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Received May 10, 1965. P.S.E.B.M., 1965, v120.

Progestin Levels in the Ovaries and Ovarian Effluent Blood in Pregnant Rabbits.* (30447)

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The mechanisms controlling the secretory activity of the corpus luteum have been attracting increasing attention in recent years. Progesterone protects the fetus during pregnancy from excessive myometrial activity and influences the onset of parturition(1). In the rabbit, the corpus luteum is essential for maintenance of the entire gestation period of 30 days. The effect of progesterone on the myometrium declines on the 29th day(2). However, the hormonal requirement of implantation varies from that required for fetal survival and prenatal development(3).

The ovarian vein blood of non-pregnant rabbits contains progesterone (preg-4-ene-3, 20-dione) and Δ^4-3-ketopregnen-20 α-ol (20 α-hydroxy-pregn-4-ene-3-one)(4,5). Progestin levels were determined in the peripheral blood serum of rabbits by the Hooker-Forbes technique(6). A rapid increase was seen during the first 12 days of pregnancy and slower rise from the 12th day until term; maximal concentration of 10 μg/ml of serum was noted on the day before expected parturition. In the ovarian vein blood the concentration of progesterone was found to rise gradually to a peak of 2.36 μg/ml at mid-pregnancy, followed by a gradual decline in the second half of pregnancy, with consistent low values of about 0.41 μg/ml 2 days prior to parturition (7).

In the present study ovarian progestin levels were estimated during pregnancy, pseudopregnancy and superovulation in the rabbit. Data were gathered simultaneously on progestin levels in ovarian effluent blood.

Materials and methods. Fifty-four adult New Zealand White rabbits were randomly assigned to one of 3 groups: pregnant, pseudopregnant, or superovulated. Pregnancy was achieved by breeding twice to fertile bucks followed by an intravenous injection of 20 IU of luteinizing hormone (HCG, Upjohn). Pseudopregnancy was achieved by breeding to a vasectomized buck. Superovulation was induced by 3 successive injections of 50 IU of pregnant mare serum (PMS, Ayerst) and one intravenous injection of 20 IU of HCG. The animals were laparotomized at different stages of pregnancy or pseudopregnancy. The number of corpora lutea and number of implantations were recorded. The animals were injected with nembutal and 10 mg heparin.

* This investigation was supported in part by USPHS research grant HD-00585-03 from Nat. Inst. of Child Health and Human Development. Scientific Paper 2660, Washington Agri. Exp. Stations, Pullman, Project 1698. Drugs were kindly donated: PMS (Equinex) by Ayerst Laboratories, Inc., New York; HCG by Upjohn Co., Kalamazoo, Mich.; Nembutal by Abbott Laboratories, North Chicago, Ill. Thanks are due to Mr. R. E. Mauer for technical assistance.

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The ovarian vein was isolated and the non-ovarian tributaries were ligated. A 1-mm polyethylene catheter was inserted in the ovarian vein and blood allowed to drip into a volumetric cylinder immersed in ice. A total of 24 to 82 ml of blood was collected in a period of 50 to 70 minutes.

Progestins in whole blood. A modification of Short's(9) method as described below was used for extraction of progesterone and 20 α-hydroxy-pregn-4-ene-3-one§ (4-3-keto-pregnen-20 α-ol) from the whole heparinized ovarian venous blood. To the fresh ovarian venous blood, 2 volumes of normal saline were added and pH adjusted to 10 by addition of 5% sodium hydroxide. A tracer amount of progesterone-4-^{14}C (about 10,000-12,000 cpm) was added and the sample equilibrated for about 15 minutes. It was extracted 4 times with equal volume of washed, redistilled ether in 250-ml certrifuge bottles. The ether portion was aspirated in 500-ml round bottom boiling flask after centrifuging for about 7 minutes at approximately 2,000 rpm and evaporated to dryness under vaccum on a flash evaporator. The residue was dissolved in 10 ml of redistilled skelleysolve B (B.P. 45-60°C) and partitioned 4 times with equal volume of 70% methanol in a 40-ml centrifuge tube. The 70% methanol was aspirated in a 300-ml round bottom boiling flask and reduced to aqueous phase on the flash evaporator maintained at 45°C. The aqueous phase was transferred to 40-ml tube and partitioned 4 times with equal volume of redistilled benzene. The benzene portion was each time aspirated in a 50-ml tube and dried. The sides of the tubes were rinsed with methanol and the final rinse dried under nitrogen.

Chromatography and elution of samples. The final residue was taken up in 4, 3, 2, or 1 drop of methanol: Chloroform (1:1) mixture and applied to 2 cm wide paper strips (Whatman No. 1). The strips were previously boiled in redistilled ethanol for 15-20 minutes prior to application of the residue. Two strips, one with authentic standards of progesterone and 20 α-ol and the other blank, were included with each set of samples. These strips were chromatographed in skelleysolve B : 90% methanol system for 3½ hours. The steroids were located on the strips by viewing under ultraviolet light and by scanning the chromatograms with a radiochromatogram scanner. The steroid areas were carefully cut for the chromatogram and eluted with redistilled methanol. Areas corresponding to progesterone and 20 α-ol on the blank were also cut and eluted. The methanol was evaporated on the Evapomix and the tubes rinsed 3 times with methanol; final drying was done under nitrogen.

The optical density of the extracts was determined at 230, 232, 240, 242, 250, and 252 millimicrons against the appropriate paper blank in a Beckman D.U. spectrophotometer. The quantity of steroid was then calculated using the method of Allen(8) compared with the optical density of authentic progesterone or 20 α-ol in methanol. A liquid scintillation counting system (Nuclear Chicago, Model 6722) was used for counting radioactive progesterone.

Progestins in ovarian tissue. Fresh ovaries were cut into small pieces and homogenized 3 times in 10-15 ml of ethyl acetate in a Tri-R tissue homogenizer. The ethyl acetate was filtered over anhydrous sodium sulphate into a 300-ml round bottom boiling flask. The filter paper and anhydrous sodium sulphate were washed twice with an additional 10-15 ml ethyl acetate. The ethyl acetate was evaporated to dryness under vacuum in a flash evaporator. The residue was taken up in 10 ml redistilled skelleysolve B and subjected to the same procedure as described for the venous blood.

Results and discussion. The whole blood contained an average of 0.7 μg progesterone /ml at day 4 and 1.3 μg/ml at day 20 of pregnancy. The fluctuations in progesterone levels from day 4 to 20 of pregnancy were inconsistent due to the wide individual variation. The level of 20 α-ol in the ovarian blood varied from 0.2 to 1.5 μg/ml with no significant correlation to the stage of pregnancy. In several cases the levels of progesterone and 20 α-ol were very low in both pregnant and pseudopregnant animals at 4 days *post-*

§ Hereinafter abbreviated as 20 α-ol.

coitum (*p.c.*). The level of progesterone and of 20 α-ol was correlated neither with the number of corpora lutea nor the number of viable or degenerating implantations in pregnant animals. Considerable variations occurred in progesterone level and rate of ovarian blood flow at time of collection. Much of this variation may have been the result of prolonged occlusion of the ovarian blood supply at the time of cannulation producing partial anoxia of the corpora lutea. Similar variations were recorded in the ewe following stimulation with gonadotropins(9).

The average weight of the two ovaries increased steadily from .43 g at 4 days *post-coitum* (*p.c.*) to .648 g at 20 days *p.c.* At 4 days *p.c.* the ovarian levels of progesterone and 20 α-ol were very low in pregnant and pseudopregnant does and in most cases progestins were not detected. In pregnant animals the level of progesterone at 6 days *p.c.* was 12.2 μg/g of fresh ovarian tissue. There was a significant rise in progesterone level at 8-9 days *p.c.* (18-21 μg/g) and at 18 days *p.c.* (18 μg/g). The total amount of progesterone in the 2 ovaries varied from 4.7 μg/g at 6 days to 13.7 μg/g at 18 days *p.c.* with no significant correlation with the stage of pregnancy. The concentration and total amount of progesterone in the ovarian tissue did not show significant trends in pseudopregnant and superovulated does. There was significant correlation for the concentration of progesterone and 20 α-ol in the ovarian tissue and that of the ovarian effluent blood during pregnancy.

Implantation in the rabbit occurs at 8 days *p.c.* At 9 days *p.c.*, the total amount of ovarian progesterone was higher in pregnant than in pseudopregnant animals. This was not the case for blood progesterone or 20 α-ol at 9 days *p.c.* With increasing number of ova, the number of implantations and the percentage of post-implantation mortality increases(10). In the present study there was no correlation between number of implantations and ovarian or blood progesterone. Correlations between number of implants in the pig and progesterone content and concentration in luteal tissue were not statistically significant(11). This may indicate that post-implantation mortality associated with overcrowding *in utero* is not due to progestin insufficiency.

It was reported that the biological technique of Hooker-Forbes will detect a progesterone concentration of 0.3 μg/ml of blood(12). Further work on better techniques for progesterone assay is needed for routine studies in reproductive physiology and clinical endocrinology. Mayer(13) reported a highly significant correlation between number of embryos implanted and conjugated and non-conjugated fractions of pregnancy in the pregnant sow urine. On the other hand, progesterone concentration of the plasma in the ewe was not related to the abnormality of the ova(14).

The patterns of progestin levels in the blood and luteal tissues during pregnancy vary with the species. Further studies are needed to establish the patterns of progestins during pregnancy and in different reproductive disorders in a variety of species. Further investigations are also needed in the ovary to evaluate the secretory activity of the interstitial cells as affected by endogenous and exogenous gonadotropins. The physiological significance and the cyclical changes of the interstitial gland tissue have been studied in detail for the human ovary (15) but little is known about these phenomena in laboratory or domestic mammals.

Summary. Progesterone and 20 α-ol were assayed chemically in ovaries and effluent blood from the ovary of pregnant, pseudopregnant and superovulated rabbits. During pregnancy, the level of progesterone varied from 0.7 to 1.3 μg/ml blood and the level of 20 α-ol varied from 0.2 to 1.5 μg/ml blood with no significant correlation to stage of pregnancy. Progestin levels were correlated neither with number of corpora lutea nor to number of viable or degenerating implantations. At 9 days *p.c.*, the total amount of ovarian progesterone was higher in pregnant than in pseudopregnant does; this was not the case for 20 α-ol. The levels and total amount of 20 α-ol in the ovaries were 2-5 times those of progesterone. There was significant correlation between the concentration of progesterone and 20 α-ol in the ovaries and

that of the blood during pregnancy.

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Received May 10, 1965. P.S.E.B.M., 1965, v120.

Effects of Gonadectomy and Sex Hormone Administration on Accessory Reproductive Organs of Protein-Deficient Rats.* (30448)

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It has been reported(1) that hypophysectomy of protein-depleted rats produced a significant decrease in weights of testis and ventral prostate, already markedly reduced as a result of protein deprivation. This finding favored the supposition that small amounts of gonadotrophins were circulating in the blood stream of intact male protein-deficient rats, especially since adrenalectomy—and loss of adrenal androgens—had no such effects on reproductive organs of these animals. The present study describes the effects of gonadectomy on accessory reproductive organs of protein-deficient rats of both sexes. The response of accessory organs to low doses of sex hormones was also investigated in order to test the sensitivity of these organs to exogenous hormones in the absence of dietary protein.

Materials and methods. Female and male Long-Evans rats were gonadectomized and placed for approximately 1 month on diets containing 0% or 20% protein.† They received distilled drinking water and were kept, singly, in cages with wire screen floors. Female rats were nulliparous, 80-85 days of age, and weighed an average of 208 g; male rats were 40-42 days old and averaged 152 g at onset. Groups of female rats, anestrous as judged by vaginal smears, received estradiol benzoate (Nutritional Biochemicals Co.) for 3 days preceding autopsy. Daily amounts ranged from 0.03 μg to 0.30 μg in 0.3 ml sesame oil and were administered subcutaneously in 3 equally divided doses. Male animals were injected subcutaneously with testosterone propionate (Matheson Coleman & Bell) for 10 days preceding sacrifice. The hormone was given in single daily doses ranging from 10 μg to 250 μg in 0.1 ml sesame oil. Vehicle-injected animals and uninjected intact rats fed purified diets served

* Supported by grant B-668 from Nat. Science Foundation.

† Composition of purified diets was reported earlier (2). The protein-deficient diet is known to reduce food intake; the effects of food restriction and protein deprivation on reproductive function in rats have been compared by paired feeding(3). In both sexes, the consequences of such protein deprivation are much more serious than those following limited consumption of a complete diet. For this reason, and because it is known that underfed rats are still responsive to low levels of sex hormones(6,8), paired feeding was not employed in the present studies.