28. ALTERATION IN THECAL ANDROSTENEDIONE SYNTHESIS IN PREANTRAL AND ANTRAL FOLLICLES
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Theca from preantral and antral follicles were isolated and incubated in vitro with 100 ng luteinizing hormone (LH) on each day of the hamster estrous cycle. On the morning of estrus (day 1) theca produced large amounts of progesterone (P) but not androstenedione (A); whereas on the afternoon of estrus large amounts of P and A were synthesized in vitro. On the morning of days 2-4 theca from antral follicles produced produced mainly A. Theca from preantral follicles produced A on the morning of day 4. Following the LH surge, theca from preantral and antral follicles produced large amounts of P but not A. Thus, the LH surge inhibits thecal A production; accounting for the decline in follicular estrogen production. The increase in thecal P production by antral follicles represents an initial event in transformation of the preovulatory follicle into a corpus luteum. The alteration in thecal A in preantral follicles induced by the LH surge may represent a signal synchronizing follicular steroidogenesis and thus follicular development. These data indicate that the LH surge alters steroidogenesis in not only antral follicles but also preantral follicles.

29. BIPHASIC PROGESTERONE SYNTHESIS BY HAMSTER PREOVULATORY FOLLICLES IN VITRO.
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LH-stimulated hamster preovulatory follicles (POF) begin to synthesize progesterone (P) within 15 min. while isolated granulosa cells (G) or theca (T) do not synthesize P until after 4-6 hr (1). In vitro time course experiments (0-9 hr) using POF showed that LH (100 ng/ml) stimulated biphasic P synthesis. Phase 1 (0-5 hr) P accumulation totaled 17 ng/POF, and phase 2 (5-9 hr) P accumulation was a further 23 ng/POF. Cyclohexamide or puromycin had no effect on LH-stimulated phase 1 P, but inhibited phase 2 P accumulation. cAMP (10^{-4}M)-stimulated follicles also exhibited biphasic P synthesis. However, cAMP phase 1 P accumulation never equalled that of the LH-phase 1 P, while phase 2 P was comparable in LH-and cAMP-stimulated follicles. Protein synthesis inhibitors inhibited phase 2 but not phase 1 P in cAMP-stimulated follicles. These results and those in the cited paper are indicative of 2 separate mechanisms in POF P synthesis. Phase 1 P synthesis is short-lived, involves a G/T interaction, is independent of de-novo protein synthesis, and may involve more than one messenger. Phase 2 P synthesis appears to reflect independent P synthesis by G and T, requires de-novo protein synthesis, with cAMP as the probable messenger.
1. Makris, A. and Ryan, K.J. Endocr. Res. Comm. 4(3 & 4), 233, 1977.

30. ESTRADIOL SYNTHESIS BY OVARIAN GRANULOSA CELLS: A ONE CELL THEORY.
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The concept that ovarian estrogen synthesis requires the interaction of at least 2 kinds of cells is more than 20yrs old. Current interpretations favor thecal-interstitial cell (TI) production of androgens, which are converted to estrogens by the aromatase of granulosa cells (GC). However, the recent finding (Steroids 38:581,1981) that GC have equal 17-hydroxylase and aromatase activity casts doubts on the validity of such a 2-cell theory. In the present study, GC were harvested from follicles of hypophysectomized immature rats 24,48 or 72h after injection of 20 IU of PMSG, and incubated in Krebs-Ringer bicarbonate buffer with or without added progesterone (P4) or pregnenolone (P5). The amount of estradiol (E2) was determined by radioimmunoassay. At 24h, GC produced 18.8±1.3 ng/mg protein/h; at 48h the rate was 20.5±0.54. The rate was linear with time up to 90 min and with the amount of cells incubated. The rate was increased by 50% when P4 was present and by 100% with P5; 17-OH-P5 was no more effective than P5, i.e. lyase was not limiting. At 72h the rate was 59.4±3.7 which increased to 99.9± 7.7 with P5 in the medium; addition of TI cells to the GC did not alter the rate of E2 production. No E2 was produced if NADP was omitted from the culture media. The results clearly show that only one cell, the GC, is necessary for estradiol production.