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The Response of Freely Growing Laboratory Populations of Prairie Deermice (Peromyscus maniculatus Bairdii) to the Administration of Synthetic FSH/LH-RH

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THE RESPONSE OF FREELY GROWING LABORATORY POPULATIONS OF PRAIRIE DEERMICE (*PEROMYSCUS MANICULATUS BAIRDII*) TO THE ADMINISTRATION OF SYNTHETIC FSH/LH-RH

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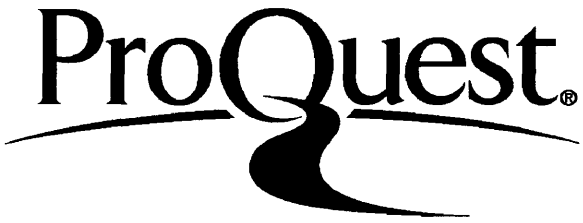

APPROVAL SHEET

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ABSTRACT

A synthetic hypothalamic release factor believed responsible for the adenohypophysial secretion of LH and FSH was administered to freely growing laboratory populations of prairie deermice (Peromyscus maniculatus bairdii). Body weights, ovarian weights, uterine weights, vaginal opening, atretic follicles, corpora lutea, and follicles of types 6, 7, and 8 were evaluated in animals which remained in the populations while receiving various doses and treatment regimes of estrogen and LRF. Across all experiments the only consistent patterns of responses noted in these animals were the occurrence of vaginal opening and the presence of type 8 follicles. Animals receiving treatments of either estrogen or estrogen plus LRF showed a significantly greater incidence of vaginal perforation than controls. The presence of type 8 follicles was significantly greater in females injected with the combination of estrogen and LRF than those receiving only estrogen or saline. This response is believed to be the result of elevated LH titers elicited by the administered release factor. Since the ovarian parameters measured are dependent on prior FSH stimulation, the inconsistent and anomalous responses observed in these experiments may reflect the absence or inappropriate secretion of this gonadotrophin. It was concluded that the reproductive endocrine systems of population animals are not refractory to appropriate hormonal stimulation.

THE RESPONSE OF FREELY GROWING LABORATORY POPULATIONS OF PRAIRIE DEERMICE (*PEROMYSCUS MANICULATUS BAIRDII*) TO THE ADMINISTRATION OF SYNTHETIC FSH/LH-RH

INTRODUCTION

Regulation of the anterior pituitary involves specialized neurons which secrete specific hypothalamic neurohormones into the primary capillary plexis of the median eminence which are then transported to the sinusoids of the anterior pituitary to stimulate the release and possible synthesis of hypophysial hormones. There appears to be at least one hypothalamic release factor for each adenohypophysial hormone and in some cases both releasing and inhibitory factors exist. Hypothalamic substances capable of stimulating the secretion of ACTH, LH, FSH, GH, MSH, and TSH, and inhibiting secretion of prolactin, growth hormone, and MSH have been purified and characterized (Schally, Arimura and Kastin, 1973).

The first direct evidence for the existence of a hypothalamic hormone regulating the release of LH was provided by McCann, Talesnik and Friedman (1960) who demonstrated that the intravenous injection of crude rat hypothalamic stalk median eminence depleted ovarian ascorbic acid in immature rats pretreated with gonadotrophins. Administration of hypothalamic extracts were found to raise LH levels both *in vivo* and *in vitro* (Schally and Bowers, 1964a). In addition, LRF activity has been demonstrated in extracts of hypothalami from rats (Nikitovitch-Winer, 1962), rabbits (Campbell, Feuer and Harris, 1964), cattle (Schally, and Bowers, 1964b), monkeys (Campbell, Feuer,

Garcia and Harris, 1964), pigs (Schally, Bowers, White and Cohen, 1967), and man (Schally, Muller, Arimura, Bowers, Saito, Redding, Sawano and Pizzolato, 1967).

The releasing factor responsible for the secretion of LH was demonstrated to be a decapeptide of the amino acid sequence (pyro) Glu-His-Trp-Gly-Leu-Arg-Pro-Gly-NH_2 (Schally, Nair, Redding and Arimura, 1971; Matsuo, Baba, Nair, Arimura and Schally, 1971). In addition to controlling the secretion of LH, both natural and synthetic LRF have been found to induce the release of FSH in vivo (Zeballos and McCann, 1974) and in vitro (Schally, Bowers, White and Cohen, 1967).

The pituitary response to natural and synthetic LRF can be significantly influenced by sex steroids which may exhibit an inhibitory of facilatory effect (Schally, Redding and Arimura, 1973; Libertun, Orias and McCann, 1974; Debeljuk, Vilchez-Martinez, Arimura and Schally, 1974). In addition, recent studies on small mammals indicate that pituitary responsiveness to LRF changes during the estrus cycle possibly mediated by variations in steroid secretion (Gordon and Reichlin, 1974; Cooper, Fawcett and McCann, 1973; Martin, Tyrey, Everett and Fellows, 1974; Arimura, Debeljuk and Schally, 1972; Vilchez, Arimura and Schally, 1974). Preovulatory estrogen levels have been implicated in the sensitization of the pituitary to LRF (Arimura and Schally, 1971; Libertun, Orias and McCann, 1974; Debeljuk, Arimura and Schally, 1972). Whether such increased pituitary sensitivity occurs concurrently with tonic or

cyclic secretion of releasing factors is unresolved. Regulation of pituitary function may eventually be found to involve a complex interaction between changing concentrations of releasing factors, gonadial hormones, and variable pituitary sensitivity.

Studies of population growth and regulation suggest that environmental factors may influence reproductive function. The importance of pheromones to the alterations of reproductive function have been indicated by Van der Lee and Boot (1956), Whitten (1965), Bruce (1956), and Terman (1968), and a transfer of pheromonal information from the nervous to the endocrine system is almost certainly mediated by changes in the secretion of release factors.

Earlier studies of free growing laboratory populations of prairie deermice (Peromyscus maniculatus bairdii) supplied with excess food and water indicated that growth regulation occurs at variable numerical levels under identical environmental conditions. This control of population growth was achieved in each population by either of two mechanisms; the failure of the young to survive or cessation of reproduction (Terman, 1965). The reproductive organs of population animals were significantly lighter in weight when compared with control animals raised as bisexual pairs. In addition, 80- to 90% of the females born into these populations and at least 100 days of age failed to reproduce (Terman, 1969). If animals in this inhibited condition are removed from the population and paired with proven mates, reproductive function is restored in 75% of the males and females within 70 and 80 days, respectively (Terman, 1973).

This recovery of nulliparous females when removed from the population indicates that the endocrine and reproductive organs are not refractory to appropriate hormonal stimulation.

It is possible that the reproductive inhibition observed in most of the young born into these populations is due to insufficient secretion of hypothalamic release factor. To validate this assumption treatment regimes were designed to simulate the pattern of endogenous secretion of both estrogen and release factor to see if the inhibition, apparently promoted by the population environment, could be overcome by intervention at specific points in the neuroendocrine pathway.

METHODS AND MATERIALS

The populations of deermice (Peromyscus maniculatus bairdii) used in this experiment were founded by animals born into a laboratory colony in which sibling matings were not permitted. The colony had been maintained for approximately 14 years with field caught mice added once a year when possible for the past 7 years. Subsequent to weaning (21 days) the mice were reared as bisexual pairs until approximately 14 weeks of age at which time four males and four females (either pregnant or nonpregnant) from different litters were used to found five populations. These populations founded by pregnant females had their first litter removed at 21 days of age.

All populations in this study were originally kept in enclosures of corrugated aluminum (floor area 20 foot^2) and were later transferred to galvanized steel cans (diameter 48.26 cm; floor area 1829.22 cm^2) at least 6 months prior to the experimental manipulation. The animals were supplied with a bedding of wood shavings; D & G lab diet (Price-Wilhoite Co.) and water were supplied ad libitum. Illumination was controlled to provide bright light from 0730 hours to 1915 hours (by four 40-watt fluorescent bulbs) and dim light from 1930 hours to 0715 hours (by four 15-watt bulbs). A 15-minute dark period separated the two lighting regimes. The temperature in the animal room ranged 21° to 30° C.

Although the populations had not stopped growing at the time of the experiment, 67% to 82% of the females born into these populations were nulliparous. Those females above the median age were placed in one subgroup and those below the median age in a second subgroup. The animals in each subgroup were randomly assigned to the various treatments. The age range of animals used in the study was between 100 and 600 days.

Design

The animals of each experiment were members of the same population and were at least 100 days of age and nulliparous when they were randomly assigned by age to the various treatment groups. Injection treatments were administered subcutaneously in a volume of 0.1 ml and consisted of either 17-B estradiol (in sesame oil) or sesame oil followed 18 to 24 hours later by either synthetic release factor (gift from NIAMD: lot# 21-103-DH, Pilot Study and Experiments I, II, III; lot# 19-192-AL, Experiment IV) or saline. The potency of the synthetic decapeptide was confirmed in a female rat for its ability to elevate serum LH as measured by radioimmunoassay. Following each treatment the animals were returned to their respective populations. Vaginal lavage was performed on perforate mice at the time of each injection and supplementary observations were made occasionally during the activity period for signs of mating behavior. At the conclusion of each experiment, the mice were killed with ether and weighed to the nearest 0.1 grams. The peritonal cavity was

opened and the whole animal was placed in 10% formalin. Subsequently the uteri and ovaries were cleaned of fat, lightly blotted, and weighed to the nearest 0.1 mg on an analytical balance.

Ovaries from experimental deermice were fixed in 10% formalin, embedded in Paraplast, serially sectioned at 10 microns, and stained with hematoxalin and eosin. The total number of corpora lutea, and follicles of type 6 or larger (Pederson and Peters, 1968) were counted in each mouse ovary. Atretic follicles (ovoid or irregular shaped follicles filled with noncellular matrix) were counted in the middle-most section of the middle row plus or minus one row of sections for each ovary. All ovarian components were evaluated under 100x magnification.

Pilot Study

Animals were randomly distributed into control and experimental groups. The experimental group received five release factor treatments of 100 ng throughout the course of the experiment. The last three such treatments were preceded 18 to 24 hours by 2 ug injections of estradiol. The control group received the corresponding vehicles only (see Figure 1).

Experiment I

Nulliparous females were randomly distributed into one of the following treatment groups: (i) 2 ug estradiol plus 100 ng LRF (E + RF), (ii) 2 ug estradiol plus saline (E), and (iii) sesame oil plus saline (C). Two estrogen-release factor treatments were given over the period of the experiment with the animals being sacrificed 48

Legend for Figure 1

Injection schedule for the E + RF animals: "E" denotes an injection of estrogen in sesame oil. "RF" denotes an injection of LRF in saline. "K" refers to the day the animals were killed. Treatments for the C and E animals were run according to the same schedule except that in the C group a sham sesame oil injection was given in lieu of estrogen and a sham saline injection was given in lieu of LRF; and for the E treatment a sham saline injection was given in lieu of the LRF. Each square denotes a 24-hour period.

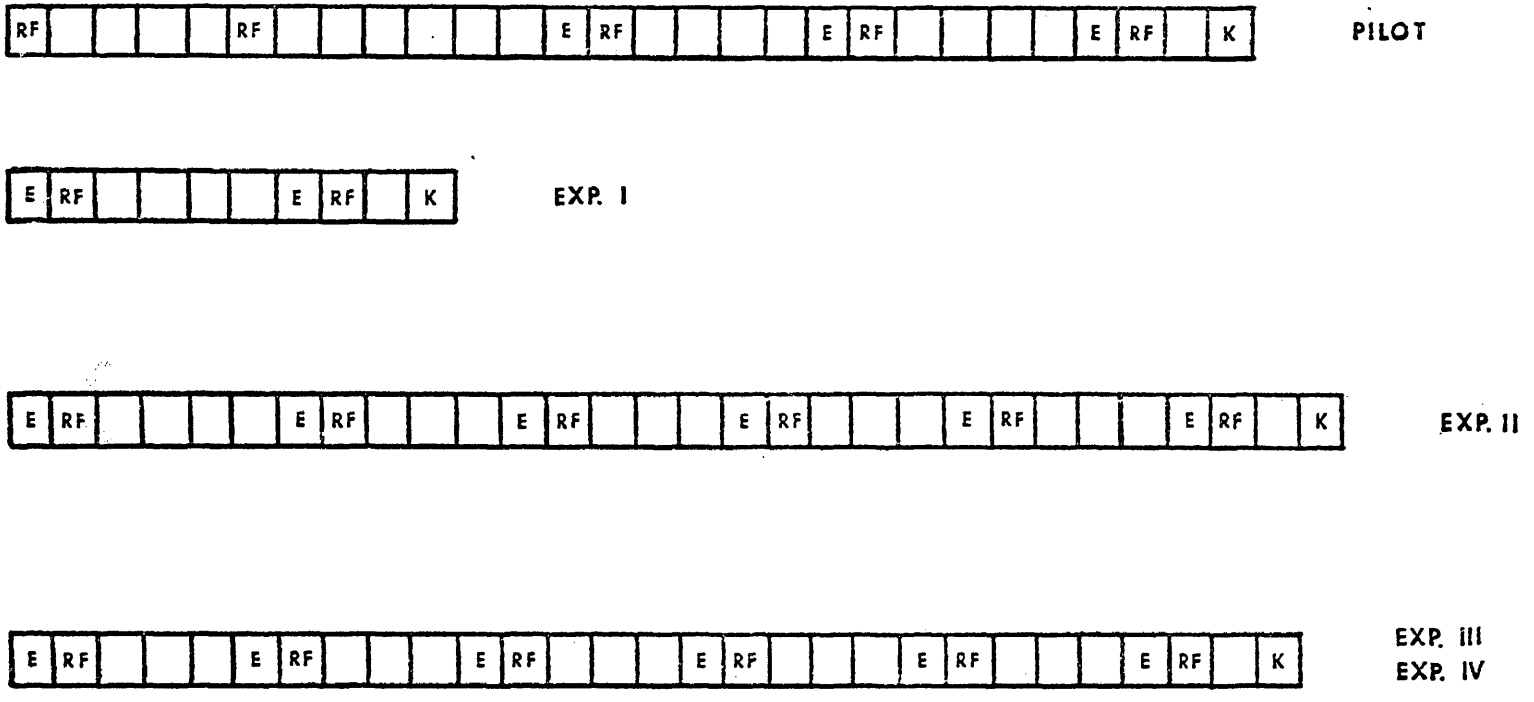

Fig. 1. Injection schedule for the E + RF animals

hours after the last release factor injection (Figure 1).

Experiment II

Experimental animals were randomly divided into three groups receiving the following: (i) 2 ug estradiol plus 200 ng release factor (E + RF), (ii) 2 ug estradiol plus saline (E), and (iii) sesame oil plus saline (C). Six such estrogen-release factor treatments were administered over the experimental period (Figure 1).

Experiment III

Animals were randomly divided into the following treatment groups: (i) 2 ug estradiol plus 200 ng release factor (E + RF), (ii) sesame oil plus 200 ng release factor (RF only), and (iii) sesame oil plus saline (C). Six such treatments were given during the experiment (Figure 1).

Experiment IV

Mice were randomly assigned to the following groups: (i) 1 ug estradiol plus 500 ng release factor (E + RF), (ii) 1 ug estradiol plus saline (E), and (iii) sesame oil plus saline (C). The treatment schedule was like that of Experiment III (Figure 1).

Statistics

Comparisons among body weights, organ weights, follicle types and number of corpora lutea were done using a Kruskal-Wallis test. If significance was obtained with this test a two sample Mann-Whitney U

test was performed. Presence or absence of various conditions were analyzed with Chi Square and Fisher exact tests. A probability value of less than 0.05 was considered significant.

RESULTS

Pilot Study

A comparison of the two treatments revealed no differences between body weights, ovary weights, the number of corpora lutea, the numbers of type 6, 7, 8, and 7 plus 8 follicles. The uterine weights of the control group were significantly lighter than the experimental group ($p < .004$) (Table 1). Sperm were found in the vaginal smears of three of the five experimental animals and no sperm were found in the control smears.

Experiment I

Comparisons made among the three treatments showed no significant differences with respect to ovarian weights, uterine weights, body weights, number of atretic follicles, number of corpora lutea and the numbers of type 6, 7, 8, and 7 plus 8 follicles. The presence or absence of type 8 follicles did not differ significantly among treatments when evaluated with a Fisher exact test.

Experiment II

Treatment comparisons revealed no differences among the three groups with respect to body weights, ovary weights, the number of atretic follicles, the number of 6 and 8 follicles. Statistical significance was obtained with a Kruskal-Wallis test across the three treatments with regard to the number of type 7 follicles, type 7 plus

TABLE 1. Mean and standard errors of body weight, ovary weight, uterine weight, and ovarian elements

Treatment	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Atretic follicles	Corpora lutea	Follicles			
						Type 6	Type 7	Type 8	Type 7 & 8
Pilot study									
Experiment	13.1 ± 0.3	6.0 ± 0.8	81.7 ± 17.2	38.0 ± 3.7	1.6 ± 0.6	0.8 ± 0.2	1.4 ± 0.4		1.6 ± 0.2
C	11.2 ± 0.4	4.0 ± 0.6	26.4 ± 9.4	39.0 ± 5.7	0.6 ± 0.6	1.0 ± 0.0	0.6 ± 0.4		0.6 ± 0.9
Experiment I									
E & RF	14.6 ± 0.8	4.8 ± 0.6	37.2 ± 7.5	86.9 ± 8.3	7.1 ± 1.5	2.5 ± 0.7	4.2 ± 0.9	1.1 ± 0.5	4.7 ± 1.2
E	15.2 ± 1.8	5.9 ± 0.8	42.7 ± 8.2	82.7 ± 15.1	8.1 ± 1.7	3.1 ± 0.5	2.8 ± 0.6	0.2 ± 0.1	3.0 ± 0.6
C	15.4 ± 1.1	7.5 ± 0.8	35.9 ± 8.2	103.8 ± 29.9	6.4 ± 2.1	3.2 ± 1.2	3.0 ± 0.8	0.2 ± 0.2	3.2 ± 0.8
Experiment II									
E & RF	14.5 ± 0.7	6.1 ± 0.7	27.8 ± 4.0	68.6 ± 8.4	8.9 ± 1.7	6.7 ± 1.0	9.6 ± 1.9	1.1 ± 0.5	10.7 ± 2.0
E	13.9 ± 0.7	4.8 ± 0.7	20.8 ± 5.6	71.8 ± 7.2	0.9 ± 0.5	5.1 ± 0.9	3.4 ± 0.5		3.4 ± 0.9
C	14.1 ± 0.4	5.7 ± 1.2	11.8 ± 2.3	102.4 ± 16.5	3.4 ± 1.5	7.9 ± 3.0	4.5 ± 1.3	0.1 ± 0.1	4.6 ± 1.3
Experiment III									
E & RF	14.8 ± 0.7	6.0 ± 0.6	27.0 ± 2.6	92.0 ± 10.9	3.4 ± 1.2	4.9 ± 1.5	5.1 ± 0.7	0.8 ± 0.1	6.0 ± 0.8
RF only	14.5 ± 1.5	4.6 ± 0.8	12.2 ± 1.8	80.1 ± 15.3	1.3 ± 0.8	4.3 ± 1.2	4.9 ± 0.9	1.0 ± 0.6	5.9 ± 1.1
C	13.9 ± 0.6	4.8 ± 2.4	22.2 ± 6.7	51.0 ± 7.1	2.6 ± 1.1	6.2 ± 1.0	4.0 ± 1.3	0.3 ± 0.3	4.2 ± 1.2
Experiment IV									
E & RF	16.7 ± 0.7	11.3 ± 2.8	61.4 ± 23.1	32.2 ± 11.8	9.0 ± 1.6	3.4 ± 1.3	4.8 ± 2.3	0.8 ± 0.3	5.6 ± 2.5
E	14.5 ± 1.1	6.0 ± 0.7	23.1 ± 9.9	65.3 ± 20.0	6.5 ± 2.1	5.5 ± 0.2	4.7 ± 1.3	0.3 ± 0.3	5.0 ± 1.4
C	14.7 ± 1.5	6.0 ± 0.3	16.5 ± 1.1	42.0 ± 7.3	3.6 ± 1.2	4.5 ± 1.1	8.8 ± 2.3		8.8 ± 2.5

8 follicles, corpora lutea, and uterine weights. Two sample comparisons were made with a Mann-Whitney U test since the above tests showed significance. The E + RF treatment was found to have significantly more type 7 plus 8 follicles than the E ($p = .001$) and C ($p = .028$) groups. The E + RF treatment had significantly more corpora lutea than both the E ($p = .04$) group and the controls ($p = .004$). The E + RF treatment had significantly more type 7 follicles than the E ($p = .004$) and C ($p = .0512$) groups. The control treatment had smaller uterine weights than both the E + RF ($p = .006$) and E ($p = .05$) treatments (Table 1).

Significantly more E + RF than control mice had type 8 follicles ($p = .02$). More animals in the E + RF group had corpora lutea than in the E treatment ($p = .018$). Comparisons between the E + RF and C treatments also reflected this trend ($p = .0512$). The number of females whose vaginae became perforate in each treatment group were compared with a Fisher exact test. The lack of the perforate condition in the C treatment differed significantly from the E + RF ($p = .00007$) and E ($p = .0007$) treatments.

Experiment III

No significant differences were found when treatment groups were compared relative to body weights, ovary weights, number of atretic follicles, number of corpora lutea, and numbers of type 6, 7, 8, and 7 plus 8 follicles. Uterine weights were larger in the E + RF group than in the RF treatment ($p < .001$) (Table 1). More animals

showed the presence of type 8 follicles in the E + RF treatment than in the C treatment ($p = .014$) (Table 2), and significantly more animals developed perforate vaginae in the E + RF group than in the C group ($p = .034$). Two animals in the RF only group became pregnant.

Experiment IV

No significant differences were noted among the three treatment groups relative to body, ovarian and uterine weights or the numbers of corpora lutea, atretic follicles, type 6, 7, 8, and 7 plus 8 follicles. Significantly more animals remained imperforate in the C group than in either the E + RF ($p = .047$) or E ($p = .047$) groups. More animals in the E + RF group (60%) had type 8 follicles than the E (25%) group, and the control group had no type 8 follicles (Table 2 and Figure 2).

Experimental Overview

Since the degree and response to each treatment varied in each population, an attempt was made to discover any trends in the data by an overview of the complete series of experiments. The following results were obtained by combining the data from Experiments I, II, III, and IV. The presence or absence of corpora lutea did not differ among the three treatments. Significantly more animals became perforate in the E + RF ($p < .001$) and E ($p < .001$) groups than in the controls. A trend was noted across the four experiments with more animals showing the presence of type 8 follicles in the E + RF group (64.4%) than in both the C (12.5%) and E (13.6%) groups (Figure 2 and

TABLE 2. Number of animals with type 8 follicles, number of animals in respective treatments and the percentage of animals in each treatment with type 8 follicles

Experiment number	Treatment								
	Control			Estrogen			E & RF		
	Number with type 8	Number in treatment	Percent with type 8	Number with type 8	Number in treatment	Percent with type 8	Number with type 8	Number in treatment	Percent with type 8
I	1	5	20.0	2	10	20.0	5	9	55.5
II	1	8	12.5	0	8		4	7	57.0
III	1	7	14.3				6	7	85.0
IV	0	4		1	4	25.0	3	5	60.0
Combined	3	24	12.5	3	22	13.6	18	28	64.4

Legend for Figure 2

Percentage of animals with type 8 follicles in the experimental treatments.

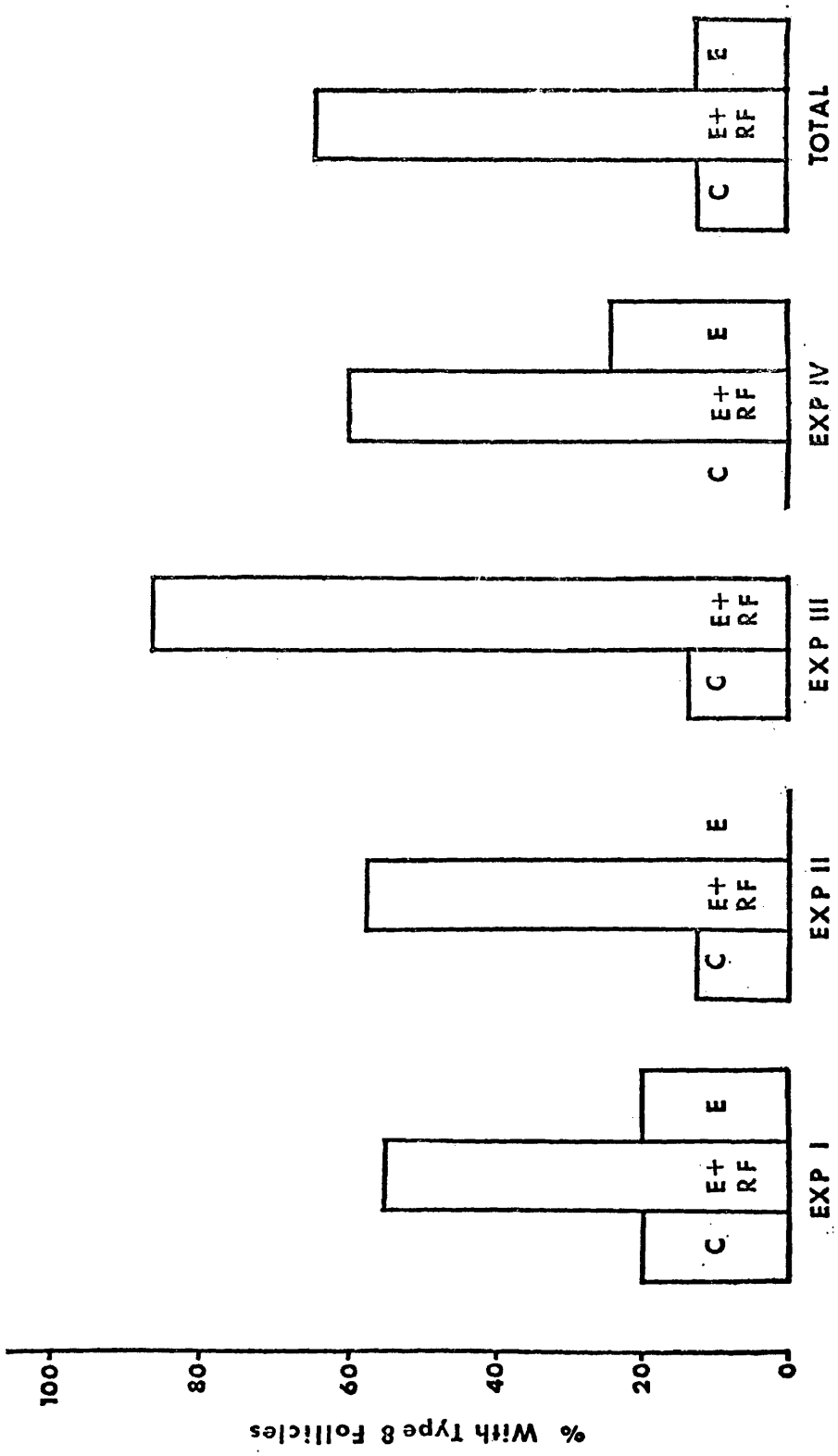

Fig. 2. Percentage of animals with type 8 follicles in experimental treatments.

Table 2).

When the frequency of animals with type 8 follicles were statistically compared, the E + RF group had significantly more animals with this follicle type than the C ($p < .001$) and E ($p < .001$) treatments.

Identical treatments were compared across Experiments I, II, III, and IV for type 8 follicles and vaginal perforation by means of a Heterogeneity Chi Square test. No differences in type 8 follicles were noted for the C, E, and E + RF treatments. Heterogeneity tests for vaginal perforation showed no differences in the E and E + RF treatments, however, significant variability was noted in the control group ($p < .01$) comparisons.

Heterogeneity tests for the other parameters were not performed on these data because of the obvious inconsistencies noted for the experimental treatments. For example, the mean numbers of corpora lutea in the estrogen groups were as follows: Exp. I = 8,1 Exp. II = .09, Exp. III = 6.5 (Table 1).

One would perhaps expect the control treatments to be similar since they received only the injection vehicles and would not contain the different doses of active principles that were administered in the other experimental groups, i.e., E + RF, E, RF only. These data indicate that this was not the case and conspicuous variability occurred throughout the control treatment (Table 1).

Comparison for the control treatments among the five experiments revealed differences with respect to mean uterine weights,

and the mean number of atretic follicles and corpora lutea (Table 1). The mean uterine weight of control animals in Experiments II and IV (16.5 mg; 11.7 mg) were much smaller than those of the Pilot Study (26 mg), Experiment I (35.5 mg), and Experiment III (22 mg). The mean numbers of atretic follicles in Experiment I (103) and Experiment II (102) were about twice that found in the other experiments (Pilot: 39; Experiment III: 51; Experiment IV: 42). Mean corpora lutea comparisons of Experiments II, III, and IV showed little variability with values of 3.3, 2.5, and 3.2, respectively. These were incongruous with the corpora lutea in the Pilot Study ($\bar{X}$ = 0.6) and Experiment I ($\bar{X}$ = 6.4). Except for one animal in Experiment IV which became perforate once, all control animals of Experiments II and IV remained imperforate throughout the treatment schedule.

DISCUSSION

This study attempted to evaluate the effects of exogenous estrogen and release factor administration on five different free-growing laboratory populations of deermice.

Animals of the Pilot Study receiving both estrogen and release factor showed greater values for the numbers of corpora lutea, the number of type 7 plus 8 follicles, uterine and ovarian weights than control animals. Reproductive behavior and inseminations occurred in the experimental group but were absent in controls. Since vaginal smears were made every 12 hours, it is possible that the associated vaginal or cervical stimulation may have promoted some of the results obtained in the E + RF treatment due to the combined action of the exogenous treatment and the neurogenic genital stimulation (Zarrow and Clark, 1968; Davidson, Smith and Bowers, 1973).

The shorter duration and fewer treatments of Experiment I (Figure 1) may have been responsible for the lack of significant response observed in the parameters measured. One trend, however, was noted in that more animals had type 8 follicles in the E + RF treatment (55%) than in both the E (20%) and C (20%) groups (Table 2, and Figure 2).

In Experiment II the release factor dose was doubled from Experiment I and six treatments were administered. Experiment II

resulted in higher values for uterine weights and the numbers of corpora lutea, type 7 follicles, and type 7 plus 8 follicles in the E + RF group compared with the C and E groups. The significantly smaller uterine weights of the control animals may reflect the absence of estrogen and the increase in uterine size of the experimentals indicates that population mice are responsive to such steroid stimulation.

In Experiment II all animals in the E + RF treatment had at least one corpus luteum compared with only 50% ($p = .0512$) of the C group and 37% ($p = .018$) of the estrogen group. In addition, the number of corpora lutea were also greater in the E + RF treatment than in either the E ($p = .004$) or C ($p = .04$) groups suggesting that more ovulations had occurred with the former treatment. Comparisons of the number of type 7 follicles in Experiment II revealed that the E + RF treatment had more of these elements than the E ($p = .001$) and C ($p = .028$) groups. It was also noted that none of the control animals ever became vaginally perforate; this was significantly different from the E + RF ($p = .00007$) and E ($p = .0007$) groups and most likely reflects the estrogen pretreatment.

Having attributed the different results obtained in Experiment I compared with Experiment II to the fact that relatively few animals were sampled in each treatment group, another experiment was performed. Although Experiment III duplicated the hormone doses and treatments of Experiment II, only uterine weight comparisons showed significant differences. The E + RF group had higher uterine weights than the

RF only treatment ($p < .001$); the high value of the control group may reflect the very high weight of one animal (Appendix A, Table 5).

Two of the animals in Experiment IV receiving only release factor became pregnant and were the only mice in this group to have corpora lutea. There appeared to be a dramatic response difference within this group; either an animal responded to release factor alone, or it did not. The two pregnant mice may have already possessed the appropriate endogenous hormone levels to produce this response which further suggests that there must be differences in reproductive states among nulliparous population animals.

Prior to this experiment it may have been argued that the lack of response to treatments observed in this study resulted from insufficient release factor stimulation and/or too much estrogen which could have reduced gonadotrophin secretion through negative feedback. To eliminate these possibilities Experiment IV was conducted using 1 ug of estrogen and 500 ng of release factor. This massive dose of LRF was ineffective in significantly altering the parameters measured which suggests that the lack of response was not due to the hormone doses. The only significant difference noted among the treatments was that more animals in the control group remained imperforate than the E ($p = .047$) and E + RF ($p = .047$) groups, which again suggests a response to estrogen. Although not significantly different, more corpora lutea and type 8 follicles (Table 1) were observed in the E + RF group than the other two treatments.

In light of the variable responses observed among the experments it was decided to examine the response of all animals in the various experiments according to treatment. Thus, the data were combined from Experiments I, II, III, and IV by treatment to obtain this overview. The statistical validity of the data derived from this approach is open to question because of the fact that the populations were different with respect to treatment, dose, time, and previous history. However, insights into the biological processes were obtained by this approach and the data are discussed from this point of view.

Combined treatments were compared for vaginal perforation and the presence of corpora lutea and type 8 follicles. No significant differences were noted among the treatments for the presence of corpora lutea suggesting that the injections of estrogen and estrogen plus release factor were ineffective in bringing about ovulations. The effectiveness of the estrogen treatment in all experiments was reflected in the fact that significantly more animals in the E + RF ($p < .001$) and E ($p < .001$) groups became vaginally perforate when compared with controls. Also, one response to estrogen and release factor that was consistently observed in all experiments was that 64% of the animals possessed type 8 follicles compared to 12.5% ($p < .001$) of the C and 13% ($p < .001$) of the E groups (Table 2 and Figure 2). It seems most probable that this final maturation of the follicle to the type 8 stage is due to an increased level of LH in response to the administered release factor (Greep, van Dyke, and

Chow, 1942). These two responses indicate that even in the presence of an inhibitory population environment, certain components of the reproductive system are functional and not refractory to appropriate hormonal stimulation. This fact may be the basis for the rapid recovery of the reproductive function observed when population animals are removed from their environment and kept as bisexual pairs (Terman, 1973).

Environmental cues are believed to cause the inhibition of reproductive function observed in these animals. The transmission of this sensory information to the endocrine system is believed to be mediated via the secretion of hypothalamic releasing and inhibiting factors and it is probable that the inhibition observed in these animals takes place at this point in the neuroendocrine pathway. The equivocal results of this study do not indicate that inhibition at this level is nonexistent, but rather that certain systems can be effected by the administration of exogenous LRF. Even though the animals remained in the population environment those receiving E + RF did respond to this treatment by having more type 8 follicles demonstrating the flexible nature of their reproductive endocrine system.

Experiments were compared with a heterogeneity test for the presence of type 8 follicles and vaginal perforation. Such tests applied to the presence of type 8 follicles revealed no significant differences among the experiments for the C, E, and E + RF treatments. The consistency of type 8 follicles in response to the combined administration of estrogen and release factor occurred in these experiments

irrespective of differences in dose, the number of treatments, and the time interval between such treatments. A similar analysis of the vaginal condition showed no significant differences for the E and E + RF groups, but the control treatment was found to be heterogeneous ($p < .01$). This seems to suggest that estrogen by itself or in combination with LRF can reduce the intrinsic population variability for vaginal perforation.

Perhaps the variability observed among the control parameters seen throughout these experiments reflects population differences related to age, method of founding, and previous history. This would support the notion that such populations are intrinsically different and suggests that they be considered as separate entities (Terman, 1973). The consistent response observed across all E + RF treatments for type 8 follicles indicates that such intrinsic differences do not play a major role in the response of populations to exogenous release factor administration.

Previous studies show that the administration of the synthetic release factor results in minimal FSH release and is not independent of LH secretion (Zeballos and McCann, 1974). Since the ovarian parameters measured are dependent on prior FSH stimulation the inconsistent and anomalous responses observed in these experiments may reflect the absence or inappropriate secretion of this gonadotrophin. Since the predominant effect produced by the synthetic release factor is a release of LH, it is reasonable that those components of the reproductive system sensitive to this gonadotrophin would be most

responsive to the treatments. The final maturation of the follicle to the type 8 stage appears to be the only consistent response to the synthetic release factor.

It therefore appears that population animals can respond to LH secretion elicited by synthetic release factor and indicates that LRF suppression at the hypothalamic level may be responsible for the lack of type 8 follicles observed in nulliparous population animals. Further insights into the location and mechanisms of population inhibition can only come from further research. Additional data on the experiments have been included in Appendix A, Tables 3 through 7.

APPENDIX

APPENDIX A

TABLE 3. Age, body weight, ovary weight, uterine weight, numbers of atretic follicles and corpora lutea, number of follicle types 6, 7, and 8 for animals in the Pilot Study

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type			
							6	7	8	7 & 8
Control treatment										
457	144	9.9	3.39	4.63	55		1	2		2
470	115	12.3	6.23	6.51	35		1			
449	271	11.6	4.30	42.21	25	3	1			
465	259	10.3	2.54	15.75	30		1	1		1
423	345	11.8	3.72	26.87	50		1			
Experimental treatment										
467	259	14.3	7.40	29.32	30	1	1	2		2
463	257	12.8	3.50	84.16	30		1	2		2
453	247	12.7	7.04	131.95	40	4	1	2		2

TABLE 3. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type			
							6	7	8	7 & 8
459	243	12.9	4.28	95.19	40	2		1		1
441	143	13.0	7.88	116.34	50	1	1			

TABLE 4. Age, body weight, ovary weight, uterine weight, numbers of atretic follicles and corpora lutea, number of follicle types 6, 7, and 8, and presence of perforate vagina for animals in Experiment I

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
Control treatment											
428	117	19.0	5.0	15	138		1	4		4	40.0
461	116	13.9	8.0	23	50	11	8				40.0
332/333	670	12.5	6.0	36	33	9	1	4		4	40.0
425	275	15.4	9.0	62	100	9	2	4		4	60.0
423/323	275	16.0	8.5	43	198	3	6	3	1	4	60.0
E Estrogen treatment											
325	685	17.8	7.5	64	64	10	2	4		4	Imp[c]
463	114	17.1	4.5	16	16	7	4			2	20.0
405	449	13.0	3.0	135	135			3	1		Imp

TABLE 4. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
441	361	18.1	7.0	127	127	8	1	5		4	60.0
343/393	538	12.3	6.0	120	120	13	4	5	1	5	40.0
391	115	18.6	11.0	55	55	17	1	2		6	40.0
465	633	14.6	6.0	41	41	6	5	4		2	Imp
335	165	13.6	4.0	148	148	8	2			4	Imp
447	150	11.5	2.0	92	92	0	2	3			40.0
381	357	15.7	8.0	28	28	12	4		4	3	40.0
E & RF Treatment											
356/357	597	18.3	6.0	63	71	14	8	5	4	9	40.0
331	248	13.5	3.0	29	75	6	3	6			40.0
335/435	235	11.5	4.0	24	107	4	1	2	2	2	20.0
387	538	15.6	2.0	17	101		1	3		5	Imp
329	670	12.1	7.0	83	82	5	2	3	1	4	40.0

TABLE 4. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
323	685	14.0	5.0	19	39	5	1	2	1	3	20.0
393	538	14.2	5.5	20	89	13	3	9		9	40.0
455	149	18.4	4.0	42	90	6	2	7	2	9	40.0
371/379	585	18.5	7.0	38	128	11	2	1		1	40.0

[a]Animals of experiment I were checked for the perforate condition five times.

[b]Percentage of time perforate.

[c]Imp. animal never became vaginally perforate.

TABLE 5. Age, body weight, ovary weight, uterine weight, number of atretic follicles and corpora lutea, number of follicle types 6, 7, and 8, and presence of perforate vagina for animals in Experiment II

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
Control treatment											
435	659	14.7	3.1	10.7	197		2	4		4	Imp[c]
409	726	12.8	2.7	5.3	67		6	1		1	Imp
489	404	14.6	7.6	13.2	79		3	6		6	Imp
429	677	13.3	1.6	10.6	62	3	4				Imp
413	275	15.6	6.7	6.6	148	4	2	2	1	3	Imp
503	344	12.1	9.6	25.3	77	11	13	9		9	Imp
497	369	14.4	3.3	6.5	99		6	10		10	Imp
531	229	15.3	11.1	16.0	90	9	27	4		4	Imp

TABLE 5. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
Estrogen treatment											
451	653	14.6	4.1	19.8	71		9	3		3	46.7
423	678	13.2	1.7	13.3	70		4	2		2	Imp
444	677	10.9	3.4	13.2	96		4	6		6	6.7
455/555	606	17.2	4.9	23.8	95	2	2	2		2	46.7
509	319	15.3	8.3	10.2	55		5	2		2	66.7
505	369	13.9	6.5	58.3	75	4	3	4		4	80.0
553	207	14.0	4.2	16.4	78	1	6	4		4	40.0
541	194	12.1	5.3	11.5	34		8	4		4	20.0
E & RF treatment											
431	665	17.0	4.0	24.7	52	1	6	6		6	46.7
439	659	15.9	8.9	32.8	41	14	4	4	1	5	100.0
445	655	15.3	3.5	15.8	76	6	7	6	2	8	40.0
505/506	319	15.9	6.5	26.2	71	13	4	10		10	73.4

TABLE 5. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
559	121	12.7	8.4	19.1	57	11	6	17		17	26.7
532	229	11.5	8.0	41.7	110	9	8	16	3	19	86.7
529	247	14.3	5.0	45.5	73	8	12	8	2	10	6.7

[a]Animals of Experiment II were checked for the perforate condition fifteen times.

[b]Percentage of time perforate.

[c]Imp. animal never became vaginally perforate.

TABLE 6. Age, body weight, ovary weight, uterine weight, number of atretic follicles and corpora lutea, number of follicle types 6, 7, and 8, and presence of perforate vagina for animals in Experiment III

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
Control treatment											
171	287	14.3	2.0	9.0	62		9	3	2	5	Imp[c]
124	427	13.8	6.0	24.0	50	8	8	7		7	26.7
187	291	13.3	2.0	8.0	33		2	3		3	6.7
125	393	13.4	6.0	15.0	71	3	7	3		3	Imp
73	513	13.9	9.0	60.0	34	4	3	1		1	33.3
117	421	15.1	4.0	17.0	76		8	10		10	Imp
97	470	14.1	5.0	27.0	32	3	7	1		1	26.7

TABLE 6. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
RF only treatment											
89	481	15.4	8.0	7.0	68		2	5	1	6	20.0
149	313	14.9	6.0	15.0	104		3	7		7	20.0
151	313	12.9	2.0	17.0	145		1	2		2	Imp
175	291	11.8	5.0	12.0	82		5	6		6	Imp
121	393	21.8	5.0	10.0	97		4	2		2	6.7
107	481	13.7	3.0	29.0	5	4	4	4	8	6	6.7
131	393	12.1	3.0	36.0	4	11	8	2	10	2	Imp
E & RF treatment											
119	421	17.3	8.0	34.0	71	9	4	6	1	7	73.3
111	537	15.7	5.0	35.0	149	5	10	6	1	7	60.0
123	393	13.0	5.0	22.0	98		9	5	1	6	66.7

TABLE 6. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
181	291	13.0	4.0	22.0	80	2	7	4	1	5	6.7
179	291	14.9	5.0	33.0	79	5	3	8	1	9	66.7
127	393	13.6	3.0	18.0	105		1	2		2	53.3
75	513	16.2	6.0	25.0	65	3		5	1	6	Imp

[a]Animals of Experiment III were checked for the perforate condition fifteen times.

[b]Percentage of time perforate.

[c]Imp. animal never became vaginally perforate.

TABLE 7. Age, body weight, ovary weight, uterine weight, number of atretic follicles and corpora lutea, number of follicle types 6, 7, and 8, and presence of perforate vagina for animals in Experiment IV

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
Control treatment											
329	389	16.9	6.38	19.0	55	3	6	11		11	5.3
317	502	15.0	5.05	14.1	47		6	4		4	Imp[c]
381	493	13.7	6.46	17.8	21	4	1	15		15	Imp
365	207	13.4	6.10	15.1	45	6	5	5		5	Imp
Estrogen treatment											
367	206	11.4	7.68	52.9	38	9	5	5		5	42.4
353	234	14.5	5.93	13.9	57	4	6	4		4	15.8
357/352	217	15.3	6.57	14.1	42	11	6	8	1	9	21.1

TABLE 7. (continued)

Animal number	Age	Body weight (gm)	Ovary weight (mg)	Uterus weight (mg)	Number atretic follicles	Number corpora lutea	Follicles by type				Vaginal perforation[a] (%)[b]
							6	7	8	7 & 8	
327	408	16.6	3.90	11.4	124	2	5	2		2	26.3
E & RF treatment											
383	493	17.5	20.45	110.4	42	13	7	12	1	13	84.2
275	691	16.5	4.26	13.9	52	4	1				73.7
359	207	14.2	10.40	45.7	16	7	3	2		2	100.0
357	234	18.2	7.27	15.0	3	9		1	2	3	100.0
349	291	17.2	14.26	122.3	68	12	6	9	1	10	68.4

[a]Animals of Experiment IV were checked for the perforate condition nineteen times.

[b]Percentage of time perforate.

[c]Imp. animal never bacame vaginally perforate.

APPENDIX B

POPULATION HISTORY

The Pilot Study was begun April 25, 1973 and was previously designated as follows: Population Cues Tactile and Visual, Experiment I, population 15.

Experiment I was begun November 8, 1973 and was previously designated as follows: Population Cues Tactile and Visual, Experiment II, population 4.

Experiment II was begun January 20, 1974 and was previously designated as follows: Pheromone, Experiment II, population 5.

Experiment III was begun April 9, 1974 and was previously designated as follows: Population Cues Tactile and Visual, Experiment II, population 11.

Experiment IV was begun July 23, 1974 and was previously designated as follows: Population Cues Tactile and Visual, Experiment II, population 3.

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