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Regulation of a Specific Uterine Protein by Estrogen and Progesterone in Ovariectomized Rabbits[1] (38091)

D. W. BULLOCK AND GARY F. WILLEN
(Introduced by G. S. Kanter)
Departments of Obstetrics and Gynecology and of Physiology, Albany Medical College, Albany, New York 12208

Several authors have reported the ability of estradiol and progesterone to influence the appearance of the specific uterine protein of early pregnancy in the rabbit which has been called "blastokinin" (1) or "uteroglobin" (2, 3). By increasing the ratio of estradiol to progesterone, Beier (4, 5) was able to reduce the level of "uteroglobin" when these steroids were administered in combination to pregnant rabbits. Sequential administration of estradiol followed by progesterone did not significantly reduce "uteroglobin" levels in early pregnancy but delayed the time of appearance of this protein (6).

Progesterone has been shown to induce "blastokinin" (7) or "uteroglobin" (8) in ovariectomized rabbits, an effect which could not be obtained with estradiol. These results have been confirmed by Arthur and Daniel (9) who showed that "blastokinin" could be stimulated by administration of progesterone to intact (nonpregnant) or ovariectomized rabbits. Although the time of appearance of "blastokinin," after 3 days of treatment with progesterone, coincided with its time of appearance in pregnancy, after 10 days of treatment "blastokinin" was still present in substantial amounts. In contrast, by day 10 of pregnancy "blastokinin" is no longer present in uterine fluid (1).

Regulation by progesterone alone cannot therefore account for the normal course of "blastokinin" secretion between days 3 and 9 of pregnancy, particularly since plasma progesterone is still rising (10) at the time of disappearance of "blastokinin." We postulated that the persistence of "blastokinin" in progesterone-treated rabbits could be explained on the basis of a high dose of progesterone or that a second hormone may be involved in the termination of "blastokinin" synthesis. That this latter hormone may be estradiol was supported by the claim that the level of "uteroglobin" in pregnancy was reduced by treatment with this steroid (6).

To test these explanations we have examined "blastokinin" in uterine flushings of ovariectomized rabbits treated with different doses of progesterone or with progesterone followed by estradiol plus progesterone. Previous investigators have not employed a regimen of progesterone followed by estradiol and this report represents an attempt to imitate the normal sequence of secretion of these steroids in early pregnancy.

Materials and Methods. Adult, female Dutch-belted rabbits were bilaterally ovariectomized and kept for at least 1 mo before use. Twenty animals were randomly allocated in groups of five to one of four treatments: A. Progesterone, 3 mg/kg/day, days 1–10; B. Progesterone, 1 mg/kg/day, days 1–10; C. Progesterone, 3 mg/kg/day, days 1–10 plus estradiol benzoate, 5 μg/kg/day, days 6–10; D. Progesterone, 1 mg/kg/day, days 1–10 plus estradiol benzoate, 5 μg/kg/day, days 6–10. Both steroids were administered subcutaneously in sesame oil.

Before treatment on day 6, fluid was col-

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lected from one uterine horn by injecting 1 ml of isotonic saline into the lumen and withdrawing the fluid into the same syringe. On day 11, fluid was collected from the opposite uterine horn of each animal in a similar manner. After centrifugation at 1000*g* for 10 min, the protein content of each uterine fluid sample was determined by the method of Lowry *et al.* (11) using rabbit serum albumin as a standard.

Each sample (approx 100 μg protein) was subjected to disc electrophoresis on 7% polyacrylamide gel in 0.02 *M* Tris–0.19 *M* glycine buffer, pH 8.4. The protein bands were fixed and stained in 1% amido black in 7% acetic acid and quantitated by scanning densitometry. The percentage of total protein represented by "blastokinin" was calculated and differences between days within treatments were examined by Student's *t* test.

Protein synthesis was investigated by means of pulse-labeling with ^{14}C-leucine. In a similar experimental design, ovariectomized rabbits were treated for 10 days with 1 mg/kg of progesterone daily or with progesterone plus estradiol superimposed for the second half of the treatment period. At the end of the first 5 days of injection, one uterine horn was flushed as before, while at the time of the last injection (i.e., 24 hr before flushing) the second horn was infused with 5 μCi 1-leucine-1-^{14}C (sp act 53.5 mCi/mmole) in 0.1 ml saline.

After centrifugation and protein determination as described above, the uterine flushings were subjected to gel filtration over a 45 × 1.5 cm column of Sephadex G-200 eluted with 0.02 *M* phosphate–citrate buffer, pH 7.4, at a flow rate of 6 ml/hr. The protein content and radioactivity of each fraction were measured and protein peaks examined by polyacrylamide gel electrophoresis. The gel from the fraction containing the peak of "blastokinin" was cut into 1.5 mm segments and the radioactivity in each segment was counted after treatment with H_2O_2 and NCS solubilizer (12).

Results. Levels of "blastokinin" as a percentage of total protein are shown in Table I. All day 5 samples contained "blastokinin" in amounts comparable to that found on day 5 of pregnancy (1), with no significant differences between groups. Treatment with 3 mg/kg/day or 1 mg/kg/day of progesterone resulted in persistence of "blastokinin" on day 10, with no significant fall in either group. Superimposition of estradiol benzoate (5 μg/kg/day) on either the 1 mg or 3 mg dose of progesterone for the last 5 days of treatment caused a significant ($p < 0.01$) decline in the percentage of "blastokinin" from day 5 to day 10.

TABLE I. "Blastokinin" as Percent of Total Uterine Fluid Protein.

Treatment	Duration 1–5 (days)	Duration 6–10 (days)	Day 6	Day 11
P^4 3 mg	x	x	27.5 ± 4.1*	18.0 ± 3.7
P^4 1 mg	x	x	31.5 ± 4.7	21.1 ± 4.1
P^4 3 mg	x	x	17.8 ± 3.7	6.2 ± 3.7
E_2 5 μg		x		
P^4 1 mg	x	x	39.3 ± 3.2	4.0 ± 2.1
E_2 5 μg		x		

* Mean ± SE.
P^4 = progesterone.
E_2 = estradiol.

Figure 1 shows representative gels from each treatment. The prominent "blastokinin" band in the postalbumin region is evident on day 5 in all treatments. On day 10 the "blastokinin" band persisted at both levels of progesterone alone whereas, in these gels, "blastokinin" was not detectable when estradiol was superimposed on either level of progesterone.

The incorporation of ^{14}C-leucine into proteins of uterine flushings is shown in Fig. 2. Three peaks of protein were detected, two of which were associated with peaks of radioactivity. Gel electrophoresis revealed that the protein peak eluting at fractions 26–32 contained mainly albumin, in which little radioactivity was present. Figure 3 shows the pattern of radioactivity in a gel from fraction 38, which has a single peak of radioactivity coincident with a single stained protein band corresponding to "blastokinin."

Table II shows data for protein composition and ^{14}C-leucine incorporation in rabbits ($n = 2$) treated with progesterone

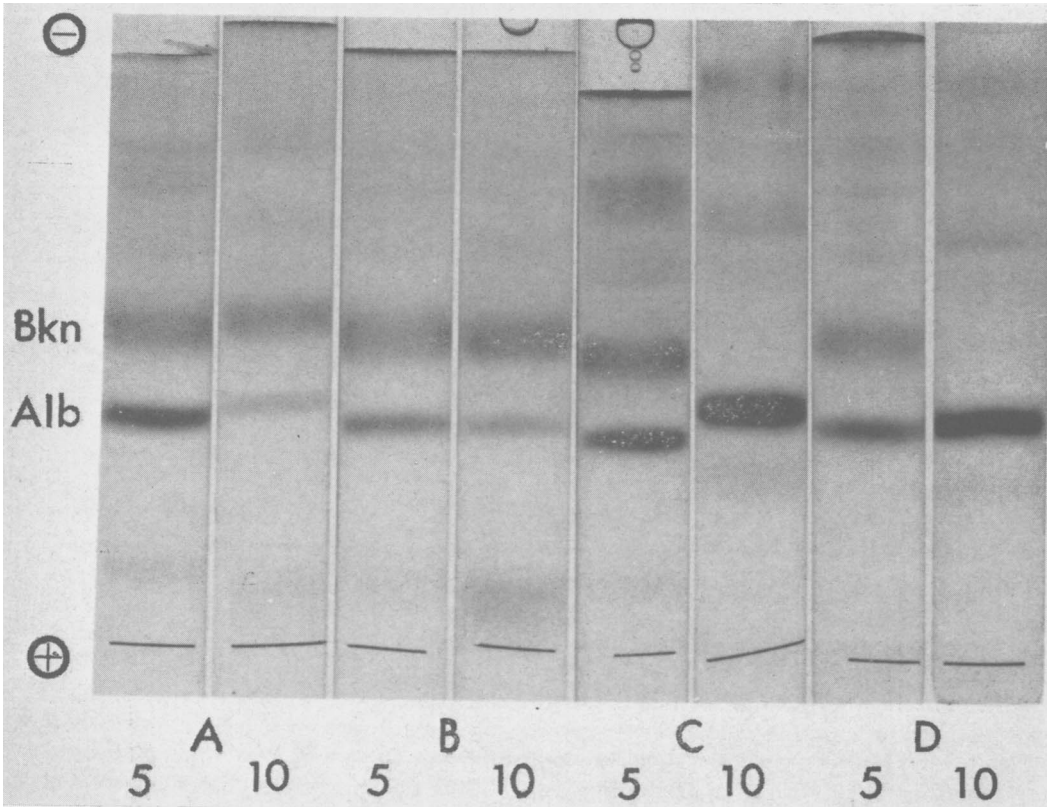

FIG. 1. Gel electrophoresis of uterine flushings. Bkn = "blastokinin"; Alb = albumin; A = progesterone, 3 mg/kg/day, days 1–10; B = progesterone, 1 mg/kg/day, days 1–10; C = progesterone as in A plus estradiol, 5 μg/kg/day, days 6–10; D = progesterone as in B plus estradiol as in C. 5 = collection after 5 days of treatment; 10 = collection after 10 days of treatment.

alone or with progesterone followed by progesterone plus estradiol. With progesterone alone, the proportion of "blastokinin" was increased after 10 days of treatment and the proportion of albumin was unchanged. Treatment with estradiol reduced the proportion of "blastokinin" and increased the proportion of albumin. The total protein specific activity, as well as the specific activity of "blastokinin," was reduced by estrogen treatment.

Discussion. The identity of the protein band isolated by gel electrophoresis with that of the protein called "blastokinin" has previously been established (13). It is recognized that density of staining is an imperfect index of protein concentration, since different proteins may differ in their ability to bind a stain such as amido black. Nevertheless, by keeping the total protein and the staining procedure uniform it is possible to arrive at a valid comparison of changes in protein concentration between treatments.

The results show that estradiol benzoate is able to lower the percentage of total protein represented by "blastokinin" in progesterone-treated, ovariectomized rabbits (Table I). It is possible that this effect could be due to a stimulation by estradiol of the synthesis of other proteins rather than to a reduction in the synthesis of "blastokinin" itself. The total protein in uterine flushings does not give a reliable measure of changes in total uterine fluid protein, since the recovery of fluid by flushing will vary from animal to animal and fluid volume

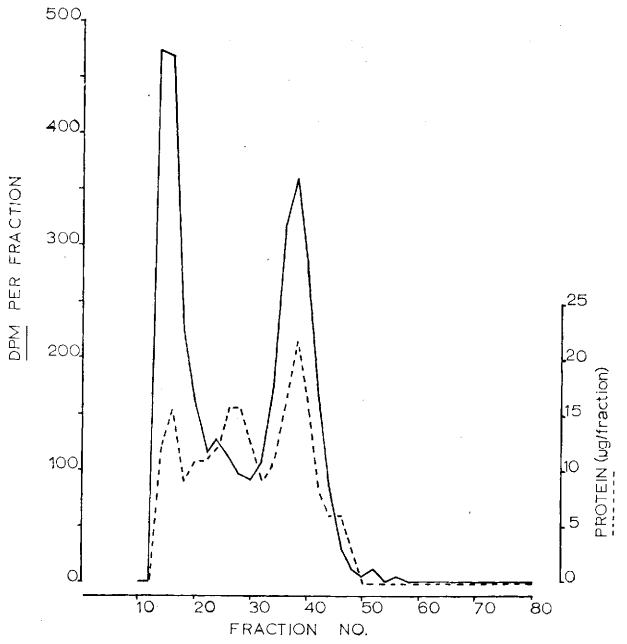

FIG. 2. Incorporation of ^{14}C-leucine into uterine fluid proteins fractionated by Sephadex G-200 gel filtration.

may itself be influenced by the condition of the uterus (14).

Although the recovered protein concentration was higher after estrogen treatment (Table II) the increase was mainly due to elevated albumin, which is an extrauterine protein. We have no indication of increased overall uterine protein synthesis when estradiol was superimposed on progesterone, nor is it likely that the decrease in "blastokinin" is a proportional change due to elevated albumin, since the specific activity of "blastokinin" was reduced by estradiol treatment. It appears possible that estrogen directly or indirectly reduces the synthesis of "blastokinin" in the progesterone-stimulated uterus. The absence of detectable "blastokinin" in some samples (Fig. 1) supports this interpretation. However, the possibility that estrogen treatment increases the degradation of "blastokinin" has not been eliminated.

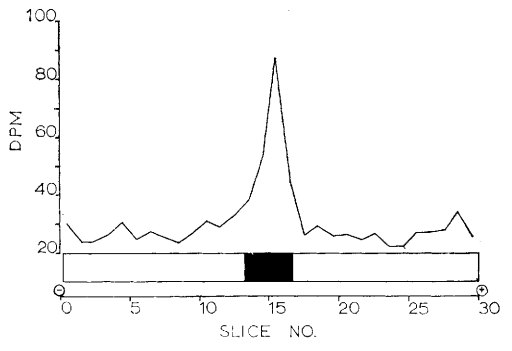

FIG. 3. Incorporation of ^{14}C-leucine into "blastokinin" after separation by gel electrophoresis.

The pulse-labeling data indicate that synthesis of "blastokinin" is still occurring after 10 days of progesterone treatment. By day 10 of pregnancy, "blastokinin" is no longer present (1) despite increasing levels of plasma progesterone (10). Available data (15, 16) suggest that levels of plasma estradiol in the rabbit begin to rise at about the time of disappearance of "blastokinin." The suppression of progesterone-induced "blastokinin" by estradiol is therefore consistent with the pattern of secretion of these steroids and the time-course of "blastokinin" in early pregnancy.

Regulation of the specific uterine protein "blastokinin" appears to be under dual hormonal control, its synthesis being stimulated by progesterone and terminated by a mech-

TABLE II. Incorporation of ^{14}C-Leucine into Uterine Fluid Proteins.

	Progesterone		Progesterone plus estradiol	
	Day 6	Day 11	Day 6	Day 11
Recovered UF protein (mg/ml)	0.90	0.65	1.65	2.14
% Blastokinin	22.1	38.7	41.2	27.1
% Albumin	30.3	30.6	18.2	33.1
Total specific activity (dpm/μg)	—	16.6	—	8.2
Blastokinin specific activity (dpm/μg)	—	19.1	—	14.5

anism directly or indirectly involving estradiol.

Summary. Ovariectomized rabbits were injected with progesterone daily for 10 days with or without estradiol superimposed for the second half of the treatment period. After 5 days of treatment, one uterine horn was flushed with saline and after 10 days the opposite horn was flushed in a similar manner. The proportion of uterine fluid protein represented by "blastokinin" was similar on day 5 and on day 10 after treatment with either 3 mg/kg or 1 mg/kg of progesterone alone. Estradiol (5 μg/kg) at either dose of progesterone significantly reduced the proportion of "blastokinin." Suppression of progesterone-induced synthesis of "blastokinin" by estradiol is consistent with the pattern of secretion of these steroids and the time-course of "blastokinin" during early pregnancy in the rabbit.

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