FACTORS INFLUENCING THE DEVELOPMENT OF DECIDUOMATA
IN PUBERAL AND ADULT RATS

Dissertation

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By

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INTRODUCTION

A. Statement of the Problem

The experimental production of deciduomata in animals has during this past half century provided both the reproductive physiologist and the anatomist with a further understanding of the factors relating to the implantation and maintenance of the fertilized ovum. The influence of proper nutrition during pregnancy is of course well recognized; however, the role of nutrition with regard to the establishment of the maternal portion of the placenta has not been fully investigated. Since the production of experimental deciduomata gives rise to tissue which is identical to that of the 'maternal placenta' a technique was available for the study of nutritional deficiencies of the factors which enable the uterus to give rise to such growth. Acute starvation, a state of maximal nutritional deficiency, was therefore studied with respect to the influence it might have on the formation of deciduomata in the adult rat. Since to my knowledge, no one had investigated the ability of the puberal rat to produce deciduomata, studies were also extended to this problem. The potentially greater susceptibility of these young animals to acute starvation also merited a comparison with the adult levels of deciduoma formation. During the course of the above investigations points of interesting digression were suggested by the problem itself. Some of these relating to the nature of pseudopregnancy were therefore followed.

This dissertation therefore does not deal exclusively with the relationship of starvation to deciduoma formation in the rat, but
includes other factors that may have an influence on the development of the decidual tissue.

B. Review of Literature

1. Deciduomata and Their Experimental Production

a. Introduction

The experimental production of deciduomata (placentoma) was first reported by Loeb in 1907. These deciduomata are comparable to the 'maternal' placenta which is derived from the uterine stromal cells under the stimulus of a fertilized ovum. Loeb (1907) showed that if the guinea pig uterus was incised during the fourth to the sixth day of pregnancy, nodules would be found several days later. These nodules arose through proliferation of the stromal cells and were noted as early as twenty-four hours following the trauma. These growths persisted until the twelfth to the fifteenth day of pregnancy or pseudopregnancy. (Loeb, 1908a; Selye et al, 1935; King et al 1949) By this time, necrosis of the tissue was virtually completed. The uterus did not respond to traumatization at all times, but showed selective periods of sensitivity during which the trauma was effective in evoking a decidual reaction. These periods of sensitivity for the pregnant or pseudopregnant rat ranged from the fourth to the seventh day following copulation or the induction of pseudopregnancy. (Allen, 1931; Loeb,
1908; Lyon et al, 1938; Peckham et al, 1947, 1950; Selye et al, 1935, 1942) In the lactating rat the sensitive period extended from the fourth to the twenty-fourth day post-partum (Corner et al, 1919; Long et al 1922; Lyon, 1939; Lyon et al 1938; Peckham et al 1950; Selye et al 1935) The castrate rat was shown by Rothchild et al (1942) to maintain a sensitivity for 3 to 20 days under continued progesterone treatment. Following the example of Loeb (1908) who elicited the decidual reaction in guinea pigs, many investigators turned to other animals. Deciduoma formation has therefore been demonstrated in the rabbit (Loeb, 1908a), the rat (Frank, 1911; Corner et al 1919; and ‘many others), the mouse (Parkes, 1929; Atkinson, 1942) and the monkey (Hisaw, 1937; Heuser et al 1941).

b. Histology

The irritation of the uterine mucosa at the proper time resulted in the proliferation of decidual cells from the uterine stroma. In a period of days, the growth of these cells caused the formation of a tumor which distended the walls of the uterus. This deciduoma consisted of two distinct regions. One of these was made up of small vacuolated cells and was situated on the mesometrial aspect of the uterus while the other was located on the antimesometrial portion and was characterized by
large binucleate cells which were eosinophilic.
(Selye et al 1935; Krehbiel, 1935, 1937; Rothchild et al 1940) The mesometrial decidua contained glycogen in appreciable quantities (Selye et al 1935; Krehbiel, 1937) whereas the antimesometrial decidua was characterized by lipid deposition. (Krehbiel, 1937)

c. Methods of Uterine Traumatization
The original type of trauma used by Loeb (1907) in which the uterus was incised in many directions was modified by Long et al (1922). These authors inserted threads transversely through the uterine lumen by means of a needle. The loose ends were tied so that the position of the trauma could later be identified. Scratching of the uterine mucosa (Corner, 1919), mechanical compression of the uterus (Schlesnyak, 1951), or the application of electricity across the transverse axis of the uterus (Krehbiel, 1936, 1937; Schlesnyak, 1952) proved to be equally effective. Recently, Chambon et al (1952) and Schlesnyak (1952) have independently shown that the introduction of histamine solutions into the uterine lumen would also produce deciduoma. Doses of 1 microgram were more effective than 10 - 100 micrograms, while the 1000 microgram level gave no response (Chambon et al, 1952). The use of antihistaminics was found successful in inhibiting the decidual response to injected
histamine. Furthermore, antihistaminics injected into the uterine lumen were also capable of inhibiting the effect of other types of stimulation such as mechanical or electrical trauma (Schlesnyak, 1952, 1954a). The suggestion has been made that it is the release of histamine by the fertilized ovum that initiates placentation (Schlesnyak, 1952).

d. Hormonal Factors Relating to Deciduoma Formation

The development of a deciduoma depended on humoral factors. (Loeb, 1908a; Krehbiel, 1939) These investigators found that decidual reactions could still take place in a uterus that had been transplanted into subcutaneous tissue or into the kidney. Conclusive evidence for the necessity of the ovary for deciduoma formation was produced by Corner et al, (1919) who showed that removal of the ovaries of the rat 24 to 48 hours after parturition, prevented the decidual response. A somewhat similar experiment was performed by Selye et al (1935) who removed the ovaries after the establishment of deciduoma during lactation. Within three days, the deciduoma that had been formed involuted completely. The importance of the ovary in deciduoma formation has been related to the presence of functional corpora lutea whose secretion, progesterone, is required to maintain the sensitivity of the uterus to traumatization. Such active corpora lutea are found in
states of lactation, pregnancy, or pseudopregnancy, and in all cases enable decidua formation to occur. Deciduomata may be formed in the castrate if uterine sensitivity is maintained by use of corpus luteum extracts, or progesterone (Astwood, 1939; Goldstein et al 1929; Nelson et al 1930; Rothchild et al 1940, 1942; Weichert, 1928) Progesterone was also effective in eliciting the decidual response in the hypophysectomized-castrate rat (Rothchild et al, 1940). However, progesterone even in large doses did not enable the uterus to maintain the decidua indefinitely (Atkinson, 1942; Selye, 1942). Other substances capable of progestational activity such as desoxycorticosterone acetate, and pregneninolone were also shown to facilitate the production of decidua in the castrate rat (Masson, 1943; Cohen et al 1940).

Since ovarian function depends upon the anterior pituitary, one may conclude a priori that hypophysectomy would be detrimental to decidua formation. That such indeed was the case for the rat was demonstrated by Evans et al (1941), who found that decidua did not form in the absence of the anterior pituitary. Administration of anterior pituitary extract to intact rats or rabbits maintained corpus luteum activity and permitted decidua development to take place (Brouha, 1926; Evans et al, 1929;
Friedman 1932). The pituitary factor responsible for the maintainence and activity of the corpora lutea has been called luteotrophin (Astwood, 1941). In the rat, this hormone appears to be similar to, or identical with, prolactin which has been shown by Evans et al (1941b) and by Sydnor, (1945) to permit decidua production through maintenance of corpora lutea activity. This pituitary principle was shown to be effective only in the presence of the ovaries (Evans et al, 1941a). Furthermore, the corpora lutea must remain under continuous stimulation of the luteotrophic hormone, for when a lapse of 20 to 30 hours was permitted between hypophysectomy and the initiation of the luteotrophin, the corpora lutea became unresponsive to the trophic hormone. (Astwood, 1941; Evans et al, 1941a) If the luteotrophic principle was administered continuously for 15 days following hypophysectomy, the corpora lutea showed activity for only 10 to 13 days. (Astwood 1941) This limited activity of the corpora lutea even in the presence of sufficient pituitary stimulation was attributed by Astwood, (1941) to an 'inherent life span' of the corpus in the presence of LTH. The additional duration of pregnancy, he believed, was mediated by hormones of chorionic origin. That this is not the complete answer is obvious from the fact that pseudo-pregnancy can be associated with extended luteal function.
and this condition is devoid of chorionic activity. Recent studies by Everett (1954) indicated that in the normally cyclic rat, luteotrophic activity was apparently kept in state of inhibition for if the pituitary was separated from the hypothalamus and transplanted into the renal capsule or the carotid region of the neck, the luteotrophin became manifest as shown by ability for deciduoma formation. This luteotrophic activity occurred at the expense of FSH and LH for the ovaries showed follicle growth to the antrum stage only.

**e. Inhibition and Augmentation of the Decidual Reaction**

Loeb (1928), Courrier (1930) and Astwood (1939) noted that estrogens inhibited the development of deciduomata. The quantitative studies on the castrate rat by Rothchild et al (1940) showed that the dosage of estradiol given with a standard level of progesterone during the posttrauma period was a very important factor, for the lower estrogen levels tended to cause an augmentation of the reaction whereas the higher doses brought about an inhibition of the reaction. Part of the diminution in size could be accounted for by a reduction in the mesometrial portion of the deciduoma. The effect of different dosages of estrogen with a constant progesterone dose during the period preceding uterine traumatization showed that as little as 0.03 to 0.15 micrograms of estradiol per day
would prevent formation of the deciduoma if it was administered for the 3.5 days prior to uterine trauma (Rothchild et al. 1942). The action of this 'pretrauma' estrogen was believed to inhibit the progesterone's sensitization of the uterus to trauma, for when the estrogen (0.15 mg/day) was given with the progesterone during the postrama period, then no inhibition of deciduoma occurred, but on the contrary, deciduoma growth was increased (Rothchild et al. 1942).

The qualitative as well as the quantitative approach to estrogen inhibition of the decidual reaction was undertaken by Velardo et al. (1951). Various amounts of different estrogens were added to a constant dose of progesterone (1.5 mg/day) and administered to castrate rats during the first three days of the postrama period. It was learned that estradiol-17-beta is the most potent inhibitor of the action of progesterone. The other estrogens, in decreasing order of inhibitory activity were: estrone, estriol, estradiol benzoate, diethylstilbestrol and equilenin. Using a level of estradiol-17-beta that did not cause inhibition, the authors were able to show that if the ratio of progesterone to this estrogen were maintained, then inhibition would not be brought about even with larger amounts of the estrogen, and the deciduo-mata formed were all about the same size.

Hisaw et al. (1951) tested the ability of other steroid
compounds to inhibit decidual development. In the posttrauma period, 1.5 mg of the progesterone plus the compound being tested was administered for three days to castrate rats. Pregnandiol, which is usually thought to be 'physiologically inactive' was shown to inhibit decidual formation. Pregnenolone, up to 6.0 mg/day was also inhibitory but above this dosage it could induce the reaction (Cohen et al 1940). Pregnenolone showed no antagonism because it too was capable of permitting the reaction. Pregnandione neither inhibited nor induced the growth, while testosterone gave no inhibition until doses of 1.0 mg/day were reached. Desoxycorticosterone acetate, though enabling decidual development with 10 mg/day doses (Masson 1943) was inhibitory when administered with progesterone in 3 mg/day amounts. Cortisone or A.C.T.H. (1.5 mg/day) tended to suppress decidual formation. This may account for the interruption of pregnancy by cortisone and A.C.T.H. observed in mice and rabbits by Robson et al (1952).

Further evidence for an antagonism between the adrenal cortex and progesterone is found in the experiments of Poumeau-Dellile (1949), who found that castrated rats maintained on 0.6 mg of progesterone per day did not form deciduomata unless the adrenals and the accessory cortical tissue were also removed. The removal of the adrenal
glands in the rat was found to have no influence on luteal function in the hypophysectomized animal treated with luteotrophin (Astwood, 1941; Evans et al, 1941). The action of selected drugs on decidua formation was investigated by Schelesnyak (1954b). He found that instillation of epinephrine tartrate, oxytocin, or atropine sulfate into the uterus after the trauma gave inhibition of decidua. These substances were ineffective subcutaneously. Pitressin and serotone creatine were ineffective in suppressing the response. The mechanisms whereby aminopterine (Velardo et al 1953), relaxin (Frieden et al, 1953) and novacain (Molina et al, 1950) inhibit the decidual reaction require further elucidation. Selye et al (1935) found that the presence of placentae or decidua in one horn of the rat uterus inhibited the development of subsequent decidua following the traumatization of the other horn. Lyon et al (1943) and Atkinson et al (1945) have performed comparable experiments and have come to the same conclusion, i.e., that the placenta, or the primary set of decidua, produced some specific inhibitor which prevented the formation of secondary decidua and by this mechanism the animal was protected against the possibility of superfetation. However, others (Chambon, 1951; Kehl et al, 1951a; Rothchild et al,
1942; Peckham et al 1947a, 1947b) do not agree with the above findings since they have found that secondary deciduomata can be induced in the presence of a primary set of deciduomata, or a unilateral pregnancy. They therefore conclude that there are no specific inhibitory effects due to pre-existing deciduomata. The decrease in size of the secondary deciduomata has been attributed by Peckham et al (1947b) to the reduced sensitivity of the uterus at the time of the secondary trauma. If the sensitivity were markedly reduced, this might explain the failure to obtain the secondary deciduomata. Peckham et al (1950) have attempted to elucidate the cause for failure of deciduoma formation in the rat after the seventh day of pregnancy. They found that when both ovaries were removed on the fifth day of a unilateral pregnancy, the decidual response, following trauma on day nine and autopsy on day twelve, was only partially restored. However, if the ovaries were removed on the fourth day of pregnancy, thereby preventing implantation, trauma on day nine and autopsy on the twelfth day showed a complete restoration of deciduoma production. The authors conclude that an inhibitor, probably estrogen, is secreted from both the maternal ovaries, and the fetal trophoblast.
f. Miscellaneous Facts Relating to Deciduomata

Enzyme studies on decidua tissue obtained between the sixth and the twelfth days of pseudopregnancy have been conducted. (Meyer, 1952) Both nucleic acids were highest on the seventh day of pseudopregnancy and declined slowly through day eleven. Alkaline phosphatase activity increased up to the seventh day. During the involutional and necrotic phases of the reaction, acid phosphatase, succinic and malic dehydrogenase and beta glucuronidase appeared to be dominant. The enzyme histaminase has been found in abundance in decidua tissue (Swanberg, 1950; Roberts et al 1953) but its role has not been clearly established.

The influence of deciduomata and/or pseudopregnancy in reducing the blood pressure of hypertensive rats appears somewhat controversial at the moment. Page et al (1941) found a definite reduction in the blood pressure of their hypertensive rats under the influence of pseudopregnancy and deciduoma formation. Grollman (1947), on the other hand, claims to have repeated the work and finds no difference in the average blood pressure before and after the induction of deciduomata. However, he does not present his data, nor does he give any indication as to the degree of the decidual responses obtained in his six rats.
The data by Page et al (1941) in light of recent evidence (see infra) gives indication of extensive decidua formation, and their results may likely be the more valid of the two. (This problem, I believe, should be re-investigated).

2. **Pseudopregnancy in the Rat**

The most important characteristic of pseudopregnancy in the rat is the presence of a functional corpus luteum. In the short, 4 - 6 day cycle of the rat this structure is believed to be without any appreciable secretory activity. (Long et al, 1922). However, upon the initiation of the state of pseudopregnancy, the corpus luteum assumes a functional status, secretes progesterone, and is responsible for the abeyance of the estrus cycles by inhibition of the luteinizing hormone (Astwood et al, 1940). The duration of the pseudopregnant interval is somewhat variable. Earlier experiments gave ranges extending from 7 to 23 days (Long et al, 1922; Slonaker, 1929). Values obtained during the last few decades however, set the duration between 10 to 19 days with means between 13 to 14 days. (Ershoff et al, 1943; Peckham et al, 1948; Olsen et al, 1951).

The corpora lutea do not depend upon 'direct' nervous control for the maintainence of their activity as shown by their ability to function even upon transplantation (Long et
al 1922). Removal of the anterior pituitary caused a cessation of luteal secretion (Evans et al 1941). The pituitary principle responsible for active maintenance of corpora lutea was not the luteinizing hormone (Astwood et al, 1940) but rather the luteotropic hormone (Astwood 1941) which in the rat appeared to be similar to the lactogenic hormone with respect to its effect on the corpora lutea (Evans et al, 1941). For continuous maintenance of function the corpora lutea could not be deprived of luteotropic stimulation for a period of more than 30 hours (Astwood, 1941). Furthermore, the corpora lutea seemed to have a limited life span of 10 to 13 days in the presence of continuous luteotrophin administration (Astwood, 1941). The prolonged activity that is apparent under certain conditions, as pregnancy or lactation, has no adequate explanation at present.

Pseudopregnancy has been induced in the rat by mechanical stimulation of the cervix by means of a glass rod (Long et al, 1922; Leonard et al, 1929; Selye, 1933; Rothchild et al, 1939) or by electrical stimulation of the cervix (Greep et al, 1938; Shelesnyak 1931, 1953). Either type of stimulation was applied during the proestrus or estrus phase of the cycle. However, pseudopregnancy has also been induced by electrical stimulation during the 'diestrus interval' (Greep et al, 1938), but this was less efficient. Once the state of pseudopregnancy
was initiated, it could not be prolonged in duration by repetition of the cervical stimulus (Greep et al 1938). Electrical stimulation through the head of rats (Harris, 1936) or rabbits (Marshall et al, 1935) also resulted in pseudopregnancy. Intranasal instillation of silver nitrate in the rat gave rise to the pseudopregnant condition (Rosen, 1937) as did also the removal of the sphenopalatine ganglion (Shlesnyak, 1940). However, in light of the work of Swingle et al (1951) these actions were probably non-specific. Copulation with a vasectomized male was capable of producing a pseudopregnancy (Long et al, 1922). No adequate explanation has been offered for the findings of Haterius (1933) who noted that abdominal sympathectomy prevented the induction of pseudopregnancy through mechanical or electrical stimulation of the cervix, but did not interfere with the induction of pseudopregnancy following infertile copulation. Stimulation of the nipples of rats brought about the pseudopregnant state (Selye et al, 1934) and so did lactation (Long et al, 1922). The duration of pseudopregnancy during lactation seemed to be a function of the litter size (Long et al, 1922). This was also true for gestation, i.e., the interval between fertilization and delivery was prolonged in the presence of a suckling litter (Weichert, 1940). The prolongation was attributed to a delay in implantation of 6 to 16 days.

The taking of vaginal smears for extended periods of time
appeared to lessen the responsiveness of rats to the
induction of pseudopregnancy by mechanical or electrical
stimulation as well as by infertile copulation (Ball, 1934;
Hall, 1950; Vogt, 1933).
The cervical stimulus for the initiation of pseudopregnancy
could be rendered ineffective by use of anaesthetics as
ether or nembutal (Jacobson et al. 1950; Meyer et al., 1929).
Rats stimulated under spinal anaesthesia also failed to
become pseudopregnant (Meyer et al, 1929). Epinephrine and
acetylcholine, as well as their antagonists, dibenamine and
atropine, were also claimed to block the induction of pseu-
dopregnancy, but no explanation was offered (Jacobson et al
1950). Direct application of acetylcholine to the exposed
rat pituitary produced the pseudopregnant state (Taubenhaus
et al, 1941). Administration of progesterone for 3 to 4 days
to adult rats was sufficient to prevent the induction of
pseudopregnancy following cervical stimulation (Astwood et
al, 1940). The authors attributed the failure to the sup-
pression of L.H. which is required for ovulation.
Bilateral pelvic nerve resection in the rat rendered the
vaginal and cervical mucosa completely anaesthetic and anal-
gesic thereby interrupting the afferent portion of the genital-
nerve-pituitary pathway. Under this condition copulatory
stimuli did not reach the pituitary since normal estrus cycles
continued without the induction of pseudopregnancy or pregnancy
The duration of pseudopregnancy has been found to be prolonged in the presence of massive deciduomata. The interval was made to approach or equal that attained during gestation by increasing the degree of deciduoma formation (Ershoff et al., 1943; Peckham et al., 1948; Olsen et al., 1951; Verlardo et al., 1953). Under these conditions luteal function was maintained and ovulation was suppressed. The corpora lutea and the metrial gland appeared to be the two persisting functional structures but no further explanation was advanced to explain the prolongation (Verlardo et al., 1953). Kamell et al. (1948) failed to find the extended pseudopregnancy following deciduomata formation in mice. However, the difference may lie in the degree of deciduomata formation produced by these investigators, as well as the fact that deciduomata degenerate rapidly after the seventh day in the mouse as compared to about fourteen days in the rat. The extended duration of the pseudopregnancy seen in the data presented by Page et al. (1941) may be indicative of more massive deciduoma formation and therefore may render their blood pressure findings (see supra) more valid than those of Grollaman (1947).

Bilateral adrenalectomy in the rat was found to give rise to the pseudopregnant state (Selye et al., 1935; Swingle et al., 1951b). Some animals continued to show several normal estrus
cycles before becoming pseudopregnant. The cycles could be returned to their normal status by administration of adrenal cortical extract (Kroc et al, 1934; Corey et al, 1934). Swingle et al. (1951b) believed that adrenalectomy caused the removal of some inhibitor of pituitary luteotrophin and the hormone could then be released to activate the corpora lutea. (This hypothesis of Swingle has some support in the recent studies by Everett (1954) who found that removal of the pituitary from its hypothalamic association and its subsequent transplantation to other body sites gave rise to luteotrophin secretion, and activation of the corpora lutea). Swingle et al (1951a) have also shown that any vigorous, non-specific stress procedure as injections of adrenalin, alloxan, insulin, tissue homogenates and formaldehyde were all capable of causing the release of luteotrophin in the rat. These agents, although not active in the ovariectomized animal, were active in the adrenal demodulated preparation. These non-specific stresses of Swingle et al. (1951a) may also explain the pseudopregnancy produced by starvation (Selye et al, 1935) and by the feeding of 0.25 to 0.5 gm of dessicated thyroid per day (Selye et al, 1935; Weichert et al, 1933).
MATERIALS AND METHODS

The animals used in these experiments were derived from the Sprague Dawley strain and were obtained from the Holtzman Rat Company of Madison, Wisconsin. Adult rats were 4 months of age while the immature animals were 22 - 24 days of age at arrival. All animals were sent by air freight, and upon arrival in our laboratories were housed in cages in which three adults or five immatures occupied a floor space of approximately 70 - 90 sq. inches in a cage volume of 400 - 700 cu. in. Diet consisted of standard Purina Dog Chow Checkers fed ad libitum. No dietary supplementation was provided. Water, of course, was available at all times. The animals were individually marked by the system of ear punches. In approximately 50% of the animals the markings were made on the 6th - 7th day after arrival. In the remaining animals the markings were applied on the 2nd day of arrival. The immature animals were examined daily for the occurrence of vaginal opening. When this state of development was attained vaginal smears were begun and continued each day. The 'smear' was taken by introducing the tip of a medicine dropper containing a drop of warm water into the vaginal orifice. The water was admitted into the vagina and then withdrawn and placed on a glass slide for immediate reading. No staining was used. Adult animals were smeared in the same manner. The presence of various types of cells in the smear served as an indication of the stage of the estrus cycle (Long et al, 1922). A smear was regarded as proestrus (Stage I), if small,
rounded epithelial cells were observed. If cornified cells were present, the designation of estrus (Stage II; III) was given. A diestrus smear (Stage V) was interpreted as consisting of epithelial cells and leucocytes.

Pseudopregnancy was induced by insertion of the medicine dropper into the vagina until resistance (cervix?) was encountered. This was done at the proestrus or estrus phase of the cycle. Withdrawal and re-insertion of the dropper was continued about 30 times in fairly rapid succession. The establishment of pseudopregnancy was verified by the persistence of the diestrus type of smear. Pseudopregnancy and decidua formation were induced in adult females, and also in puberal ones at successive estrus following the occurrence of vaginal opening, i.e. at the first, or the second, or the third, or the fourth, or the fifth or the sixth. Decidua formation was induced by uterine traumatization on the fourth day of diestrus. The uterus was reached by a ventral abdominal incision. A burred needle was inserted into the uterine lumen at the bifurcation of the cornu. The needle was advanced toward the ovarian end of the uterus until the resistance of the utero-tubal junction was encountered. The antimesometrial wall was scratched as the needle was withdrawn. The process was repeated for the other horn. Autopsy was performed five days later i.e. the ninth day of diestrus (see Fig. 1, "Basic Plan"). Animals were killed by overanaesthetization with ether. The uterus, ovaries and adrenals were removed, cleaned, and weighed. The uterus was weighed on a beam balance with a sensitivity of 0.1 mg while the ovaries and adrenals
were weighed on a Roller-Smith balance whose sensitivity permitted readings of 0.1 mg. The ovaries and uteri were then fixed in Bouin's solution for histological study.

The periods of starvation which were employed varied in duration according to the scheme presented in Fig. 1. The term starvation is taken to mean the complete withdrawal of food. Water was available. Each animal was isolated until the end of the starvation period. The starvation period was begun immediately after vaginal smearing late in the A.M. and continued until the same time of the morning of one of the subsequent days.
Figure 1. Basic scheme and periods of starvation
RESULTS

A. Deciduoma Formation in the Puberal vs. the Adult Rat

Maturation of the puberal animal brought with it an increased ability for deciduoma formation. The animal at the time of vaginal opening was capable of becoming pseudopregnant like the adult; however, its capacity for deciduoma formation, measured on an absolute weight basis, was found to be only 60% of that achieved in adulthood. On a relative weight basis the response between puberal and adult was the same, i.e. about 1.7 mg/100 gms body weight. By the time the 6th estrus cycle was reached sufficient maturation had occurred to enable the adult type of response in terms of absolute weight. (See Fig. 2) Increases in ovarian as well as adrenal weight accompanied the general body weight rise throughout this period (see Table I.).

B. Influence of Starvation on the Deciduoma Response of Puberal and Adult Rats

1. Pretrauma vs. Posttrauma Starvation

In the puberal animal a definite inhibition of deciduoma formation was noted following pretrauma (day 0-4) starvation. (see Table 2, 5) This inhibition was accompanied by a significant (p=0.05) reduction in adrenal weight at the time of autopsy. The body weights at the beginning and end of the experiment were about the same, indicating that the 30% loss in initial body weight as a result of pretrauma starvation was recovered during the refeeding period. Even with advance in maturity, the animal at the 6th estrus cycle level was still unable to produce deciduomata to the same degree as was the adult (see Fig. 2) The latter animal
appeared to be unaffected by the pretrauma starvation, for its response was like that of its non-starved control. Table 4 shows that the decidual response of the pretrauma starved adult was adequate following pretrauma starvation, while ovarian, adrenal, thyroid and pituitary weights were all depressed to some extent. Liver weight recovered its loss during postrauma feeding.

Postrauma starvation in puberal rats did not reduce the decidual response to the same degree as did the pretrauma starvation (Table 3). The younger animals showed a greater depression than the more mature individuals. Nevertheless, at the same level of maturity postrauma starvation permitted an increase of almost 100% over that achieved by pretrauma starvation (Table 5). This was surprising in light of the fact that these puberal animals grew these tissues during a period of complete starvation, in which their own body weights underwent a loss of 36% of the weight they possessed at the time of trauma (day 4). The full grown adult was comparable to the animals at the level of maturity attained at the 6th estrus (see Table 3). Both types of animals gave responses of the order of 2.6 to 2.9 gms. This was still below the non-starved level of 4.0 gms. As a result of the postrauma starvation, the ovarian weights were reduced in all animals, puberal and fully matured (see Table 4,5). The adrenal weight however was maintained. The slight increase was not significant (p=0.60). This maintenance of weight may be indicative
Figure 2. Effect of starvation on deciduoma formation of the rat at different levels of maturity
of a hyperactivity. The full grown adult, following posttrauma starvation, was also characterized by a reduction in thyroid, pituitary, and especially liver weight. These same reductions may have also occurred in the younger animals but these organs were not studied for them.

One group of adult animals was starved for both the pretrauma and the posttrauma periods (i.e. days 0-9). Deciduoma formation was possible (see Table 4) and was not depressed much below that obtained by posttrauma starvation alone. A further reduction of ovarian, thyroid, pituitary, and liver weights below the 4-9 day starvation level was also noted. Again the adrenals appear to have maintained normal weight. The puberal animals were not studied under this prolonged (0-9) starvation as they succumbed before the end of the experiment.

2. Effect of 4 Days of Starvation in Animals at the First Estrus

The effect of four days of starvation on deciduoma formation at the pseudopregnancy induced at the first estrus, depended on where the starvation was situated with respect to either the pretrauma or the posttrauma period. When the bulk of the starvation occurred in the pretrauma phase (days 0-4, 1-5, 2-6) there was greater inhibition than when it included more of the posttrauma period (days 3-7, 4-8). (see Table 6) Starvation from days 4-8 at the first estrus permitted somewhat better growth (significant at 5.0% level) of deciduoma than
3. Effect of Limited Starvation Within the Pretrauma Period of
Rats at the First Estrus

When the pretrauma period of starvation was shortened from
days 0-4 of pseudopregnancy to days 0-3, or 0-2 or 0-1 of
pseudopregnancy an inhibition was found only if the starva-
tion included the 3rd and/or the 4th day of pseudopregnancy.
That is, starvation on days 0-1 or 0-2 gave no interference
with deciduoma formation but starvation from days 0-3 showed
some inhibition which was more pronounced as the starvation
extended to days 0-4 (see Table 7).

A single day of starvation during the pretrauma period gave
no suppression of deciduoma (see Table 8). That is, starva-
tion from days 0-1, 1-2, 2-3 or 3-4 gave results no different
from the non-starved animals. The animals starved from days
2-3 of pseudopregnancy showed a slight reduction in response
but the difference was not significant (p=0.10). The slight
elevation in response noted with days 3-4 starvation was also
not significant (p=0.30).

A starvation period of two days i.e. from days 0-2, 2-4, or
4-6 of pseudopregnancy gave inhibition only with the 2-4 day
group (Table 9). The response of the animals starved from
days 0-2 or days 4-6 was not different from that of the non-
starved controls while the 2-4 day group showed a 50% reduc-
tion in deciduoma formation (see Table 9). (Using a paired
test of comparison this difference was significant at the 1% level).

C. Effect of Progesterone Administration During the Pretrauma Starvation Period

Daily administration of 2 mg of progesterone during days 1-4 inclusive of the pretrauma period gave no improvement in the size of the decidual reaction of the non-starved animal (see Fig. 3). The pre-trauma starved (days 0-4) animal appeared to show a slight improvement with the progesterone treatment, but the difference was not significant (p=0.40) (see Fig. 3).

D. Spontaneous Occurrence of Deciduomata

In the course of obtaining daily vaginal smears, several animals were found with traces of blood in the vagina. In over 1000 animals who had been followed daily for a period ranging from one to twenty cycles, there were found only ten such animals with a bloody smear. Five of these animals were autopsied immediately and the presence of deciduoma, either in nodular or extensive form was established. The animal in each case was noted to be in a state of spontaneous pseudopregnancy.

E. Pilot Experiments on Miscellaneous Factors Possibly Relating to Deciduoma Formation

1. Histamine and Deciduoma Formation

The injection of 0.5 cc of histamine acid phosphate (1.35 mg) into one lumen of a rat on the fourth day of pseudopregnancy resulted in extensive formation of deciduoma in that horn. The contralateral horn received the same volume of saline and showed a nodule only at the site of entrance of the
Figure 3. Influence of progesterone on deciduoma formation of the non-castrate rat (pseudopregnancy induced at the time of vaginal opening)
syringe needle. Earlier attention to the possible use of histamine in the initiation of the decidual reaction had been focused upon an explanation for the observed hyperemia that accompanied the trauma to the uterus, no matter how the trauma was applied. An attempt to induce the decidual growth by simulation of the hyperemic state via intraluminal injection of the cholinergic agent mecholy chloride, was unsuccessful. In a total of 11 animals, doses ranging from 0.125 mg to 2.0 mg per cornu were ineffective. The material did enter the systemic circulation since extensive salivation and lacrimation were noted.

2. Adrenalectomy and Deciduoma Formation

Removal of both adrenals from adult rats on the fourth day of pseudopregnancy followed by maintenance on 1% NaCl, enabled normal deciduoma production in all four animals. If, however, the adrenals were removed on day 0, soon after cervical stimulation, then an impairment of deciduoma production was noted. The mean for the five animals was now 3.0 gms instead of 4.0 gms usually achieved by intact non-treated adults. In all cases, the status of the accessory adrenals was not determined for these adrenalectomized animals.

3. Unilateral Castration and the Decidual Reaction

Right hemicastration performed on day 0 or day 4 of pseudopregnancy still enabled deciduoma formation whose means were not statistically significant from that of the controls. The
remaining ovary of these hemicastrates contained from 2 to 7 corpora lutea each (gross examination), and the weight of the ovary was not significantly different from the weight of the left ovary of the control animals. Although statistically the response obtained following hemicastration on day 4 is not significant, the number of animals used in this group was too small. That is, four animals were hemicastrated on day 4, while 8 animals were hemicastrated on day 0. The non-castrate group also contained 8 animals. A repetition of the experiment is probably worthwhile.

4. **Bilateral Castration and the Degree of Decidual Response Attained with Estrogen - Progesterone Combinations**

In a series of experiments (4 adults each) deciduoma formation in the castrate was tested as follows:

a. **Long Term Castrate**

Animals gonadectomized from one to two months before use were primed with a single dose of 1 microgram of estradiol. On the day following vaginal cornification doses of 10 mg or 20 mg of progesterone were administered daily (the total dose given in two subcutaneous injections), until day 8. Trauma and autopsy were performed as in "Methods" above. The 10 mg level gave a response of $1.77 \pm 0.19$ gms while the 20 mg progesterone dose gave a response of $2.84 \pm 0.45$ gms. Although these differences between the 10 and 20 mg level were not significant ($p=0.10$), the
values for each were well below those achieved by the non-castrate adult.

b. **Castrate Day 0**
The pretrauma dose of progesterone was maintained at 2 mg per day in these animals gonadectomized on day 0. The postrauma dose however, contained not only 2 mg of progesterone but also sufficient estradiol to give various ratios of estrogen to progesterone. The ratios and results were as follows: (Normal non-castrate response was about 4.0 gms).

1. **Postrauma Progesterone Alone**
   (2 mg) gave a decidual reaction of \(2.56 \pm 0.14\) gms. This was definitely below the normal level but was comparable to the response achieved by 20 mg progesterone in long term castrates.

2. **Postrauma Estrogen - Progesterone** *(ratio: 1/20000)*
The response here was \(2.93 \pm 0.37\). Not much improvement was seen over the 2 mg progesterone. The response still was below the normal level.

3. **Postrauma Estrogen - Progesterone** *(ratio: 1/1500)*
Development now approached that of the normal, for response reached \(3.5 \pm 0.26\) gms. This difference was significant \((p=0.05)\) over that found for 2 mg progesterone alone.

4. **Postrauma Estrogen - Progesterone** *(ratio: 1/10000)*
A value of \(3.12 \pm 0.14\) gms was reached by this treatment. This value receded from that of the normal, indicating perhaps possible estrogen inhibition.
F. Studies on the State of Pseudopregnancy

1. Occurrence of Spontaneous Pseudopregnancy at the Time of the First Vaginal Smearings (Puberal and Adult Rats)

In a population of 250 puberal animals smeared daily from the time of vaginal opening, 37% were found to possess first cycles whose duration was comparable to that obtained upon deliberate induction of pseudopregnancy. That these spontaneously prolonged cycles were true pseudopregnant cycles was established by the presence of decidualata after uterine traumatization on the fourth day of vaginal leukocytes.

In a total of 306 adults (age 4 months) pseudopregnant cycles appeared in 30% of the animals following the first or second estrus of smearing. This high incidence was due to the inclusion of a group of 145 animals, arriving in July, 1952, most of which became pseudopregnant. If this group were not included, then the spontaneous pseudopregnant incidence for the 161 remaining adult animals would be 15%. Many of the 306 adult rats were already in the prolonged cycle when the smearing was begun.

2. Prolongation of the Estrus Cycle as a Result of Starvation (No Cervical Stimulation)

In an attempt to determine whether starvation itself was capable of initiating a pseudopregnant period, a group of 6 adult rats were starved for four days commencing on the day of estrus. No cervical stimulation was given. It was found that in 4 of the 6 animals a prolongation of the immediate
cycle occurred. This prolongation amounted to cycle durations of 8, 10, 13 and 15 days respectively. (Unfortunately, no attempt was made to confirm the pseudopregnancy by decidual formation). The remaining 2 animals showed two or three cycles during this time.

3. Duration of Induced Pseudopregnancy Following Pretrauma Starvation

Of the 66 animals that were taken at the first six estrus cycles and were cervically stimulated, and then subjected to pretrauma starvation, twenty-five (38%) were found to have the induced pseudopregnancy interrupted by the eighth or ninth day following the cervical stimulus. Of 62 non-starved animals at the first six estrus cycles, only 2 (3%) showed an interruption of the pseudopregnancy. The interference with pseudopregnancy in the starved animals was more consistent in those rats maintained under normal temperature conditions. A considerable reduction in the number of interrupted pseudopregnancies was noted in animals maintained at temperatures of 85 to 90 degrees F.

Eight animals maintained at the 85 - 90 degrees temperature were taken at the time of first estrus, cervically stimulated, and then subjected to pretrauma starvation. These animals continued through the induced pseudopregnancy without interruption. Furthermore, at the termination of the induced pseudopregnancy, 5 of the 8 animals, (60%) proceeded into another pseudopregnancy spontaneously.
4. Duration of Pseudopregnancy as Influenced by the Presence of Deciduomata

Six animals at the estrus of vaginal opening were made pseudopregnant and the uterus was traumatized for deciduoma formation. Instead of autopsy, however, the animals were allowed to continue in the state of pseudopregnancy until the animals returned to estrus. The pseudopregnancy under these conditions was not terminated at the usual time (mean is usually between 13 and 14 days). Instead, a prolongation occurred in which the pseudopregnant interval now showed a mean for the six animals of 20.2±0.32 days. Each of the animals had a pseudopregnant length ranging between 19 and 21 days. The latter interval, interestingly enough, is also the approximate duration of the gestation period.
DISCUSSION

A. Deciduoma Formation in Puberal vs. Adult Rats

Although the immature rat (18 - 26 days) has been found to show the decidual response under proper hormonal treatment (King et al, 1949; Shelesnyak, 1931, 1933a, 1933b), no studies have been made on the puberal animal. This present series of experiments has established that at the time of vaginal opening, the Sprague Dawley rat was capable of a deciduoma response (absolute weight) which was 80% of that obtained in the fully matured animal. (On a relative weight basis the response was equal to that of the adult.) Sufficient maturation of the puberal animal was achieved by the 6th estrus cycle, at which time it produced deciduomata equal to those of the adult (absolute weight). The animals at this time were about 69 days of age (autopsy) and weighed 186 gms. Ovarian and adrenal weights were also at the adult (4 - 6 month) level. This particular index of reproductive maturity (deciduoma response) shows some agreement with other indices used for the guinea pig (Ford et al, 1953) and the mouse (Mills et al, 1936). In the guinea pig, Ford, (1953) found that the vaginal opening which occurred at each estrus period in this animal was considerably prolonged at the early cycles. The adult duration of opening was noted by the time the animals reached the 5th cycle. Mills et al, (1936) found that full fecundity was not established in the mouse until the 6th or 7th cycle had been reached.

No explanation can be offered at present for the reduced deciduoma response in early puberty and its improvement with maturity. Several possibilities present themselves however. These are: (1) a
reduced corpus luteum activity either as a result of a deficiency in luteotrophin production or release, or else a diminished sensitivity to the trophic hormone. (2) a decreased sensitivity of the uterus to the luteal hormones. (3) the amount of uterine tissue which is available for participation in the deciduoma response may be submaximal in the puberal rat.

B. The Deciduoma Response as Influenced by Starvation

No studies have as yet been reported, aside from the author's, on the influence of starvation on the decidual reaction. In 1935, Selye et al mentioned that the diestrus resulting from reduced food intake enabled the formation of microscopic deciduomata following uterine traumatization. In the present investigation, periods of starvation which included all of the four days prior to uterine trauma or periods of starvation that included only the five days after trauma were studied. The four days of the pretrauma period are associated with uterine 'preparation' for implantation of the ovum (or deciduoma response to induced trauma). The preparation is possible through the action of progesterone. This period is also characterized by a marked sensitivity to estrogen, small amounts of which are capable of vitiating the progestational effect necessary for implantation (Rothchild et al, 1942). The postrauma period represents the period of actual growth of the deciduoma. During this time, progesterone is required. Furthermore, doses of estrogen which caused inhibition of the sensitivity of the uterus to progesterone now have no inhibiting effect, (Rothchild et al, 1942). Actually, an augmentation of the decidual response may be achieved.
In the present experiment it is concluded that the marked diminution in the decidual reaction apparent after pretrauma starvation was the result of a loss of uterine sensitivity. This is based on the finding that postrauma starvation which was of a longer duration than that of the pretrauma starvation was nevertheless compatible with a greater degree of deciduoma formation. Apparently the postrauma starved animal was still capable of growing deciduomata from its own body stores in spite of a concomitant loss of 36% of its body weight. The pretrauma starved rat, however, although it had exogenous food available during the postrauma period, still gave an inhibited response. The nature of the proposed loss of uterine sensitivity is as yet undetermined. However, several possibilities may be considered:

1. a reduction in hypophyseal luteotrophin as a result of starvation (Meites et al, 1949, have reported a reduced luteotrophin formation in female rats following caloric restriction). The diminution in luteotrophin would also lower ovarian progesterone production which is needed for uterine sensitivity. (2) an increase of adrenal cortical activity as a result of the stress of starvation. (Selye (1946) has shown that starvation may constitute a 'stress' in which an adrenal hypertrophy occurs. The type of starvation is important however for the hypertrophy does not appear in chronically undernourished rats. Baker (1953) compared the effect of ACTH and chronic inanition on the zona fascicuMa and found an hypertrophy with ACTH but an atrophy following inanition. The atrophy he attributed to a direct denial of nutriment to body tissues in general and not to a stress reaction involving the pituitary - adrenal axis. Complete inhibition of the
decidual reaction in castrate rats was noted following administra-
tion of 1.5 mg/day of cortisone or ACTH given simultaneously with
progesterone during the postrauma period (Hisaw et al, 1951).

Robson et al, (1952) have found that cortisone or ACTH interrupted
the pregnancy of mice and rabbits. An increase in adrenal cortical
activity which has been assumed for the present experiments involv-
ing starvation may have affected the pretrauma sensitivity to pro-
gesterone and in that way reduced deciduoma formation. (3) an in-
sability on the part of the liver to inactivate estrogens in the face
of starvation. A subsequent increase in estrogen titer would be
capable of inhibiting the sensitivity to progesterone as shown by
Rothchild et al (1942). (4) the possible utilization of progesterone
by the stress reaction and therefore its relative lack of availability
for adequate uterine sensitization. (Progesterone can prolong the life
of the adrenalectomized animal (Emery et al, 1940; Corey, 1941)).

This explanation however, appears unlikely since the administration
of 2 mg of progesterone daily during the pretrauma starvation gave no
improvement in the decidual response.

Deciduoma formation in the postrauma starved rat was reduced when
compared with the non-starved controls. But, this reduction was much
less than that noted with pretrauma starvation. The lessened degree
of deciduoma response of postrauma starved animals could not have been
due to a loss of sensitivity for the sensitivity had already been
established before the starvation was initiated. The diminution may
be attributed to: (1) a reduced progesterone output by the corpora
lutea and therefore reduced maintenance of the decidua (2) a 'reluc-
tance' on the part of the animal economy to satisfy this new tissue
with all its demands, especially since they have to be met at the expense of other body tissues (3) a concomitant removal of tissue from the decidua at a slightly lower rate than it is being deposited. The catabolic action of the cortical hormones which may be increased during this period of starvation may be partially responsible.

In the adult postrauma starved animal the deciduoma response is similar to that of the more mature, puberal rat. Pretrauma starvation in the adult was without effect on the degree of deciduoma formed i.e., both pretrauma starved and control gave the same values. If the starvation extended from the time of cervical stimulation until autopsy i.e., included both the pretrauma and postrauma periods, the deciduoma formation achieved was not much reduced below that of the postrauma starvation level. The adult animal therefore, unlike the puberal one can not be said to undergo a loss of uterine sensitivity as a result of the pretrauma starvation. Lee et al, (1952) found that a diet low in protein (5%) caused a termination of pregnancy in mice. A protein free diet, started on the day of breeding, resulted in 90 - 100% resorption. Fetal death usually occurred on the 9th or 10th day of gestation (Nelson et al, 1953). Perhaps in these cases of protein restriction the effect may have been mediated via an involvement of the decidua.

C. Effect of Progesterone During Pretrauma Starvation

The failure of 2 mg of progesterone during the pretrauma period of starvation to improve the decidual response may be indicative of either an interruption at the pituitary level followed by lack of proper luteal maintenance during the postrauma period, or else the presence of some additional substance during the pretrauma period that negates the
sensitizing action of progesterone. Such a substance might be an adrenal cortical hormone. It remains to be seen whether this material can exert its influence during the pretrauma period. The interference with deciduoma formation when administered during the postrauma period has already been demonstrated (Hisaw et al, 1951).

D. Spontaneous Deciduoma Formation

Previous to the report of Selye et al (1934) on the occurrence of spontaneous deciduomata during the pseudopregnancies which were induced by irritation of the nipples of rats, the only other accounts of spontaneous deciduoma formation were those noted in the presence of Vitamin E deficiency. (Bishop et al, 1926; Evans, 1926) Evans (1928) showed that 60% of animals maintained on vitamin E deficient diets formed deciduomata spontaneously. Furthermore, he found that they were not induced by the descent of non-fertilized eggs into the uterine lumen for removal of the tip of the uterus did not reduce the incidence of their appearance. He also noted that the size of deciduoma following deliberate uterine traumatization was greater if the animals were maintained on a low rather than a high vitamin E diet. In the present experiments, a low rate (1%) of spontaneously formed deciduoma was noted. The spontaneous presence of these growths in our animals does not invalidate any of the findings herein reported, for the deliberate traumatization of the uterus gives rise to deciduoma whose size is believed to be maximal for the given experimental conditions.

E. Pilot Experiments

1. Histamine and Deciduoma Formation
The manner in which histamine induced the growth of deciduomata is not known. In these experiments, the failure of the cholinergic agent 'Mecholyl' to mimic this action of histamine may indicate that initiation was not mediated through vascular alterations. Shelesnyak (1954b) found that if at the time of uterine trauma he injected 0.1 ml of Ringer's solution containing epinephrine tartrate (0.01 - 0.13 mg) or atropine sulfate (5 - 50 mg) he obtained a suppression of the deciduoma response. Injection of intrauterine, local anaesthetics were ineffective in inhibiting the response. He concluded that the mechanism for decidual cell initiation involved a "triad of an adrenergic-cholinergic-histaminic system". It would indeed be interesting to know how a single administration of histamine can serve to stimulate the growth of cells over a period of several days. Once initiated, does the reaction continue on its own by providing histamine?

Deciduomata have been found to contain histaminase in high concentration (Roberts et al, 1953). The function of this enzyme is at present speculative but some believe (Roberts et al, 1953) that it may serve to protect the uterus against histamine released via tissue breakdown.

2. Adrenalectomy and Deciduoma Formation

The present experiments have shown that removal of the adrenals in pseudopregnant rats on day 0, plus maintenance on NaCl, caused a reduced deciduoma response. Poumeau-Delille (1949) on the otherhand concluded that an antagonism existed between
the adrenal cortex and progesterone. This conclusion was based on the finding that he could obtain deciduomata in castrate - adrenalectomized rats using a dose of progesterone (0.6 mg) which was ineffective in the castrate - non adrenalectomized rat. The discrepancy between his results and those found here concerning an inhibition following adrenalectomy, may be related to the fact that removal of the adrenals caused an increase in ACTH which may have influenced the pattern or the output of luteal hormone in the non-castrate pseudopregnant animal. The possibility that ACTH may stimulate the corpora lutea is based on the report of Meyer (1952) who found that a pregnancy in the rat could be maintained by as few as two corpora lutea. If he removed the adrenals, in such a preparation, on the 12th day of gestation, the pregnancy not only continued, but the corpora lutea were found to hypertrophy from a usual maximum of 5 mg (during pregnancy) to one of 7 or 8 mg. He therefore was tempted to think in terms of stimulation of the corpora lutea by ACTH.

3. Unilateral Castration and Deciduoma Formation

In an attempt to determine whether or not a reciprocity existed between pituitary luteotrophin and active corpora lutea it was found that hemicastration on day 0 gave no interference in deciduoma formation. This may be indicative of either a reciprocity or else an output of hormone by the corpora lutea which is in excess of that needed for deciduoma formation. The results achieved by hemicastration on day 4 were not conclusive because
of the small group, but if the suspected trend toward inhibition is substantiated, it may indicate that a reciprocity is possible only under certain conditions and that the output of luteotrophin may be 'set' early in the pseudopregnancy.

4. Bilateral Castration and Deciduoma Formation

The deciduoma response in the long term castrate rat receiving daily progesterone was still below that achieved in the normal intact rat. Doses as large as 20 mg progesterone per day were still incapable of duplicating the normal response. Rothchild et al (1939) found that the addition of 0.6 mg of estradiol to 3.0 mg of progesterone during the postrauma period of rats castrated at the time of uterine trauma gave responses that were 'normal' in size. This ratio of 1/5000 appears comparable to that of 1/15000 for the present experiments. The discrepancy may be attributed to the difference in the time of castration (in the present experiments castration was performed on day 0) as well as the use of different end points for determination of degree of response. In the former, diameters (antimesometrial - mesometrial) were used while in the latter the total uterine weight served as an index of the deciduoma response. The important fact indicated by both types of data is that in studies of deciduoma formation involving the castrate animal, it would be best to use the proper ratio of estrogen/progesterone during the postrauma period instead of progesterone alone, thereby achieving a closer basis of comparison with the non castrate.
F. Studies on the State of Pseudopregnancy

1. Spontaneous Pseudopregnancy at First Vaginal Smearings

The high incidence of spontaneous pseudopregnancy at the first cycles following vaginal smearing may have been indicative of a stress like response due to unfamiliarity of the animal with this type of handling. It may also have had its inception in whatever stress resulted from 'ear marking' for many of the adults appeared to be in a state of pseudopregnancy at the time the smearing began. Although the ear punches were made as early as a week before the smearings were initiated, there is good evidence in the experiments of Greep et al (1938) Swingle et al (1951) and Everett (1952) that the stimulus to the induction of a pseudopregnancy may take place quite some time before the actual pseudopregnancy appears. In the meantime, one or more complete cycles may be seen. The large number of initial spontaneous pseudopregnancies in the adult rats which arrived in July, may be related to the stress of summer heat combined with a possible inadequate water supply during transit.

2. Prolongation of the Estrus Cycle as a Result of Starvation

In adult rats receiving no cervical stimulation, the prolonged duration of the estrus cycle as a result of days 0 - 4 starvation may be the equivalent of a true pseudopregnancy. (This was not tested by deciduoma formation.) If so, these findings would agree with those of Selye et al (1935) who found that uterine traumatization in rats subjected to chronic inanition gave rise to 'microscopic deciduoma' (and therefore pseudopregnancy). In the present experiment the prolonged cycle continued
even after food was returned to the animals. The starvation may have initiated the pseudopregnancy in the same manner (mechanism unknown) as the non-specific stresses of Swingle et al (1951).

The puberal animals which were maintained at high temperature (85 - 90 degrees) and were starved during the first four days of the induced pseudopregnancy showed, another pseudopregnancy (spontaneous) at the termination of the current one. It would be interesting to ascertain if one could continue to produce a succession of pseudopregnancies in rats merely by starvation during the first four days of the preceding pseudopregnancy. No explanation is available for the production of pseudopregnancy by starvation or any other means; however, the experiments of Everett (1954) indicate that part of the mechanism may be a removal of some inhibitor of the pituitary luteotrophin.

3. Duration of Pseudopregnancy Following Pretrauma Starvation

The effect of pretrauma starvation in causing an interruption of pseudopregnancy was most prevalent in those animals maintained under normal temperature conditions. The animals kept at temperatures that varied between 85 and 90 degrees showed fewer interruptions of pseudopregnancy as a result of pretrauma starvation. (Deciduoma formation, however, appeared to be uninflueneced by these higher temperatures.) Since an increase in temperature is accompanied by a decrease in thyroid activity (Dempsey et al, 1943) the resultant hypothyroid state, postulated in the present experiments, may have yielded some protec-
tion for the maintenance of the pseudopregnancy. (Janes, 1950, 1954, found that there was a greater degree of luteinization of follicles in the rat as a result of increased sensitivity to gonadotrophins during the hypothyroid state.) The interruption of pseudopregnancy at normal temperatures while under the stress of starvation may then be related to an interference in luteotrophin secretion or production. (If the control of hypophyseal luteotrophin is similar to that of the other gonadotrophins, then it would appear that there is a failure in the ability to 'secrete the hormone from the hypophysis', for Rinaldini (1949) has shown that during chronic inanition the gonadotrophic potency of the pituitary, measured as potency per milligram of tissue is actually increased. Since circulating gonadotrophin is at low levels, he concluded that it was the release of the gonadotrophin which was impaired and therefore it was the failure in the secretory mechanism and not the reduction in pituitary gonadotrophin production which was responsible for the initial gonadal atrophy.) It is unlikely that the interruption of pseudopregnancy can be attributed to a decrease in sensitivity of the ovary for the gonads have been shown to retain their normal sensitivity to gonadotrophin during inanition (Werner, 1939; Maddock et al, 1947; Rinaldini, 1949).

Cauterization of the corpora lutea (McKeown, et al, 1938) during a pseudopregnancy, or the removal of the young from a lactating rat (Long et al, 1922) caused a return to estrus
in four days. In the present experiment, the return to estrus (interruption of pseudopregnancy) by the eighth or the ninth day might be evidence of a withdrawal of luteal function by means of the pretrauma (0 - 4) starvation.

4. Duration of Pseudopregnancy as Influenced by Deciduomata

No explanation is available at present for the manner in which massive deciduoma formation caused an extension of the pseudopregnant duration. This duration approached the true gestation interval. The same phenomenon has been observed in adult rats (Ershoff et al, 1943; Peckham et al, 1948; Olsen et al, 1951; Velardo et al, 1953). Therefore whatever explanation is accepted as accounting for the prolonged duration in the adult, will also have to be adequate to explain the situation for the puberal animal which produces a decidual response which is only 60% of that of the adult.

Bradbury et al, (1950) noted an extended pseudopregnancy in rats following hysterectomy. He believed that the endometrium exerted an antagonistic or luteolytic action on corpora lutea.
Deciduoma formation although possible at the pseudopregnancy induced at the time of vaginal opening, did not attain the adult size until the animals had reached the 6th estrus cycle. The animal at puberty was found to be susceptible to the effects of pre-trauma starvation. The susceptibility may be attributed to a loss of uterine sensitivity resulting from the stress of starvation.

The first estrus cycle of the rat was characterized by a high incidence of spontaneous pseudopregnancy. A pseudopregnancy induced at puberty could be interrupted by pretrauma starvation, or could be prolonged by the presence of deciduoma.
SUMMARY

1. Pseudopregnancy and deciduoma formation were induced in the rat at the time of puberty.

2. The deciduoma response (as determined by total uterine weight) of the puberal rat was at the adult level when the animal reached the 6th estrus cycle.

3. Pretrauma starvation in the puberal rat inhibited deciduoma formation by causing a loss of uterine sensitivity.

4. During the pretrauma starvation of puberal rats, a greater sensitivity to starvation was noted by removal of food for days 2 - 4. Removal for days 0 - 2 had no effect.

5. Administration of 2 mg of progesterone during the pretrauma starvation period of puberal rats gave no improvement in the response.

6. Postrauma starvation in puberal rats reduced deciduoma formation but not to the level of pretrauma starved animals.

7. Pretrauma starvation in the adult rat was without effect on the deciduoma reaction.

8. Postrauma starvation in the adult rat caused a decrease in deciduoma response.

9. A more prolonged starvation of the adult, incorporating both pre and post trauma periods did not reduce deciduoma size much below that achieved by postrauma starvation alone.

10. Deciduoma formation was found to occur spontaneously in about 1% of all animals.
11. Histamine acid phosphate injected into the uterine lumen gave rise to deciduoma formation. The cholinergic substance 'Mecholyl' was incapable of producing the deciduoma response.

12. Bilateral adrenalectomy on day 4 was still compatible with normal deciduoma formation. Removal of the adrenals on day 0 gave an impaired response.

13. Hemicastration on day 0 of pseudopregnancy gave adequate deciduoma formation. Removal on day 4 gave results which indicate a possible interference with the response.

14. The long term castrate rat does not give a normal degree of deciduoma formation even when treated with as much as 20 mg progesterone per day.

15. The deciduoma response of animals castrated on day 0 approached that of normal animals if estrodiol was added to the posttrauma progesterone in a ratio of about 1/15000.

16. Thirty seven percent of puberal animals were found to have a spontaneous pseudopregnant cycle following the first vaginal estrus.

17. Thirty percent of adult animals showed a propensity for pseudopregnant cycles at the time of the first vaginal smears.

18. Pretrauma starvation caused an interruption of pseudopregnancy in 38% of the puberal animals. The incidence of interruption in non-starved puberal animals was only 3%.

19. Starvation of adult rats for four days caused a prolongation of the cycle. This may be a true pseudopregnancy.

20. Pseudopregnant, puberal animals undergoing starvation for four
days developed a spontaneous pseudopregnant-like cycle after the expiration of the 'induced' pseudopregnancy.

21. Extensive deciduoma formation prolonged the duration of pseudopregnancy in puberal rats. The pseudopregnant period so achieved was almost comparable to the actual gestation period.
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<td>47.0±0.84*</td>
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<td>140±3.2</td>
<td>2.47±0.08</td>
<td>30.4±1.50</td>
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<td>4-6 mo.</td>
<td>240±5.1</td>
<td>258±3.7</td>
<td>4.29±0.26</td>
<td>43.0±2.34</td>
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</table>

**DECIDUOMA FORMATION (#) AS A FUNCTION OF MATURITY (CYCLE): NON STARVED RAT**

* Mean Wt. + Standard Error.
\# Weight of Uterus bearing deciduomata.

**TABLE I**
<table>
<thead>
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DECIUDOMA FORMATION (*#) AS A FUNCTION OF MATURITY (CYCLE): PRETRAUMA STARVED RAT

* Mean Wt. ± Standard Error.
# Weight of Uterus bearing deciduomata
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<th>Number of Animals</th>
<th>Age at Autopsy</th>
<th>Body Weight (Gms)</th>
<th>Uterus Wt. (Gms.)</th>
<th>Ovary Wt. (Gms.)</th>
<th>Adrenal Wt. (Gms.)</th>
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<td>114 ± 3.6</td>
<td>1.07 ± 0.20</td>
<td>28.8 ± 3.75</td>
<td>33.4 ± 6.42</td>
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<td>57.5 ± 2.01</td>
<td>138 ± 2.1</td>
<td>1.56 ± 0.10</td>
<td>30.6 ± 3.11</td>
<td>37.3 ± 4.60</td>
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<td>7</td>
<td>62.6 ± 1.29</td>
<td>149 ± 5.0</td>
<td>1.95 ± 0.08</td>
<td>35.4 ± 10.0</td>
<td>34.8 ± 3.45</td>
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<td>6</td>
<td>5</td>
<td>72.6 ± 2.11</td>
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<td>2.62 ± 0.26</td>
<td>44.4 ± 6.20</td>
<td>41.0 ± 2.28</td>
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<td>ADULT</td>
<td>9</td>
<td>4 - 6 mo.</td>
<td>227 ± 3.3</td>
<td>2.89 ± 0.32</td>
<td>47.4 ± 1.79</td>
<td>45.4 ± 1.90</td>
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**DECIDUOMA FORMATION (#) AS A FUNCTION OF MATURITY (CYCLE): POSTTRAUMA STARVED RAT**

* = Mean Wt. ± Standard Error

# = Weight of Uterus bearing deciduomata

**TABLE III**
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<tr>
<td>Non-starved</td>
<td>8</td>
<td>240±5.1</td>
<td>258±3.7</td>
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<td>62.5±2.50</td>
<td>43.0±2.34</td>
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<td>228±4.6</td>
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<td>227±3.3</td>
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<td>47.4±1.79</td>
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<td>11.2±0.89</td>
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**EFFECT OF STARVATION ON DECIDUOMA FORMATION (#) (ADULT RATS)**

*Mean Wt. ± Standard Error

# Weight of Uterus bearing deciduomata

**TABLE IV**
## Table V

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals</th>
<th>Age Autopsy Days</th>
<th>Body Weight Initial Gm.</th>
<th>Body Weight Final Gm.</th>
<th>Uterine Weight Gm.</th>
<th>Ovarian Weight Mg.</th>
<th>Adrenal Weight Mg.</th>
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<tbody>
<tr>
<td>Non-starved</td>
<td>18</td>
<td>47.0±0.84*</td>
<td>115±2.8</td>
<td>140±3.2</td>
<td>2.47±0.08</td>
<td>40.9±0.92</td>
<td>30.4±1.50</td>
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<tr>
<td>0-4 Starved</td>
<td>11</td>
<td>53.0±2.75</td>
<td>122±4.2</td>
<td>115±5.7</td>
<td>0.50±0.21</td>
<td>31.5±2.68</td>
<td>26.5±0.79</td>
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<td>4-9 Starved</td>
<td>9</td>
<td>49.6±2.09</td>
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<td>1.07±0.20</td>
<td>28.8±3.75</td>
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**EFFECT OF EITHER PRETRAUMA OR POSTTRAUMA STARVATION ON DECIDUOMA FORMATION (#)**

(PSEUDOPREGNANCY INDUCED AT THE TIME OF VAGINAL OPENING)

*Mean Wt. + Standard Error

#Weight of Uterus bearing deciduomata
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals</th>
<th>Age Autopsy Days</th>
<th>Body Weights</th>
<th>Uterine Weight #</th>
<th>Ovarian Weight</th>
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<tr>
<td></td>
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<td>Final Gm.</td>
<td>Gm.</td>
<td>Mg.</td>
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<td>115±2.8</td>
<td>140±3.2</td>
<td>2.47±0.08</td>
<td>40.9±0.92</td>
</tr>
<tr>
<td>0-4 Starved</td>
<td>11</td>
<td>53.0±2.75</td>
<td>122±4.2</td>
<td>115±5.7</td>
<td>0.50±0.21</td>
<td>31.5±2.68</td>
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<tr>
<td>1-5 Starved</td>
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<td>47.1±1.52</td>
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<td>110±1.3</td>
<td>0.25±0.02</td>
<td>36.0±2.0</td>
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<tr>
<td>3-7 Starved</td>
<td>9</td>
<td>47.1±1.59</td>
<td>113±4.1</td>
<td>110±1.2</td>
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<td>44.7±0.44</td>
<td>110±1.9</td>
<td>105±4.6</td>
<td>1.70±0.11</td>
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**EFFECT OF 4 DAYS OF STARVATION ON DECIDUOMA FORMATION (#)**
*(PREGNANCY INDUCED AT TIME OF VAGINAL OPENING)*

*Mean Wt. ± Standard Error
f=Weight of Uterus bearing deciduomata

**TABLE VI**
<table>
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<tr>
<th>Treatment</th>
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<th>Uterine Weight</th>
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<td>Weight Gm.</td>
<td>Weight Mg.</td>
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<td>1.27±0.27</td>
<td>39.8±7.17</td>
</tr>
<tr>
<td>0-4 Starved</td>
<td>11</td>
<td>53.0±2.75±</td>
<td>122±4.2</td>
<td>115±5.7</td>
<td>0.50±0.21</td>
<td>31.5±2.68</td>
</tr>
</tbody>
</table>

DECIDUOMA FORMATION (f) AS INFLUENCED BY STARVATION WITHIN THE PREGNANT PERIOD (PSEUDOPREGNANCY INDUCED AT THE TIME OF VAGINAL OPENING)

*Mean Wt. + Standard Error
f=Weight of Uterus bearing deciduomata

TABLE VII
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals</th>
<th>Age Autopsy Days</th>
<th>Body Weight</th>
<th>Uterine Weight#</th>
<th>Ovarian Weight</th>
<th>Adrenal Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-starved</td>
<td>18</td>
<td>47.0±0.84*</td>
<td>115±2.8</td>
<td>140±3.2</td>
<td>2.47±0.08</td>
<td>40.9±0.92</td>
</tr>
<tr>
<td>1-2 Starved</td>
<td>7</td>
<td>44.4±0.49</td>
<td>105±2.9</td>
<td>121±4.8</td>
<td>2.47±0.13</td>
<td>38.7±8.41</td>
</tr>
<tr>
<td>2-3 Starved</td>
<td>7</td>
<td>45.0±0.95</td>
<td>112±2.7</td>
<td>128±3.9</td>
<td>2.11±0.20</td>
<td>36.3±5.36</td>
</tr>
<tr>
<td>3-4 Starved</td>
<td>9</td>
<td>46.0±0.77</td>
<td>110±3.7</td>
<td>130±3.7</td>
<td>2.76±0.16</td>
<td>40.5±3.62</td>
</tr>
</tbody>
</table>

DECIDUOMA FORMATION (#) AS INFLUENCED BY 1 DAY OF STARVATION DURING THE PRETRAUMA PERIOD (PSEUDOPREGNANCY INDUCED AT THE TIME OF VAGINAL OPENING)

* = Mean Wt. ± Standard Error
# = Weight of Uterus bearing deciduomata

TABLE VIII
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals</th>
<th>Age Autopsy Days</th>
<th>Body Weight</th>
<th>Uterine Weight</th>
<th>Ovarian Weight</th>
<th>Adrenal Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Weight #</td>
<td>Weight Mg.</td>
</tr>
<tr>
<td>Non-starved</td>
<td>18</td>
<td>47.0±0.84#</td>
<td>115±2.8</td>
<td>140±3.2</td>
<td>2.47±0.08</td>
<td>40.9±0.92</td>
</tr>
<tr>
<td>0-2 Starved</td>
<td>9</td>
<td>45.0±0.44</td>
<td>109±1.8</td>
<td>125±2.7</td>
<td>2.44±0.08</td>
<td>39.6±3.40</td>
</tr>
<tr>
<td>2-4 Starved</td>
<td>9</td>
<td>45.6±0.71</td>
<td>112±4.2</td>
<td>124±7.5</td>
<td>1.38±0.35</td>
<td>38.0±3.22</td>
</tr>
<tr>
<td>4-6 Starved</td>
<td>9</td>
<td>44.7±0.41</td>
<td>107±1.3</td>
<td>137±2.4</td>
<td>2.49±0.20</td>
<td>39.1±6.20</td>
</tr>
</tbody>
</table>

**DECIDUOMA FORMATION (#) AS INFLUENCED BY 2 DAYS OF STARVATION DURING PRETRAUMA OR POSTTRAUMA PERIOD (PSEUDOPREGNANCY INDUCED AT THE TIME OF VAGINAL OPENING)**

- Mean Wt. ± Standard Error
- Weight of Uterus bearing deciduomata

**TABLE IX**
I, Vincent Joseph De Feo, was born in New York, New York, October 1, 1925. My secondary school education was received in the public schools of Newark, New Jersey and Irvington, New Jersey. I obtained my undergraduate training from Juniata College from which I received the degree Bachelor of Science in 1949. I attended Rutgers University from 1949 to 1950 and received the degree Master of Science in 1951. While in residence at Rutgers University, I served as Graduate Assistant in the Department of Zoology. Research toward the Master's degree involved a study on the effects of protein restriction on the maturation of the immature mouse gonad. This work was done under the guidance of Dr. James H. Leathem. (see Leathem et al 1952) In 1950 I entered the Department of Physiology at the Ohio State University for studies leading toward the degree Doctor of Philosophy. The following appointments were held between 1951 and 1954: Teaching Assistant (1951 - 1952); Research Fellow (1952 - 1954); Assistant, and Assistant Instructor (1954). Research involving a study on deciduoma formation in the rat was conducted under the guidance of Dr. Irving Rothchild. (see De Feo and Rothchild 1952, 1953)