fetus was studied in the progeny of mice uninephrectomized on day 14 of pregnancy. Fetal kidneys removed on day 17 and at birth (day 19) and the comparable solitary maternal kidney were analyzed for content of RNA and DNA. The ratio RNA/DNA (RNA/av cell) increased about 22% in the maternal kidney compared with sham-operated controls, but no change was found in fetal RNA/DNA. Therefore, either no humoral stimulus is produced as a consequence of maternal nephrectomy, or the fetal kidney cannot respond to it.

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Sexual Receptivity in Weanling Rats Treated with PMS and Gonadal Hormones* (35601)

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Many attempts have been made to induce artificial precocious sexual maturity in immature female animals by gonadotropins. It has been reported that pregnant mare's serum gonadotropin produces ovulation in the immature rat (1–3). It was observed (1) that immature rats could become pregnant if treated with pregnant mare's serum (PMS). Ovulation was induced in 26–31-day-old rats with a single injection of PMS (4). Also mating was produced in immature animals by treating them with low doses, 3 RU, of PMS at 22 days of age. These observations were extended and it was concluded that the pituitary and the central nervous system were involved in the induction of ovulation in immature female rats (5). It was further observed that a single injection of 20 IU of PMS on day 30 and 1.2 IU of HCG given intravenously on day 32 caused superovulation in rats and if these rats were mated on the evening of day 32, an average of 22.8 implantation sites was counted on days 10 to 13 of pregnancy (6). It has also been shown that ovulation can be induced consistently in 24-day-old rats (7) and that a dose of 3 IU of PMS in 22-day-old rats produces ovulation consistently.

The work reported here is a part of a program, the purpose of which is to investigate the hormonal factors involved in the attainment of sexual maturity in the female rat. The report describes specifically the data concerned with the induction of sexual receptivity, ovulation, embryo implantation and maintenance of pregnancy in the 22-day-old rat treated with PMS.

Materials and Methods. Twenty-one-day-old immature rats, weighing 50–55 g, were received from the Holtzman Company, Madison, Wisconsin. They were kept 8–10 per cage under conditions of 14 hr light and 10 hr dark daily. The lights were turned on at 5:00 a.m. Central Standard Time (CST)

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and off at 7:00 p.m. (CST). The room temperature was 25 ± 1.5°. Water and Rockland rat diet were available *ad libitum*. All rats were given a subcutaneous injection of 3 IU of PMS (Antex Leo) in 0.25 ml of physiological saline between 7:00 and 7:30 a.m. on day 22. One hundred μg of progesterone + 2 μg of estradiol-17β in 0.12 ml of corn oil was injected subcutaneously at 3:00 p.m. on day 23. Controls were not injected. On day 24, groups of 5 females were placed in a cage with 5 experienced, adult males (100–150 days old) weighing 300–400 g. The vaginas were examined and if they were not patent, they were gently opened by means of a blunt forceps. The following morning (day 25) between 7:30 and 9:30 a.m. the females were checked for mating by the presence of a vaginal plug or spermatozoa. Females which were sperm negative were kept with the males for 1 more day and examined again on day 26, after which those which were still negative were autopsied. Females given progesterone and estradiol, if sperm negative on days 25 and 26, were kept with males on day 26 also, then examined and autopsied on day 27. When the females which had not mated were autopsied, the ovaries were weighed, the oviducts were dissected out and gently pressed between two microscope slides and ova were counted under low power magnification while still in the oviduct.

The day spermatozoa were found was designated as Day 1 of pregnancy. On Day 9 a laparotomy was performed under ether anesthesia and the number of implantation sites was recorded. The uteri without implantation sites were flushed with normal saline and the blastocysts were counted. Statistical analysis was made using the normal approximation to the binomial distribution (8).

Results and Discussion. The results show that 56% of the rats did not mate, but 76.3% of those animals ovulated (Table I). This is in close agreement with the 81.1% reported by McCormack and Meyer (9) for the incidence of ovulation in 22-day-old rats. It also shows that female rats mate at the age of 24 days. The percentage of mating was low (38 of 84 rats) or 44%, but it was

TABLE I. Induction of Ovulation and Pregnancy by 3 IU of PMS in 22-Day-Old Immature Female Rats.

Expt. no.	No. of animals	Rats mated[a]	Rats ovulated[a]	Av no. of ova (± SE)	Av ovarian wt. (mg)	No. of rats checked for implantation sites	Rats with implantation sites (%)	Rats with blastocysts (%)	Av blastocyst	Rats not pregnant (%)
1	27	59.2 ± 17	75.0 ± 23	4.2 ± 0.5	23.8	15[c]	13.3	53.3	3.25	33.3
2	19	21.0 ± 12	100.0	3.7 ± 1.7	27.3	4	50.0	—[b]		
3	5	0	80.0 ± 33	3.5 ± 0.8	23.7	0	—	—	—	—
4	10	30.0 ± 15	71.4 ± 27	2.8 ± 0.9	23.5	3	0	100.0	4.0	0
5	2	50.0 ± 98	0	0 ± 0	34.0	1	0	100.0	2.0	0
6	10	70.0 ± 27	100.0	6.0 ± 0.7	25.8	7	14.3	—[b]		
7	11	54.5 ± 29	60.0 ± 39	2.6 ± 0.7	21.6	6	33.3	0	0	66.6
Total	84	44.0 ± 9	76.3 ± 13		25.0	36	19.4 ± 11	48.0 ± 19		36.0 ± 18

[a] (%) Rats ± 95% confidence interval, according to Steel and Torrie (8), p. 353.
[b] Estrogen was given on day 9, therefore blastocysts could not be counted.
[c] One animal was found with spermatozoa when autopsied for counting ova.

similar to the results of Cole (4) who found 33% mating in 22-day-old females which were treated with 3 RU of PMS.

It is postulated that the low degree of receptivity of 22-day-old immature rats treated with 3 IU of PMS is due to a low level of circulating progesterone and estrogen. Boling and Blandau (10) reported that both estrogen and progesterone are required for sexual receptivity in the female rat. On the basis of their data we postulated that the administration of these steroids on day 23, in addition to 3 IU of PMS on day 22, would improve the receptivity of the female. It is also probable that progesterone would increase the incidence of ovulation, since McCormack and Meyer (11) have shown that progesterone facilitates ovulation in 22-day-old rats injected with PMS.

The results show that 100 μg of progesterone + 2 μg of estradiol-17β given on day 23 increased the percentage of mating from 44 to 80%, and the percentage of ovulation from 76.3 to 96.6% in those rats which did not mate (Table II). The results are statistically significant at $p<.05$ for both kinds of data.

The percentage of animals that ovulated was based on those which did not mate (Tables I and II). It was postulated that the incidence of ovulation in the mated rats was at least as great as that found for the animals that did not mate. The assumption was tested in a group of nine animals in which sperms were found in the vaginas. All of them had ovulated and fertilized ova were observed in the oviducts. Therefore, the number of animals that mated was added to the number that ovulated but did not mate. This method of calculation increases the percentage of ovulation to 86% in control and 98% in treated rats. A similar method of calculation was used to determine the incidence of ovulation in pregnant rats after PMS or PMS and steroid treatment. In both groups the number of nonmating animals which ovulated was added to the sum of the animals that had implantation sites and those in which implantation was delayed. The data calcu-

TABLE II. Effect of 100 μg of Progesterone and 2 μg of Estradiol-17β on Sexual Receptivity in 22-Day-Old Female Rats Treated with 3 IU of PMS.

Expt. no.	No. of animals	Rats mated (%)	Rats ovulated (%[a])	Av no. of ova (± SE)	Av ovarian wt. (mg)	No. of rats checked for implantation sites[b]	Rats with implantation sites (%)	Rats with blastocysts (%)	Av blastocyst	Rats not pregnant (%)
1	10	70.0	100.0	4.0 ± 0.7	31.5	7	42.8	42.8	3.0	14.3
2	10	60.0	100.0	4.2 ± 0.8	32.0	6	16.6	83.2	3.6	0
3	32	81.2	100.0	4.5 ± 0.4	35.5	6	100.0	0	—	0
4	15	73.3	100.0	5.0 ± 0.1	29.6	5	40.0	60.0	3.3	0
5	30	83.3	100.0	5.4 ± 1.4	20.0	5	40.0	0	0	60.0
6	22	86.3	100.0	5.7 ± 1.2	23.6	19	21.0	57.8	4.6	21.0
7	32	87.5	75.0	5.0 ± 3.0	24.2	8	0.0	87.5	3.5	12.5
Total or av		80.8	96.5		29.8	56	32.1 ± 11	51.8 ± 12		16.1 ± 9

[a] Percentage is of those animals which did not mate and were checked for ovulation.
[b] Random sample from mated rats.

lated in this way show that 66% of the rats given PMS ovulated while 87% of those given both PMS and steroids ovulated. The difference is statistically significant at $p<.05$. It is concluded that there is an increase in the percentage of ovulation in the progesterone–estradiol treated animals as compared to the controls. This is probably due to the secretion of more ovulating hormone (OH) from the anterior pituitary due to the facilitative action of progesterone.

The incidence of mating (80.8%) agrees with that reported by Strauss (12) who showed that 86% of a group of 30-day-old rats treated with PMS mated. Moreover, spontaneous implantation occurred in 18 of 56 PMS-steroid treated rats compared to 7 of 36 in the PMS controls (Table I). Delayed blastocysts were found in 51.8% of the animals. Barnes and Meyer (13) also obtained a high incidence of delayed implantation in adult rats given Provera at the time of mating and throughout pregnancy. It is noteworthy, however, that the delayed implantation observed in our experiments was produced without the administration of steroids after mating. It is postulated that the amount of progesterone is sufficient to maintain the embryo in the viable but undeveloped condition, but there is not enough progesterone and/or estrogen to promote implantation.

Our data show that 38.0% of the control (PMS-treated) animals mated on the night of day 24 and 9.6% mated on the night of day 25 (Table III). PMS-steroid treatment shifted the time of estrus 1 day later. Only 1.3% of the experimental animals mated on the night of day 24 whereas 79.0% mated the next night. If ovulation occurs during estrus, it can be postulated that ovulation had also been delayed 1 day in the experimental animals. This hypothesis was supported by the data which showed that not one animal of 24 examined had ovulated by the morning of day 25.

Summary. Precocious sexual receptivity and ovulation were induced in 22-day-old rats by injecting 3 IU of PMS, but the level of receptivity was low. Improvement in mating was obtained with 100 μg of progesterone and 2 μg of estradiol-17β, administered on day 23 after a priming dose of 3 IU of PMS on day 22. This treatment postponed mating 1 day and induced ovulation in 98% of the animals. Delayed implantation was observed without administering steroids after mating in 29 of 56 rats examined for implantation sites.

TABLE III. Effect of Progesterone and Estradiol-17β on the Time of Sexual Receptivity in Female Rats Treated with 3 IU of PMS.

	Experimental				Control		
	Mated on day				Mated on day		
Expt. no.	24	25	26	Total	24	25	Total
1	0	7	—	7/12	15	1	16/27
2	1	5	—	6/10	4	0	4/19
3	0	24	2	26/32	0	0	0/5
4	0	11	0	11/15	3	0	3/10
5	1	24	0	25/30	0	1	1/2
6	0	19	0	19/22	4	3	7/10
7	0	28	0	28/32	6	—	6/11
Total	2	118	2	122/151	32	5	37/84
(%)[a]	1.3	79.0	6.0		38.0	9.6	
(%)[b]	1.6	96.7 ± 9	1.6		86.0 ± 9	14.0	

[a] Out of total animals put for mating.
[b] Out of total animals mated.

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Isolation of Nalorphine Ethereal Sulfate and Nalorphine Glucuronide from Urine of Cat and Rabbit* (35602)

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In a recent paper (1), we reported the isolation of morphine-3-ethereal sulfate, a major metabolite, from the urine of chicken and cats given morphine. The general value of the isolation procedure was demonstrated by crystallization of morphine-3-glucuronide from the urine of the rabbit.

In the present study, the above method of isolation is used to crystallize two metabolites of nalorphine, one from the urine of the cat and another from the urine of the rabbit. The material isolated from the cat is shown to be nalorphine-3-ethereal sulfate and that from the rabbit nalorphine-3-glucuronide. This latter compound also appears to be present in the urine of the cat. This study forms an integral part of our investigation of the renal tubular transport and metabolism of narcotic analgesics (2).

Methods. Two male cats (2.9 and 2.1 kg), fasted overnight, were each given 30 mg(0.9 ml) nalorphine hydrochloride, s.c. At 4.5 hr, doses of 200 and 150 mg, i.p., were given to the larger and smaller cat, respectively. On the second and third days, doses of 200 mg, i.p., were repeated. The urine was collected (1) for several days after the last dose and the samples were pooled.

The primary method used for the purification process was described for the isolation of the metabolites of morphine (1) and only details essential to the immediate procedure are given below. After filtration through diatomaceous earth, the volume of the urine was 590 ml. Portions of 100, 100, 140, and 150 ml were run through the Amberlite XAD-2 resin columns. The main fractions from the 4 runs were pooled (230 ml) and extracted with 100 ml of petroleum ether. The ether phase was discarded. The aqueous phase was adjusted to pH 8.5 by dropwise addition of concentrated ammonium hydroxide and the free nalorphine was removed by extracting twice with a mixture of 100 ml of chloroform and 5 ml of isoamyl alcohol. The aqueous phase was evaporated under vacuum to a tar-like residue. The residue was taken up in 5 ml of water and 50 ml of methanol; 0.5 ml of glacial acetic acid was added. Addition of acetone and methanol yielded a brown grey precipitate (I) that weighed 35 mg after filtration and drying. The filtrate was evaporated to dryness and the resulting tar-like

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