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Maintenance of Pregnancy with Subcutaneous Pellets of Progesterone in Ovariectomized Mice.* (28567)

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Bilateral ovariectomy of the mouse at any stage of pregnancy is promptly followed by death or abortion of the fetuses(1,2). Functional corpora lutea are essential for maintenance of pregnancy in the mouse(3). However, if bilateral ovariectomy of this animal is preceded and followed by adequate administration of progesterone, pregnancy can continue(4,5,6,7,8,9). Some question remains as to the minimal amount of exogenous progesterone necessary at various stages of pregnancy to forestall loss of some or all of the fetuses. The experiments to be described were addressed primarily to this problem. To simulate the uninterrupted release of progestin supposedly occurring in the intact pregnant animal, pellets of progesterone were implanted subcutaneously for continuous absorption.

Materials and methods. Adult virgin female mice of the CHI and Brown Belt (BB) stocks were mated with males of corresponding stocks. The females were inspected each morning for vaginal plugs; the day of finding the plug was counted as day 1 of pregnancy. Mice later found not to be pregnant were excluded from the experiment. All animals received Purina Lab Chow and water *ad libitum.*

Sixty pregnant CHI and 89 pregnant BB mice served as controls. Of these, 33 CHI and 52 BB mice, intact and untreated, were allowed to go to term and deliver their young. Eight CHI and 9 BB mice, also intact and untreated, were killed on day 19 and were inspected for live and dead fetuses. Sham ovariectomies (exposure and manipulation, but not removal, of ovaries) were performed *via* a dorsal skin incision on days 10, 14, or 16 of pregnancy in 9 CHI and 13 BB mice; no pellets were given. Body weights were recorded daily, and these animals were killed on day 19. Finally, 10 CHI and 15 BB mice were ovariectomized on days 10, 14, or 16 and received no pellets. Body weights were also determined daily for these mice, which were killed on day 19.

Cylindrical pellets were made by the compression of crystalline progesterone or of 17*a*-hydroxyprogesterone in a device which ensures that each pellet is subjected to the same amount of compression(10). One or more pellets, usually of 9-11 mg each, were weighed and then were discharged from a large (#13) hypodermic needle under the dorsal thoracic or cervical skin of each experimental mouse during light ether anesthesia. Two days later the recipient was bilaterally ovariectomized, care being taken not to disturb the pellet(s). Implantation of hormone and removal of ovaries were carried out on days 8-10, 9-11, 10-12, 11-13, 12-14, and 14-16 of pregnancy. Seventy-five CHI mice and 26 BB mice received ½, 1, 2, 4, or 6 progesterone pellets. Two or 4 pellets of 17*a*-hydroxyprogesterone were given to 22 BB mice.

At autopsy of control and experimental mice the live and dead fetuses were removed, counted, freed from membranes and placentae, lightly blotted, and weighed individually. The uteri were inspected for implantation sites. The percent of living fetuses in a total representing live fetuses plus dead fetuses plus

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TABLE I. Effect of Subcutaneous Pellets on Fetal Survival in Ovariectomized Mice.

CHI mice: Progesterone					Brown Belt mice: Progesterone				
No. of adult mice	Treatment (days)	No. of pellets	mg/day absorbed*	% survival of fetuses*	No. of adult mice	Treatment (days)	No. of pellets	mg/day absorbed*	% survival of fetuses*
					10	8–10	2	.40 ±.03	59 ± 14
					10	"	1	.24 ±.01	31 ± 13
					5	"	½	.13 ±.02	0
					6	9–11	6	1.39 ±.10	100
					8	"	4	.91 ±.06	90 ± 6
					6	"	2	.37 ±.03	24 ± 14
					6	"	1	.26 ±.02	3 ± 3
5	10–12	4	.82 ±.03	30 ± 20	11	10–12	2	.44 ±.02	76 ± 11
5	"	2	.40 ±.02	0	11	"	1	.28 ±.02	35 ± 13
9	11–13	2	.40 ±.03	67 ± 17	15	11–13	2	.45 ±.05	64 ± 12
8	"	1	.18 ±.01	0	9	"	1	.29 ±.03	81 ± 12
					9	"	½	.13 ±.02	22 ± 15
5	12–14	4	.77 ±.07	96 ± 5	6	12–14	2	.27 ±.03	91 ± 7
6	"	2	.48 ±.04	95 ± 3	7	"	1	.32 ±.02	74 ± 15
9	"	1	.21 ±.03	27 ± 12	7	"	½	.12 ±.01	14 ± 12
5	14–16	4	—	98 ± 2	8	14–16	2	.41 ±.04	95 ± 3
6	"	2	.47 ±.07	78 ± 16	8	"	1	.40 ±.01	96 ± 3
12	"	1	.24 ±.03	72 ± 13	6	"	½	.14 ±.01	94 ± 4
			Brown Belt mice: 17α-OH-Progesterone						
5	12–14	4	.69 ±.10	0	6	14–16	4	.64 ±.05	0
6	"	2	.39 ±.10	0	5	"	2	.46 ±.06	0

* Mean ± standard error.

aborted or resorbed fetuses (as indicated by sites) was calculated for each pregnant animal. The pellets were recovered, cleaned, blotted, dried to constant weight in a desiccator, and weighed. The net weight loss of pellets for each animal was determined, and average daily absorption of hormone was estimated.

Results. Of the intact CHI mice allowed to go to term, 12 delivered on day 19, 17 on day 20, and 4 on day 21 of pregnancy. Of the BB mice in this group, 4 delivered on day 19, 28 on day 20, and 20 on day 21 of pregnancy.

In both the 8 intact CHI and the 9 intact BB mice killed on day 19, 98% of the fetuses were alive.

No dead fetuses were found in the 9 CHI and 13 BB mice which were killed on day 19 after undergoing sham ovariectomy on days 10, 14, or 16. There were 3 to 9 live fetuses in each animal.

No live fetuses were found on day 19 in the 10 CHI and 15 BB mice which were ovariectomized on day 10, 14, or 16. Judging from significant reductions in the body weights, recorded daily, abortion or resorption occurred (but was not necessarily complete) by day 11 after ovariectomy on day 10, by day 15 or 16 after ovariectomy on day 14, and by day 17 after ovariectomy on day 16.

17α-Hydroxyprogesterone failed completely to maintain pregnancy (Table I).

Under progesterone treatment, the dose-response relationship was fairly regular within each group. The amount of exogenous progesterone needed to maintain a given percent of fetal survival clearly diminished as pregnancy progressed. Variation in litter size from 2 to 7 fetuses contributed to the relatively large standard errors in mean percent survival of fetuses. A fetal survival rate of 90% or more was associated with an average estimated daily absorption of at least 0.91 mg of progesterone (BB) when ovariectomy had been performed on day 11, of 0.77 (CHI) or 0.27 (BB) mg when on day 14, and of 0.14 mg (BB) when on day 16. A fetal survival rate of 59% or more was associated with absorption of exogenous hormone at a mean rate of 0.40 mg (BB) when the ovaries were removed on day 10, 0.91 mg

(BB) on day 11, 0.44 mg (BB) on day 12, 0.40 (CHI) or 0.29 (BB) mg on day 13, 0.48 (CHI) or 0.32 (BB) mg on day 14, and 0.24 (CHI) or 0.14 (BB) mg on day 16. The requirements for a given survival rate after ovariectomy on a given day appeared to be less in BB than in CHI mice.

Statistical analysis of fetal weights revealed no trend which could be related consistently to experimental treatment.

Discussion. The results of ovariectomies and sham ovariectomies in CHI and BB mice not given progestin confirmed earlier observations that this surgical trauma alone does not result in abortion or resorption and that removal of the ovaries on day 10 of pregnancy or later is followed promptly by loss of the fetuses.

Courrier and Kehl(11) pointed out that if progesterone therapy is to be given to the ovariectomized pregnant rabbit, the injections should be begun on the day before operation so as to obviate even a brief interruption in availability of the hormone. In the present experiment, progesterone pellets were implanted 2 days before ovariectomy, to allow time for effective absorption of the hormone to begin.

In the Hooker-Forbes bio-assay, unesterified 17α-hydroxyprogesterone was highly active in the Rockland-Swiss mouse(12,13) but inactive in the CHI mouse(14). It is of interest that the absorption of relatively large amounts of the hormone failed completely to maintain pregnancy in the BB mouse.

Progestin deficiency does not seem to have influenced significantly the fetal body weight at term.

Injection of 1 mg daily of progesterone has maintained pregnancy in mice ovariectomized in the later stages of gestation(4). Injection of 0.4 mg of the hormone daily did not achieve this result. Pregnancy was not interrupted if daily administration of 1 mg progesterone began on day 14 in pregnant mice ovariectomized on day 15(5). In a similar experiment, injection of 1 mg a day resulted in viable fetuses at term in 16 of 30 castrated mothers. Daily injection of 0.5 mg of the hormone was followed by survival of living young at term in only 4 of 20 mothers (9). Castrated pregnant mice receiving 1 and 2 mg daily went to term(7). Crosett (personal communication) found that daily injection of 4 mg of progesterone into mice of the A strain would maintain pregnancy after ovariectomy on day 6 of pregnancy, 2 mg after ovariectomy on day 8, and 1 mg after ovariectomy on day 12 or 14. The need for exogenous progesterone thus diminishes as pregnancy advances in ovariectomized mice.

Our results support and extend the latter observation. Thus far, the reason for the general decline in the requirement for administered progesterone is not entirely clear, but two possible factors suggest themselves. An earlier study(15) of activity levels of circulating progestin in plasma of mice of the A strain suggested that luteal production of progestin declines sharply after day 10 and that placental production of the hormone is high on days 13-15. If placental secretion of progestin is also high on days 13-15 in CHI and BB mice, this may help to explain why less exogenous progesterone is required after ovariectomy during this period. A second factor is suggested by the low level of progestin in plasma of the intact A mouse on days 16-19. If a similar low level exists in the CHI and BB mouse, relatively little progestin would seem to be required on the last 4 days of pregnancy. Actually, it has been shown that exogenous progesterone may be toxic to the newborn mouse(16).

The results of others indicate that 1 mg or more of progesterone must be injected to prevent abortion in the mouse ovariectomized during the latter half of pregnancy. Replacement therapy with pellets showed that good results were usually obtained if daily absorption were somewhat less than 1 mg (Table I), confirming earlier observations that, weight for weight, implantation of pellets is the more efficient method of administering the hormone.

Summary. Mice of the CHI and Brown Belt stocks received subcutaneous pellets of 17α-hydroxyprogesterone or progesterone and then were ovariectomized at various stages of pregnancy. The average daily absorption of progesterone from the pellets which was required to ensure fetal survival to term di-

minished as pregnancy progressed. 17α-Hydroxyprogesterone pellets were ineffective in maintaining pregnancy in castrate mice. When the day of finding the vaginal plug was counted as day 1, duration of pregnancy in the intact untreated CHI mouse was usually 19 or 20 days. In the Brown Belt mouse, it was usually 20 or 21 days.

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Inhibition of Renal Growth Following Unilateral Nephrectomy in the Rat.* (28568)

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Unilateral nephrectomy in the rat stimulates growth of the remaining kidney. The growth response is manifested within 24 hours by a substantial increase in dry weight (1) and within 48 hours by an appreciable increase in mitotic activity in the remaining kidney(2). Unilateral ureteral transection (ureteroperitoneostomy), a procedure thought to be equivalent to unilateral nephrectomy in accomplishing transitory reduction of renal excretory function, does not elicit the growth response(3,4). This observation has been the basis for rejection of the hypothesis that the growth response of the remaining kidney following unilateral nephrectomy is initiated by reduction of renal excretory function. An alternative hypothesis, namely, renal growth response is dependent on reduction of non-excretory (endocrine) renal function has supplanted it. Repetition of the unilateral ureteroperitoneostomy studies in this laboratory revealed that the procedure was uniformly accompanied by a striking inflammatory reaction within the peritoneal cavity. This was not observed following unilateral neprectomy or sham operation. Furthermore, animals with unilateral ureteroperitoneostomy often lost more weight during the experimental period than did control animals.

These observations suggested that failure of growth stimulation following unilateral ureteroperitoneostomy may be related to peritoneal inflammation and/or decreased post-operative food and water intake. The following studies were designed to evaluate this this possibility.

Methods. Non-fasted Holtzman male rats were laparotomized under ether anesthesia between 9:00 A.M. and 11:00 A.M. Either left nephrectomy, sham nephrectomy (manipulation of viscera to expose left kidney)

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