tor in causation of fetal anomalies. Responses of the uterus to estrogenic and progestational hormones may be the end result of interaction between hormones, target and bacteria. Finally, it would appear that Uterone may contain substances which favor growth of some bacteria while inhibiting others.

Summary. Microorganisms were demonstrated by direct smears and cultures of uterine fluid in immature and mature mice and in uterine tissue sections. Incidence of uterine contamination varied from strain to strain. Contamination persisted in the luminal fluid of hydrouteri. However, Gram-negative bacteria increased and Gram-positive organisms decreased during accumulation of uterine fluid.

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Stimulation of Progestin Release from Rabbit Ovary *in vivo*.* (26877)

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The principal functional roles ascribed to progesterone have been related to pregnancy, and the primary site of synthesis of this steroid has been considered to be the corpus luteum of the ovary. This would imply that ovarian progesterone is not released in the absence of functioning corpora lutea. However, Forbes(1) detected elevations in progestational activity of peripheral blood drawn from female rabbits shortly after mating, several hours before ovulation could be expected; and Edgar(2) reported that the progesterone content of ewe ovarian vein blood could not be correlated with the number of functioning corpora lutea in the cannulated ovary. Zander(3) found high concentrations of progesterone in the human Graafian follicle shortly before ovulation and also isolated the progesterone metabolite, Δ^4-3-ketopregnene-20 α-ol (20α hydroxy-pregn-4-en-3-one) from the mature human follicles(4). Recently Taylor(5) has demonstrated the presence of appreciable progesterone in ovarian tissue of immature rats. Progesterone and its α 20-ol metabolite have also been found in the adrenal venous blood of calves of both sexes(6).

This paper gives further evidence that release of progesterone and Δ^4-3-ketopregnene-20 α-ol from rabbit ovaries *in vivo* is independent of the presence of corpora lutea, and that release of these steroids is rapidly activated following coitus or injection of hormonal agents which induce ovulation. Rabbits were chosen for this study since this species ordinarily ovulates only in response to coitus, and presence or absence of corpora lutea can easily be controlled.

Materials and methods. Steroid assays were made of ovarian blood of more than 60 New Zealand white rabbits. Sexually mature females (3.5-5 kg) were anesthetized with pentobarbital (approx. 25 mg/kg body wt), and 500 USP units/kg heparin were injected *i.v.* immediately before a polyethylene cannula (o.d. = 0.060″) was inserted into the ovarian vein. Blood samples were collected for specific time intervals in order that progestin content could be expressed in units

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of output; *i.e.*, μg/gland/hour.

Following initial collection of a control sample, hormones were administered intravenously in the following dosages: luteinizing hormone (LH) 1-5 Armour units; follicle stimulating hormone (FSH) 2.25 and 5.0 Armour units; lactogenic hormone (LGH) 200 I.U.; growth hormone (STH) 5 U.S.P. units;‡ thyroid stimulating hormone (TSH) 10 U.S.P. units;§ corticotropin (ACTH) 4 I.U.;‖ chorionic gonadotropin (HCG) 250 I.U.;¶ and pregnant mare's serum (PMS) 250 I.U.** To prevent shock from blood loss, alternate collections of 10-15 minutes duration were reinjected and blood volume was maintained by addition of physiological saline or 5% dextrose in saline.

Each blood sample was measured, hemolyzed with distilled water and extracted overnight with 3 volumes of ether and twice the next morning with 2 additional 50 ml of ether. The final ether extraction and blood buffer coat were washed twice with 0.1 N NaOH and distilled water. The 3 ether washings were then combined and evaporated to dryness, and the remaining residue was partitioned twice between 10 ml absolute methanol and 10 ml n-heptane. The methanol phase, which now contains the steroid, was evaporated to dryness and the residue redissolved in 1 ml ethyl acetate, half of which was chromatogrammed on Whatman #1 paper strips 7 × 22 inches. A descending system was used. Strips were equilibrated overnight at room temperature in a closed tank containing approximately 500 ml of stationary phase, Methanol:water::4:1. The mobile phase, n-heptane:ethyl acetate 5:2 was added the next morning.

As standards, progesterone 5, 10, 15 μg and Δ^4-3-ketopregnene-20-ol†† 50, 75 and 100 μg were also applied to the paper and run with each blood assay. After development (3-4 hours) purple fluorescent spots were identified with a Mineralight UV lamp and the paper was sprayed with a filtered solution of 2,4-dinitrophenylhydrazine in 1% HCl in ethanol. After washing in warm tap water to remove background color, strips were dried and steroid areas were located against the standards, cut out and eluted in 2 successive half-hour ethanol washings (with frequent shaking) of 6 and 4 ml respectively. Color was developed by addition of 0.5 ml 2 N NaOH to the combined washings and read in the Klett colorimeter (540 green filter) with the paper background blank set at zero. Criteria of progesterone identification were 1) solvent partitioning, 2) UV fluorescence and 3) paper chromatography.

Results. On our solvent system, progesterone and its 2 active metabolites, Δ^4-3-ketopregnene-20 β-ol and Δ^4-3 ketopregnene-20 α-ol, had chromatogram Rf values of 0.6, 0.48 and 0.35 respectively. Whereas Δ^4-3-ketopregnene-20 β-ol was not found in rabbit ovarian vein blood, appreciable output of Δ^4-3-ketopregnene-20 α-ol, as identified by Rf value ranged from 50-100 μg/gland/hour, and small amounts of progesterone could frequently be detected in 15 or 20 minute blood collections from non-pregnant, estrous rabbits without corpora lutea.

Within minutes following mating (4 cases) or injection of a gonadotropin which induced ovulation (LH, 5 cases; FSH, 5 cases; PMS, 5 cases; HCG, 8 cases) progestin levels rose abruptly, often to exceed an output of 1 mg/gland/hour (Fig. 1). Similar results were encountered following injections of TSH (Armour 216-174-1 and Thytropar) which were obviously contaminated with gonadotropin since ovulation ensued. A highly purified TSH preparation§ which induced thyroid stimulation without concomitant ovulation was also ineffective in stimulating progestin release. This output of progestin was independent of follicular rupture since ovulation in the rabbit follows 15-20 hours after the ovulating stimulus. Administration of pituitary hormones which did not induce ovulation (*i.e.*, ACTH, 8 cases; TSH, 2 cases; STH, 2 cases; LGH, 10 cases) caused no

‡ All by courtesy of J. D. Fisher, Armour Pharmaceutical Co.

§ Courtesy of Drs. John Pierce and Mary Carsten, Department of Physiological Chemistry, UCLA Medical School.

‖ Parke-Davis.

¶ "Follutein," Squibb.

** "Equinex," Ayerst.

†† Courtesy of Dr. D. Perlman, Squibb Inst.

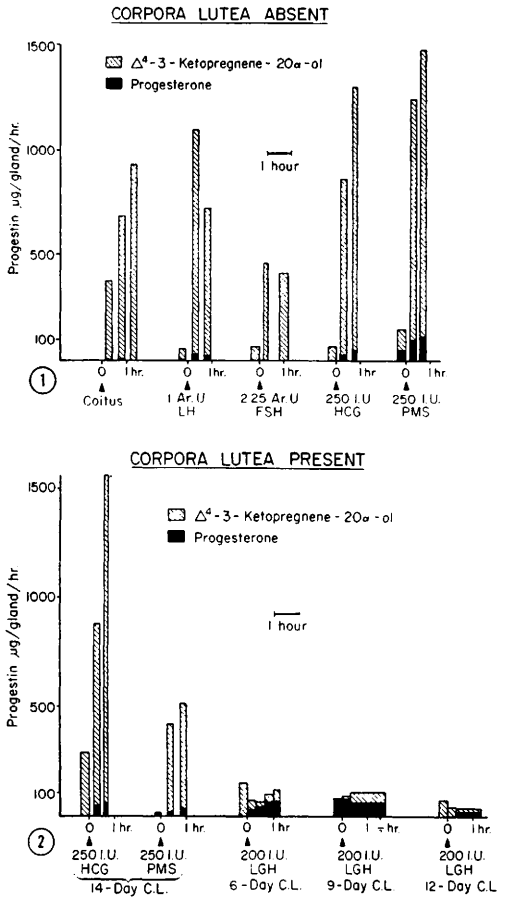

FIG. 1. Typical effects of coitus and treatment with gonadotropins on progestin release from non-luteinized rabbit ovary. The width of each bar indicates duration of ovarian blood collection for that sample.

FIG. 2. Typical effects of treatment with placental gonadotropins and lactogenic hormone (LGH) on progestin release from rabbit ovary during pregnancy or pseudopregnancy.

significant changes in progestin release. Similarly no progestin release was recorded in 2 cases of coitus followed by failure of ovulation and one case of a non-ovulatory injection of LH in a sexually immature rabbit.

In the pregnant or pseudopregnant rabbit with vascularized corpora lutea (14 day) HCG and PMS both effectively stimulated progestin release. This was in contrast to LGH which, in doses of 200 I.U., was consistently ineffective (Fig. 2).

Discussion. The principal progestin found in the ovarian vein blood of adult New Zealand white rabbits is Δ^4-3-ketopregnene-20 α-ol. This biologically active metabolite of progesterone (1/3-1/2 as active as progesterone in the rabbit uterus test) has been found in high concentrations in human Graafian follicles and placenta and is oxidized to progesterone *in vitro* by corpora lutea or in ovarian tissue without corpora lutea(4).

The rapid elevations in progesterone and Δ^4-3-ketopregnene-20 α-ol release from the ovaries of estrous rabbits following gonadotropic stimulation show clearly that functional corpora lutea are not essential to the elaboration of these steroids. The results also suggest that lactogenic hormone (LGH) may not be luteotropic in the rabbit, confirming the proposals of Klein and Mayer(7) and others in this respect.

A probable precursor of ovarian hormones has been suggested by Claesson, Hillarp and Högberg(8), who noted a marked fall in the esterified cholesterol of rabbit interstitial tissue shortly after administration of gonadotropin. Although these workers concluded that cholesterol must be the principal precursor of ovarian estrogen, our results imply that the rapid changes in interstitial gland cholesterol may be correlated with progestin output.

Summary. 1) Progesterone and its metabolite Δ^4-3-ketopregnene-20 α-ol are present in the ovarian vein blood of estrous rabbits without functional corpora lutea. 2) After mating or administration of a gonadotropin which induces ovulation, the output of these steroids is immediately elevated. 3) Gonadotropins, but not lactogenic hormone (LGH), also activate progestin release from the ovary containing functional corpora lutea of pregnancy or pseudopregnancy.

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