cation of 16-ketoestradiol, estriol, epiestriol, estrone, estradiol-17α and estradiol-17β. It was found that as the ovary increases in weight at 1-3 weeks before spawning, there is a gradual increase for all free plasma estrogens. In order of their increasing concentration, in μg/100 ml of plasma, the free plasma estrogens were found to be: estradiol-17α, estrone, estradiol-17β, epiestriol, estriol and 16-ketoestradiol.

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Free Plasma Estrogens in the Deer.*† (30704)

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Recent advances in the separation and quantitative determination of plasma estrogens have resulted in isolation and characterization of several estrogenic steroids in various species. Heretofore, however, concentration of plasma estrogens during menstrual cycle and pregnancy have been measured only in the human(1-5).

The present study was undertaken to identify and measure levels of free plasma estrogens during estrus and pregnancy in the white-tailed Texas deer, *Odocoilus virginianus texanus*.

Materials and methods. Blood was collected from 25 adult female white-tailed Texas deer during estrus and at stages of pregnancy ranging from 6 to 23 weeks. Number of samples collected for estrus were 3, for 6 to 8 weeks of pregnancy 6, for 11 to 14 weeks 5, for 15 to 18 weeks 7 and for 20 to 23 weeks 4. The samples were collected in the field at the wildlife game preserve in Sinton, Texas. Plasma was obtained by centrifugation and frozen for later analysis.

Initial identification of the free plasma estrogens was made on six 300 ml samples of plasma. Extraction of the estrogens was made using a modification of the technique described by Veenhuizen *et al*(6). The plasma was extracted 3 times with an equal volume of ether. The extracts were pooled and dried *in vacuo* under nitrogen at 40°C. The residue was taken up in 10 ml of toluene and washed 3 times with 5 ml aliquots of 5% NaOH. The pooled NaOH fractions then were backwashed with 5 ml of toluene. The pH of the NaOH fraction was lowered to 7-9 with 6 N H_2SO_4. This neutralized fraction

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then was extracted 3 times with an equal volume of benzene. The benzene was removed *in vacuo* under nitrogen at 40°C and the estrogen residue taken up in 0.1 ml of chloroform-methanol (1:1).

The estrogens were separated and identified by using thin-layer chromatography on silica gel G (Merck, Germany). The sample was run 2-dimensionally in a hexane-ethyl acetate (1:1) solvent system along with various known estrogens. The plates were developed by spraying with 10% phosphomolybdic acid in absolute ethanol and heating at 110°C for 20 minutes. Initial identification was made by comparing the Rf values of the sample and known estrogens. Further identification was made by determining Rf values of acetylated products of the estrogen extract and known estrogens on TLC silica gel G plates run in hexane-ethyl acetate (1:1). U.V. absorption(7) of the various isolated estrogens was also used as a final identification procedure.

After the estrogens were identified, 15 ml samples of the plasma from the estrus and pregnant deer were extracted as described previously after addition of known amounts of labeled C^{14}-4-estrone. The extract then was run 2-dimensionally on silica gel G TLC plates in hexane-ethyl acetate (1:1) along with the appropriate standard estrogens. The estrogens then were scraped off the plate individually and extracted 2 times with 3 ml of chloroform-methanol (1:1). After the chloroform-methanol was dried, the estrone sample was dissolved in 1 ml of absolute ethanol. A 0.1 ml aliquot of this was taken and added to 10 ml of scintillation fluid and counted in a Packard Tri-Carb model 3314 Liquid Scintillation Counter to measure the per cent recovery. The remaining 0.9 ml of ethanol was dried and fluorescent measurements were made by a modification of the method described by McAnally *et al*(8). The estrogens were dissolved in 0.1 ml of absolute ethanol to which 0.7 ml of 90% H_2SO_4 was added. The samples were shaken and then incubated for 20 minutes at 80°C. The samples then were cooled and 4.3 ml of 65% H_2SO_4 was added. Fluorescent readings were made in a Turner model 110 Filter Fluorometer with a

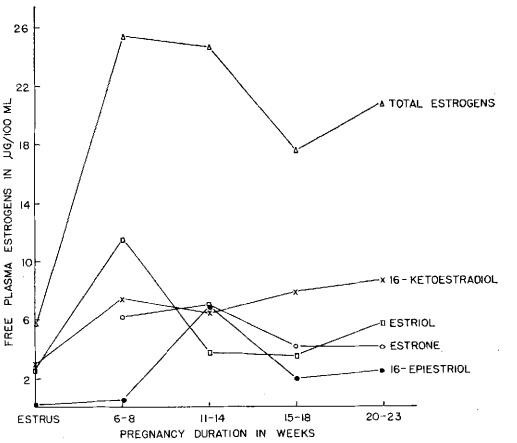

FIG. 1. Mean levels of total and individual free plasma estrogens (µg/100 ml) in female deer during estrus and pregnancy. Each point represents analyses of 3 to 7 female deer.

primary filter system consisting of a Kodak #47B plus a #2A and a Kodak #2A-12 plus a 10% neutral density filter for the secondary filters. The values obtained were extrapolated from a standard curve and corrected to 100 ml of plasma and 100% recovery. Values then were plotted as a function of time of pregnancy for each of the individual estrogens and for total free estrogens (Fig. 1).

Results and discussion. Thin-layer chromatography in hexane-ethyl acetate (1:1) solvent system, U.V. absorption, acetylation and methylation of unknown and standard estrogens established that the free plasma estrogens in the white-tailed Texas deer are estradiol-17α, estradiol-17β, estrone, estriol, 16-epiestriol and 16-ketoestradiol-17β. Because of the small quantity and difficulty in recovery of estradiol-17α and estradiol-17β, these two hormones were not measured quantitatively. Also estrone was not detectable during estrus and, therefore, does not appear in the graph (Fig. 1). In order of decreasing concentration during pregnancy the other estrogens were: 16-ketoestradiol-17β, estriol, estrone and 16-epiestriol.

Results indicate that during pregnancy there is an overall significant rise ($p<.001$) in all estrogens measured when compared to estrus (Fig. 1). This is emphasized more clearly when all the estrogens are combined. Although there is some variation in individ-

ual estrogens during pregnancy, the means of the total estrogens remain without significant change between 6 and 23 weeks of pregnancy. Total estrogens exhibit an overall 5-fold increase during pregnancy when compared to estrus. Of the individual estrogens, 16-epiestriol exhibits the most marked increase from a value of 0.2 μg/100 ml plasma during estrus to a mean of 0.47 μg to 6.93 μg/100 ml plasma during 6 to 23 weeks of pregnancy. The other estrogens exhibit a 2-fold to 3-fold increase during pregnancy.

The waning and waxing of individual estrogens during pregnancy has been noted by previous investigators, especially in the human(5). This variation may possibly reflect differential utilization and removal of these estrogens from circulation. However, a more comprehensive interpretation will be possible when plasma-bound estrogen analyses are completed for this species.

Summary. Isolation of free plasma estrogens in the white-tailed Texas deer, *Odocoilius virginianus texanus* led to identification of estradiol-17α, estradiol-17β, estrone, estriol, epiestriol and 16-ketoestradiol-17β. Because of extremely low quantities and poor recovery no measurements were made for estradiol-17α and estradiol-17β. However, in order of decreasing concentration during pregnancy, from 6 to 23 weeks, the 4 remaining estrogens were: 16-ketoestradiol-17β, estriol, estrone and 16-epiestriol. In general, there was a significant ($p<.001$) rise in total free plasma estrogens during pregnancy when compared to estrus. No significant variation was observed in total free estrogens during pregnancy from 6 to 23 weeks.

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The Association of Murine Lymphoma with Reovirus Type 3 Infection.* (30705)

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We have recently reported on the nature of the acute and chronic disease established in mice following neonatal infection with reoviruses(1,2,3). The available evidence points to a chronic immunological injury which proceeds in the absence of infective virus but in the presence of antibody to the virus. In more detailed communications, we are reporting on the clinical picture and the nature of the lesions of a large number of these mice with the "late" chronic disease and on the effects of passage of their lymphoid cells into newly-born mice of the same strain. We report here the observations with only one mouse (2731/6/272) which is also the subject of the associated communication on electron microscopic findings(4).

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Materials and methods. These are identical with the procedures outlined in detail in previous communications(1,2,3).

Results. The mouse was one of a litter of eight infected by reovirus type 3 (HEV) by contact when 1 day old. The mouse showed