among mouse large lymphoid cells, possibly reflecting a species difference in their potential. Large lymphoid cells account for approximately 20% of all lymphoid cells in mouse marrow[6], but 45% of all lymphoid cells in guinea-pig marrow[7]. Some cells in guinea-pig marrow, classified morphologically as large lymphoid cells, may be destined to produce cells other than small lymphocytes, accounting for the relatively high proportion of large lymphoid cells. On the other hand, the observation in mice of all ages that some large lymphoid cells remain unlabeled after 4–5 days continuous ^{3}H-thymidine infusion suggests that, even in mouse marrow, not all large lymphoid cells are engaged in the continuous production of small lymphocytes. Some may comprise a noncycling subpopulation of cells, possibly 'resting' progenitor cells, as postulated for the guinea-pig[9].

The attenuation of prostaglandin-induced luteolysis in decidual tissue-bearing pseudopregnant rats[1,2]

V. D. Castracane[3] and I. Rothchild

Department of Reproductive Biology, Case Western Reserve University School of Medicine, Cleveland (Ohio 44106, USA), 22 June 1977

Summary. Decidual tissue (DT)-bearing pseudopregnant (PSP) rats, in contrast to hysterectomized PSP rats, were resistant to a luteolytic regimen of either $PGF_2\alpha$ or PGE_2 when examined for the duration of PSP diestrus. The PG treatments, however, caused a marked fall in the serum progesterone levels on days 10 and 12, although the levels in DT-bearing rats on day 10 were significantly higher than in the hysterectomized rats.

Melampy et al.[4] suggested that the decidualized uterus prolonged pseudopregnancy (PSP) in the rat in the same manner as hysterectomy, that is, by preventing the production of a uterine luteolysin. Recent evidence has suggested that this luteolysin in the rat, as in some other species, may be a prostaglandin (PG)[5-7]. Reports from this laboratory[8-10] and from Melampy's[11] have indicated that decidual tissue (DT) may have a luteotrophic effect which is distinct from whatever ability it may have to prevent luteolysis. Other reports have shown that decidualization does not cause a decrease in the production of PGs by the uterus[12-14]. To see whether DT interferes with PG-induced luteolysis, the effect of PG treatment on progesterone secretion in DT-bearing PSP rats was compared with its effect in hysterectomized PSP rats. The duration of vaginal diestrus was used as a measure of the maintenance of progesterone secretion and the change in peripheral serum progesterone levels as a measure of change in rate of progesterone secretion since, in the rat, a change in the former closely parallels one in the latter[15].

Materials and methods. Regularly cyclic, 250–300 g female Holtzman (Sprague-Dawley) rats were maintained at 24.5 °C under a 14:10 light:dark schedule, with free access to Purina rat chow and water. Pseudopregnancy was induced by mechanical stimulation of the cervix with a glass rod on the days of vaginal proestrus and estrus. Day 1 of PSP was the day of ovulation (estrus).

On day 5 of the PSP, rats were divided into 2 groups, at which time 1 group was hysterectomized, and the other had their uteri scratched with a barbed needle to induce DT formation[13]. On the morning of day 9, each rat was laparotomized; this was done primarily to confirm the formation of DT in the latter rats, and as a control procedure, therefore, in the hysterectomized ones. Each group was then divided into 2 subgroups. In one, the rats were injected with PGE_2 (1 mg) twice daily on days 9 and 10, and in the other, with the tromethamine salt of $PGE_{2\alpha}$ (400 µg) each morning, on days 9 and 10. Each dose was given s.c. in 0.2 ml of 25% ethanol:saline. Vehicle controls were not included, but data from uninjected controls were available from other studies done at the same time[9,17].

Blood was collected by direct jugular venipuncture from each rat just before the PG injection on days 9 and 10, and again on day 12. Light ether anesthesia was used to facilitate the blood sampling. The blood samples were allowed to clot, and were centrifuged at 4 °C; the serum was then stored at − 20 °C until assayed for progesterone. Vaginal smears were recorded daily for each rat for the duration of the experiment to determine the length of PSP; in all the rats, an estrous cycle of normal duration followed the end of PSP.

Progesterone was assayed at first by the protein binding displacement (PBD) method, as used in this laboratory[18], and in later experiments by radioimmunoassay (RIA) with a sheep antiserum to 11β-hydroxyprogesterone complexed to BSA (GDN-337). The details of the assay, evidence for its specificity, sensitivity, and variability, and a comparison with the PBD method, are described

1 Acknowledgment. The authors wish to thank Helen Wilk and Edward Butler for their help in animal care; Ellen O'Laughlin-Phillips for the progesterone assays; Rosa Garnett, Jo Fletcher, and Aileen Svaty for editorial and secretarial help; Dr John E. Pike of the Upjohn Company for the gifts of PGE_2 and $PGF_{2\alpha}$; and Dr G. N. Niswender for the gift of antiserum GDN-337 used in the RIA for progesterone.

2 Aided in part by NIH Training Grant HD-00024, Ford Foundation Grant 670–0135A, and NIH Program Project HD-07640.

3 Postdoctoral trainee in Reproductive Biology. Present address: Southwest Foundation for Research and Education, P. O. Box 28147, 8848 West Commerce Street, San Antonio, Texas 78284.

4 R. M. Melampy, L. L. Anderson and C. L. Kragt, Nature *74*, 501 (1964).

5 J. A. McCracken, J. C. Carlson, M. E. Glew, J. R. Goding, D. T. Baird, K. Green and B. Samuelsson, New Biol. *238*, 129 (1972).

6 B. B. Pharriss, S. A. Tillson and R. R. Erickson, Recent Prog. Horm. Res. *28*, 51 (1972).

7 J. Hilliard, Biol. Reprod. *8*, 203 (1973).

8 G. Gibori, I. Rothchild, G. J. Pepe, W. K. Morishige and P. Lam, Endocrinology *95*, 1113 (1974).

9 I. Rothchild and G. Gibori, Endocrinology *97*, 838 (1975).

10 V. D. Castracane and I. Rothchild, Biol. Reprod. *15*, 497 (1976).

11 I. Hashimoto, D. M. Hendricks, L. L. Anderson and R. M. Melampy, Endocrinology *82*, 333 (1968).

12 V. D. Castracane and A. A. Shaikh, J. Reprod. Fertil. *46*, 101 (1976).

13 C. W. Weems, J. E. Pexton, R. L. Butcher and E. K. Inskeep, Biol. Reprod. *13*, 282 (1975).

14 S. O. Anteby, S. Bauminger, U. Zor and H. R. Lindner, Prostaglandins *10*, 991 (1975).

15 G. J. Pepe and I. Rothchild, Endocrinology *93*, 1200 (1973).

16 J. M. Yochim and V. J. DeFeo, Endocrinology *71*, 134 (1972).

17 J. Akaka, E. O'Laughlin-Phillips, E. Antczak and I. Rothchild, Endocrinology *100*, 1334 (1977).

18 G. Pepe and I. Rothchild, Endocrinology *91*, 1380 (1972).

The effect of prostaglandin (PG) treatment of decidual tissue (DT)-bearing and of hysterectomized pseudopregnant (PSP) rats, on days 9 and 10 of PSP, on the duration of PSP, and on the serum progesterone level on days 10 and 12

Type of PSP rat[a]	PG treatment[b]	Serum progesterone level (ng/ml) on			Duration of PSP diestrus (days)
		day 9	day 10	day 12	
DT-bearing	None[c]	100.1 ± 11.5	90.8 ± 6.3	68.8 ± 8.0 (4)	19.4 ± 1.2 (7)[e]
	$PGF_{2\alpha}$	68.1 ± 3.0[d]	22.3 ± 3.7	19.0 ± 5.0	17.3 ± 1.7
	PGE_2		35.0 ± 3.8	13.2 ± 4.3	16.1 ± 0.6
Hysterectomized	None[c]	65.7 ± 7.9	72.4 ± 8.9	63.2 ± 4.7	20.7 ± 0.5 (39)[f]
	$PGF_{2\alpha}$	42.0 ± 3.5[d]	9.3 ± 1.5	10.1 ± 2.0	11.7 ± 1.3
	PGE_2		15.2 ± 2.5 (7)	8.1 ± 1.7 (7)	11.7 ± 0.4 (7)

The values are shown as mean ± SE; N was 10–13 except as indicated otherwise in parentheses. [a] DT induction, or hysterectomy was done on day 5 of PSP. [b] $PGF_{2\alpha}$ (400 µg) was injected once/day in the morning, and PGE_2 (1.0 mg) twice/day, on days 9 and 10; each dose was given in 0.2 ml of 25% ethanol:saline. [c] Progesterone values from Rothchild and Gibori[9]; although the rats from which these values were derived were untreated, we have found that the alcohol vehicle, used in other experiments, has never had any effect on the serum progesterone level. [d] The day 9 means were determined on samples obtained before the first injection of either PG from approximately equal numbers of rats assigned to each PG treatment. [e] Data from Lam and Rothchild (submitted to Endocrinology). [f] From Akaka et al.[17]. A similar value for the duration of PSP in untreated hysterectomized rats [19.1 ± 0.7 (N = 13)] was found by Lam and Rothchild (submitted to Endocrinology). Statistical comparisons: Day 10 serum progesterone, $PGF_{2\alpha}$, DT vs. hysterectomized < 0.005. Day 10 serum progesterone, PGE_2, DT vs. hysterectomized < 0.001. Diestrus, DT, no treatment vs. $PGF_{2\alpha}$ NS. Diestrus, DT. no treatment vs. $PGE_2 < 0.02$. Diestrus, hysterectomized, no treatment vs. $PGF_{2\alpha}$ or $PGE_2 < 0.00001$. Diestrus, $PGF_{2\alpha}$, DT vs. hysterectomized < 0.02. Diestrus, PGE_2, DT vs. hysterectomized < 0.001

elsewhere[19]. The RIA yielded values statistically similar to those obtained by PBD. The specificity of the antiserum obviated the need for more than a hexane extraction of each serum sample. Sensitivity was 0.10 ng/assay tube, or the equivalent of 1.0 ng/ml of serum. Progesterone could not be measured in the serum of ovariectomized-adrenalectomized rats, and progesterone added to such serum could be fully recovered. The coefficients of variation for intra-assay and inter-assay variability were 10.9 ± 1.2% and 13.6 ± 3.1%, respectively.

Student's t-test was used for statistical comparisons between group means; p-values < 0.05 were considered to indicate insignificant differences.

Results. PSP Diestrus. The duration of PSP in the hysterectomized rats was markedly shortened ($p < 0.00001$), from 20.7 ± 0.5 days in controls, to 11.7 ± 1.3 and 11.7 ± 0.4, in $PGF_{2\alpha}$- and PGE_2-treated animals, respectively. Only a slight (PGE_2-treated animals, $p < 0.02$) or no effect ($PGF_{2\alpha}$-treated animals) on the duration of PSP was noted in the DT-bearing rats (table).

Serum Progesterone Level. The PG's induced a marked fall in the serum progesterone level in both types of PSP rats on the day after the 1 injection; the levels in DT-bearing rats (22.3 ± 3.7 and 35.0 ± 3.8 ng/ml in $PGF_{2\alpha}$- and PGE_2-treated rats, respectively), however, were significantly greater than those in hysterectomized rats (9.3 ± 1.5 and 15.2 ± 2.5 ng/ml, respectively). By day 12, the levels in the PG-treated groups were significantly lower than those of untreated PSP rats and were not significantly different from one another, although the DT-bearing rats tended to have values slightly higher than those of the hysterectomized ones (table).

Discussion. The almost negligible effect of PG treatment on the duration of PSP in the DT-bearing rats seems to be inconsistent with the depressant effect of the treatment on progesterone secretion. $PGF_{2\alpha}$ treatment has also been shown, however, to markedly decrease progesterone secretion without interfering with the maintenance of pregnancy[20]. The significant shortening of PSP in the hysterectomized rats probably means, therefore, that progesterone secretion was reduced by the PGs below a level which could prevent ovulation; in the DT-bearing rats, on the other hand, the PG treatment apparently did not reduce progesterone secretion enough to permit ovulation to occur much before its expected time.

These findings help to emphasize the difference between the way hysterectomy and decidualization prolong PSP. Removal of the uterus reduced luteolysis by removing the source of a luteolytic agent, presumably a PG; and its effect, therefore, can be reversed by administration of PGs in rats (table), and in other species[21]. DT, in contrast, seems to prevent the luteolytic effect of PGs, since PSP is prolonged as much by decidualization as by hysterectomy[21] without any reduction in the production or release of uterine PGs[12–14]. Our findings support this interpretation by showing that the presence of DT reduced the luteolytic effect of exogenous PGs.

DT also has a luteotrophic effect distinct from merely preventing luteolysis, since rats bearing DT secrete progesterone at a higher rate than do hysterectomized ones[9,11] and continue to secrete progesterone for a much longer time than do hysterectomized rats, after prolactin secretion has been reduced[8,10]. It is unlikely that this action by itself explains the ability of DT to prolong PSP. For example, the importance of prolactin as a luteotrophin in the rat is unquestioned, yet the continuous secretion of prolactin by a pituitary homotransplanted to an intact PSP rat has only a very slight effect on the duration of PSP[22].

The luteotrophic effect of DT does not depend on the maintenance of the normal connections between the uterine and ovarian circulations[10], while the ability of DT to prevent the uterine luteolytic effect does depend on these connections[23]. This difference suggests that DT may produce 2 substances, one of which reaches the ovary through the general circulation to exert its luteotrophic action, while the other reaches the ovary through the 'local pathway' between the uterus and ovary to prevent luteolysis. Aside from this, we know nothing about any possible differences between the luteotrophic and the 'anti-luteolytic' effects of DT.

19 G. Gibori, E. Antczak and I. Rothchild, Endocrinology, in press (1977).
20 A. R. Fuchs, E. Mok and K. Sundaram, Acta endocr. *76*, 583 (1974).
21 L. L. Anderson, in: Handbook of Physiology, section 7, vol. II, part 2, p. 69, (1973).
22 I. Rothchild, Vitam. Horm. *23*, 209 (1965).
23 L. Wilson, Jr, R. L. Butcher and E. K. Inskeep, Biol. Reprod. *3*, 342 (1970).