time is not measured. In principle, tissue breakdown might differ in different states (i.e. in prolactinoma-bearing rats vs controls). The participation of PRL in the disrupting effect of estradiol on TIDA neurons is difficult to evaluate, for estrogens increase PRL release, and long-lasting hyperprolactinemia can itself prove toxic to TIDA neurons[18]. In the experiments of Demarest et al.[12] long-term treatment with estradiol reduced the activity of TIDA neurons whereas continuously elevated PRL induced by chronic haloperidol administration increased dopamine turnover in the same neurons[24]. On the other hand the recent demonstration that chronic elevation of PRL produces a decline in TIDA function[25] in the absence of any rise in plasma estrogens, suggests that PRL per se may have a disrupting effect.

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A daily rhythm in hCG binding to ovarian follicles of the cyclic hamster

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Summary. On each day of the estrous cycle hCG binding to follicle increased from 09.00 to 21.00 h; then hCG binding was static until 09.00 h of the next day. FSH binding did not exhibit rhythmicity. This pattern of hCG binding may be related to the pulsing of LH on each cycle day.
Key words. hCG receptor; FSH receptor; rhythm; follicles; steroids.

There is evidence of circadian rhythms in secretion patterns of most central nervous system controlled pituitary hormones, e.g. thyrotropin, prolactin, growth hormone and luteinizing hormone[2,3], whose adaptive role is poorly understood. Everett and Sawyer[4] reported that barbiturates prevent ovulation, if administered at 14.00 h on proestrus. Subsequently several studies[5-7] suggested a critical period during proestrus when administration of blocking agents prevents ovulation. Everett[8] defined the critical period in proestrous rats between 14.00 h to 16.00 h. Other investigators have reported critical periods for LH release on other cycle days[9,10]. Dominguez and Smith[9] noted that pentobarbital, a blocker of LH secretion, was effective in delaying ovulation for one day if the injection was given at 12.45 h on any day of the cycle while injections at 09.00 und 17.00 h were ineffective.

Bolton[11] has shown that serum LH levels in female hamsters were significantly elevated at 13.00 h on day 1, 19.00 h on day 2 and 16.00 h on day 4; a high level of LH in serum was also noted at 19.00 h on day 3 although it was not statistically significant from lower values on that day thus, a circadian pattern in LH release appeared evident on each day of the cycle. Since LH alters the number of LH receptors in the ovary[12] and since serum LH levels in hamsters exhibit a circadian rhythm, we were interested in determining whether follicular LH receptors exhibited circadian alteration throughout development of the 4-day estrous cycle. An additional aim was to determine whether follicular steroid levels coincided with any change in gonadotropin receptors.

Materials and methods. Cyclic hamsters, weighing 80–100 g, were maintained on a 14-h light: 10 h dark schedule with lights on from 05.00 to 19.00 h in a room of 21–23 °C. Day 1 (the day of ovulation, estrus) was determined by the characteristic vaginal discharge. Three consecutive 4-day cycles were monitored before using the animals.

Classification of follicles. Follicles were classified according to a previous description[13]. The stages are: stage III: preantral follicles with 6–7 layers of granulosa cells; stage IV: preantral follicles with eight or more layers of granulosa cells; stage V: follicles

with early signs of antral cavity formation; small lacunae appearing between the granulosa cells but not a single large antrum; stage VI: antral follicles characterized by a single large antrum.

Collection of follicles. Hamsters were anesthetized with ether and the ovaries removed from the body cavity and placed in ice cold (4 °C) homogenizing buffer (HB; 0.05 M Tris-HCl containing 0.01 M $CaCl_2$ and 0.075 M $MgCl_2$, pH 7.2). Using a dissecting microscope and extra-fine tipped jewelers forceps, the follicles were removed, dissected and cleaned of adhering interstitial tissues. On proestrus and estrus, the largest preantral follicles (stages III and IV; diameters: 300–400 µm) were collected. On diestrus I and diestrus II the largest follicles were stages V and VI with diameters of 400–450 µm. 20–30 follicles were isolated from 2 to 3 animals each day at 09.00 h, 15.00 h and 21.00 h with a total of 4–6 groups. Follicles were pooled by group and snap frozen in 1.0 ml HB. Progesterone, androstenedione and estradiol concentrations and hCG and FSH binding were determined in homogenized follicular tissue by radioimmunoassay and radioreceptor assay, respectively.

Steroid radioimmunoassay. Progesterone, androstenedione and estradiol were determined by radioimmunoassay as described previously[15–17]. The intra and interassay coefficients of variation were less than 10.3% for all assays.

FSH and hCG binding assays. Binding of iodinated ovine FSH (I-1 from the National Pituitary Agency, Baltimore, MD, USA) and hCG (CR-119 from the same agency) to follicular homogenates was tested in vitro as described previously[18]. Radioiodination was performed using lactoperoxidase[19].

Statistics. Data were analyzed by one or two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test, where appropriate.

Results. 1) FSH and hCG receptors binding in growing follicles. The most interesting observation was the rhythmicity of hCG binding in follicles on each cycle day (fig. 1). On each day hCG binding to follicles significantly ($p < 0.05$) increased from 09.00 h to 21.00 h; however, either no significant change or a decrease in the hCG binding occurred from 21.00 h to 09.00 h of the next day. FSH binding increased from 09.00 to 21.00 h on each day except day 2. In contrast to hCG binding, a significant increase in FSH binding was also observed from 21.00 to 09.00 h (of the next day) except days 2–3.

2. Steroid content of growing follicles. Progesterone (P_4), androstenedione (A) and estradiol (E_2) contents of growing follicles are shown in figure 2. On day 4, P_4 content of preantral follicles (stages III and IV) increased ($p < 0.01$) from 09.00 h to 21.00 h and subsequently decreased ($p < 0.01$) from 21.00 h on day 4 to 09.00 h on day 1. Changes in hCG binding paralleled changes in preantral follicular P_4 only on day 4. Androstenedione content in preantral follicles on day 4 increased ($p < 0.01$) immediately with the serum LH surge (15.00 h) but returned to low levels after the surge and remained low until 09.00 on day 1. Androstenedione increased again on day 1 from 09.00 h to 15.00 h but remained elevated and unchanged on days 2 and 3. The E_2 content of follicles did not show any rhythmic changes. Follicular E_2 content showed a gradual increase from preantral stages late on Day 1 to the antral stage on Day 3 and this paralleled increases in FSH binding ($r = 0.77$).

The slope of the hCG binding per follicle from 09.00 h to 21.00 h increased on each day of the cycle beginning with day 4 (preantral follicles) to day 3 (antral follicles) (fig. 3).

Discussion. The present study clearly shows a rhythmicity of hCG binding to growing follicles of the cyclic hamster. On each day hCG binding significantly increased from 09.00 h to 21.00 h and then became static between 21.00 h of the same day and 09.00 h of the next day. This was not observed for FSH binding. Three factors may be important in the hCG rhythmicity. 1) A periodicity in serum LH secretion during the estrous cycle in hamsters has been reported[11,20]. 2) Pulses of LH in serum down-regulate LH receptors in the ovary[12]. 3) hCG (LH) can augment FSH induction of LH receptors in granulosa cells of developing follicles[21]. Therefore, it is hypothesized that the LH surges on each day of the cycle are causal in producing a biphasic pattern in follicular hCG binding. LH may directly induce down-regula-

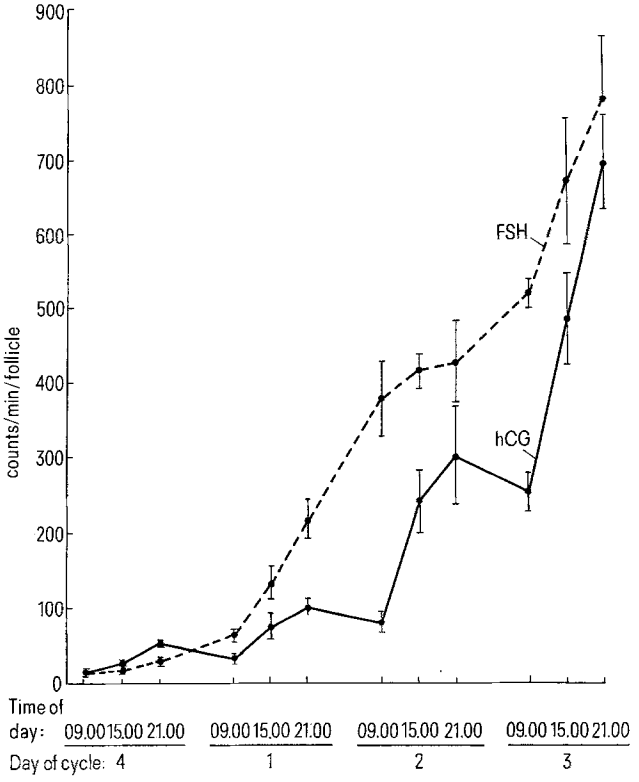

Figure 1. Follicular FSH and hCG binding on each cycle day at 09.00 h, 15.00 h and 21.00 h.

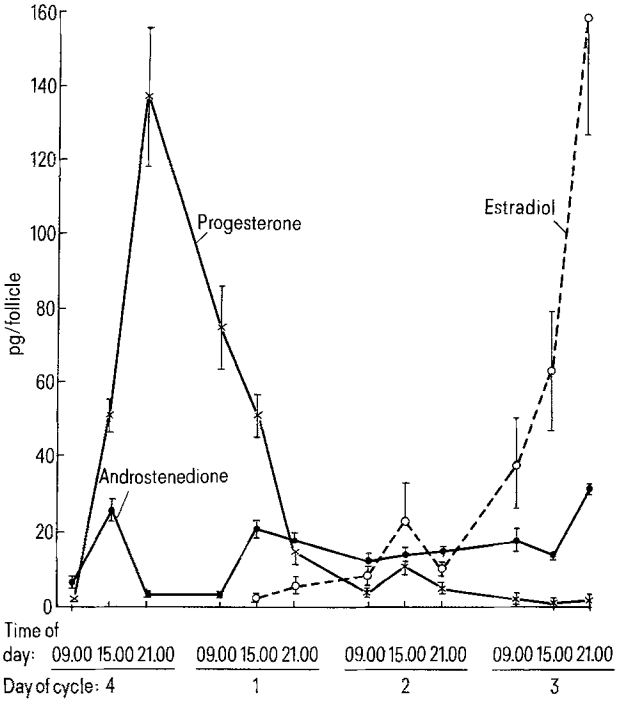

Figure 2. Progesterone, androstenedione and estradiol content of follicles on various days of the cycle.

tion of LH receptors in growing follicles[12] with a lag period of 9–12 h[22,23]. A further decline in LH receptors has been observed 24 h after LH stimulation[23]. Since serum LH increases from 13.00 h to 19.00 h on each day during the estrous cycle[11], a decrease in LH receptor would be expected to occur between 22.00 (of the same day as the increase in serum LH) and 01.00 h of the next day. The decrease might even be evident by 13.00–19.00 h of the next day; thus, the decrease (or stasis) in hCG binding observed on each day between 21.00 h and 09.00 h in the present study is in agreement with the time lag for disappearance of the LH receptor as reported by Conti et al.[22] and Schwall and Erickson[23].

The serum FSH level is acutely and transiently elevated at 16.00 h on day 4 (i.e. the proestrous surge of FSH) and at 08.00 h on day 1 (the estrus FSH surge) but on days 2 and 3 the serum FSH profile is less variable than LH and it is nonfluctuating[24,25]. FSH induces a small transient increase in LH receptor within 24 h in hypophysectomized-estradiol primed immature rats[21]; however, if hCG is given 12 h before FSH there is an interval of 24–36 h between hCG injection and a subsequent 10–16-fold increase in LH receptors. Thus the large increase in hCG binding may be due to the synergism with FSH approximately 12–24 h earlier. Unlike hCG, FSH binding did not exhibit rhythmicity, suggesting an independent development of LH and FSH receptors in growing follicles. There is no evidence of disappearance of FSH receptors due to injections of FSH or LH. FSH binding increased significantly on each day except between 21.00 h on day 2 and 09.00 h on day 3. The close correlation between the increase in E_2 content of the follicles and FSH binding indicates that E_2 might be controlling FSH receptor as reported for rats[26]. However, estrogen does not stimulate formation of large preantral/preovulatory follicles in the hamster[27].

Since present data are expressed per follicle one may argue that the pattern of hCG binding could be due to rhythmicity in cell division. No evidence of such rhythmicity of cell division has been reported. Cell-cycle times are usually 39–260 h except for fast growing bone marrow cells with a cell-cycle time of 18 h[28]. hCG binding in these follicles is localized primarily to the theca in preantral follicles on days 4 and 1 and in the theca and granulosa on days 2 and 3[29]. Therefore, only 1 compartment, theca, contributes to the changes on days 4 and 1 whereas on days 2 and 3 both theca and granulosa are responsible for that profile.

No statistical correlation existed between changes in hCG binding and changes in P_4, A and E_2 content of follicles. The lack of correlation between follicular steroids and hCG binding indicates that the effect of LH and/or FSH on changes in hCG binding occur(s) by mechanisms independent of steroids.

The slope of hCG binding per follicle from 09.00 h to 21.00 h increased on each day of the cycle (fig. 3). As follicles grow the rate of increase in hCG binding appears logarithmic. This pattern of hCG binding may reflect the growth promoting effects of daily LH surges (in synergy with FSH) on the final stages of follicular development. Pulsatile secretion of LH preceding the preovulatory LH surge is an important determinant of follicular maturation and subsequent corpus luteum function in the ewe[30]. The reason for the alternating high and low slopes of FSH binding on days 1, 3, and 4, 2, respectively, is unknown. However, it is well known that FSH receptors are localized to only the granulosa cells in the hamster[29]. Thus this pattern of FSH binding may reflect functional changes in the granulosa cell population.

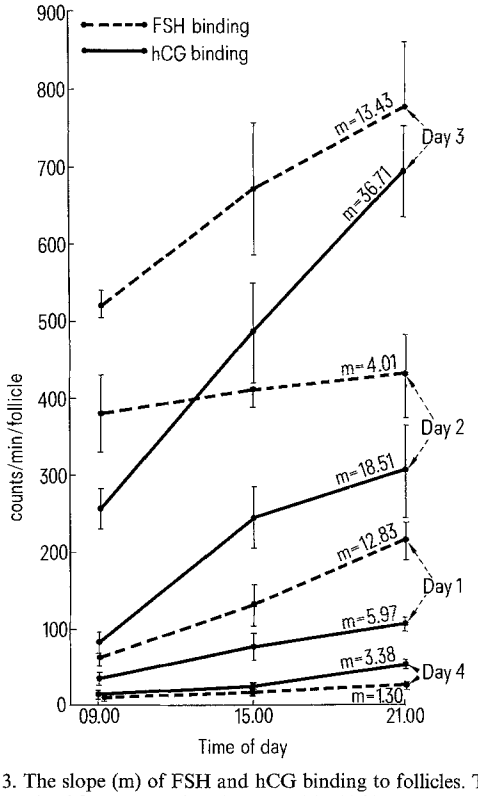

Figure 3. The slope (m) of FSH and hCG binding to follicles. The slope was determined from two points (09.00 and 21.00 h).

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