

POST-COITAL ORAL
ADMINISTRATION OF SYNTHETIC
PROGESTINS AND ESTROGENS TO THE WHITE RAT

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ADMINISTRATION OF SYNTHETIC
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RESPECTFULLY DEDICATED TO

MY MOTHER,

MRS. RUTH H. BURDEN

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ABSTRACT

Hubert White Burden. POST-COITAL ORAL ADMINISTRATION OF SYNTHETIC PROGESTINS AND ESTROGENS TO THE WHITE RAT. (Under the direction of Everett C. Simpson) Department of Biology, August, 1967.

Non-pregnant female rats were treated with five oral contraceptive pills ("Norlestrin," Parke, Davis & Co.) given at 24 hour intervals beginning at metestrus. A pseudopregnancy interval of 13 days was induced. A similar dosage was given to pregnant rats before and after nidation. The representative estrogen (ethinyl estradiol) and progestin (norethindrone acetate) components of the pill were given separately to two groups of pregnant animals after implantation.

Five pills given to pregnant rats before implantation rendered the gravid females sterile. When given after implantation, the pills resulted in varying degrees of fetal resorption by day 15. At term delayed partuition and reduced litter size was observed. There was a significant reduction of normal fetuses at day 15 and young at term in estrogen-treated females. At day 15 all progesterone treated females exhibited fetuses not unlike controls; however, there was a significant reduction in the number born. A delay in partuition was noted in estrogen and progesterone-treated animals.

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INTRODUCTION

Orally active inhibitors of ovulation are at present widely used for the control of conception. All of the preparations currently in use consist of a progestin in combination with an estrogen in either the sequential or combined form.

The most widespread use of the progestin-estrogen compound is for preventing conception by inhibiting ovulation. It should be noted however, that the effectiveness of oral administration of progestins and estrogens in contraceptive pills may involve more than suppressing the discharge of a mature, unfertilized ovum from the graafian follicle of the ovary (Pincus, 1965). It is known that estrogens and progestins can induce an antifertility condition by influencing either tubal transport of eggs, blastocyst development, impaired implantation, or perhaps all three. This research is concerned with some effects of an orally administered post-coital progestin-estrogen tablet on the implantation processes and subsequent blastocyst development in the albino laboratory rat.

The possibility of reduced fecundity and/or sterility seems well worth investigating, as a similar action in humans would appear to offer efficient alternatives to the present contraceptive practice of taking synthetic steroids on day 5 through 24 of the menstrual cycle.

REVIEW OF LITERATURE

Although an appreciable number of compounds are capable of interfering with mammalian fertility, very few, with the exception of certain steroidal compounds, have been of value in studying this phenomenon. It is known that pregnancy interruption may result from a special qualitative and/or quantitative steroidal effect on certain reproductive organs and functions. The role that the ovarian steroid hormones, estrogen and progesterone, play in reproductive physiology has been comprehensively studied and reviewed during the past 40 years.

The investigation of the mechanism whereby pregnancy is affected by exogenous steroids began in the mid 1920's. Parkes and Bellerby (1926) first observed the estrogen-induced interruption of pregnancy in rats and mice. They found that the injection of estrin during the early stages of pregnancy, such as two days after copulation, invariably caused termination of pregnancy and the rapid reappearance of estrus. In addition, the same investigators noted that in later stages of pregnancy, (day 8) injection in sufficient amounts nearly always terminated pregnancy, but the amount required was approximately twice as great.

These findings initiated a very active and fruitful period of research during the following decade.

Burdick (1937, 1938) reported that small amounts of estrogen caused retention of ova in the oviducts of the mouse while large amounts accelerated ova transport through the tubes. Dreisbach (1959)

showed that as little as 0.02 mg/kg estrone interrupted pregnancy when administered on day 3 post coitum during the period of tubal transport in rats while 0.1 mg/kg was necessary to prevent implantation after the ovum was free in the uterus (days 5-7). Later it was ascertained that a single dose of 10 or 15 μ g of estrone per rat given on day 1 of pregnancy partially inhibited implantation while a dose of 20 μ g expelled the eggs from the oviducts to the uterus in 20-24 hours with a consequential later expulsion into the vagina (Banik and Pincus, 1964). Estrone administered to the rat on day 2 and 3 post coitus at 40 μ g in a single injection will inhibit pregnancy (Edgren et al., 1961) whereas from day 7 on, when the blastocyst has already implanted, doses up to 160 μ g were ineffective. Greenwald (1961b) showed that the effect of 10 μ g of estradiol cyclopentylpropionate (ECP) administered to rats on day 1 of pregnancy was to increase tubal and uterine motility, so that the fertilized ova were expelled from the uterus by 48 hours post coitum.

In the rabbit, Pincus and Kirsch (1936) found ova retained in the fallopian tubes after administering estrone and related compounds during preimplantation stages. Black and Asdell (1959) gave 2.5 mg estradiol to mated rabbits, but did not record, in contrast, any retention of eggs in the oviducts. They hypothesized that a mechanism is present at the lower end of the oviduct which prevents the entry of ova into the uterus until the reproductive organs come under the influence of progesterone. Whitney and Burdick (1938) reported that a massive single postcoital injection of estrogen in the rabbit (5,000 R. U.) resulted in acceleration of the rate of tubal

passage of the eggs and ultimate disintegration of the eggs within a few hours after entering the uterus. Noyes et al. (1959) lost many transferred rabbit eggs in estradiol-treated hosts, twenty-three per cent passing into the vagina 10 to 78 hours after transfer. Greenwald (1961b) reported that ovum transport in the rabbit is accelerated with 25 μ g of estradiol cyclopentylpropionate (ECP) whereas with 250 μ g of ECP, ova are retained at the ampullary-isthmic junction for as long as six days. Chang and Harper (1966) concluded that exogenous estrogen (ethinyl estradiol) administered after ovulation caused rapid egg transport from the tube to the uterus ultimately resulting in egg degeneration or expulsion from the uterus.

In guinea pigs, low doses of estradiol benzoate, given immediately after mating, inhibit pregnancy. This is not due to retention of eggs in the oviducts but to their expulsion (Deanesly, 1963).

Progesterone has been intensely studied as to its effects on reproduction. Early workers (Corner, 1928; Wislocki, 1933) reported that progesterone speeded the transportation of eggs into the uterus. However, it is felt by many workers today that progesterone does not markedly speed up ovum transport and is effective only when it can overcome the effect of endogenous estrogen (Black and Asdell, 1959). From this point of view it is interesting to note that during the luteal phase of the rat more than one part of estrogen nullifies the action of 1000 parts of progesterone (Velardo and Hisaw, 1951). Alden (1942) observed that progesterone administered to castrate rats carrying fertilized eggs caused accelerated ova passage through the oviducts and consequent premature expulsion into the uterus. Davis (1963a)

showed that a single injection of 0.9 mg of norethynodrel on day 2 of pregnancy suppressed fertility in the rat. Chang (1966) found that the administration of medroxyprogesterone acetate (MPA, 2 mg) after ovulation did not speed the transportation of eggs into the uterus in the rabbit. Wakeling (1965) has concluded that the rate of passage of free ova through the oviduct on days 2 and 3 post coitum is more susceptible to the action of norethynodrel than are the processes of either ovulation or fertilization in the rat.

From the foregoing documentation, one may infer that the rate of ova transport is an especially vulnerable period in the reproductive process for interrupting pregnancy.

The implantation of the blastocyst to the endometrium of the uterus is not a single event but a sequence of integrated mechanisms. For simplicity, these mechanisms may be grouped as the muscular mechanisms which transport and space and immobilize the blastocysts with respect to the length of the uterus; the adhesive mechanisms which attach to the endometrium first the noncellular membranes of the blastocyst and then the trophoblast; and the invasive mechanisms by which the trophoblast penetrates the uterine epithelium (Böving, 1958).

Nidation is a rigidly conditioned process occurring at a critical time under delicately balanced conditions. One way of unbalancing such a state is to have an excess of any one of its constituents. Normally estrogen and progesterone act synergistically in delicately balanced quantitative proportions upon the uterus to prepare it for implantation, although progesterone alone may accomplish this in some species (Pincus, 1965). The quantity of exogenous hormone administered

at the time of nidation is proportional to the number of implants. Hence, the lower the dosage, the nearer to unity is the implantation ratio (number of implantations/number of corpora lutea) (Pincus and Kirsch, 1936). An upset of hormonal balance essential to progestational proliferation may be accompanied by an excess of estrogens (Alden, 1942) or an excess of progesterone (Cochrane and Meyer, 1957). Thus, disturbance of implantation may be due to an interference with maintenance of a progestational endometrium (Segal and Nelson, 1958). Pincus and Kirsch (1936) observed complete prevention of implantation in the rabbit with daily injections of 150 R. U. of estrone-in-oil on days 3 and 4 post coitum. Burdick et al. (1937) administered estrogens to rats after the ova had arrived in the uterus. They proved that the resultant interference with implantation was not due to a direct toxic effect of the steroid on the ova. Large doses of the synthetic progestin, norethynodrel, will inhibit implantation when administered postcoitally to the intact rat (Davis, 1963a). The same investigator also reported that norethynodrel may inhibit implantation in ovariectomized rats receiving pregnancy-maintaining doses of estrogen and progesterone (1963b). Saunders (1964) pointed out that norethynodrel given postcoitally to rats may act as an antigonadotrophin to the pituitary and thus depress ovarian function and consequent sustainment of pregnancy.

That cleavage and subsequent blastocyst development and fetal growth are effected by exogenous estrogens and progestins has been verified by several investigators. Implanted embryos are susceptible to abortion by an upset in hormonal balance. Estrone injections into bred doe rabbits before and during the period of cleavage did not effect

the cleavage rate, however, the embryos always died in the early blastocyst stage (Pincus and Kirsch, 1936). Davis (1963a) found that 10 mg doses of norethynodrel administered daily from day 10 through 12 in the rat allowed some fetal development to proceed unimpaired, though litter size was significantly decreased, while 10 mg doses of the steroid given on days 9 through 13 caused complete fetal resorption. It is hypothesized that effects on the conceptus result from a specific action of the progesterone on a metabolite. Chang (1966) observed degeneration of eggs when 0.05 mg/rabbit/day of ethinyl estradiol was fed for three days after ovulation. Greenwald (1957) has shown that treatment of rabbits with estradiol 48 hours post coitum reduced the mucin layer surrounding the egg and interrupted pregnancy. The same investigator noted that when estradiol treatment was initiated later, pregnancy was interrupted only by much larger doses of the steroid.

Several estrogens, namely estradiol 17_B, stilbestrol, ethinyl estradiol, and mestranol will not only prevent implantation, but when given to the rabbit later in larger doses, will also result in placental separation and death of the fetus. Microscopic sections show changes at the site of implantation with necrosis, loss of decidua, vacuolization, and degeneration of the endometrium (Morris and Wagenen, 1966). D'Amour et al. (1933) felt that the effect of estrone during the early post-implantation period was directly on the fetus, although no evidence for this view was presented.

The effect of estradiol 17_B on fertilized rabbit eggs in vitro has been studied extensively by McGaughey and Daniel (1966). They found that when estradiol was present at a concentration of 25 $\mu\text{g/ml}$

single-celled eggs typically failed to cleave and amphimixis appeared to be inhibited. When the concentration of estradiol was reduced to 10 $\mu\text{g/ml}$ the eggs tended to fragment instead of undergoing normal cleavage. This was presemably an effect on the cell membrane. Two and four-celled stages were much more resistant to the estrogen than were single-celled ones. It should be kept in mind that, at present, it is difficult to correlate the results obtained from experiments involving steroid effects on mammalian embryos in vivo and in vitro.

Experience with the use of progestin-estrogen preparations as oral contraceptives indicates that they inhibit fertility by preventing ovulation and that this action is clearly a physiological process. The purpose of this research was to establish some possible effects on pregnancy of the post coital (post ovulation) administration of an oral contraceptive tablet ("Norlestrin," Parke, Davis and Co.) containing 0.05 mg of ethinyl estradiol (synthetic estrogen) and 2.5 mg of norethindrone acetate (synthetic progesterone) given per os. It should be pointed out that the observations in this research are in no way incongruous with the established efficiency of "Norlestrin" as an antifertility agent.

MATERIALS AND METHODS

Animals

Albino nulliparous female rats (200-310 gm) of the Holtzman strain were used in this investigation. In the fertility studies, males of proven fertility were employed. The animals were housed in a room with 14 hours of light (5:00 A. M. - 7:00 P. M.) and 10 hours of darkness (7:00 P. M. - 5:00 A. M.). Because of limited facilities, females and males were housed separately up to four individuals per wire bottom cage. All animals had free access to water and received Purina Laboratory Chow ad lib.

Vaginal lavages for all females were obtained daily between 8:00 and 10:00 A. M. Only mature females having demonstrated two or more successive 4-day estrus cycles were used in these experiments.

Design of Experiment

Part I of the investigation was designed to determine some effects of the oral contraceptives on the reproductive behavior of non-pregnant females. It was felt that a working knowledge of the pill's effect on non-pregnant females was a prerequisite to post coital studies. In the initial phase the animals were divided into three groups, each group receiving a total of five pills. Groups 1 and 2 were administered pills at 24 hour intervals. The pill regimen was started on the first day after estrus (metestrus) in Group 1 and on the day of estrus in Group 2. The animals in Group 3 were given pills at 48 hour intervals, the initial dosage commencing the first day after

estrus (metestrus). Daily lavages were taken to establish the effects of the exogenous hormones on the vaginal cytology. Several females were bred during the first heat following treatment with oral contraceptives to determine if a fertile mating would occur.

In another phase of this experiment, uterine and ovarian weights of pseudopregnant (control) and experimental animals were compared and contrasted at necropsy. The experimental animals received a total of five pills at 24 hour intervals beginning on the second day after estrus (diestrus). They were sacrificed on day 10 following termination of the pill regimen. The control females were sacrificed on day 12 of pseudopregnancy since this represents the time at which the pseudopregnant condition would be at or near its peak. Based on previous work by this investigator, it was known that the hormonal induced pseudopregnant state attained a pinnacle on day 10 subsequent to the terminal tablet of a five pill regimen.

Part II of the study was involved with ascertaining certain consequences of the post-coital administration of the pill on fecundity. Intact females weighing 200 to 250 g were caged overnight with fertile males. Presence of spermatozoa in vaginal lavages or copulatory plugs the following morning established day 1 of the experiment and presumably of pregnancy. The inseminated rats were allotted in a random fashion to five different groups of 10 animals each. Autopsy and in some instances, laparotomy, was performed on one half of all groups of animals on days 6 or 15. The remaining half of the pregnant animals were allowed to go to term in both experimental and control groups. Groups 1 and 2 were non-hormonal treated animals which were sacrificed on days

6 and 15 respectively, and are hereafter referred to as 6 day control and 15 day control. Groups 3 and 4 were given a total of five pills at 24 hour intervals beginning on day 1 of pregnancy and autopsy or laparotomy was performed on days 6 (Group 3) or day 15 (Group 4). Group 5 animals received a total of five pills at 24 hour intervals beginning on day 6. These animals were sacrificed on day 15 for postmortem examination and study. Observations recorded included:

(1) Gross and microscopic examination of uteri at days 6 and 15, including weights, (2) Evaluation of fetuses at 15 days for absorption, fetal death, and runting. Runting was defined as fetal weight less than the mean weight minus two standard deviations for control fetuses of similar age (Rosenkrantz et al., 1967). (3) Weight and analysis of young at term for runting, (4) The assesment of skeletal development and possible anomalies by the alizirin red S technique at 15 days and at term.

Part III was aimed at determining whether the effects seen in Part II were a synergistic expression of the estrogen and progesterone or a consequence which could be simulated or altered by administering the two hormones separately. Two groups of test animals were used. Ethinyl estradiol (synthetic estrogen) and norethindrone acetate (synthetic progesterone) were dissolved separately in cottonseed oil to give concentrations of 0.1 and 5.0 mg/ml, respectively. Dosages of each steroid mimicking the amounts found in the pill ($\frac{1}{2}$ ml of each oil solution) was administered separately to two groups of nulliparous females. A total of five consecutive doses were given by gavage at 24 hour intervals commencing on day 6 of pregnancy. A control group

received the vehicle alone.

Experimental Procedures

Vaginal Lavages

Vaginal lavages were taken daily on all animals and cell stages were determined according to Turner (1966). Approximately $\frac{1}{4}$ cc of warm water was introduced into the vagina by means of a small pipette and this was aspirated three or four times in order to obtain the maximum amount of sloughed cells. One drop of water containing these sloughed cells was then placed on a clean microscope slide. Since the rats were numbered, several vaginal lavages were placed on the same slide. Caution was taken in order to prevent the smears from running together. Each lavage was examined microscopically under 100X and the results were recorded.

The presence of spermatozoa in vaginal washings or of a copulatory plug established day 1 of pregnancy.

Administration of Tablets

The animals were subjected to ether until they acquired a noticeable stupor. At this point (before the animal was totally unconscious) it was removed from the ether jar, and its mouth held open with the thumb and index fingers while a pill was placed on the back of the tongue. The pill was quickly pushed down the esophagus (often into the stomach) with a polished glass rod. By this time the animal was usually well recovered from the ether. The rat was placed in a clean plastic bucket for fifteen minutes following the administration of the pill. This was done to be assured that the pill was swallowed

and retained. At first whole pills were given; however it was later found that the size of the pill (5.5 mm diameter, 1.5 mm thick) warranted it being administered in halves. The smaller sections were more easily accommodated by the esophagus.

Gavage Technique

The animals were anesthetized with ether as described previously, its mouth held open with the thumb and index finger, and a 13 guage needle with a rounded, polished point was gently pushed down the esophagus and the 0.5 ml solution released from the syringe into the rat's stomach.

Pseudopregnancy Induction

The rats were made pseudopregnant during estrus by cervical stimulation with a glass rod. This method has been employed successfully by many investigators in the field.

Laparotomy Technique

The animals were anesthetized with an intraperitoneal injection of aqueous chloral hydrate (120 mg/cc; dosage = 30 mg/100 g body weight) and placed on their back on the operating table. The pelvic region of the abdomen was clipped, and swabbed with 70% alcohol. A mid-ventral longitudinal incision about 2.5 cm in length was made slightly anterior to the pelvic region and the uterine horns were exteriorized for the purpose of gross examination. The exposed viscera was periodically swabbed with physiological saline (0.9%). Following examination the cornua were replaced into the abdominal cavity, several sutures were taken in the muscle wall, and the skin incision was closed with surgical clamps (11 mm).

Autopsy

The animals were sacrificed by an overdose of ether. Reproductive organs obtained for further study were stripped of fat, weighed immediately on a torsion balance, and fixed in Bouin's fluid.

Histological Studies

Ovaries and uteri were serially sectioned at 7 microns and stained with haematoxylin and eosin Y.

Pictures

Photographs of selected organs in toto were taken with a "Pentax" 35 mm camera using Kodak "Plus-X" film (ASA 125). The same camera with a microscope adapter was employed for photomicrographs.

RESULTS

Part I

Effects of Pills on Non-Pregnant Females

A summary of the data from three groups of non-pregnant females is given in Table 1. A total of five pills administered at 24 or 48 hour intervals induced pseudopregnancy regardless of the stage of the cycle

Table 1. Summary of data from three groups of non-pregnant animals that received pills

<u>Group no.</u>	<u>No. of rats</u>	<u>Treatment</u>	<u>No. days pseudopregnant*</u>
1	10	Total of five pills administered at 24 hour intervals beginning at metestrus	13.0 $\pm$ 0.76**
2	9	Total of five pills administered at 24 hour intervals beginning at estrus	10.4 $\pm$ 0.29
3	10	Total of five pills administered at 48 hour intervals beginning at metestrus	12.0 $\pm$ 1.63

*Day 1 of pseudopregnancy was the first day after the termination of the five day pill regimen.

**Mean $\pm$ standard error

in which the pill regimen was initiated. It was noted that the length of the hormonally induced pseudopregnant state of Group 1 animals more closely mimicked the pseudopregnant condition induced by mechanical stimulation of the cervix. Mechanical trauma of the cervix is the method

normally employed for inducing pseudopregnancy in this laboratory, and the condition usually lasts about 13 days (Turner, 1966). The rats of Group 2 exhibited pseudopregnant periods which were relatively shortened when compared to both the mechanically induced pseudopregnant animals and the animals of Group 1. Animals of Group 3 showed a high degree of variability and this is reflected by the comparatively high standard error. Thus it was ascertained that a total of five pills administered at 24 hour intervals beginning at metestrus was adequate to simulate a mechanically induced pseudopregnancy.

Daily aqueous vaginal lavages reflected the apparent upset in hormonal balance during the period of treatment with pills. Cytological examination showed a uniform hormonal induced change in the vaginal epithelium of the rats involved in Group 1. Day 1 of the pill treatment exhibited predominately leucocytic cells, with a few cornified cells (metestrus), day 2 had leucocytic cells (diestrus), day 3 had cornified epithelial cells (estrus), and days 4 and 5 showed vaginal cytology which consisted of a mixture of rounded nucleated and enucleated epithelial cells (hormonal effect). After the terminal pill, the lavage invariably yielded leucocytes until the termination of the pseudopregnant period.

The vaginal cytology of the animals of Groups 2 and 3 was highly variable during treatment with pills. However, in both groups of females, the lavages always consisted of leucocytes alone by two days after the fifth pill and this cell type was predominant throughout the duration of the pseudopregnant interval. A detailed account of the vaginal cell types of these rats is considered equivocal at this point. Complete

data on the cellular patterns of Groups 2 and 3 during the treatment periods are given in Appendices C and D.

It was meaningful to know if a fertile mating would occur during the first estrus period following treatment with oral contraceptives. Four females from Group 1 that were bred during the first heat following a pill induced pseudopregnancy were allowed to go to term. Litters of 9, 10, 13, and 13 offspring were observed. Both the mothers and young appeared normal in all respects.

Two groups of animals were sacrificed to determine if there was any difference in weights of certain reproductive organs of animals made pseudopregnant by pills and those made pseudopregnant by cervical stimulation. Table 2 relates comparative summaries of data on uterine

Table 2. Summary of data on uterine and ovarian weights of two groups of pseudopregnant females

Treatment	Uterine weight*	Ovarian weight*
Pseudopregnant by mechanical stimulation of cervix (control)	145.1 $\pm$ 2.86**	26.2 $\pm$ 1.66
Pseudopregnant by administering 5 pills at 24 hour intervals (experimental)	113.6 $\pm$ 2.78	25.9 $\pm$ 1.12

*Weight is expressed as mg/100 g body weight

**Mean $\pm$ standard error

and ovarian weights of these two groups of pseudopregnant animals.

Complete data forms are in Appendices E and F. It can be readily seen

that the mean uterine weights of the hormone-treated rats is less than that of females made pseudopregnant by mechanical stimulation of the cervix. However, this difference is not statistically significant.

Part II

Effects of Pills on Pregnant Females

Females sacrificed on day 6 of pregnancy

Untampered controls and rats which received pills on days 1 through 5 of pregnancy were sacrificed on day 6 and the number of implantation sites and corpora lutea were recorded. The large vascular corpora lutea of pregnancy were distinguished from older, regressing corpora lutea by the use of a 25X stereoscopic binocular dissecting microscope.

Table 3. Females sacrificed on day 6 of pregnancy

Treatment	Animal no.	Condition	Body wt.(g)	Uterine wt.(mg)	No. corpora lutea	No. implantation sites
Untampered control (Group 1)	6	fertile	337	538	16	13
	14	fertile	298	552	10	10
	20	fertile	290	527	15	13
	24	fertile	294	489	14	10
	28	fertile	298	505	16	14
Pills days 1-5 of pregnancy (Group 3)	40	sterile	261	493	13	0
	43	sterile	231	466	15	0
	48	sterile	259	418	14	0
	33	sterile	212	454	12	0

The results shown in Table 3 indicate that implantation was inhibited in animals which received pills on days 1 through 5 of pregnancy.

Microscopic examination of uteri at day 6 revealed some differentiation between control (Group 1) and experimental animals (Group 3). The endometrial histology of Group 3 showed less tortuosity and more regression of the uterine glands.

Females sacrificed on day 15 of pregnancy

On day 15 three groups of females were sacrificed and the uterine weights and number of corpora lutea were recorded. In addition, the products of conception were examined. By referring to Table 4, it can be seen that animals which received pills on days 1 through 5 (Group 4) of pregnancy were made sterile. Rats which were treated with pills on days 6 through 10, after implantation had occurred, exhibited aberrant fetal conditions which ranged from varying degrees of resorption to normal size.

Histological studies showed that Group 4 females had an endometrium which was generally regressed. Both the uterine glands and the endometrial epithelium appeared to be in various stages of involution. A minimal amount of tortuosity was manifested.

Sections of uteri were made of the animals of Group 5 between fetuses and through resorbing fetuses. Areas between fetuses demonstrated a slight progestational condition similar to that which normally prevails during pseudopregnancy. During pseudopregnancy, the uterus becomes tortuous, and uterine glands become conspicuous. Sections through resorbing fetuses revealed extensive degeneration of the conceptus.

Table 4. Females sacrificed on day 15 of pregnancy

Treat- ment	Animal no.	No. products of conception	No. normal fetuses	No. live runts	No. dead fetuses*	No. resorp- tions
Con- trol (Group 2)	29	14	14	0	0	0
	2	13	13	0	0	0
	5	14	14	0	0	0
	21**	0	-	-	-	-
Pills days 1-5 of preg- nancy (Group 4)	69	0	-	-	-	-
	68	0	-	-	-	-
	53	0	-	-	-	-
	71	0	-	-	-	-
	58	0	-	-	-	-
Pills days 6-10 of preg- nancy (Group 5)	50	13	7	0	0	6
	34	0	-	-	-	-
	38	3	0	0	0	3
	55	10	2	2	2	4
	35	12	6	0	0	6

*Based on absence of heart beat

**This animal had previously received local injections of steroids in the periovarian capsule in earlier experiments. The oviducts were cystic and enlarged. Salpingitis was indicated.

Females allowed to go to term

One half of the females in each group were allowed to go to term. Animals which were treated with pills on days 1 through 5 did not, in any case, give birth to young. This was to be expected

since it had been determined previously that females receiving treatment on days 1 through 5 were sterile on days 6 and 15.

Based on the results presented in Table 5, it can be said that:

- (1) Partuition was delayed in two-thirds of the treated mothers that

Table 5. Females allowed to go to term. Data at partuition

	Untampered controls (Groups 1 and 2)								Pills days 6-10 of pregnancy (Group 5)				
Animal no.	26	21	9	29	28	32	13	30	74	72	56	57	77
Day of partuition	22	22	23	22	23	22	22	21	23	24	24	*	*
No. offpsring delivered	14	14	12	14	10	9	13	9	8	4	6		
No. normal offspring	13	12	12	13	10	9	11	9	3	2	2		
No. live runts	0	0	0	0	0	0	0	0	0	0	0		
No. dead offspring**	1	3	0	1	0	0	2	0	0	0	0		
No. offspring with edema	0	0	0	0	0	0	0	0	0	2	1		
No. still-born***	0	0	0	0	0	0	0	0	5	0	3		

*No partuition observed. Hypothesize that fetuses were completely resorbed before term.

**Not known to have been born dead. May have died after partuition

***Known to have been born dead

went to term. (2) The number of offspring which were delivered in the treated group was significantly lower than in the controls ($p < 0.01$). (3) In treated rats with the largest litters, numbers 74 and 56, 63% and 50%, respectively, of the young were stillborn.

(4) The treated rat with the least number of young at partuition (No. 72) had the greatest percentage (100%) of viable offspring. (5) Edema was associated with a small percentage of offpsring whose mothers had received pills during pregnancy. (6) Apparently two rats (Nos. 57 and 77) completely resorbed their fetuses if these were ever present, because no young were delivered. From data presented here, it can be inferred that a total of five contraceptive pills given daily to pregnant rats on days 6-10 of pregnancy is a differential hormone dosage, capable of inducing variable results.

By comparing Tables 4 and 5 it can be seen that the number of normal offspring noted at partuition is lower than the number of normal fetuses at day 15 in the animals of Group 5. However, this difference is not statistically significant.

All of the offspring which were born alive to hormonal treated mothers died within 48 hours after partuition. This effect was apparently caused by the exogenous hormones, since no deaths were observed in the young from controls housed in an identical environment.

Part III

Effects of Estrogen and Progestin Components of Pills Given Separately to Two Groups of Pregnant Females

Females sacrificed on day 15 of pregnancy

The effects of ethinyl estradiol and norethindrone acetate given separately to two groups of pregnant animals are summarized in Table 6. There was no significant difference in the number of live normal young between oil-treated controls and progesterone-treated animals. The number of live normal fetuses found in estrogen-treated

animals was much less than the number found in the controls and progesterone-treated groups. The differences between estrogen-treated vs. control and estrogen-treated vs. progesterone-treated

Table 6. Females sacrificed on day 15 of pregnancy

Treat- ment	Animal no.	No. products of conception	No. normal fetuses	No. live runts	No. dead fetuses*	No. resorp- tions
Oil days 6- 10 of preg- nancy (vehicle control)	105	10	9	0	0	1
	87	14	13	1	0	0
	44	9	8	1	0	0
	49	13	13	0	0	0
	53	15	5	0	0	10
0.05 mg estrogen (ethinyl estradiol) days 6-10 of preg- nancy	88	0	-	-	-	-
	91	14	12	0	0	2
	89	0	-	-	-	-
	90	0	-	-	-	-
	86	0	-	-	-	-
2.5 mg proges- terone (nor- ethin- drone acetate) days 6- 10 of preg- nancy	70	12	9	0	0	3
	73	12	12	0	0	0
	51	9	9	0	0	0
	67	12	10	0	0	2
	42	11	5	0	1	5

*Based on absence of heart beat

are statistically significant at 0.01% and 0.05% levels respectively.

The uterine microanatomy of females receiving estrogen on days 6 through 10 was examined. Hyper-stimulation of the endometrium was indicated. Sections through resorbing fetuses showed extensive progressive deterioration of these fetuses.

Females allowed to go to term

Table 7 presents data that demonstrates several effects of ethinyl estradiol and norethindrone acetate on females that went to term. Some important deductions that can be determined by examination of table 8 include: (1) Partuition was delayed in all hormone-treated mothers. (2) The number of offspring delivered in estrogen and progesterone-treated females was significantly lower than controls ($p < 0.01$). (3) The number of normal offspring delivered by progesterone-treated females was significantly less ($p < 0.01$) than the number of normal fetuses observed at day 15. (4) As with pill-treated mothers, there was a wide range of conditions noted in the few young delivered, ranging from stillborn to normal individuals. (5) The number of normal young born to estrogen-treated mothers was not statistically different from the number of normal young born to progesterone-treated mothers.

None of the young delivered to estrogen and progesterone-treated mothers lived more than 48 hours. This same effect was noted previously in Part II.

Representative fifteen day old fetuses and young at term from all hormonal treatment groups (Parts II and III) were assayed for skeletal anomalies and compared with controls of similar age. The alizarin red S technique was employed. No skeletal anomalies were noted.

Table 7. Females allowed to go to term. Data at partuition

	Oil days 6-10 of pregnancy (vehicle control)				2.5 mg progesterone days 6-10 of pregnancy					0.05 mg estrogen days 6-10 of pregnancy				
Animal no.	82	100	75	92	60	94	98	84	85	47	79	45	54	83
Day of partuition	23	23	23	23	+	+	24	+	25	+	+	25	24	25
No. off- spring del- ivered	12	10	9	13	-	-	1	-	2	-	-	4	7*	#
No. normal offspring	12	9	5	9	-	-	1	-	0	-	-	4	2	-
No. live runts	0	0	1	0	-	-	0	-	0	-	-	0	3	-
No. dead offspring [†]	0	1	2	0	-	-	0	-	0	-	-	0	2	-
No. off- spring with edema	0	0	0	0	-	-	0	-	0	-	-	0	0	-
No. still- born ^{##}	0	0	0	0	-	-	0	-	0	-	-	0	0	-

[†]No partuition observed. Hypothesized that fetuses were completely resorbed before term

*No offspring in cage 24 hours post partuition. Hypothesized that five living animals died and mother devoured them

#Vagina and cage very bloody on day of expected partuition. Hypothesized that young were stillborn and mother ate them.

[†]Not known to have been born dead. May have died after partuition

^{##}Known to have been born dead

Figure 1. Reproductive tract from a 6 day pregnant rat (control).
Arrow indicates decidual tissue forming at implantation site.

Figure 2. Reproductive tract from a 15 day pregnant rat (control).

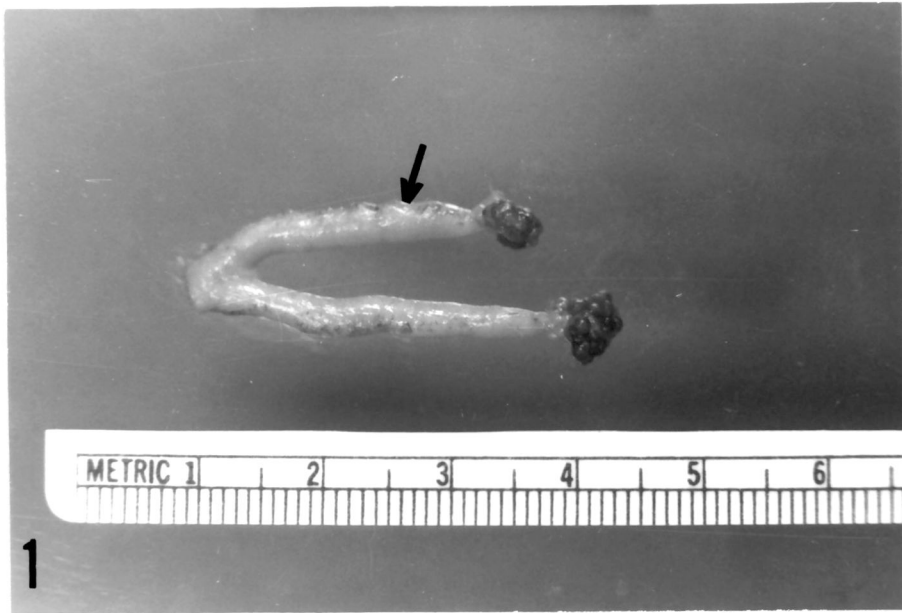


Figure 3. Representative reproductive tracts from two 15 day pregnant rats that had received "Norlestrin" tablets on days 1 through 5 of pregnancy. No fetuses were present, although some decidual tissue was noted (arrow).

Figure 4. Representative reproductive tracts from two 15 day pregnant rats that had received "Norlestrin" tablets on days 6 through 10 of pregnancy. Arrows indicate resorbing fetuses.

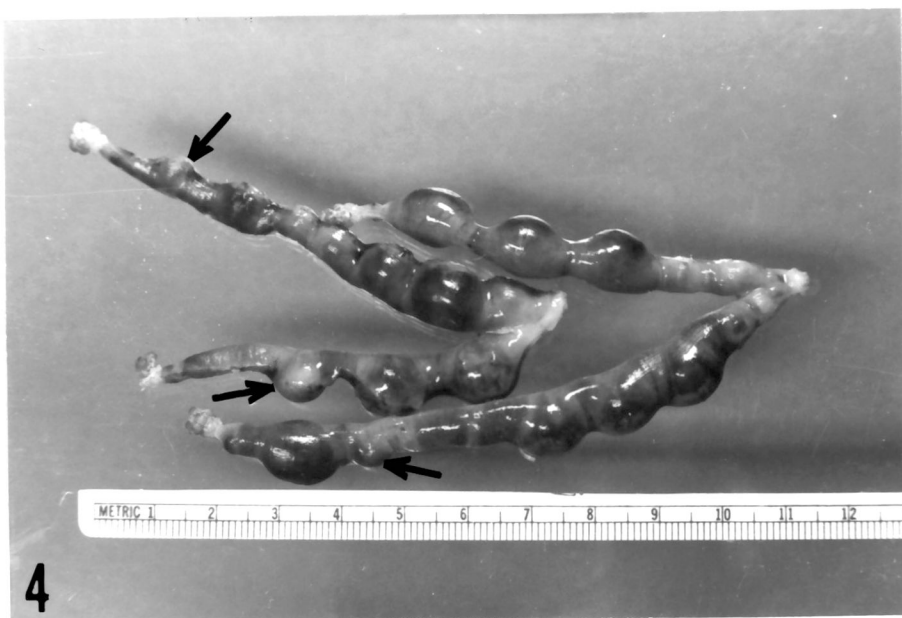
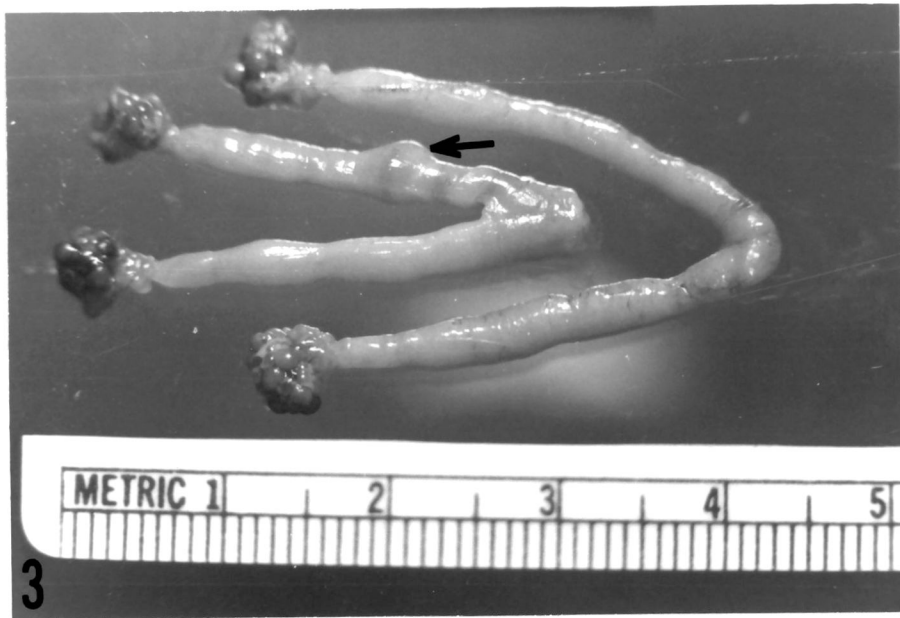


Figure 5. Representative reproductive tract from a 15 day pregnant rat that had received the estrogen component (ethinyl estradiol) in "Norlestrin" on days 6-10. Note resorbing fetal remnants.

Figure 6. A section of uterus from a 6 day pregnant rat (control). Note moderate degree of tortuosity and glandular development. Arrow indicates basal vacuole of endometrial gland. Magnification: X35.



Figure 7. A section of uterus from a 6 day pregnant rat that received a "Norlestrin" tablet on days 1 through 5 of pregnancy. Note reduced tortuosity. Magnification: X35.

Figure 8. A section of uterus from a 15 day pregnant rat that received a "Norlestrin" tablet on days 1 through 5 of pregnancy. Note decreased tortuosity. Arrows indicate suppressed endometrial glands. Magnification: X35.

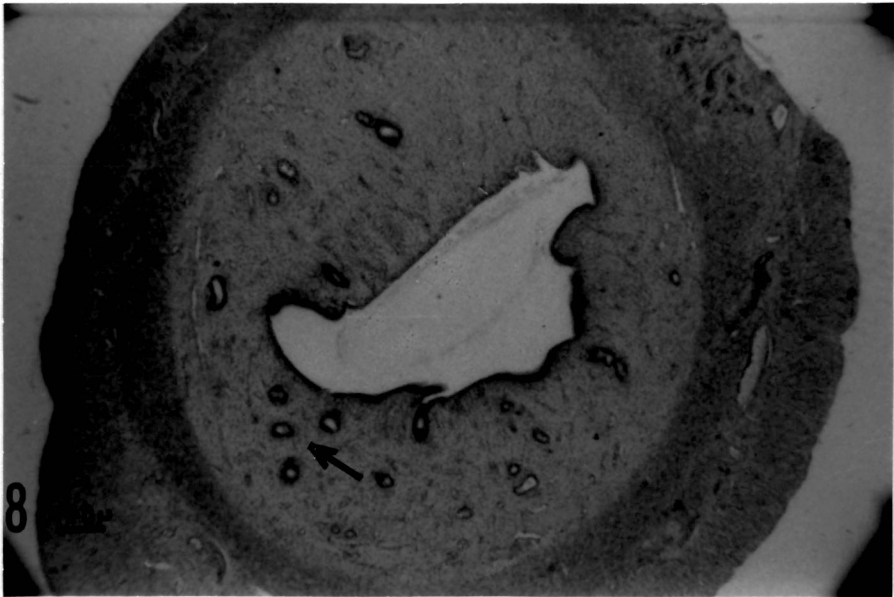
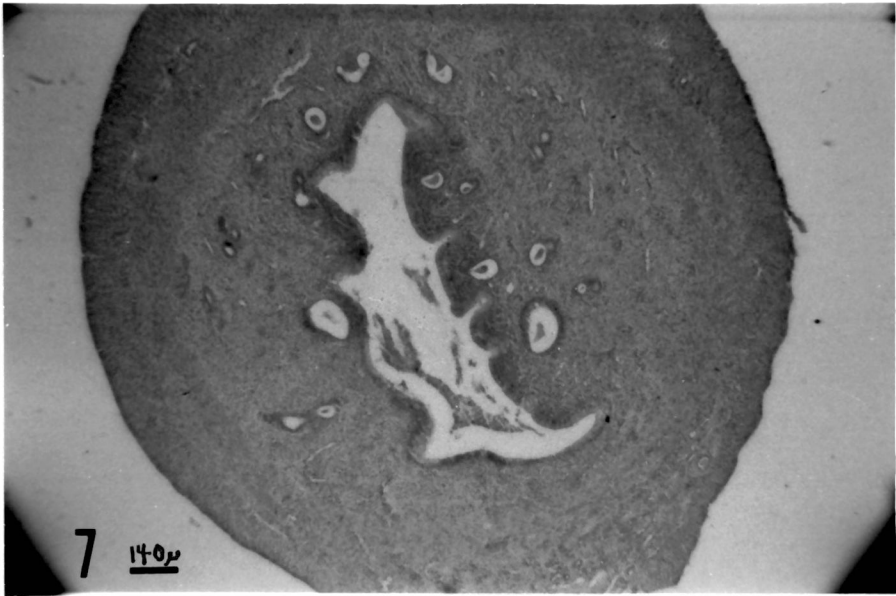
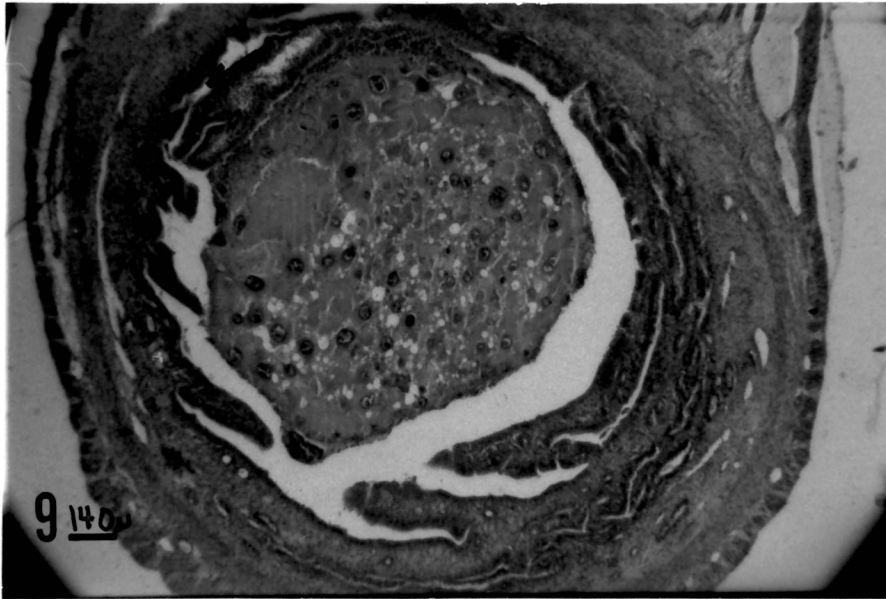


Figure 9. A section through resorbing fetal material (day 15). The mother received "Norlestrin" pills on days 6 through 10 of pregnancy. Arrow indicates region of loosely organized placental tissue. Magnification: X35.

Figure 10. A section of uterus from a 15 day pregnant rat that received ethinyl estradiol on days 6 through 10 of pregnancy. Note excessive tortuosity of endometrium. Magnification: X35.



DISCUSSION

Effects of Hormones on Non-pregnant Females

Induction of Pseudopregnancy

The fact that the oral contraceptive pills instituted a pseudopregnant state is to be expected. The hormonal induction of pseudopregnancy in rats has previously been described with injection of prolactin (Ascheim, 1954), injection of estrogen (Alloiteau, 1957), injection of progesterone (Rothchild and Schubert, 1963; Everett, 1963), and injection of estrogen followed by progesterone (Banik *et al.*, 1965). Single subcutaneous injections of 25 mg of norethindrone acetate (the synthetic progestin in "Norlestrin") inhibits estrus cycles for 30 days in adult female rats (unpublished laboratory data, Research Laboratories, Parke Davis & Company). Thus it might be inferred that 12.5 mg of norethindrone acetate (2.5 mg per tablet, 5 tablets) would probably inhibit estrus cycles for a lesser period. This was indeed found to be the case.

The relative lengths of the pseudopregnant states in the three groups of nonpregnant females deserves a word of explanation. Group 2 animals (pills started at estrus) showed shorter periods of cycle inhibition than Group 1 animals (pills started at metestrus). Apparently this effect is due to the fact that treatment was initiated at different times in the estrus cycle. When the pill regimen was begun at estrus the pseudopregnancy was comparatively shortened. Estrus represents the period when endogenous estrogen is at its peak, hence it is hypothesized that the relatively high level of estrogens sup-

pressed the action of the exogenous synthetic steroids, thereby shortening the pseudopregnant state. When the pill regimen was begun at metestrus, (Group 1) as endogenous estrogen is descending, the actions of the synthetic hormones were relatively uninhibited. Hence the length of the pseudopregnant interim was increased.

The variable vaginal cytological effects seen in the animals of Group 3 was considered due in part to the interval of the administration of the pills. Giving the five pills at 48-hour intervals probably allowed for interactions of endogenous hormones with the exogenous hormones during the 48-hour interlude between pill treatments rather than a suppression of the actions of the endogenous steroids. Individual physiological interactions were suggested by the highly variable vaginal cytology (Appendix D). The length of pseudopregnancy was correspondingly variable.

Vaginal Cytology

The fact that the vaginal cellular patterns were affected by the pill treatment was to be expected. A regular estrus cycle, as assessed by vaginal cytology, depends on the harmonious functioning of the entire hypothalamic-pituitary-gonadal-vaginal system (Duncan and Forbes, 1966). That the cyclic sequence of changes in the vaginal cytology was altered by the pills, is strong evidence that this system was being antagonized.

The pills in general produced regressive changes in the vaginal epithelium which became progressively more accentuated toward the end of pill treatment and after the last pill. The "hormonal effect," a characterization employed by this investigator to designate rounded nucleate and enucleate epithelial cell, was predominant in most

hormonally treated non-pregnant animals toward the end of the pill regimen. Rounded enucleate epithelial cells are not generally encountered in the lavage of normally cycling mature females. Since this condition was observed, it is hypothesized that the enucleate cells were chronically pyknotic, and represented an advanced state of regressed vaginal epithelium. The "hormonal effect" may possible have been cellular evidence of a transitory state which exists between a regressing condition and the completely pseudopregnant state.

Breeding Performance After Discontinuance of Oral Contraceptives

All females that were bred during the first heat following a hormonally induced pseudopregnancy littered. The average litter size was 11.0. The fact that these females gave birth to normal young after treatment is probably to be expected. The research for this investigation unveiled no information on the pregnancy rate in rats after discontinuance of oral contraceptives. However, there is a plethora of data in the literature attesting to the prompt and regular return of ovulation in women during the first cycle after suspending an oral contraceptive regimen (Rice-Wray et al., 1967).

Effects of Oral Contraceptives on Organ Weight

Uterine and ovarian weights of rats made pseudopregnant by treatment (experimental) and those made pseudopregnant by cervical stimulation (control) were compared at necropsy. No statistically significant differences in organ weights were ascertained. However, it has been found that 2 mg of norethindrone acetate administered daily for 14 days produced definite depression of the weights of ovaries in rats (unpublished laboratory data, Research Laboratories, Parke Davis & Co.).

The amount of norethindrone acetate utilized in this regimen was 2.5 mg daily for five days, less than half the aforementioned dosage. Since a progressive increase in endometrial glandular suppression is known to occur with increasing doses of certain synthetic progestagens (Rudel et al., 1967), it is probable that uterine weights might be reduced with a 10 or 15 day regimen of pills. It is evident that further work should be done.

Effects of Hormones on Pregnant Females

A pill, or the representative estrogen and progestin components of a pill, was administered daily to each of the pregnant experimental animals over a five day period. It is meaningful to recall that this was the dosage which induced pseudopregnancy in non-pregnant animals. This dosage was administered to pregnant rats at different stages of pregnancy.

Females Sacrificed On Day 6 of Pregnancy

The period of ova transport through the oviducts on days 1 through 4 is an especially vulnerable time in the reproductive cycle for interrupting pregnancy in the rat (Greenwald, 1961a). Eggs enter the uterus early on the 5th day of pregnancy and implantation usually takes place that afternoon (Christie, 1966). In this research five oral contraceptive tablets administered to rats daily starting on day 1 of pregnancy totally inhibited implantation, presumably by speeding the rate of ova transport through the tubes. This presumption is based on the knowledge that large doses of progesterone (Alden, 1942) and estrogen (Burdick, 1938; Banik and Pincus, 1964) will accelerate ovum passage through the tubes and cause premature expulsion into the uterus.

Histological examination of uteri indicated some regression of the endometrium. The uterine glands appeared to be in early stages of regression. The basal vacuoles of the glands were present, but generally had a smaller diameter than those of controls. The overall tortuosity was reduced. These observations (Figure 7) are interpreted as evidences of some inhibition of normal glandular development that is usually associated with a six day pregnant rat. It is suggested that the norethindrone acetate component of the pill induced the aforementioned changes. Norethindrone, the parent compound of norethindrone acetate, is known to produce endometrial glandular inhibition quite consistently (Rudel *et al.*, 1967a).

Females Sacrificed on Day 15 of Pregnancy

It should be recalled that four groups of experimental females were sacrificed on day 15 of pregnancy, namely: (1) Animals which received pills on days 1 through 5 of pregnancy, (2) Animals which received pills on days 6 through 10 of pregnancy, (3) Animals which received the estrogen component (ethinyl estradiol) of the pill on days 6 through 10 and (4) Animals which received the progestin component (norethindrone acetate) of the pill on days 6 through 10.

As was expected, animals which received pills on days 1 through 5 were made sterile, apparently because of premature expulsion of the eggs from the oviducts as explained earlier. Histological studies of uteri showed extreme glandular involution and an abnormally low amount of tortuosity. These effects are illustrated in Figure 8.

From Figures 4 and 5 it can be seen that the steroid hormones induced variable effects on fecundity when administered after implantation

had occurred. However, these effects were generally less drastic than the results recorded when hormones were administered before nidation. Of the animals receiving pills on days 6 through 10, there was a variation in the fetal condition. By referring to Table 4 it can be seen that approximately 50% of the products of conception were in stages of resorption at day 15 while 40% were considered normal. The dead fetuses recorded in one animal probably represent an early stage of resorption. The occurrence of live runts in one animal is of equivocal significance. Such a condition could indicate an effect on thyroid function or perhaps a degree of resorption.

Progestational proliferation was observed in those animals which were administered the pill subsequent to implantation. This condition is to be expected because of the influence of normal fetuses in close proximity. Sections through extensively resorbed fetuses showed loosely organized deteriorating fetal tissue (Figure 9).

The administration of the components of the pill separately to two groups of rats produced some noteworthy consequences. Table 6 shows that only one rat of five that received ethinyl estradiol exhibited normal fetuses at day 15. Apparently ethinyl estradiol exerted a powerful effect on maternal and fetal tissue. Sections of uteri of rats made sterile by ethinyl estradiol had extensive hyper-tortuosity of the lining (Figure 10). Remnants of fetal tissue were loosely organized and showed advanced cellular degeneration. Probably a synergistic action of the exogenous synthetic ethinyl estradiol with the endogenous estrogen and progesterone accounted for the hypertrophy and hyperplasia of the uterine lining. It is hypothesized that the degenerate condition of the fetal remnants was

due to the over-riding effect of exogenous ethinyl estradiol. This is in accordance with other investigators who have described the detrimental effects of large doses of exogenous synthetic and natural estrogens on the fetus (Morris and Wagenen, 1966).

Norethindrone acetate, administered after implantation, did not affect the number of normal fetuses at day 15 when compared to controls. Obviously the physiological tolerance level for this exogenous synthetic progestin after nidation is much higher than that for estrogens. This finding is in accordance with the literature. Black and Asdell (1959) have suggested that generally exogenous progestins are effective only when they can overcome the effects of endogenous estrogens. It has been shown that 1 part of endogenous estrogen is capable of nullifying the action of 1000 parts of progesterone (Velardo and Hisaw, 1951). Thus it may be inferred that the following statements singly or in combination may be explanatory: (1) Detrimental effects of the exogenous progesterone may have been nullified by the endogenous estrogen. (2) The excess progesterone may have had no injurious effects, since progesterone is required for maintenance of pregnancy.

Females Allowed to Go to Term

One half of all pregnant animals, including both control and experimental, were allowed to go to term. In the experimental groups, partuition was noted only in rats which received hormones after nidation, *i. e.* animals which were treated on days 6 through 10.

Table 5 shows that only three rats which received pills after implantation littered. Of the 18 pups born to three mothers, only seven

(39%) were evaluated as normal. Three animals had conspicuous edema and eight were stillborn. By referring back to Table 4, it can be seen that of the 38 products of conception noted at day 15 in identically treated animals, only 16 fetuses (42%) were considered normal. Based on this limited data, it can be hypothesized that if fetuses are normal at day 15, in animals treated with five oral contraceptive pills shortly after implantation, they will be normal at term.

Only two rats which received the progesterone component of the pill gave birth to young, two stillborn and one living pup, respectively. Thus only three pups were born to five pregnant females that were treated with norethindrone acetate. It should be recalled that there was no statistical difference in the number of normal fetuses at day 15 between controls and progesterone treated. However, in progesterone treated females that went to term, a highly significant reduction ($p < 0.01$) in number of young was observed. This research indicated that norethindrone acetate exerts a powerful detrimental effect on fetuses between day 15 and term. These findings concur with those of Huiming (1961; quoted in Pincus, 1965) who observed that norethindrone, the parent compound of norethindrone acetate, would at high doses cause fetal death. It is hypothesized that the large number of normal fetuses noted at day 15 had not yet come under the influence of norethindrone acetate because of the overriding effect of endogenous steroids. Perhaps it should be noted that although norethindrone acetate is a potent inhibitor of ovulation (Pincus et al., 1956) it is not a true progestin because it will not maintain pregnancy in the ovariectomized rabbit (Pincus, 1965). The intended role of norethindrone acetate in the

oral contraceptives employed is anti-ovulatory. It should be pointed out that there is no necessary correlation between ovulation-inhibiting and progestational actions in synthetic progestins (Pincus, 1965).

Five females which received estrogen (ethinyl estradiol) went to term. Only two females delivered young. Litters of four and seven were recorded. Three pups from the litter of seven were evaluated as runts.

Both synthetic and natural estrogens will cause fetal death when administered in large doses to gravid females (Morris and Wagenen, 1966). Dreisbach (1959) observed that fetal death was commonly induced by single injections of 0.4 mg/kg estrone on days 8, 9, or 10. Apparently the dosage of ethinyl estradiol administered in this investigation (0.05 mg on days 6 through 10 of pregnancy, total of 0.25 mg) was a differential dosage. Although five rats were presumably pregnant, only two littered, thus complete resorption is indicated in three rats. It should be recalled that complete resorption was indicated on day 15 in four out of five females identically treated, while one female exhibited 12 normal fetuses. Based on these observations at day 15, one would probably expect one or two of the five females to litter at term. This was indeed the case. It is important to note that the litter size was reduced in the two females at term. It is hypothesized that there is continued resorption between day 15 and term.

One observation which was common to all steroid-treated pregnant females that went to term was the delayed partuition. These findings are in agreement with earlier investigations. It is known that

various steroids will delay or prevent partuition in rats and other laboratory animals (Stucki, 1961). Dreisbach (1959) noted that delayed partuition of surviving fetuses was almost invariably produced by subcutaneous injection of 0.4 mg/kg oestrone on days 10 and 11.

It is generally held that there is a decrease in progesterone production near the terminus of pregnancy (Turner, 1966). Such a decrease probably results in response of the uterus to the posterior pituitary hormone, oxytocin, with subsequent expulsion of the fetus (Grotta et al., 1967). Delayed partuition was noted in this investigation in pill and estrogen-treated females as well as in progesterone-treated. It is hypothesized that delayed partuition in all cases was due to abnormally high levels of synthetic steroid hormones which probably acted as follows: (1) The high level of exogenous hormones prevented the uterus from responding to oxytocin at the regular time and/or (2) The high level of circulating exogenous hormones prevented the release of oxytocin from the posterior pituitary gland at the regular time.

Previous investigators have recorded that the experimental prolongation of pregnancy in most test animals usually leads to intra-uterine fetal death due to ischemia. The ischemia, whether uterine or uteroplacental, is considered to be due to an unknown vasoconstrictive mechanism governed by progesterone and estrogen (Moore, 1964). Stillbirths are also associated with pregnancy prolongation (Nelson, 1930). In this investigation several stillbirths were recorded. However, the fact that live pups born to hormonally treated

mothers died within 48 hours after partuition was not explained.

The results obtained in this investigation indicate that oral contraceptive pills are anti-ovulatory in nonpregnant rats and may abortive in pregnant rats. The progestin and estrogen components of the pills, given separately to different pregnant animals, were also found to be differentially abortive. The detrimental effects observed were attributed to the hormonal imbalance induced by the exogenous steroids. The concept of hormonal imbalance has received quite a bit of attention lately in the non-pregnant human female as a solution to the problem of population control. Rudel et al. (1967b) assert that there may be no reason to interrupt the hypothalamic-pituitary axis and ovulation to block conception, when temporarily imbalancing the cyclic estrogen-progestogen relationships might suffice. The results of this study indicate that a hormonal imbalance between conception and implantation induces complete sterility in the rat, while an imbalance created after implantation results in varying degrees of fetal resorption.

SUMMARY

The effects of "Norlestrin," an oral contraceptive pill, were studied on both non-pregnant and pregnant white laboratory rats. The experiment was divided into three parts: (I) Effects of Pills on Non-Pregnant Females, (II) Effects of Pills on Pregnant Females, (III) Effects of Estrogen and Progestin Components of Pills Given Separately to Two Groups of Pregnant Females.

Part I was concerned with the determination of the dosage necessary to produce a pseudopregnancy length comparable to that of a pseudopregnancy induced by mechanical stimulation of the cervix. It was found that a total of five pills administered at 24 hour intervals beginning at metestrus would achieve this.

In Part II, this same dosage (total of five pills at 24 hour intervals) was given to pregnant rats before and after implantation. When given before implantation these hormones rendered gravid females sterile. When given after implantation, the pills resulted in varying degrees of fetal resorption by day 15. However, the effects were generally less drastic than the results recorded when hormones were administered before nidation. When five rats were allowed to go to term, only three littered. A total of 18 pups were delivered, with eight stillborn and three with conspicuous edema. Thus only seven pups were evaluated as normal. All viable neonates died within 48 hours. In contrast, five control rats had an average of 12 pups per litter.

In Part III, the estrogen and progestin components of the pills (ethinyl estradiol and norethindrone acetate respectively) were

administered to separate groups of pregnant rats after implantation. The dosage mimicked that in the pill for each steroid. At day 15 only one of five estrogen treated females exhibited normal fetuses while at term only two of five similarly treated animals littered. At day 15 all progesterone treated females exhibited fetuses not unlike controls, however at term only two of five similarly treated animals littered a total of three offspring.

The aberrant maternal and fetal conditions observed in each treatment are evaluated in relation to the temporary hormonal imbalance created by the exogenous synthetic hormones at critical times during gestation.

APPENDIX A

MATERIALS FOR MAINTENANCE AND TREATMENT OF ANIMALS

Purina Laboratory Chow

Crude protein not less than 23.0%

Crude fat not less than 4.5%

Crude fiber not more than 6.0%

Ash not more than 9.0%

The ingredients of the food included meat and bone meal, dried skimmed milk, fish meal, animal liver meal, dried beet pulp, corn, oats, soybean, and alfalfa products, cane molasses, and vitamin supplements.

Obtained from: Ralston Purina Company
Wilson, North Carolina

Oral Contraceptive Pills

"Norlestrin," (Parke, Davis & Co.) a progestational-estrogenic combination for use in control of conception. Each tablet contains: norethindrone acetate, 2.5 mg; ethinyl estradiol, 0.05 mg

Obtained from: Bissette's Drug Store
Greenville, North Carolina

Ethinyl Estradiol

The experimental solution was made by dissolving ethinyl estradiol in cottonseed oil to a concentration of 0.1 milligram per milliliter.

Obtained from: Parke, Davis & Co., Research Div.
Detroit 32, Michigan

APPENDIX A (continued)

Norethindrone Acetate

The experimental solution was made by dissolving norethindrone acetate in cottonseed oil to a concentration of 5.0 milligrams per milliliter.

Obtained from: Parke, Davis and Co., Research Div.

Detroit 32, Michigan

APPENDIX B

PART I. DATA FROM THREE GROUPS OF NON-PREGNANT FEMALES ADMINISTERED FIVE PILLS

Rat no.	Group no.	Day of cycle pill regimen initiated	Time interval between pills	No. days pseudopregnant
1	1	metestrus	24 hrs.	15
3	1	metestrus	24 hrs.	14
6*	1	metestrus	24 hrs.	
8	1	metestrus	24 hrs.	14
10	1	metestrus	24 hrs.	11
11*	1	metestrus	24 hrs.	
12	1	metestrus	24 hrs.	14
13	1	metestrus	24 hrs.	12
15	1	metestrus	24 hrs.	15
16	1	metestrus	24 hrs.	9
				$\bar{X}=13.0 \pm 0.76^{**}$
33	2	estrus	24 hrs.	9
43	2	estrus	24 hrs.	10
47	2	estrus	24 hrs.	12
48	2	estrus	24 hrs.	11
49	2	estrus	24 hrs.	11
56	2	estrus	24 hrs.	11
57	2	estrus	24 hrs.	10
58	2	estrus	24 hrs.	10
60	2	estrus	24 hrs.	10
				$\bar{X}=10.4 \pm 0.29$
2	3	metestrus	48 hrs.	15
4	3	metestrus	48 hrs.	14
5	3	metestrus	48 hrs.	22
7	3	metestrus	48 hrs.	2
9	3	metestrus	48 hrs.	10
14	3	metestrus	48 hrs.	12
17	3	metestrus	48 hrs.	10
18	3	metestrus	48 hrs.	10
19	3	metestrus	48 hrs.	15
20	3	metestrus	48 hrs.	10
				$\bar{X}=12.0 \pm 1.63$

*Accidental death from overdose of ether while administering pill

**Mean $\pm$ standard error

APPENDIX C

PART I. DATA ON VAGINAL CYTOLOGY OF GROUP 2 ANIMALS (FIVE PILLS AT 24 HOUR INTERVALS BEGINNING AT ESTRUS) WHILE ON PILLS AND TWO DAYS AFTER END OF PILL TREATMENT

	E=cornified epithelium (estrus) M=rounded nucleated and enucleated epithelial cells (hormonal effect) D=leucocytes (diestrus, pseudopregnant) Days: Day 1 = day of first pill, therefore animals received pills on days 1, 2, 3, 4, and 5						
Animal no.	1	2	3	4	5	6	7
33	E	E	M	M	D	D	D
43	E	E	M	M	D	D	D
47	E	E	M	M	D	D	D
48	E	E	M	M	M	D	D
49	E	E	M	M	M	D	D
56	E	E	M	M	D	D	D
57	E	E	M	M	M	D	D
58	E	M	M	D	D	D	D
60	E	M	M	D	D	D	D

APPENDIX D

PART I. DATA ON VAGINAL CYTOLOGY OF GROUP 3 ANIMALS (FIVE PILLS AT 48 HOUR INTERVALS BEGINNING AT METESTRUS) WHILE ON PILLS AND TWO DAYS AFTER END OF PILL TREATMENT

[illegible]

APPENDIX E

PART I. DATA ON UTERINE AND OVARIAN WEIGHTS OF ANIMALS MADE PSEUDO-PREGNANT BY MECHANICAL STIMULATION OF THE CERVIX (CONTROL)

Animal no.	Body wt. (g)	Uterine wt. (mg)	Ovarian wt. (mg)	Uterine wt. expressed as mg/100 g body wt.	Ovarian wt. expressed as mg/100 g body wt.
7	296	448.6	74.5	151.5	25.1
25	295	437.4	73.2	148.3	24.8
12	268	492.4	78.0	183.7	29.1
13	279	361.2	68.0	129.4	24.3
27	302	451.2	107.2	149.4	35.4
30	349	401.5	62.0	115.0	17.7
27	295	286.2	77.5	97.0 7	26.2
31	273	448.3	60.7	164.2	22.2
32	277	464.0	87.7	167.5	31.6
33	<u>283</u> $\bar{X}=291.6$	1700.0 7	58.0	<u>600.7</u> $\bar{X}=145.1 \pm 2.86^*$	<u>20.4</u> $\bar{X}=26.2 \pm 1.66$

~~7~~No characteristic vascularization

~~77~~Two spontaneous deciduoma present. Not included in $\bar{X}$ figure

*Mean $\pm$ standard error

APPENDIX F

PART I. DATA ON UTERINE AND OVARIAN WEIGHTS OF ANIMALS MADE PSEUDO-PREGNANT BY ADMINISTERING FIVE PILLS AT 24 HOUR INTERVALS (EXPERIMENTAL)

Animal no.	Body wt. (g)	Uterine wt. (mg)	Ovarian wt. (mg)	Uterine wt. expressed as mg/100 g body wt.	Ovarian wt. expressed as mg/100 g body wt.
4	287	318.0	86.4	110.8	30.1
9	303	355.0	83.5	117.1	27.5
16	280	399.0	80.5	142.5	28.7
30	288	404.0	85.9	140.2	29.8
1	295	331.8	70.5	112.2	23.8
2	293	209.0	60.0	71.3	20.4
8	294	318.5	77.6	108.3	26.3
15	289	286.7	58.4	99.2	20.2
19	313	238.2	84.8	76.7	27.0
20	307	486.5	77.9	158.4	25.3
	$\bar{X}=294.9$			$\bar{X}=113.6 \pm 2.78^*$	$\bar{X}=25.9 \pm 1.12$

*Mean $\pm$ standard error

APPENDIX G

"T" VALUES FROM STATISTICAL ANALYSIS OF THE EFFECTS OF FIVE DIFFERENT TREATMENTS ON THE NUMBER OF NORMAL FETUSES SURVIVING AT DAY 15 AND THE NUMBER OF NORMAL YOUNG AT PARTUITION

Groups	Age	"T" value	Significance
Control vs. Pills 1-5*	15 days	14.119	$p < 0.01$
	Term	18.883	$p < 0.01$
Control vs. Pills 6-10	15 days	6.625	$p < 0.01$
	Term	10.545	$p < 0.01$
Control vs. Estrogen 6-10	15 days	3.969	$p < 0.01$
	Term	13.663	$p < 0.01$
Control vs. Progesterone 6-10	15 days	0.556	$p > 0.05$
	Term	9.631	$p < 0.01$
Pills 1-5 vs. Pills 6-10	15 days	1.720	$p > 0.05$
	Term	2.333	$p < 0.05$
Pills 1-5 vs. Estrogen 6-10	15 days	0.990	$p > 0.05$
	Term	1.000	$p > 0.05$
Pills 1-5 vs. Progesterone 6-10	15 days	9.268	$p < 0.01$
	Term	1.500	$p > 0.05$
Pills 6-10 vs. Estrogen 6-10	15 days	0.073	$p > 0.05$
	Term	1.896	$p > 0.05$
Pills 6-10 vs. Progesterone 6-10	15 days	4.044	$p < 0.01$
	Term	0.199	$p > 0.05$
Estrogen 6-10 vs. Progesterone 6-10	15 days	2.412	$p < 0.05$
	Term	1.212	$p > 0.05$

*Numbers denote days of pregnancy treatment was administered; a duration of five days in all instances

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