. It was demonstrated that daily oral administration of MAP commenced at any stage of the estrous cycle successfully maintained the most recently formed corpora lutea in a histological functional state. MAP apparently also had a stimulatory influence on the corpora lutea of previous ovulations. The oral administration of 6-chloro-17-α-acetoxy progesterone (CAP) and subcutaneous administration of crystalline progesterone daily did not maintain corpora lutea in a histological functional state.

Daily vaginal smears were obtained from intact rats treated with MAP and crystalline progesterone. There was no suppression of the cyclic occurance of the estrogenicity as indicated by vaginal smears. However, the recurrence of estrogenicity was not rhythmical and could not be predicted.

Similar observations were made when ovariectomized rats were treated with MAP.

A successful decidual response was obtained in intact or ovariectomized rats on a dosage level of MAP capable of maintaining corpora lutea. There was considerable variation in response to uterine trauma, which could be correlated to the levels of endogenously secreted estrogen at the time of trauma. Further support for this conception was obtained when it was noted that the rats which exhibited a massive decidual response had an estrogenic type smear on the day their uteri were traumatized.

In conclusion it can be stated that daily administration of MAP maintains corpora lutea in rats beyond their expected functional life. The estrogenic surges as evidenced by the vaginal smear does not subside completely under progestin treatment in intact or ovariectomized rats. It is postulated that uterus itself might be the source of this phenomenon.

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By

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

The series of studies described in this thesis were initiated subsequent to the observation that an orally administered synthetic progestational agent apparently had the ability to induce maintenance of corpora lutea of rats beyond their expected functional life. This observation was of considerable interest since it did not appear to conform to a normal or "reciprocal control mechanism" of endocrine gland control. This mechanism implies that the products or hormones secreted by an endocrine organ either directly or indirectly regulate further production of that hormone (84). There are no obvious exceptions to this rule to be found in the literature. In fact, normal or high levels of hormone in the blood tend to suppress further production of the hormone and often result in slight or marked involution of the endocrine gland. An apparent anomaly existed with the progestational agent under discussion, since it could if administered at any stage of the estrous cycle support the structural integrity of the corpora lutea far beyond the brief period of two to three days noted in the normal estrous cycle.

It is appropriate, therefore, in the introduction to this thesis to consider the many factors that are known to

influence the functional life of the corpora lutea of a variety of animals; the relationship of the synthetic progestational steroids utilized in these studies to natural progestins, the possibility of their conversion to other gonadal mimetic hormones and the various means available for researchers to estimate morphological and functional maintenance of the corpus luteum when animals are being administered steroid.

Life of the Corpus Luteum

The functional life of the corpus luteum varies in different species and is the primary factor determining the length of the normal reproductive cycle. In the four to five day estrous cycle of the rat and mouse the corpora lutea are essentially non-functional and do not secret appreciable amounts of progesterone (69,2). In animals exhibiting longer reproductive cycles, as the primate (139), ewe (43), goat (50), mare (49), sow (26), or cattle (46), the corpora lutea secrete progesterone for a considerable portion of the cycle. In any one cycle the follicular phase of the cycle remains relatively constant (three to six days) and the difference in cycle lengths can be attributed to differences in secretory life of the corpora lutea. In the primate and domestic animals, where the total cycle length is from sixteen to twenty-eight days, the functional life of the corpora lutea is approximately thirteen to eighteen days,

whereas, in the dog, with a cycle length of six months, the functional life of the corpora lutea is approximately two months (74,38). In animals, as the rat and mouse, with very short estrous cycles, or in animals that do not ovulate spontaneously, false mating or stimulation of the genital tract by a variety of means may result in "pseudopregnancy," in which case the animal ovulates and the corpora lutea resulting from this ovulation become functional (69,65,120). The length of time from the induction of pseudopregnancy to the next spontaneous ovulation now approaches an interval that approximates animals demonstrating a functional luteal phase in their normal estrous cycle, somewhere between fourteen and nineteen days. It can, therefore, be concluded that, with the exception of the domestic dog, primates and the majority of the domestic animals in the non-pregnant state maintain corpora lutea for a maximum of fourteen to nineteen days.

The maintenance of corpora lutea in the domestic and wild animal species studied is prolonged over that of the normal reproductive cycle if conception occurs following ovulation. It cannot be stated, however, as with the length of the estrous cycle, that the differences in the length of gestation among animals can be attributed to the difference in the functional life of the corpora lutea of pregnancy. Although corpora lutea of pregnancy outlive those of the normal estrous cycle, they do not necessarily persist in a

functional state until physiological term. There is marked regression of the corpora lutea prior to the last trimester of pregnancy in man (131), baboons (140), cats (27), horses (25, 64,4), and sheep (43). Progesterone is necessary for maintenance of pregnancy in these animals but it is produced in extra-ovarian sites, primarily in the placenta (3, 5).

Following parturition and assuming livability of the young, the act of nursing in several species is associated with the development and maintenance of functional corpora lutea in the ovaries. These corpora lutea are not the corpora carried throughout the term of pregnancy but result from ovulations subsequent to parturition (28,80,27). Thus, in the cat, mouse, and rat corpora lutea are maintained in the ovaries during the period of lactation and this is associated with a period of lactation anestrus or infertility. A lactation anestrous does exist in the sow, but this is not concomittant with the presence of functional corpora lutea in the ovaries. Although estrus without ovulation may occur a few days after parturition in the sow new corpora lutea are not formed and the corpora lutea of pregnancy undergo regression (130).

Luteotrophic Factors

Four essentially different physiological states are therefore normally associated with functional activity of corpora lutea: i.e., non-pregnant reproductive cycling

activity; limited or extended functional corpora lutea of pregnancy; corpora lutea of pseudopregnancy and corpora lutea of lactation. Are these related in the sense that they only represent quantitative differences in the factors responsible for the maintenance of secretory activity of the corpora lutea? This would appear to be an acceptable thesis. It is generally agreed that the life of the corpora lutea under each of these conditions is regulated by a gonadotropic hormone from the anterior pituitary. In regard to follicular growth, ovulation and establishment of the corpora lutea, researchers are in general agreement with the following concepts. Follicular growth in the ovaries is believed to be stimulated by a follicle stimulating hormone of anterior pituitary origin (82). This hormone will not stimulate final maturation or ovulation of the follicle, but development of the follicle to the tertiary stage (82). An additional hormone, luteinizing or interstitial cell stimulating hormone (LH-ICSH), brings about the final maturation of the follicules and whatever changes ensue in the process of ovulation (84, 63). From this development onward there is considerable controversy. There are diverse mechanisms proposed for the formation and maintenance of corpora lutea, the main differences being noted between the rodents and other species. In normal cycling rats, following ovulation, the corpora lutea are histologically similar to functional corpora lutea of other animals secreting sufficient progesterone to block

follicular growth in the ovaries. However, the rat does not secrete appreciable amounts of progesterone from the corpora lutea when in the non-pregnant condition. It would appear that in the rodent, unlike other species studied, there are separate hormones responsible for structural integrity and functional activity of corpora lutea. ICSH stimulates final maturation of follicles, ovulation and development of corpora lutea but not, the functional activity of the corpora lutea. On the other hand in the majority of the other animals it appears that ICSH is not only ovulation provoking and luteinizing but luteotropic. That is, it induces ovulation, development of the corpora lutea and secretions of progestins by the corpora lutea (41, 55, 64, 57, 17, 77, 60). In the rodent, at least one other hormone of pituitary origin is necessary to stimulate the fully developed corpora lutea to secrete progestin (39,92), prolactin or lactogenic hormone. It is, therefore, of considerable importance when discussing the hormone responsible for the secretion of progestins by corpora lutea not to use the term luteotrophic hormone loosely, since it implies ICSH in the majority of animals and lactogenic hormone in rodents. The length of life of the corpora lutea of normal cycling animals can therefore be attributed to the presence or absence of a luteotrophic stimulus to the ovaries. This information, however, arises from proof obtained indirectly.

Prolactin is not luteotrophic in the rabbit (77), monkey (55,19), or man (64,57,17). Moor and Nalbandov (81,90), however, reported that when prolactin was injected into anestrous ewes in which corpora lutea had been artificially induced, progesterone secretion resulted, whereas similar prolactin treatment in ewes with corpora lutea of spontaneous ovulation induced progesterone secretion in only 50% of the treated animals.

Marked variation between species has been reported with respect to the gonadotropic activity of the adenohypophysis during pregnancy; it has been reported to increase, decrease or to show little or no change. Within species, the results have not always been consistent primarily due to different procedures used in assaying the glands. In a notable example, the pituitary glands of pregnant cattle were observed to contain increased gonadotropic content over those of non-pregnant animals (13), but in a later study (89) the potency was found to decline steadily throughout pregnancy. Similarly it was reported (104) that the gonadotropic potency of the pregnant sow decreases throughout gestation. There is agreement that in man the pituitary gonadotropic potency falls during the second month of gestation and remains barely detectible until a few days post partum (18). It has been assumed in many that a placental gonadotropin (HCG) prolongs the luteal production of hormones until the placenta becomes capable of secreting the high amounts of

gonadal steroids required for maintenance of pregnancy. The pregnant mare, however, also has a rich extrapituitary supply of gonadotropin during pregnancy yet shows no decline in pituitary gonadotropic potency (53).

Extensive research on pseudopregnancy and luteal function has been restricted to the rat. Sterile mating and several other procedures involving neural stimulation will induce rats to become pseudopregnant. Stimulation of the cervix by mechanical means (69) or electric shock (119) have become standard methods. Pseudopregnancy is also invoked by continuous stimulation of the nipples for several days (118). Each of these stimuli is believed to result in release and increased blood levels of luteotropic hormone (LTH), which activates the corpora lutea.

It has been reported in non-suckled lactating mice, that injections of oxytocin retard the involution of mammary glands, whereas there was complete involution of the mammary glands in control groups receiving saline injections (15). Since it had been reported earlier that prolactin retards mammary involution (143), it was concluded that oxytocin stimulates prolactin release from the hypophysis.

Estrogens directly or indirectly prolong the corpus luteum function. Estrogens have a direct luteotropic action in the rabbit (102,103,47). The effect does not depend on the hypophysis and has been produced by implantation of estrogen crystals within corpora lutea (48,47). However, the

important influence of estrogen on the corpora lutea of rats is largely indirect and requires the presence of hypophysis. Massive treatment with estrogen, commencing soon after ovulation in rats, results in the enlargement of the corpora lutea and the production of sufficient amounts of progesterone to mucify the vaginal mucosa (117,133,82). It has also been reported that continued estrogen administration in normal rats resulted in diestrous periods of varying lengths (66, 72,73). These effects are now assumed to be the result of estrogen induced liberation of hypophysial luteotropin. Similar effects have been reported after administration of androgens (79,134). Recently it has been reported that injections of stilbesterol (1 to 3 mg.) during luteal phase in sows, suppressed development of ovarian follicles and prolonged the life of the corpus luteum (93).

Progesterone is postulated to influence the gonadotropic function of the anterior pituitary gland in two ways. It is assumed that progesterone promotes the secretion of LTH (positive feed back) (107,109), thus causing persistence of the pseudopregnant state, at least for some time. Secondly, it may decrease the secretion of FSH and LH and hence retard follicular maturation and inhibit ovulation (99,100). Recently it has been reported that progesterone treatment or pseudopregnancy increases pituitary FSH and LH levels. This increase has been attributed to inhibited release of both gonadotropins (101).

Luteolytic Factors

The consideration so far has been with direct or indirect luteotropic factors affecting the corpora lutea. There is also good evidence that luteolytic factors play a role in the life of the corpora lutea.

The period of pseudopregnancy in rats is from ten to fourteen days. The state of pseudopregnancy can be further extended approximately six days by hysterectomy (17,52,18). In cycling guinea pigs, the life of the corpus luteum is prolonged if hysterectomy is performed following estrus (67,111). These corpora lutea are maintained as long as three months, a period equal to that of pregnancy. Hysterectomy on the seventh day of the estrous cycle in gilts prolonged the life of the corpora lutea for a duration approximating the period of gestation, whereas hysterectomy in pregnant gilts (seventh to thirtieth day of pregnancy) resulted in maintenance of the corpora lutea for a length of time exceeding the average gestation period (126,33). Similar results have been obtained in ewes and heifers (132).

Various hypothesis have been suggested on the mechanism responsible for the regression of the corpus luteum. It has been reported that LH is responsible for the anatomical disappearance of the corpus luteum (20,44,45,30), since persistence of large corpora lutea in the hypophysectomized rat

is curtailed by the administration of gonadotropic preparations with luteinizing properties.

It has been reported that oxytocin is capable of inhibiting the development and function of the corpus luteum in dairy heifers (8,141). The corpora lutea formed during oxytocin treatment failed to attain the expected size, contained a decreased number of normal luteal cells and an increased proportion of connective tissue elements.

The luteotropic influence of progesterone has been discussed. However, a more important property of progesterone is usually considered to be its luteolytic action. Progesterone is reported to cause the regression of the corpus luteum by inhibiting the release of luteotropic agent from the hypophysis (115,138,70). Evidence for this conclusion is based upon the following observations. Complete regression or great reduction in size of the corpora was observed when large doses of progesterone were administered to swine, sheep and heifers during pregnancy. It is postulated that the extent to which the corpora lutea are damaged may depend upon the time of pregnancy when injections are administered to gilts (115), ewes (138), and heifers (70). However, no gross or microscopic effects were noted, when progesterone was administered to pregnant rats (114,1). Treatment with progesterone in mice one or two days after mating, however, resulted in luteal regression (21). Luteal regression was also reported in pregnant as well as

hysterectomized gilts, where the treatment was started the tenth to twenty-fifth day following mating (126). This study indicates that progesterone action is independent of participation of the uterus.

A luteolytic endometrial factor is also apparently involved in the regression of the corpus luteum (22,21,110,634). It has been reported that the initiation of luteal regression during pseudopregnancy is shortened as the amount of non-traumatized endometrium is increased (81). An inverse relationship between the quantity of uterine endometrial autotransplants and the duration of pseudopregnancy, suggests a production of luteolytic substance by the endometrium. When the endometrium is converted to decidual tissue, the ability to produce a luteolytic substance is not present (81).

A neural factor has also been implicated in determining the life of the corpus luteum (116,85,54). In sheep the implantation of plastic beads in uterus during the early luteal phase shortened the cycle by several days. Successive cycles tended to be unusually short. When the uterine segments containing the beads were denervated, however, the cycles were essentially normal suggesting that the uterus influences the ovary by way of the nervous system (85).

Measurement of Corpus Luteum Function

Morphological criteria of corpora lutea have not been closely correlated with secretory activity. In the rat the corpora lutea can be of functional size and yet in the absence of prolactin the gland does not secrete progesterone (39). On the other hand, in animals where ICSH is believed responsible for both structural and functional maintenance, it is generally assumed that a fully developed gland maintained for any length of time is secreting progesterone. In the present study, it was of considerable interest to determine whether corpora lutea maintained in the face of high levels of exogenous progesterone treatment were secreting progesterone. There are several methods available to estimate the secretory activity of the corpora lutea and they include direct ovarian venous or systemic blood assay of progesterone or its metabolic products; histological and histochemical estimation of steroid procursors in the corpora lutea and estimation of changing levels of circulating progesterone as evidenced by progestational influence in genital tract proliferation or vaginal cytology.

By far, the most desirable method of estimating secretory activity of the corpora lutea would be to determine the amounts of progesterone released from the ovary into the ovarian vein (37), and second to assay progesterone content in the systemic circulation. This second method, however, raises a problem since endogenously and exogenously

administered progesterone disappear from the blood very quickly in the human female and in laboratory animals (dog, rabbit and mouse (24,95)). A third method is to determine the amount of progesterone present in the luteal tissue (35). Unfortunately, these procedures were not available to the candidate.

A consideration of the various biological actions of progesterone provide several methods of estimating the amounts of progesterone to which the animal is being exposed. Several methods have been utilized.

(1) Vaginal cytology is generally used by most investigators, especially when the experimental animal is a rat. The presence of a smear typical of diestrous, i.e., basophilic squamous epithelial cells and leukocytes, is indicative of the presence of functional corpora lutea.

(2) As mentioned earlier, the histological appearance of the corpus luteum is not always a useful criterion to estimate functional activity. For example, the sheep corpus luteum synthesizes and releases progesterone into the ovarian vein for almost the entire duration of the cycle (111), and yet this is not reconciled with the histological examination of the corpus luteum, since degenerative changes are noted as early as day ten, and by day fourteen the luteal cells are very atrophic. In the guinea pig, however, the progesterone concentration begins to decline on day six and this coincides with the histological evidence of degeneration of the luteal cells (111).

(3) There are histochemical techniques consisting of Oil Red O staining for fat or Sudan IV Stain and Schultz stain for cholesterol and its esters. It has been reported that LH is responsible for the mobilization and deposition of cholesterol in the corpora lutea of rats. However, in the rat at least, unless there is LTH released, the corpora lutea do not produce progesterone (146). Corpora lutea of the normal estrous cycle are Schultz negative as are the corpora of pregnancy in rats. These corpora can be rendered Schultz positive by a single injection of LH. Thus, it is clear that the presence of the precursors of progesterone, cholesterol and its esters, does not always estimate the functional ability of the corpora.

(4) The influence of endogenous progesterone on the genital tract has been utilized to estimate the corpus luteum function. Under the influence of progesterone the endometrium increases in thickness, the deep uterine glands grow rapidly in diameter and length, become extremely branched and convoluted. However, there is a lag, probably of from one to three days (188), between the time when the corpus luteum begins to secrete its hormone and the time the effects of this hormone are seen on the endometrium. Similarly, the corpora wane long before the effect of the decline of progesterone is noted on the uterus. It is known that in pseudopregnant rats, when the uterus is traumatized on the fourth day, there is an induction of corpus luteum function

(69,29). However, in this case, the uterus is sensitive only on day four of the estrous cycle and the presence of the decidual growth further stimulates maintenance of the corpus luteum.

The substance of this thesis is on the influence of progestins on maintenance of corpus luteum function. Progestins include a variety of steroids that exhibit some or all of the biological properties of progesterone, regardless of whether they are natural gonadal hormones, derivatives of testosterone, adrenal hormones or synthetic steroids. Progesterone is the principal progestational substance secreted by the corpus luteum. Although the luteal cells of the ovulatory corpus luteum are generally accepted as the source of ovarian progesterone in the non-pregnant female, progesterone secretion can result from luteal transformation of preovulatory follicles (19,3), and from the corpora lutea of the preceeding cycle, as appears to be the case in the sheep (105,106). Progesterone secretion from non-luteinized ovarian tissue has been demonstrated by the progesterone content of follicular fluid (sow, cow, rabbits and women) (59,40,36) and cyclic preovulatory changes in the progesterone content of peripheral and ovarian venous blood (51).

In the human, the chimpanzee, goat and rabbit (139), progesterone is metabolized to biologically inert pregnanediol and excreted into the urine as pregnanediol glucuronidate. In other animals (rodents and probably monkey) progesterone

is apparently eliminated by other routes, since pregnanediol appears only in traces in the urine (139). It is now clearly established that pregnanediol, like estrogen, persists in the urine after ovariectomy add that in subjects with healthy adrenals pregnanediol excretion is increased by administration of corticotropin (62,87,11). Significant quantities of progesterone are also produced by the adrenal cortex (14,110), placenta (113,122) and testes (68). In all steroid synthesizing organs progesterone serves as a precursor in the synthetic pathways for formation of hormones with androgenic (32), estrogenic (124,112), glucocorticoidal and mineralocorticoidal (31) activity. Interpretation of results following administration of progestational agents, therefore, whether the natural steroid or one of the many synthetic compounds cannot obviate the possibility that the resulting physiological phenomena are the result of other than normal progestational activities (97).

In recent years synthetic progestational compounds have become available, some very similar to progesterone in structure and others modifications of natural glucocorticoids and androgens. The advantage of utilization of synthetic steroid is not only due to the availability and lower price of synthesis but to the increased effecacy and potencies by both parenteral and oral administration.

It has been reported that 6-α-methyl 17-α-acetoxy progesterone (MAP) is a highly active progestin based

on its ability to promote a secretory endometrium and to prevent ovulation in rabbits. Orally, 6-α-methyl-17-α-acetoxy progesterone is sixty to seventy-five times as active as 17-α-acetoxy progesterone in the McPhail assay (98). The potency of the six methyl compound in inhibiting ovulation in rabbits is at least twenty times that of subcutaneously administered progesterone while 17-α-acetoxy progesterone is seven times as active as progesterone (98,12). Either orally or parenterally, 6-chloro-17-acetoxy progesterone (CAP) is ten to forty times more potent than MAP.

While most other synthetic steroids have inherent estrogenic or androgenic properties, as far as is known MAP (98) and CAP (61) have no estrogenic or androgenic effects.

In summary, gonadotropins, estrogens, progesterone and the uterus have a pronounced influence on corpora lutea function. The introduction commenced with the observation that in the current studies administration of a progestational compound resulted in maintenance of corpora lutea. This observation is not entirely without precedence since under very limited conditions crystalline progesterone injection has been reported to stimulate maintenance of corpora lutea (109). The purpose of this study is to further clarify the role of exogenous progestins in the maintenance of the corpus luteum.

METHODS AND PROCEDURES

Mature cycling Long-Evans strain rats from the Endocrine Research Unit were utilized in these studies. The rats were housed under controlled lighting conditions of twelve hours of light and twelve hours of darkness at a controlled mean temperature of 72 - 73 degrees Farenheit.

The synthetic progestational compounds (6-α-methyl 17-acetoxy progesterone, "MAP"[1] and 6-chloro-17-acetoxy progesterone, "CAP"[2]) were administered in the daily ration. Mature cycling rats in the colony consumed a minimum of ten grams of feed per day.[3] The feed was prepared in order that each ten grams of feed contained the desired levels of crystalline MAP or CAP. Crystalline progesterone dissolved in corn oil was administered subcutaneously. Animals being treated with MAP and CAP were housed in individual cages throughout the experiment.

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[1] Supplied by the Upjohn Company, Kalamazoo, Michigan.

[2] Supplied by the Eli Lilly Company, Indianapolis, Indiana.

[3] Mature cycling rats weighing 200 to 250 grams were placed in individual cages or in groups of five and their daily feed consumption was recorded. These rats consumed from ten to fourteen grams of feed daily. It was on this basis that experimental rats were allotted 10 gm. of ration per day.

In order to study changes in vaginal cytology, vaginal smears were obtained with a standard laboratory spatula and spread on a clean histological slide. Whenever the smears could not be obtained, during diestrous periods or during progestational treatment, because of the dryness of the vaginal epithelium, the tip of the spatula was coated with a drop of water to moisten the vagina. The smears were immediately fixed in a solution of ether and ninety-five percent alcohol (1:1 by volume). After a period of not less than five minutes or more than three days, the smears were removed from the fixative and carried through the Papanicolau's staining procedure.

Vaginal smears were routinely obtained for at least one complete estrous cycle prior to treatment in order to establish normal cyclic ovarian activity in the experimental animals. During the next estrous cycle, at the time of estrus, the rats were separated into individual cages. On the following day rats not showing a metestrous vaginal smear were assumed not to have ovulated and were eliminated from the study.

When the time of ovulation had been estimated by vaginal cytology, the newly formed corpora lutea were marked by the following technique: The ovaries of anesthetized rats were bilaterally exteriorized through flank incisions. The fresh corpora lutea were exposed by rupture of the ovarian bursa and the tip of a twenty-seven gauge needle

previously dipped into India ink was inserted to approximately the center of each corpora. The India ink marks were easily identifiable in the ovary for at least 22 days.

Hysterectomy was performed through a mid-line incision on the abdomen under ether anesthesia. Post-operative rats were allowed a recovery period of five days. Normal post-operative ovarian activity was estimated by vaginal cytology and only rats showing a normal cyclic ovarian activity were utilized.

In order to obtain pregnant rats, female rats placed in a cage with male rats were examined daily for the presence of a copulation plug or sperms in the vaginal smear. The day on which a copulation plug in the vagina or sperm in the smear was observed was recorded as day one of pregnancy.

An endometrial scratch method was used as a deciduous inducing stimulus. The rats were anesthetized with ether and one horn of the uterus was exteriorized through a mid-line incision. A 20 gauge one and one-half inch long needle was inserted into the lumen at approximately the middle of the length of the uterus. The needle was then guided the entire length on both the ovarian and cervical end of the uterus and trauma was inflicted on the endometrium by scratching with the tip of the needle as the needle was withdrawn.

At the termination of the hormone treatment, rats were sacrificed with chloroform. During autopsy, body and

adrenal weights, and a record of the gross appearance of the ovaries were obtained. The ovaries and uteri were removed for histological examination.

The ovaries utilized for cholesterol and lipid content measurements were quick frozen by placement on the quick freeze bar in the cryostat chamber. They were sectioned at 20 to 30 micra and stained with Oil Red O fat stain (74) or Schultz's stain (95). Tissues prepared for routine histological examination were fixed in Carnoy's fixative, embedded in paraffin, sectioned at 5-7 micra and stained with Papanicolau's stain or Hematoxyline and eosin (83,7).

Statistical analysis of the data were made using the "t" test in case of equal variances and the modified "t" test when the variances were not the same.

Experiments

Experiment 1 was designed to determine the influence of oral administration of 10, 15 or 18 mg. of MAP/day on corpora lutea maintenance. Twenty-two rats were utilized in this experiment, 6 rats received 18 mg. of MAP/day, 8 rats 15 mg. of MAP/day, and the remaining 2 rats 10 mg. of MAP/day for a period of 6 to 10 days. At the time of metestrus, as indicated by vaginal cytology, the ovulatory corpora lutea were marked with India ink, and the rats were placed on steroid treatment. The animals were autopsied while on treatment, 8 to 10 days later (Table 1).

The results of experiment 1 suggest that less than 10 mg. of MAP/day was sufficient to maintain the ovulatory corpora lutea. In order to estimate the minimum effective levels of orally administered MAP for corpora lutea maintenance, a dosage range of 3, 2, 1 and 0.5 mg. of MAP/day was administered in experiment 2. A total of 29 cycling rats were utilized. The corpora lutea were marked with India ink on the day that the vaginal cytology indicated that the rats were in metestrus. On the same day the rats were placed on steroid treatment for a period of 8 to 22 days. The animals were autopsied while on treatment 8 to 22 days later (Table 1).

Experiment 3 was designed to compare the influence of daily administration of MAP and subcutaneously injected progesterone on corpora lutea maintenance. Twenty-three rats were subcutaneously administered 5, 10, and 15 mg. progesterone in oil per day starting on the day of metestrus for a period of from 5 to 10 days. An additional 10 rats were orally administered 5 mg. of MAP/day for 10 days. The animals were autopsied while on treatment 5 to 10 days later (Table 1).

Experiment 3 demonstrated that subcutaneously administered progesterone in amounts as high as 10 mg. was effective in maintenance of the corpora lutea for only a short duration of time.

Experiment 4 was designed to determine the efficacy of 6-chloro-17-acetoxy progesterone (CAP) on corpora lutea maintenance. Since CAP is reported to be a more effective progestational steroid by oral route than MAP for inhibition of ovulation, dosages of CAP lower than those utilized for MAP were administered in this experiment. Ten rats were orally administered 0.277 mg. of CAP/day for 14 days, 15 rats were orally administered 5 mg. of MAP/day for 14 days and an additional 15 rats were orally administered 0.5 mg. of MAP/day for 14 days. The rats were placed on treatment on the day of metestrus. The corpora lutea were not marked. The rats were autopsied while on treatment 14 days later (Table 1).

Experiment 5 was designed to determine whether corpora lutea of pregnancy were influenced by high levels of MAP treatment. Seven pregnant rats, at approximately 10 days of pregnancy, were orally administered 5, 10, or 15 mg. of MAP/day for 8 days. The influence of treatment on the corpora lutea of these animals was compared to non-treated pregnant control rats and to 3 groups of non-pregnant rats receiving 5, 10, and 15 mg. of MAP/day for a period of 8 days. The non-pregnant controls were at the diestrous stage of the estrous cycle when placed on treatment (Table 2).

Experiment 6 was designed in order to determine whether the maintenance of corpora lutea during MAP treatment was dependent upon the presence of the uterus. Twelve

hysterectomized rats were orally administered 15 mg. of MAP/ day for 11 to 22 days, starting at metestrus. Four additional hysterectomized rats, of comparable age and weight, and hysterectomized on the same day were autopsied to obtain control adrenal weights. Autopsy was performed in the treated rats while on treatment, 11 to 22 days later (Table 3).

Experiment 7 was designed to determine the influence of chronic steroid treatment on the return of the rats to normal cyclic ovarian activity, subsequent to treatment. This was estimated by the recurrence of estrus (Table 4).

Experiment 8 was initiated to determine whether a single administration of MAP, by oral route, would result in induction of pseudopregnancy as reported by Rothchild (109) following a single injection of crystalline progesterone. It was also of importance to determine whether the induction of pseudopregnancy was related to the stage of the cycle when the treatment was initiated. Five rats received 5 mg. of MAP at 8:30 p.m. on the evening of the day of proestrus and an additional five rats received 5 mg. of MAP at 9:00 a.m. on the day of estrus. In order to insure immediate consumption of feed, the rats were denied feed for 12 hours prior to treatment. An additional group of 10 rats were administered a subcutaneous injection of 10 mg. progesterone in oil at 9:00 a.m. on the day of estrus. A fourth group of 5 rats received a single dosage of 0.277 mg. of CAP, by oral

route, at 9:00 a.m. on the day of estrus. One uterine horn of each rat receiving a single treatment of 10 mg. of MAP was traumatized with a needle on the fifth day following estrus. The non-traumatized uterine horn served as control (Table 5).

Two criteria were used to determine whether pseudopregnancy was induced by the treatment. (1) The length of diestrous period subsequent to a single administration of the steroid, as estimated by vaginal cytology; (2) Successful induction of a deciduoma in response to endometrial trauma, applied on the fifth day following estrus.

Experiment 9 (Table 6) was designed to determine the effectiveness of MAP on deciduoma induction in response to trauma. Earlier pilot studies demonstrated that rats not receiving progestin treatment following trauma did not develop deciduoma. Three dosage levels were administered, 15 mg., 1 mg. and 0.5 mg. of MAP/day. Animals receiving 1 mg. and 15 mg. level of MAP treatment were placed on treatment on the day of metestrus and their uteri were traumatized while on treatment 4 to 13 days later. They were autopsied 5 days subsequent to uterine trauma while still on treatment. Rats on the 0.5 mg. regime were placed on treatment on the day of estrus. Both uteri were traumatized while on treatment 5 days later and autopsy occurred the third day of post-trauma treatment. It has been reported (29,121) that rats traumatized on the fourth day of pseudopregnancy develop massive

deciduoma. The purpose of this study was to determine if the response to deciduoma inducing stimuli was dependent upon the stage of the cycle when MAP treatment or trauma were initiated. At autopsy, the weights of the adrenal glands were recorded in milligrams per 100 grams of body weight (Table 6).

Experiment 9 (Table 6) suggested that uterine trauma should be induced on the fourth diestrous day in order to obtain a maximum decidual response. Experiment 9, however, did not clarify whether the maximum decidual response was dependent upon the stage of the estrous cycle when treatment was initiated. Experiment 10 was designed to determine the decidual responses resulting during oral MAP treatment initiated on the second or third day following estrus. The rats were administered 0.5 mg. and 10 mg. of MAP/day at metestrus, i.e., second day following estrus and at diestrus, i.e., third day following estrus. One uterine horn of each rat was traumatized on the fifth day following estrus, i.e., third and fourth day respectively following the start of the treatment. The other uterine horn served as a control for each rat. The uterine response was compared to uteri of rats placed on MAP treatment on the day of estrus, i.e., day one, and traumatized on the fifth day after estrus. An additional group of rats received 0.5 mg. and 10 mg. of MAP at various stages of estrous cycle and the uteri were traumatized 8, 9 or 11 days after the initiation of hormone

treatment. The rats were autopsied 4 to 5 days after traumatization of the uterus. Each group of rats remained on hormone treatment until autopsy. Body weights and the weights of the uteri and adrenal glands were obtained at autopsy. Net gain in the traumatized uterine horn was calculated by subtracting the weight of the control horn from that of the traumatized horn (Table 7).

Experiment 11. Experiments 1 and 4 demonstrated that a dosage range of 0.5 mg. to 18 mg. of MAP/day maintained corpora lutea in normal cycling rats. Experiments 9 and 10 demonstrated that normal cycling rats administered 0.5 mg. to 15 mg. of MAP/day exhibited a decidual response following uterine traumatization. However, it was not known whether the progestin dependent deciduoma formation was supported by the exogenous steroid or by progesterone secretion from the maintained corpora lutea, or by both mechanisms. Experiment 11 (Table 8) was designed to determine whether administration of 0.5 mg. of MAP/day would support a deciduoma in ovariectomized rats. Thirty-two ovariectomized rats were placed on 0.5 mg. and 10 mg. of MAP/day, 6 days following ovariectomy. One uterine horn of each of these rats was traumatized on the fifth day after the start of the treatment. Vaginal smears were obtained at the time of trauma in order to determine whether there was any relationship between the condition of the genital tract (estrogenic type smear or non-estrogenic type smear) and extent of decidual response (Table 8).

INTRODUCTION TO RESULTS

The terminology utilized in the result section is based on the gross appearance of corpora lutea as regards size and color and histological description of the luteal cells and other elements within the corpora lutea. It includes an estimation of the uniformity of cytoplasmic staining, cytoplasmic vacuolations, and chromatin patterns within the nuclei of the luteal cells. For these reasons a description of the corpora lutea of normal cycling rats and pregnant rats is outlined in detail.

The Normal Cycling Rat

The normal cycling rat has an estrous cycle of 4 to 5 days. The structures in the ovary at various stages of estrous cycle of concern to this study are as follows:

1. Proestrus: During proestrus, the beginning of the follicular phase, the ovaries contain numerous declining corpora lutea (which range from 0.2 to 1.3 mm. in diameter) formed at the previous ovulations, and follicles at varying stages of development. The size of the follicles range from 0.1 to less than 0.72 mm. in diameter. Histologically the largest corpora lutea appear functional. There are small numbers of stromal cells in the substance of the corpora

lutea. The luteal cells are large with dispersed chromatin in the nuclei and eosinophilic cytoplasm. Other corpora lutea show various degrees of regression as indicated by the cell size as well as by the amount of connective tissue elements found in the substance of the corpora lutea. The cytoplasm of the luteal cells is faintly eosinophilic to strongly eosinophilic. There is a condensation of chromatin material in the nuclei of other corpora in further stages of involution.

2. Estrus: At estrus, the corpora lutea of the previous cycle are still quite large, measuring about 1.3 mm. in diameter, although some invasion by the connective tissue elements is noted. The cytoplasm of the luteal cells is eosinophilic and the chromatin material still appears dispersed.

3. Metestrus: At metestrus new corpora lutea are formed. They reach their maximum size within 12 to 24 hours following ovulation. The first marked signs of the degeneration of corpora lutea of previous cycles are obvious. There is a marked invasion of stromal connective tissue elements into the extracellular space of the corpus luteum, cells which occupy a major portion of the regressing corpus luteum twelve to fifteen days later. The corpora lutea of previous ovulation exhibit obvious degenerative changes following ovulation and the formation of a new set of corpora lutea.

4. Metestrus to Proestrus: From metestrus to proestrus the transition is gradual and major change is in increased follicular growth in the ovaries. The most recent corpora lutea appeared histologically functional.

The Ovary of the Pregnant Animal

Following ovulation and conception in the rat, the corpora lutea of ovulation attain their maximum size at approximately the twelfth day of gestation. At this time the ovary consists of very highly vascularized corpora lutea appearing red to pink in color. Follicular growth in the ovaries is not entirely inhibited. Follicles measuring as large as 0.5 mm. in diameter are noted in the ovaries. Whereas the diameter of the corpora lutea of the normal cycle average 0.90 mm., the corpora lutea of mid-pregnancy have reached a size of 0.90 mm. to 2 mm. in diameter (mean 1.49 mm.). The individual luteal cells of functional corpora lutea are larger than those of the normal estrous cycle. The nuclei are round, the chromatin dispersed, and the granular cytoplasm stains uniformly eosinophilic. Degenerative changes in corpus luteum of pregnancy are not noted until after the post-partum ovulation in the rat.

In the following result section, the gross observations on the corpora lutea include their general color and the size of the corpora lutea. The term grey indicates that the corpora lutea were not entirely white, red or pink. The

color was intermediate to these extremes. The grey corpora lutea exhibit a few capillaries on the surface, whereas, the corpora lutea referred to as white were completely avascular in appearance. The corpora are divided into large, medium and small. Corpora lutea above 0.90 mm. were classified as large, those ranging between 0.51 and 0.90 mm. in diameter were considered as medium. The small category contains corpora lutea from 0.2 to 0.5 mm. in diameter.

In the tables the "medium" category for corpora lutea is within the range of corpora lutea size following normal ovulation. Therefore, corpora lutea described at autopsy during treatment or "large" were maintained in a higher functional state than corpora of the normal cycle; corpora lutea classed as medium presumably underwent little if any gross change in size from the time of ovulation; whereas corpora classified "small" category had undergone moderate or marked changes in diameter from the time of ovulation.

RESULTS

Experiment 1.

The influence of orally administered MAP of 10, 15 and 18 mg. of MAP/day is recorded in Table 1. This preliminary experiment demonstrated that daily treatment with either 10 or 18 mg. of MAP/day was sufficient to maintain corpora lutea in the ovaries for at least 9 to 10 days. At autopsy on the sixth, seventh, eighth, ninth, or tenth day of treatment, the corpora lutea protruded from the surface of the ovary and appeared grey in color. Cross section measurements demonstrated that the maintained corpora lutea ranged from 0.6 to 13. mm. in diameter with a mean of 0.90 mm. This is somewhat smaller than the range of 0.90 to 2 mm. in diameter in the ovaries of rats at 12 to 14 days of pregnancy (mean 1.49 mm.). The color of the corpora lutea of pregnancy were pink or intermediate between pink and red.

Histological analysis of luteal cells maintained for 9 to 10 days on treatment demonstrated that they were very similar to those of the pregnant rat and to smaller luteal cells of diestrous rats. Although there was very little difference in the size of the cells of pregnant and treated rats, the cytoplasm of the luteal cells from treated rats was much less eosinophilic. The nuclei were large and round

with dispersed chromatin. There was no indication of penetration of the corpora lutea of treated rats by connective tissue as noted in involuting corpora lutea. It also appeared that the corpora lutea of the cycles previous to treatment were arrested in their process of involution, since even the smallest corpora lutea in the ovaries did not exhibit marked invasion by stromal cells. On the contrary, these smaller corpora lutea appeared to be stimulated. There were new, healthy masses of luteal tissue surrounded by connective tissue in the corpora which otherwise would have been called corpora albicans. This suggests that luteal cells from the previous cycles as well as those from most recent ovulations were maintained in a histological functional state for 9 to 10 days.

Follicular growth was minimal in rats on progestin treatment and histological analysis demonstrated that the majority of the follicles in the ovary were atretic. This is in contrast to the rather limited, but continuous follicular growth seen in pregnant animals.

Experiment 2.

The results of experiment 2 (Table 1) demonstrated that corpora lutea were maintained when rats received the lowest dosage of MAP utilized, i.e., 0.5 mg./day. this dosage, however, did not maintain corpora lutea in all rats placed on treatment. Only 2 of 5 rats receiving 0.5 mg. of

MAP/day exhibited ovaries that contained medium size corpora lutea after 11 days of treatment. All of the rats administered 0.5 mg. of MAP/day for 8 days exhibited medium size corpora lutea. As the dosage levels were increased from 0.5 mg. to 1, 2, and 3 mg. of MAP/day, a greater proportion of animals maintained corpora lutea of near normal (medium) or larger than normal size (large) for longer periods of time. With one exception (1 mg. of MAP/day for 22 days) the corpora lutea of rats administered MAP for beyond 18 days had undergone some involution (small). Histologically, the medium to large corpora lutea in each treatment group appeared histologically functional. The individual luteal cells had round nuclei, dispersed chromatin, uniform cytoplasm, while small corpora contained some luteal cells in a high functional state and others undergoing various degrees of atrophy. Although there was limited invasion of the small and medium corpora lutea by stromal connective tissue cells, the infiltrating connective tissue was usually localized in one portion of the corpus luteum rather than being dispersed throughout the whole corpus. This indicated there were foci of degenerative changes. The types of involution noted in the medium and small corpora lutea was not similar to the shrinkage seen in the decline of the corpus luteum of the normal cycle. There was a marked vacuolation and high fat content of the degenerating cells. In the corpora lutea of each size maintained for 14 days at higher dosage levels, degenerative

changes were apparent. This again was not a uniform fatty degeneration of luteal cells, but a rather localized destruction of cells in discrete portions of the corpora lutea.

Experiment 3.

It was apparent that daily injections of crystalline progesterone maintain corpora lutea, and that the size and histological functional state at 9 days of treatment were comparable to rats with MAP maintained corpora lutea. Progesterone treated rats, however, maintained corpora lutea for a considerably shorter duration of time. A comparison of size and histological appearance of each treatment demonstrated that MAP treated rat corpora were maintained in a higher functional state at 5, 6, or 9 days of treatment. At the ninth day of treatment, the corpora lutea of progesterone treated rats had undergone considerable involution. The corpora lutea of progesterone treated rats were red or hemorrhagic as compared to the grey appearance of corpora lutea of MAP treated rats. Histologically, although the diameter of the corpus luteum maintained for 6 days on progesterone was similar to that of MAP treated groups, there was marked invasion of the corpus luteum by connective tissue and at 9 days on progesterone treatment, the corpora lutea resembled those found in later stages of degeneration in normal cycling rats.

Experiment 4.

Daily administration of 0.277 mg. of CAP, an amount equivalent to 5 mg. of MAP in ovulation blocking activity, maintained corpora lutea in the rat (Table 1). The corpora were small and red in color. Histologically they exhibited various degrees of degenerative changes. In contrast, the corpora lutea of 8 of 15 rats in 0.5 mg. of MAP/day and 5 mg. of MAP/day treated groups were maintained for 14 days, although they were not as large as those at higher levels of treatment in the previous experiments. There is a possibility that there might have been a greater degree of maintenance for a short duration in CAP treated rats, since the rats were not autopsied prior to the fourteenth day of treatment.

Experiment 5.

Experiment 5 was designed to determine whether there was any obvious influence of administration of MAP on the size or functional histology of corpora lutea of pregnant animals (Table 2). The corpora lutea of pregnant rats administered 5, 10 and 15 mg. of MAP for 8 days appeared grey in color. This is in contrast to the pink colored corpora of non-treated pregnant rats. There were no other obvious differences as regards the size of the corpora. Normal cycling rats administered 5, 10, and 15 mg. of MAP/day starting at the diestrous phase of the cycle maintained corpora

lutea in the medium size range. Histological examination did not suggest the MAP treatment induced any marked decrease in cell size or any increased infiltration of stromal connective tissue. Histochemical treatment of frozen sections of corpora lutea of treated rats with Oil Red O Stain, however, demonstrated that the pregnant control rat corpora and corpora of normal cycling rats contained less concentration of fat than corpora lutea of MAP treated pregnant and non-pregnant rats. The corpora of normal cycling rats autopsied at various stages of estrous cycle were Schultz negative as were the corpora lutea of pregnant non-treated control rats. However, the corpora lutea of pregnant MAP treated rats were slightly Schultz positive and corpora lutea of non-pregnant treated rats showed a strong positive Schultz reaction.

The adrenal glands of MAP treated rats, whether pregnant or non-pregnant, weighed considerably less when compared to pregnant control rat adrenals. The mean adrenal weight, calculated per 100 gms. of body weight, of the pregnant treated rats at 5, 10 and 15 mg. of MAP/day together was 11.7 mg. This can be compared to 22 mg. in the pregnant controls.

Experiment 6.

Experiment 6 was designed to determine the length of time that higher levels of MAP treatment would maintain functional appearing corpora lutea in hysterectomized cycling

rats. Table 3 demonstrates that corpora lutea were maintained for at least 11 to 17 days of treatment in all rats receiving MAP. The ovaries and corpora lutea of 6 of 7 rats administered MAP for 22 days were of smaller size than the rats treated for shorter intervals. The white appearance of corpora lutea is typical of corpora of advanced stage of degeneration. However, the size of the corpora lutea in this experiment were considerably larger than the corpora albicans of the normal cycling or pregnant rats.

Histological examination demonstrated that there was progressive atrophy of the corpora lutea as the duration of treatment was extended. The white corpora contained marked fatty vacuolations and extensive foci of degeneration in the substance of the corpora lutea.

Earlier experiments demonstrated a definite relationship between dosage of MAP and adrenal atrophy during the same duration of treatment. Although the data are limited this experiment demonstrated progressive adrenal atrophy at a constant dosage of MAP with increased duration of treatment.

Experiment 7.

Experiment 7 was designed to estimate the effectiveness of MAP in blocking ovulation in the normal cycling rat. These data demonstrate that rats fed the same level of MAP for 8 days did not return to cyclic activity subsequent to treatment as soon as rats placed on MAP for 1, 2, and 3 days.

With the exception of 1 rat fed MAP for 1 day, the length of time until the return to estrus subsequent to treatment was not different in rats fed MAP for 1, 2, and 3 days. It was assumed that the long duration in this one rat was the result of induction of pseudopregnancy. The mean post-treatment estrus following 8, 3, 2, and 1 days of treatment was 9.0, 5.0, 6.2 and 6.2 days, respectively. (One rat on treatment for one day was judged pseudopregnant and was not included for calculating the mean.)

Experiment 8.

Table 5 includes the results on the influence of a single administration of MAP and crystalline progesterone on the length of the subsequent estrous cycle. Only 1 rat administered 5 mg. of MAP showed a prolonged interval from the end of treatment to the next estrus, i.e., 12 days. This suggested that pseudopregnancy was induced in this animal. The interval from the single treatment of 5 and 10 mg., at either proestrus or estrus, in the remaining rats was 5 and 6 days. Only 1 of 7 rats receiving a subcutaneous injection of 10 mg. of crystalline progesterone at estrus, demonstrated a prolonged interval until the next estrous period, i.e., 13 days. The remainder of the progesterone treated rats came in estrus 6 to 10 days from the time of treatment, i.e., 6 of 7 progesterone treated rats showed a delayed interval from the end of treatment to next estrus. None of the rats

administered a single treatment of 0.277 mg. of CAP showed a prolonged interval to the next estrous period. This study also demonstrated that a single administration of 5 or 10 mg. MAP did not result in formation of the dedicuomata, when the uterus was traumatized on the fourth diestrous day.

Experiment 9.

The results recorded in Table 6 demonstrate that all rats placed on 1 or 5 mg. of orally administered MAP treatment on the day of metestrus and traumatized 4 to 13 days later gave a decidual response. The uterine weights (both horns) ranged from 390 to 610 mg. Normal non-traumatized uterine weights did not exceed 200 mg. (Table 7). Rats placed on 0.5 mg. of oral administration of MAP on the day of estrus, and where the uteri traumatized on the fifth day following estrus, developed a massive deciduoma (the uterine weights, both horns, ranged from 1670 to 2400 mg.). If the rats on 1 mg. and 15 mg. level are maintained for the same duration, it would be expected that the adrenal weights of rats on 1 mg. treatment would be heavier than those on 15 mg. treatment. However, Table 6 demonstrates that adrenal weights of rats on 1 mg. level are lighter. Since the rats on 1 mg. level were treated for a longer length of time this indicates a progressive decline in adrenal weight with the length of duration of treatment.

Experiment 10.

In Table 7, the decidual response has been expressed in two ways: (1) the wet weight of the traumatized horn in milligrams and (2) the net gain in uterine weight calculated by subtracting the weight of the control horn of the uterus from the traumatized horn. Contrary to data in Experiment 9, (rats numbers 13 through 15, Table 6), all rats placed on 0.5 mg. of MAP treatment on the day of estrus (rats numbers 14 through 24) did not exhibit an extensive decidual response. The decidual response in terms of net gain in uterine weight varied from 56 mg. to 1285 mg. per uterine horn. Only rat numbers 16, 17, and 22 had a massive decidual response as expected. Similarly, there was no uniform pattern in group of rats treated with either 0.5 mg. or 10 mg. of MAP/day, starting from second or third day following estrus, and only rats numbers 7 and 12 exhibited a massive decidual response. These data (rat numbers 30 through 40) indicate that the dose of MAP administered and also the number of days of treatment prior to traumatization apparently did not have a uniform effect on decidual response. However, the effect of the level of dosage of MAP and the duration of treatment is reflected by the decline in weights of the adrenal glands.

The effect of duration of treatment and adrenal atrophy was clearly demonstrated. The mean adrenal weight

of rats on 0.5 mg. of MAP/day for 7 to 8 days was 17.4 mg./ 100 gm. body weight. The mean adrenal weights of rats on a similar regime, but treated for 12 to 15 days was 15.8 mg./ 100 gm. body weight. These means were not significantly different. Likewise, the effect of level of MAP treatment was evident in the two groups of rats receiving 0.5 mg. of MAP for 7 to 8 days and 10 mg. of MAP for 8 days. The mean adrenal weights calculated per 100 gm. body weight were 17.4 and 13.8 mg. respectively ($P < .05$).

Experiment 11.

The data in Table 8 demonstrate that all rats developed deciduomas. There was a positive relationship between the magnitude of the estrogenic conditioning, as indicated by vaginal cytology at the time of trauma, and the subsequent decidual response. It was not possible to quantitate the extent of "estrogen surge" by means of the vaginal cytology alone. It was noted that rat numbers 6, 7, 8, 11, 12, 13, 14, 15, 23, 24, 25, 29, 32, etc., exhibited a more estrogenic type of vaginal smear at the time of trauma. These rats exhibited a more extensive decidual response as estimated by the net gain in uterine weight.

Although there was no demonstrable difference in the decidual response attributed to the dose of MAP administered, the mean weights of the adrenal glands demonstrate that the adrenal glands of the rats receiving 10 mg.

of MAP/day were lighter (highly significantly ($P < .01$)) than those receiving 0.5 mg. of MAP/day (mean adrenal weight per 100 gm. body weight was 16 mg. at 0.5 mg. treatment and 13.8 mg. at 10 mg. treatment).

Vaginal Cytology in Ovariectomized and Progestin Treated Rats

Vaginal smears were obtained at various levels of MAP administration in order to determine changes in vaginal cytology. Contrary to expectations, at each level of MAP treatment (from 0.5 mg. to 18 mg./day) the vaginal smears did not exhibit a uniform or typical diestrous cytology. There were recurrent peaks of precornified or a mixture of precornified and cornified epithelial cells at intervals of between 4 and 8 days. This typical follicular phase smear was followed by the appearance of leucocyte-like cells. The leucocyte-like cells were compact and round and sometimes contained an eosinophilic cytoplasm. The presence of these cells with the few precornified cells mimics the vaginal cytology typical of the "metestrous stage" of the normal estrous cycle. The appearance of the leucocyte-like cells was much easier to detect than changes in the appearance of precornified or cornified epithelial cells. These recurrent peaks of mixture of precornified and cornified cells were never associated with estrus. The vaginal cytology between recurrent peaks was not typical of any distinct phase of the

normal estrous cycle of the rat. There were basophilic and precornified epithelial cells but very few fully cornified epithelial cells. With the exception of the time of surges of new leucocyte-like cells, fragmented and degenerating leucocyte-like cells were present in the smears at all times. Histological examination of the ovary indicated that follicular growth was at a minimum in rats receiving oral administration of MAP. However, when the vaginal cytology of the ovariectomized rats and ovariectomized MAP treated rats were examined a similar pattern was noted. The recurrent cytological cycle in ovariectomized rats, however, was of longer duration and not as pronounced. Similar results were obtained when crystalline progesterone was administered subcutaneously in oil in intact normal cycling rats. Crystalline progesterone administration, as high as 10 mg./day, did not alter the pattern noted above.

Table 1. Corpora lutea maintenance in normal intact rats.

No. of Rats	Experiment	Level of Progestin Treatment (mg./day)[1]		Days on Treatment[2]	Observation on Corpora Lutea	
					Color[3]	Size[4]
2	1	18	MAP	6	Grey	Large
2	1	18	"	7	Grey	Large
2	1	18	"	8	Grey	Large
2	1	15	"	6	Grey	Large
2	1	15	"	7	Grey	Large
2	1	15	"	8	Grey	Large
2	1	15	"	9	Grey	Large
4	1	10	"	9	Grey	Large
4	1	10	"	10	Grey	Large
3	2	3	"	14	Grey	Large
2	2	3	"	18	Grey	Small
2	2	2	"	10	Grey	Large
2	2	2	"	13	Grey	Large
2	2	2	"	18	Grey	Small
1	2	2	"	18	Grey	Large
3	2	1	"	14	Grey	Medium
1	2	1	"	14	Grey	Small
1	2	1	"	8	Grey	Small
2	2	1	"	22	White	Small
1	2	1	"	22	Grey	Medium
10	3	5	"	10	Grey	Large

8	4	5	"	14	Grey	Medium
7	4	5	"	14	Grey	Small
2	2	0.5	"	11	Grey	Medium
3	2	0.5	"	11	Grey	Small
4	2	0.5	"	8	Grey	Medium
8	4	0.5	"	14	Grey	Medium
7	4	0.5	"	14	Grey	Small
2	3	10	Prog.	5	Red	Medium
2	3	10	"	6	Red	Medium
6	3	10	"	8	Red	Small
4	3	10	"	10	Red	Small
5	3	15	"	9	Red	Small
4	3	5	"	9	Red	Small
10	4	0.277	CAP	14	Red	Small

[1]6-α-methyl-17-α-acetoxy progesterone (MAP) by oral route.
6-α-chloro-17-α-acetoxy progesterone (CAP) by oral route.
Progesterone (Prog.), Subcutaneous in oil.

[2]On treatment on the day of metestrus counted as day one and the day before sacrifice counted as the last day of treatment.

[3]Gross appearance of corpora lutea.

[4]Large -- 0.90 mm. and above.
Medium -- 0.51 to 0.90 mm.
Small -- 0.2 to 0.50 mm.

Table 2. Corpus luteum maintenance in pregnant rats.

No. of Rats	Condition	Level of MAP Treatment[1] (mg./day)	Observation of Corpus Luteum Color	Size	Mean Adrenal Wt. mg./100gm. body weight
3	pregnant	15	Grey	Large	13.3
2	non-pregnant	15	Grey	Medium	11.0
2	pregnant	10	Grey	Large	9.5
2	non-pregnant	10	Grey	Medium	12.5
2	pregnant	5	Grey	Large	11.5
2	non-pregnant	5	Grey	Medium	10.5
4	pregnant	no treatment	Pink	Large	22.0

[1]MAP treatment by oral route, from the 10th day of pregnancy for 8 days or for 8 days in the non-pregnant rats.

Table 3. Corpus luteum maintenance in hysterectomized rats.

No. of Rats	Level of MAP Treatment[1] (mg./day)	Days on Treatment	Observations on Corpus Luteum Color	Size	Mean Adrenal Wt. mg./100 gm. body weight
2	15	11 & 12 days	Grey	Medium	12
2	15	16	Grey	Medium	10
1	15	17	Grey	Medium	8
1	15	22	Grey	Medium	8
6	15	22	White	Small	
4	no treatment	--	-----	------	18.3

[1]MAP treatment, by oral route, from the day of metestrus until the day prior to autopsy.

Table 4. Ovarian response to MAP treatment of various lengths of time.

No. of Rats	Level of MAP Treatment (mg./day)	Days on Treatment[1]	Post-Treatment Estrus (days)[2]	Length of Cycle (days)[3]
2	5	8	8	16
2	5	8	9	17
1	5	8	11	19
2	5	3	6	9
3	5	3	5	8
2	5	2	7	9
2	5	2	6	8
1	5	2	5	7
3	5	1	6	7
1	5	1	7	8
1	5	1	10	11

[1]MAP treatment, by oral route, initiated on the day of estrus (9-10 a.m.).

[2]From the day following the last day of treatment.

[3]Interval from the estrus of the first day of treatment to the first estrus following the end of treatment.

Table 5. Ovarian activity after a single treatment with steroids on the day of estrus or proestrus.

No. of Rats	Level of Progestin Treatment[1] (mg./day)	Day of Treatment	Post-Treatment Estrus[2] (days)	Condition
4	5 mg. MAP	Proestrus (8:30 p.m.)	5	not delayed
1	5 mg. MAP	Proestrus (8:30 p.m.)	12	delayed
5[3]	10 mg. MAP	Proestrus (8:30 p.m.)	--	no deciduoma
5	5 mg. MAP	Estrus (9:00 a.m.)	6	not delayed
5[4]	10 mg. MAP	Estrus (9:00 a.m.)	--	no deciduoma
1	10 mg. Prog.	Estrus (9:00 a.m.)	13	delayed
2	10 mg. Prog.	Estrus (9:00 a.m.)	9	delayed
2	10 mg. Prog.	Estrus (9:00 a.m.)	8	delayed
2	10 mg. Prog.	Estrus (9:00 a.m.)	5	not delayed
5	0.277 mg. CAP	Estrus (9:00 a.m.)	5	not delayed

[1]MAP orally administered and crystalline progesterone administered in single injection subcutaneous in oil.

[2]Interval from the day of treatment, until the next estrus. For rats treated at proestrus this interval is one-half day longer.

[3,4]Uterus traumatized on the fourth day of diestrus and the rat sacrificed five days later.

Table 6. Deciduoma induction in MAP treated rats.

Rat No.	Day of Estrous Cycle MAP Treatment Started[1]	Level of Map Treatment (mg./day)	Uterine Trauma (days)[2]	Day of Autopsy[3]	Uterine Weight (mg.)[4]	Adrenal Wt. mg./100 gm. Body Weight
1	2	1	13	17	500	11
2	2	1	13	17	550	10
3	2	1	12	16	610	9
4	2	1	12	16	580	9
5	2	15	4	8	600	10
6	2	15	4	8	490	15
7	2	15	4	8	390	11
8	2	15	4	8	432	13
9	2	15	4	8	800	15
10	2	15	5	9	700	13
11	2	15	5	9	650	15
12	2	15	5	9	500	15
13	1	0.5	5	7	1800	16
14	1	0.5	5	7	2400	14
15	1	0.5	5	7	1670	14

[1]MAP treatment by oral route
Estrus = day 1; Metestrus = day 2; Diestrus = day 3.

[2]Number of days from the day when oral administration of MAP treatment started until trauma.

[3]Total number of days on treatment until autopsy.

[4]Wet weight of the uterus in milligrams.

Table 7. Deciduoma induction in MAP treated rats.

Rat No.	Day of Estrous Cycle MAP Treatment Started[1]	Level of MAP Treatment (mg./day)	Uterine Trauma (day)[2]	Day of Autopsy[3]	Uterine Wt. (mg.)[4] Control Horn	Uterine Wt. (mg.)[4] Trauma Horn	Difference[5] Trauma Horn-Control Horn (mg.)	Adrenal Wt. mg./100 gm. Body Weight
1	2	0.5	4	8	126	171	45	18
2	2	0.5	4	8	110	473	363	17
3	2	0.5	4	8	153	260	107	17
4	2	0.5	4	8	201	273	72	18
5	2	0.5	4	8	152	226	74	18
6	2	0.5	2	6	138	200	62	16
7	2	0.5	4	8	142	1110	968	19
8	2	10	4	8	130	425	295	14
9	2	10	4	8	121	602	481	18
10	2	10	4	8	98	212	114	12
11	2	10	4	8	106	304	198	14
12	2	10	4	8	280	1531	1251	11
13	2	10	4	8	121	211	90	14
14	1	0.5	4	8	95	412	317	17
15	1	0.5	4	8	275	845	570	15
16	1	0.5	3	6	185	1470	1285	18
17	1	0.5	3	6	148	1417	1269	15
18	1	0.5	2	6	123	353	230	16
19	1	0.5	3	7	122	230	108	18
20								
21	1	0.5	3	7	127	418	291	17
22	1	0.5	4	8	132	1023	891	18
23	1	0.5	4	8	142	198	56	17
24	1	0.5	4	8	101	353	252	18
25	3	0.5	3	6	130	160	30	18
26	3	0.5	3	6	101	400	299	18
27	3	0.5	3	6	101	865	764	18

28	3	0.5	2	6	187	275	88	13
29	3	0.5	4	8	101	172	71	17
30	3	0.5	8	12	180	445	265	17
31	3	0.5	8	12	103	400	297	17
32	3	0.5	8	12	158	202	44	18
33	3	0.5	8	12	153	181	28	19
34	2	0.5	11	15	136	215	79	16
35	4	0.5	11	15	114	175	61	15
36	3	0.5	11	15	170	205	35	12
37	4	0.5	11	15	149	225	76	12
38	3	10	9	14	95	165	70	11
39	3	10	9	14	105	192	87	13
40	4	10	9	14	117	600	483	11

[1]Estrus = day 1; Metestrus = day 2; Diestrus = day 3; Proestrus = day 4.

[2]Number of days from first oral administration of MAP until trauma. MAP treatment, by oral route.

[3]Total number of days on treatment until autopsy.

[4]Wet weight of the uterus in milligrams.

[5]Net gain in uterine weight due to successful induction of deciduoma.

Table 8. Deciduoma induction in ovariectomized MAP treated rats.

Rat No.	Level of MAP Treatment mg./day[1]	Uterine Trauma[2] (day)	Day of Autopsy[3]	Uterine Wt. (mg.)[4] Control Horn	Trauma. Horn	Difference (mg.) Trauma Horn-Control Horn	Adrenal Wt. mg./100 gm. Body Weight
1	0.5	5	9	90	187	97	17
2	0.5	5	9	103	104	1	15
3	0.5	5	9	107	183	76	14
4	0.5	5	9	129	176	47	17
5	0.5	5	9	95	241	146	20
6	0.5	5	9	123	448	325	16
7	0.5	5	9	155	473	318	16
8	0.5	5	9	210	572	362	15
9	0.5	5	9	78	310	232	15
10	0.5	5	9	81	226	145	17
11	0.5	5	9	104	547	442	17
12	0.5	5	9	124	662	538	17
13	0.5	5	9	110	521	411	11
14	0.5	5	9	79	536	457	14
15	0.5	5	9	110	644	534	19
16	10	5	9	118	247	129	13
17	10	5	9	80	150	70	12
18	10	5	9	130	368	238	14
19	10	5	9	92	335	243	10
20	10	5	9	72	252	180	17
21	10	5	9	98	332	134	15
22	10	5	9	85	177	92	14
23	10	5	9	105	532	427	13

24	10	5	9	132	648	506	12
25	10	5	9	98	400	302	13
26	10	5	9	87	228	140	16
27	10	5	9	103	335	232	13
28	10	5	9	103	658	555	11
29	10	5	9	96	410	314	15
30	10	5	9	66	166	100	14
31	10	5	9	92	257	165	17
32	10	5	9	89	395	306	15

[1]**MAP** treatment, by oral route, for 5 days starting on the 6th day following ovariectomy.

[2]Number of days on **MAP** treatment until uterine trauma.

[3]Number of days on **MAP** treatment until autopsy.

[4]Wet weight of the uterus in milligrams.

DISCUSSION

The results of the current studies have demonstrated that: Oral administration of MAP resulted in the maintenance of recently formed corpora lutea in a histological functional state for at least 14 days; Corpora lutea of previous ovulations, present in the ovaries at the time of treatment, appeared to be stimulated or at least arrested in their involution by MAP treatment; It was necessary to administer MAP daily in order to maintain corpora lutea in the ovaries; The administration of MAP at any stage of the estrous cycle resulted in maintenance of the most recently formed corpora in the ovaries and that during the period of corpora lutea maintenance by MAP treatment, follicular growth in the ovaries was minimal. It was also demonstrated that although extremely low dosages of MAP resulted in maintenance of the corpora lutea, dosages higher than 5 mg. had a greater stimulatory influence on corpora lutea.

Several hypotheses have been advanced in the literature to explain the maintenance of the functional life of the corpus luteum. It is theorized that corpora lutea are maintained if there is a secretion of a luteotrophic substance (LTH) from the pituitary, if there is an inhibition of secretion of a luteolytic substance from either

the uterus or the pituitary, or by a direct stimulatory influence of the steroid on the luteal cells of the ovary. Although each of these is attractive, it would appear that the results of the current study with MAP treatment cannot be fully interpreted within any one hypothesis.

Considerable indirect support has been suggested for the hypothesis that progestins stimulate pituitary secretions of prolactin. It has been recognized for a considerable length of time that the nervous system, through neurohumoral pathways, chronically inhibits the secretion of pituitary luteotrophin in the rat (39,136,107,78). An effective means of removing pituitary gland from hypothalamic control is by transplanting the anterior pituitary in the kidney capsule. Under these conditions the release of the pituitary luteotrophic factor continues for an indefinite length of time and consequently, the corpora lutea are maintained for months (92). It has also been demonstrated that certain stimuli, that is electrical, mechanical or chemical, administered at specific phases of the estrous cycle initiate a continuous release of pituitary luteotrophic factor for a definite period of time. The duration of release of the pituitary luteotrophic factor under these conditions can be further prolonged in pseudopregnant rats by hysterectomy, the injection of oxytocin or by the suckling stimulus. Considering pseudopregnancy in the rat, Rothchild (107) has suggested that progesterone may be a factor that modifies hypothalamic

centers and maintains the secretion of LTH. This would be classed as positive feed back mechanism. A single subcutaneous injection of 10 mg. crystalline progesterone in oil on the day of estrus resulted in the induction of pseudopregnancy in 75% of the treated rats (107). It is interesting that a similar treatment initiated during proestrus resulted in pseudopregnancy in only 25% of the treated rats. The inability of progesterone injection to initiate pseudopregnancy at all stages of the estrous cycle has placed some doubt on the feasibility of this proposed mechanism of action, since mechanical stimulation of the cervix either at proestrus or estrus results in the development of the pseudopregnant state. If endogenous progesterone stimulated the further release of LTH (positive feed back theory) the question remains as to why exogenously administered progesterone does not act in a similar fashion, if administration is initiated at all stages of the cycle. There is also some question as to whether Rothchild induced pseudopregnancy in all of the treated animals, since the other criteria for pseudopregnancy utilized in this study, that is, the length of the diestrous period following single injection of progesterone, is not entirely reliable.

It has also been reported that massive treatment with estrogen, commencing soon after ovulation, resulted in the enlargement of corpora lutea and production of sufficient amounts of endogenous progesterone to mucify the vaginal

mucosa (117,133,82). The administration of estrogen, however, was not immediately followed by the pseudopregnant state. Rats treated with massive doses of estrogen exhibited an estrogenic vaginal smear for a few days before demonstrating the typical diestrous smear characteristic of pseudopregnancy. It would, therefore, appear that with estrogenic treatment the animal is exposed to conditions resembling that of estrus before pseudopregnancy ensues. Merckel and Nelson (82) reported that the continuous administration of estrin started at estrus maintains the corpora as long as the normal length of gestation, at which time the rat again exhibits an estrus smear. Massive doses of estrogen on the day of estrus also prolong the life of the corpora lutea for about 14 to 15 days. It would therefore appear that the mechanism of maintenance of corpora lutea by progesterone or estrogen exhibit some similarity. That is, Rothchild injected progesterone when the animal was in the estrous stage. On the other hand, Merckel and Nelson (82) imposed an estrogenic state on the luteal phase animal or administered estrogen on the day of estrus with subsequent release of endogenous progesterons.

A consideration of the experimental data of this thesis demonstrates that there are notable differences from that reported by other workers. In the current study, corpora lutea are maintained only as long as the rats were on MAP treatment. Whatever mechanism MAP is affecting,

unlike a single injection of progesterone, this stimulus has to be continuously applied. Another difference is that MAP administration at any stage of the estrous cycle resulted in the maintenance of corpora lutea in the ovaries. Estrogen administration or progesterone injection was only successful if limited to a specific stage of the estrous cycle. It could be reasoned that MAP is acting only at a specific phase of the estrous cycle and that continuous treatment would logically apply the stimulus at the proper time. However, experiment 8 demonstrated that a single administration of MAP, by oral route, during proestrus or during estrus did not result in pseudopregnancy, whereas 5 of 7 rats administered a single injection of 10 mg. crystalline progesterone in oil on the day of estrus did exhibit an extended diestrous period. On the basis of Rothchild's definition of pseudopregnancy, therefore, the single administration of MAP during proestrus or estrus did not result in pseudopregnancy, whereas the majority of the rats injected with crystalline progesterone at estrus exhibited pseudopregnancy.

The major portion of this study was concerned with the administration of levels of MAP at 0.5 mg. per day or greater. The last experiment conducted in this study, on a very limited number of animals was an attempt to determine the minimal level of daily administration of MAP that would maintain corpora lutea in the ovaries or support a decidual response. This was of considerable importance since levels 0.5

to 18 mg. maintain corpora lutea in the intact animal and supported decidual response in both the intact and ovariectomized rats. Five rats were placed on treatment of 0.1 mg. of MAP daily and 3 rats on 0.3 mg. of MAP daily. The rats were placed on treatment during diestrus, and were autopsied on the 10th day of treatment. These levels of MAP treatment did not result in the maintenance of corpora lutea in the ovaries. The ovaries contained small corpus albicans and haemorrhagic follicles. The uteri of each rat were estrogenic which suggested that while ovulation was being inhibited during treatment follicular growth was probably continuous. Two additional groups of 4 rats each were placed on daily oral administration of 0.3 and 0.1 mg. of MAP and uteri were traumatized 4 days following the initiation of treatment. The rats were autopsied on the 5th day following traumatization. A decisive decidual response was obtained in only one animal at 0.1 mg. treatment level. The response was segmental and did not involve the entire uterine horn. the increase in uterine weight over the control horn was 217 mg. The decidual response at 0.3 mg. of MAP treatment involved the entire uterine horn but was considerably less than that observed with 0.5 mg. of MAP. The increase in weight over the controls ranged from 183 to 324 mg. Although conducted with a limited number of animals, this experiment clearly demonstrated that there were marked differences in response in maintenance of decidual reaction between

the 0.1, 0.3, and 0.5 mg. of MAP. This also established that of the dosages utilized, that 0.5 mg. of MAP daily appeared optimal, both in maintenance of corpora lutea and in supporting a maximal decidual response. For these reasons it is considered that levels of MAP treatment between 0.5 and 5 mg. daily are within the range of what might be considered as physiological.

It is recognized that oral or subcutaneous administration of progestin are not comparable, and that similar blood progestin levels may not be attained by each method of treatment. Although it is difficult to equate progestational efficacies of different progestational agents administered by different routes, the following estimates can be made. Daily subcutaneous injections of 1.5 mg. of progesterone are capable of indefinitely delaying the next expected estrus if the treatment is initiated during early diestrus. The same result has been reported following daily oral administration of 1 mg. of MAP (144,96). Daily subcutaneous injection of 1 mg. progesterone in the ovariectomized female rats was sufficient to obtain maximal decidual response subsequent to traumatization of the uterus. The present study has demonstrated that amounts of MAP daily administered greater than 0.3 mg. are necessary to obtain a maximal decidual response in ovariectomized female rats.

It was also apparent that levels of MAP above .3 mg. daily were necessary to maintain functional corpora lutea in

the ovaries. Therefore, as regards ovulation blocking ability and maintenance of decidual reaction, 0.3 to 0.5 mg. of daily orally administered MAP are equivalent to the daily subcutaneously administered 1.5 mg. of progesterone on a weight basis. Therefore, MAP by oral route is three times as progestationally active as subcutaneously injected progesterone. It would appear that the 5 and 10 mg. of orally administered MAP, utilized in the current studies, is equivalent to one and one-half times and 3 times respectively the amount of progestational activity as the 10 mg. progesterone administered subcutaneously in oil by Rothchild. The mechanism of action of MAP in maintaining corpora lutea in the ovaries could be quite different than that found following estrogen treatment, the injection of progesterone at estrus, following sterile matings or stimulation of the cervix at estrus. The induction of pseudopregnancy in the latter instances appear to be an all or none phenomena. That is, if these stimuli are sufficient, the central nervous system block of luteotrophic factor secretion from the pituitary is released and apparently luteotrophic hormone is continuously secreted for the entire stage of pseudopregnancy of 8 to 14 days. At this time the central nervous system resumes its chronic inhibition of pituitary secretion. The necessity for daily administration of MAP in order to maintain corpora lutea suggests that this was not acting on all or none basis. Rather than facilitating the release of a pituitary

gonadotropin, the action of MAP appears more similar to that noted in the chronic maintenance of corpus luteum following hypophysectomy. That is, the action of MAP might be mediated through a complete block of gonadotropin release.

A second consideration of a possible action of MAP on the maintenance of corpus luteum in the ovaries could be by the inhibition of luteolytic factors of either uterine or pituitary origin. The role of uterus has been implicated in the maintenance of a corpus luteum. The state of pseudopregnancy in the rat can be extended for approximately 6 days by hysterectomy (18,52,17). The life of the corpus luteum in guinea pig is prolonged if hysterectomy is performed at estrus (69,111). Similar results have been obtained in the sow, ewe and heifers (132). Melampy (81) demonstrated that an inverse relationship exists between the quantity of uterine endometrial autotransplants and duration of pseudopregnancy. This suggested that the intact uterus is producing a luteolytic substance which hastens the involution of corpus luteum on the ovary. That this influence is mediated by the nervous system was suggested by Nalbandov (85) since denervation of uterine segments containing plastic beads prevented the shortening of the estrous cycle noted in the animal with beads placed in the intact uterus. It would appear, however, that the maintenance of corpora lutea of the ovaries of rats under MAP administration is not dependent on the intact uterus. Experiment 6 demonstrated that the

maintenance of corpora lutea and adrenal atrophy was not modified in MAP treated hysterectomized rats. It has long been recognized that the uterus, as a steroid secreting gland, contains enzymes capable of marked modification of steroids reaching the uterus in the blood stream. One concern in this study was of possibility that orally administered MAP might be converted in the body to other steroidal compounds. Although this possibility has not been absolved, experiment 6 demonstrated that whatever role the uterus might have in the modification of administered MAP, that the MAP corpora lutea maintaining activity is not dependent upon this action.

It is not unreasonable to assume that pituitary gonadotropins other than those considered to be luteotrophic, have a luteolytic action on the life of the corpus luteum, particularly since the injection of chorionic gonadotropins or pituitary LH have been reported to decrease the life of functional corpora lutea in the ovaries (127,125). It has been reported that in the normal cycling animal progesterone secreted by the corpus luteum inhibits the production of FSH (negative feed back mechanism) (84). It has also been demonstrated that the majority of synthetic progestins are capable of suppressing pituitary secretions (42). Although progesterone has a dampening effect on the response of the ovary to the injection of ovulatory gonadotropins, this is considered secondary to its ability to inhibit gonadotropin

release from the pituitary (108). The similarity between the histologically functional maintained corpora lutea in MAP treated rats and in the hypophysectomized rats strongly suggest that the action of progesterone on the pituitary might not be to affect the release of luteotropic factor, but to inhibit release of normal luteolytic factors.

It has been demonstrated that one of the gonadal hormones, that is estrogen, has a direct effect upon the ovary (102,145). The possibility that progesterone also has a direct effect upon the ovary cannot be ignored. It has been demonstrated that progestin administered with estrogen has a dampening effect on the response of the ovary to the injection of pituitary gonadotropins (71). With this in mind, it would be rather surprising to find that MAP has a direct stimulating effect on luteal cells of the ovary. The result of experiment 7 on the relationship of duration of MAP treatment to the rebound, or the follicular growth phase subsequent to treatment suggests the opposite. The delay in follicular growth subsequent to treatment could be due to one or both of two factors. It is not unreasonable to assume that the pituitary gland of a rat on high levels of chronic MAP might be incapable of releasing sufficient gonadotropins subsequent to treatment to affect ovulation. It has been reported that the synthesis of pituitary FSH and LH is not decreased during progestin treatment or during pseudopregnancy, although the release of gonadotropins is inhibited (108).

Numerous studies have indicated that the amount of gonadotropins released from the pituitary subsequent to progesterone treatment is higher than normal levels and may, in fact, result in superovulation. For these reasons it would not appear that prolonged suppression of pituitary gland is responsible for the delay of return to normal cyclic activity following MAP treatment. It is more likely that constant suppression of the ovarian response to gonadotropins under MAP treatment results in a condition of ovarian "sluggishness," the extent of inhibition being dependent upon dose and duration of progestational treatment. It is extremely difficult to resolve this problem.

In the consideration of the results of the present study, the term "histological functional state" has been utilized to a considerable extent. The reasons for the use of this terminology has been earlier clarified. That is, a rat in contrast to other animals can maintain a fully developed corpus luteum for short periods of time in the normal estrous cycle in leiu of progesterone secretion by this tissue. A disturbing enigma has been present throughout all of these studies. How can one demonstrate the production of progesterone by maintained corpora lutea when the animal is on exogenous progesterone treatment. The only accurate means would be by the measurement of ovarian vein or systemic blood progestins, in order to determine whether the circulating levels during the period of corpora lutea

maintenance were in excess of that of ovariectomized rats on similar MAP treatment. The histochemical analysis of the corpora lutea of rats maintained by MAP treatment demonstrated that they contained a greater content of free lipids than either the normal cycling or pregnant rat. Although lipid content has not been accurately correlated with the secretory activity of the ovary, it is not unreasonable to assume that accumulation of lipids in a steroid secreting gland representing a non-secretory state. Studies on the relationship of adrenal gland lipid and secretion of corticosteroids have demonstrated that the more inactive adrenal gland contains the greatest quantity of lipids in the rat (142). The staining for Schultz positive substances, that is, primarily cholesterol and its derivatives, suggested that the corpora lutea of both normal cycling and pregnant rats placed on MAP treatment contain higher quantities of cholesterol than the control normal cycling or non-treated pregnant rats. This would again suggest that there is an accumulation of cholesterol and its esters in either non-pregnant or pregnant rats on MAP treatment. These observations, although limited, would support the hypothesis that the action of MAP is to maintain the integrity of the corpus luteum in a highly functional state, but not secreting progesterone.

At all levels of MAP utilized in this study, 0.5 to 18 mg. daily by oral route, the amount of exogenous progestin

was in excess of the minimal amount necessary to maintain a decidual response in ovariectomized rats. In the non-estrogen treated ovariectomized progestin treated rat the extent of decidual response cannot be accurately correlated with MAP dosage level. For example, daily subcutaneous injection of 0.5 mg. of crystalline progesterone in the ovariectomized female rat results in a decidual response approaching 500 mg. Levels of progesterone administered above this level that is, at 1 mg. or above, maintains a decidual response of approximately 1400 mg. (135). In the present study, therefore, daily administration of 0.5 mg. of MAP in the ovariectomized or normal cycling rat resulted in the maintenance of deciduomata ranging from 200 to 1110 and 104 to 662 respectively. The marked variation in response at the separate dosage levels, in both the ovariectomized and normal cycling rats (standard deviation in ovariectomized rats was 181 to 0.5 mg. level and 144 at 10 mg. level), obviated the possibility of estimating any additional estrogen of endogenous origin. It is, therefore, on the basis of the high cholesterol content and the high fat content that the corpora lutea maintained by MAP treatments are assumed to be secreting less progesterone than the functional corpora lutea of the non-treated pregnant animals.

The large variation in the decidual response in both ovariectomized rats or normal cycling rats placed on MAP treatment is not considered to be the normal variations of a

standard animal preparation to progestin treatment. It would appear that the decidual response in these animals was either being inhibited or augmented by unknown endogenous factors. It is recognized that if the uteri of pseudopregnant rats are traumatized on the third or fifth day of pseudopregnancy no decidual respons is obtained (29). However, if the uteri are traumatized on the fourth day of pseudopregnancy a massive decidual response results. Shelesnyak (121) has suggested that an endogenous estrogen surge is responsible for this difference in response to trauma. On the basis of this theory, a transient surge of estrogen occurs on the fourth day of pseudopregnancy and this surge or increased amount of estrogen is prerequisite for the establishment of a uterine deciduomata. It is suggested that this estrogen surge takes place during the latter part of day 3 of pseudopregnancy and not prior or later than this time. This surge is probably responsible for the receptivity of the uterus for the ova and subsequent changes for implantation. It has also been demonstrated that decidua can be produced in pseudopregnant rats ovariectomized on the 4th day of pseudopregnancy and administerd daily injections of estrone or progesterone (an estrogen/progesterone ratio of about 1:2000 (134)). Maximal sensitivity was observed when the dose of estrone was limited to between 0.4 and 1 micrograms per day per rat. It is not unreasonable to assume, therefore, that in the intact MAP

treated rat, that there might be variations in estrogen secretion during treatment period, thus accounting in difference in decidual response obtained after traumatization. Vaginal smearing of rats at each level of treatment demonstrated that there were recurring periods of proliferation of the superficial epithelium and decreases in the leucocytes in the smear. The extent of vaginal proliferation was not comparable to that seen in the non-treated estrus rat. However, the change from the atypical diestrous smear to one of increased mucification suggested that the animal is being exposed to recurrent periods of endogenous estrogenic influence. This was surprising since follicular growth of the treated rats whether at 0.5 or 18 mg. per day of MAP suggested that follicular growth in the ovaries was kept at minimal during the treatment period. In order to determine whether the ovary was the source of the endogenous estrogen, ovariectomized rats were smeared following surgery. There is no question that in these rats there were recurring periods of estrogenic stimulation as noted by vaginal cytology. These intervals were not uniform in any single rat. Thus, the period of occurance of a estrogenic type of smear could not be predicted. These intervals varied from 4 to 8 days.

Nelson (91), Swezy and Evans (129) and Zeiner (137) have presented evidence that there is persistance of cyclic activity during gestation. In fact, Nelson reported

recurrence of the estrus and acceptance of the male in pregnant rats. In contrast, Long and Evans reported that there were no estrus changes occuring in the cellular content of the vaginal smear of the rat during the period of pregnancy (69). Mandl (75), on the other hand, reported the same cyclic vaginal patterns in ovariectomized rats, the length of the recurrent cycles in 19 spayed rats was 114 hours as compared with 104 hours in normal intact animals. This author concluded that the source of the estrogenic compound in the ovariectomized rats might be due to secretion of adrenal cortex. In the present study ovariectomized non-treated and ovariectomized MAP treated rats retained inherent recurrent vaginal changes that strongly suggested estrogenic surges throughout the treatment period. It is of considerable interest to speculate as to the source of the estrogen since, in the present study at high levels of MAP treatment the adrenal glands underwent marked involution. Although it is possible that a shift to the production of an estrogenic hormone could occur it is not likely that an atrophic gland would be secreting large amounts of estrogen. It would not appear that the recurrent vaginal smears are governed by circadian rhythms secretions by the adrenal glands, since the cyclic variation of the estrogenic activity on the vagina was not exhibited in a regular rhythm. It is not unreasonable to assume that a portion of MAP is being converted to estrogen in the treated animals. However, if

this was true, one would expect to find a constant estrogenic influence on the vagina as long as the animal was on MAP treatment. It was also demonstrated that this recurrent estrogenic surge occurred in the ovariectomized non-treated animals.

There was no uniformity in the extent of decidual response within the animals at 0.5 or 10 mg. level of MAP treatment. Although it is difficult to quantitate these responses it was noted that animals exhibiting the greatest decidual response also exhibited an estrogenic smear at the time of uterine traumatization (Table 8, rat numbers 6, 7, 8, 11, 12, 14, 15, 24, 25, 29, 32, etc.). Since in intact rats the estrogenic surge is shifted due to treatment, Table 7 demonstrated that maximum decidual response was not related to the number of days from estrus when trauma occurred, as in pseudopregnant untreated rats. Although the ovary is not necessary for this surge, this would suggest that the intact ovary augments this response.

These findings support the hypothesis that ovariectomized rats have recurring phases of estrogenic uterine conditioning, and establish that the levels 0.5 to 10 mg. of MAP treatment do not inhibit the cyclic changes. These results, however, are not in agreement with Mandl's report that estrogenic surges occur at repeating predictable intervals. It is also unlikely that the source of these estrogens

is of adrenal gland origin. It is suggested that the uterus itself might be responsible for this phenomena.

CONCLUSIONS

The influence of orally administered 6-α-methyl 17-α- acetoxy-progesterone (MAP) or 6-α-chloro-17-α-acetoxy progesterone (CAP) and subcutaneously administered crystalline progesterone on the maintenance of corpora lutea was studied on rats. Dosage levels of MAP above 0.3 mg./day, that is 0.5 to 18 mg./day maintained recently formed corpora lutea in a histological functional state. Corpora lutea of previous ovulations which were in the ovaries at the time of MAP treatment, appeared to be stimulated or at least arrested in their involution. It was necessary to administer MAP daily in order to maintain corpora lutea in the ovaries. The administration of MAP at any stage of the estrous cycle resulted in maintenance of the most recently formed corpora in the ovaries. During the period of corpora lutea maintenance by MAP treatment, follicular growth in the ovaries was minimal. Although extremely low dosages of MAP resulted in maintenance of the corpora lutea (.5 mg.), levels higher than 5 mg. resulted in a greater stimulatory influence on corpora lutea. Similar studies utilizing oral administration of .277 mg. of CAP, biologically equivalent to 5 mg. of orally administered MAP and subcutaneous administration of crystalline progesterone in oil as high as 10 mg. per day

(biologically equivalent to 3 to 4 mg. of orally administered MAP) did not maintain corpora lutea. A single subcutaneous injection of crystalline progesterone, however, administered on the day of estrus delayed the next estrus by 8 to 10 days. It was apparent that the mechanism of action of MAP was different than that of progesterone. It is suggested that the action of MAP may be more similar to that noted in the chronic maintenance of corpus luteum following hypophysectomy. That is, the action of MAP might be mediated through a complete block of gonadotropin release and not by facilitating the release of a pituitary gonadotropin (LTH). It is also postulated that the role of MAP in the maintenance of the corpus luteum in the rat may be due to inhibition of a luteolytic factor of pituitary origin.

Corpora lutea were also maintained when hysterectomized cycling rats were treated with MAP. This demonstrated that the action of MAP on the maintenance of corpora lutea was not mediated through the uterus.

Additional findings of this work are that the cyclic variations in the vaginal smears of the normal cycling rats persist in rats placed on MAP or progesterone treatment. These cyclic variations did not occur at regular intervals nor were they predictable. The source of this variation in the vaginal smears was not governed by the ovary. The recurring changes in the vaginal cytology were not abolished in ovariectomized or MAP treated rats. The possibility that

adrenal glands might be involved in this phenomenon of the vaginal cytology was discounted, since the changes in vaginal cytology did not conform to the circadian rhythm of the adrenal secretions, and there was also considerable atrophy of the adrenal glands in animals under MAP treatment. It is not likely that atrophic adrenal glands would secrete sufficient quantities of estrogen to affect vaginal cytology.

Studies on deciduoma formation demonstrated that dosage levels as low as 0.5 mg. of MAP per day were sufficient for a maximal decidual response. However, the response was not uniform. That is, there were great variations in response to trauma from animal to animal. Ovariectomized MAP treated rats, traumatized on the day they exhibited an estrogenic type of smear, had a greater decidual response. This finding further supports the conception that cyclic changes of estrogenicity occur in MAP treated intact rats or ovariectomized rats.

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