at birth can either prevent or abolish the state of immunologic unresponsiveness normally induced by the same dose of antigen alone.

The mechanism of this interference may be explained in several ways.

1. The injection of these lymphoid cells may merely serve to increase the number of immunologically competent cells early in the life of the recipient animal, and thereby change the ratio of lymphoid cells to antigen. If unresponsiveness is the result of reaching every immunologically competent cell with a critical concentration of antigen, it can be expected that, as the number of cells is increased, the dose of antigen required to affect every cell increases statistically.

2. The intraperitoneal injection of cells may result in a less effective contact between the reactive tissue and the paralyzing antigen than would normally be the case, *i.e.*, lymphoid cells become present in areas where they are normally lacking in a newborn animal.

3. The maturation of the cells from the lymphoid organs may have been affected by their dislocation from the supporting stroma. This last possibility has to be considered particularly in view of the fact that a difference has been reported(9) in the effectiveness of whole thymus and thymic cell suspensions in restoring the immunologic competence to thymectomized mice.

Further studies are under way to determine the relative effectiveness of thymus and spleen cells from newborn animals, and thymus cells from newborn and adult animals, in interfering with the establishment of immunologic unresponsiveness.

Summary. Injection of 5 to 15 $\times$ 10^6 newborn lymphoid cells into newborn mice, 2 to 6 hours following injection of a tolerance-inducing dose of BSA, resulted in immunologic responsiveness in 13 out of 17 mice upon challenge 30 to 40 days later. It is felt that quantity and distribution of lymphoid tissue are of major importance in determining the ease with which immunologic unresponsiveness can be established.

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Effect of Estrogen Administration *in vivo* on Prolactin Release by Rat Pituitary *in vitro*.*† (27935)

ALBERT RATNER, P. K. TALWALKER, AND JOSEPH MEITES
Department of Physiology and Pharmacology, Michigan State University, East Lansing

In vitro cultures of anterior pituitary tissue for a number of days produce considerable quantities of prolactin(1). Such cultures are capable of synthesizing and releasing many times more prolactin than is found in fresh anterior pituitary tissue. Recently we reported(2) net prolactin synthesis by rat anterior pituitaries incubated in a Dubnoff shaker for only 2 hours. This short term incubation procedure has been found to be particularly appropriate to investigate problems concerned with control of prolactin release.

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In vivo experiments have shown that estrogens can increase pituitary prolactin content and induce mammary growth and lactation in a variety of species(3). By the use of *in vitro* cultures, Nicoll and Meites(4) demonstrated that addition of estradiol to the culture medium can directly stimulate anterior pituitary cells to increase prolactin secretion. This does not exclude the possibility that *in vivo,* estrogens may also stimulate pituitary prolactin secretion indirectly through other mechanisms. The present study was undertaken to determine whether estrogen administered to rats *in vivo* could influence the release of prolactin *in vitro.*

Methods and materials. Mature females of the Sprague-Dawley strain, weighing 200-300 g each, were housed in a constant temperature room (74 ± 1°F) and fed a basal laboratory ration. The experimental animals were injected subcutaneously with 50 μg estradiol benzoate daily for 6 days, while the controls were injected with an equal volume of the solvent. The estradiol benzoate was diluted with sesame oil and daily injection volume was 0.1 ml.

On the 7th day the animals were sacrificed by decapitation and the pituitaries were removed as quickly as possible. The neural lobes were discarded and the anterior pituitaries were cut into halves. Three anterior pituitaries (6 halves) were weighed and placed into a 25 cc Erlenmeyer flask containing 2 cc of synthetic medium "199" at pH 7.4. Nine pairs of flasks were used in all, comprising a total of 27 experimental and 27 control pituitaries. One flask from each pair contained the anterior pituitaries from the estradiol-treated rats and the other, the anterior pituitaries from the control rats. The flasks were incubated in a Dubnoff metabolic shaker at 37°C in an atmosphere of 95% O_2-5% CO_2 for 2 hours.

After incubation the medium from each flask was assayed for prolactin activity in 5-7-week-old White King squabs by the intradermal method of Lyons(5) as modified by Reece and Turner(6). The medium from each flask of a given pair was assayed in the same birds, the estrogen medium being injected over one side of the crop sac and the control medium over the other side. This permitted each bird to serve as its own control. The amount of prolactin (IU) released into the medium from each flask was calculated on a per pituitary and per 100 mg pituitary basis. The percentage difference in prolactin content of the medium between both flasks of a given pair was considered to reflect the effect of estradiol on prolactin release. To determine the *in vivo* effects of estradiol on pituitary weight and prolactin content, 10 fresh anterior pituitaries from an estradiol benzoate-treated group and 10 from a control group were assayed in 10 birds.

TABLE I. Influence of Estradiol Benzoate (50 μg) Injected for 6 Days *in vivo* on Pituitary Prolactin Content of Rats.

		Avg prolactin content		
	Avg AP wt (mg)	IU per AP	IU per 100 mg AP	P
Control	11.1	.4	3.2	
Estradiol	17.1	1.6	9.2	<.01
Avg % diff.	+45	+315	+187	

AP = Anterior pituitary.

Results and discussion. Table I shows that administration of estradiol benzoate increased anterior pituitary weight by 45% over that of controls. Prolactin content was increased 315% on a per pituitary basis and 187% on a 100 mg pituitary weight basis. This is in agreement with other reports on the effects of estrogen on pituitary weight and prolactin content in rats(7).

Table II shows that in 8 out of 9 flask pairs, the amount of prolactin released into the medium from the estradiol benzoate-treated rats was significantly greater than the controls on a per pituitary and per 100 mg pituitary basis ($P<0.01$). The pituitaries from the estrogen treated rats released an average of 96.4% more prolactin per pituitary and an average of 47.0% more per 100 mg of pituitary, than the pituitaries from the control rats.

These results demonstrate that estradiol benzoate administered *in vivo* increases the capacity of the anterior pituitary to release prolactin *in vitro.* This is in agreement with the results of *in vitro* cultures, in which estradiol was added to the medium containing

TABLE II. Prolactin Release *in vitro* by Anterior Pituitaries from Rats Pre-Treated with Estradiol Benzoate (50 μg) for 6 Days.

Flask pair No.	I.U. per AP*			I.U. per 100 mg AP†		
	Control	Estradiol	% diff.	Control	Estradiol	% diff.
1	.048	.090	+ 88	.40	.52	+ 30
2	.013	.041	+215	.12	.25	+108
3	.041	.084	+104	.37	.46	+ 24
4	.041	.048	+ 17	.35	.29	— 20
5	.048	.090	+ 88	.45	.55	+ 22
6	.152	.287	+ 89	1.23	1.72	+ 40
7	.108	.130	+ 20	.92	.98	+ 7
8	.155	.285	+ 84	1.29	2.00	+ 55
9	.130	.342	+163	1.10	2.40	+118
			Avg 96.4 ± 20.7‡			Avg 47.0 ± 13.0‡

AP = anterior pituitary.
* = C vs E: $P < .01$.
† = C vs E: $P < .01$.
‡ = Mean ± standard error of mean.

pituitary tissue(4). It should be emphasized that only one dose level of estradiol was used in this study, and higher doses might have a different effect than reported here. Thus high doses of estrogen administered *in vivo* have been shown to have relatively little ability to increase pituitary prolactin content(8), and did not increase prolactin release when rat anterior pituitary was cultured *in vitro*(9). Estrogens have also been shown to stimulate or inhibit gonadotropin release, depending on the dose administered(10). Van Rees(11) used a short-term incubation system to study the effects of gonadal hormones upon FSH release, and reported that the amount of FSH released into the medium was influenced by the quantity of FSH stored in the pituitary. Whether the increased release of prolactin demonstrated in this study is due merely to the greater initial content remains to be determined. Most *in vivo* studies suggest that an increased pituitary prolactin content is associated with increased prolactin release(7, 8).

Summary. Twenty-seven mature female rats were injected daily with 50 μg of estradiol benzoate in oil for 6 days, while 27 controls received only oil injections. On the seventh day the pituitaries were removed and cut into halves. Three anterior pituitaries from the estrogen-treated rats and 3 from the controls were placed in separate flasks (9 pairs of flasks) containing 2 cc of synthetic medium "199," and incubated for 2 hours in a Dubnoff metabolic shaker at 37°C in an atmosphere of 95% O_2-5% CO_2. After incubation the medium from each flask was assayed for prolactin. The pituitaries from the estradiol benzoate-treated rats released 96% more prolactin into the medium than the pituitaries from control rats on a per pituitary basis, and 47% more on a 100 mg pituitary weight basis. Administration of estradiol benzoate increased the average weight of the anterior pituitaries by 45% and the average pituitary prolactin content by 187%. These results demonstrate that administration of estradiol benzoate *in vivo* increases the capacity of the anterior pituitary to release prolactin *in vitro*.

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Effect of Injecting Heterologous Glomerular Basement Membrane Preparations in Pregnant Sheep.* (27936)

RAYMOND WILLIAM STEBLAY (Introduced by M. Edward Davis)
Department of Obstetrics and Gynecology, University of Chicago, Chicago, Ill.

We have previously shown that injections of heterologous glomerular basement membrane (GBM) and Freund's complete adjuvant into adult female sheep every 2 weeks produces a fatal, fulminating glomerulonephritis within 27 to 90 days after the first injection, presumably by an autoimmune mechanism(1).

We decided to inject pregnant sheep in the last third of their pregnancy (gestation is approximately 150 days) to observe the effect of pregnancy on the course of the induced nephritis and to observe the effect of the injections of heterologous GBM on maternal and fetal kidneys. Toxemias of pregnancy occur only in the last third of human pregnancies.

This paper reports that immunization of sheep with heterologous GBM and Freund's adjuvant in the last third of their pregnancy produces a fatal glomerulonephritis in the mother without any demonstrable effect on the fetal kidneys.

Methods. Human, monkey and rat GBM were prepared by the method previously described(2). The GBM was homogenized in isotonic saline with the sonic oscillator† and made up to a concentration of 50 mg (wet weight) per ml. The homogenized suspension was slowly added to Freund's complete adjuvant‡ to form a 1:1 water-in-oil emulsion. The final concentration of GBM in the emulsion was 25 mg/ml.

Three Hampshire sheep in the last third of pregnancy were obtained from different herds through a local veterinarian. After having been found free of overt disease and with normal urine and blood urea nitrogen (BUN), the sheep were injected every 2 weeks with 7 ml of emulsion. Each sheep was injected with antigen from a single species. The injections were given by a combination of intramuscular, subcutaneous and intradermal routes at each administration. For example, 7 cc of emulsion was divided as follows: 3 ml intramuscularly in one hind leg, 2 ml subcutaneously in the axillary and inguinal regions on the same side, and 1 ml intradermally in the skin of the dorsum of the neck.

Periodic urinalyses and BUN determinations were performed on the sheep during the immunization. Serum cholesterol was determined prior to injection and at death.

At death complete autopsies were performed, bladder urine analyzed and the kidneys carefully examined in the gross and microscopically. Kidney tissue was fixed in ice cold 10% formalin and stained with hematoxylin and eosin, periodic acid-Schiff reagent with alcian blue and Masson's trichrome stain.

Results. The results are summarized in Tables I and II. All 3 pregnant sheep died from a fatal fulminating glomerulonephritis in 45, 46 and 54 days after the first injection of antigen. All the criteria for nephritis

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† Raytheon Sonic Oscillator Model S102A, 50 watt, 9 kc, Raytheon Manufacturing Co., Waltham, Mass.

‡ Freund's complete adjuvant: Arlacel A (Atlas Powder Co., Wilmington) 15.0 ml; bayol F (Penola Division, Detroit) 85.0 ml; *Mycobacteriumbutyricum* (Difco) 100 mg.