THE primary aim of this paper is to present a series of experiments designed to study the mechanisms which control the onset of puberty in the rat. A second major goal is to discuss these results in relation to observations in other mammalian and certain non-mammalian species in an attempt to develop a generalized concept for the mechanisms controlling the onset of puberty or reproductive capability.

Throughout the animal kingdom regulatory mechanisms for precise control of gonadal differentiation, maturation and function have evolved to insure that reproduction is possible. The degree of complexity of these mechanisms increases as one proceeds from phylogenetically lower animals to mammals (Hagadorn, 1967; Rothballer, 1954). Neurosecretory cells have been identified in all invertebrate phyla that possess a well defined nervous system. Even though such cells do not exist in coelenterates, specialized hypostomal nerve cells do play a role in the initiation of growth, sexuality and regeneration. In echinoderms, such as the starfish, the circumoral ring and radial nerves contain neurosecretory cells which are thought to release substances which stimulate and inhibit the release of ripe gametes. In polychaete and oligochaete worms surgical removal of cerebral ganglia produces premature gamete formation. These regulatory mechanisms relate to what Hagadorn calls a "First order neuroendocrine reflex" that involves a direct action of central nervous system products on the target or gonad.

Inhibitory nerves from the subpedunculate lobe of the brain of the octopus regulate the release of gonadotropic hormones from a specialized optic gland. Elaborate regulatory mechanisms also exist in arthropods. X organs produce gonadal inhibitory substances and Y organs produce molting hormones and gonadal stimulating materials in crustacea. In insects, juvenile hormone is essential for yolk deposition in eggs and for formation of spermatophores. Juvenile hormone inhibits the action of molting hormone. Removal of the corpora allata prevents production of ripe eggs. Corpora allata are supplied by both excitatory and inhibitory nerves from the brain. These "Second-order-reflexes" involve the insertion of a non-neural endocrine gland between the nervous system and the final target. No feedback signal exists from the target in these species.

Detailed knowledge of the structural relationship between the central nervous system (CNS) and the hypophysis has helped expand our understanding of the neural control of reproduction in chordates (Green, 1951; Wingstrand, 1966). Little or no neural control over gonadotropin secretion exists in cyclostome fishes (Gorbman, 1965). In fishes the fibers of hypothalamic magnocellular nuclei ramify and pass in large numbers to the pars intermedia and pars distalis. In higher vertebrates these fibers typically terminate in close proximity to blood vessels within the pars nervosa. In teleosts (perch and trout) no direct hypothalamic innervation of the adenohypophysis exists, however, the neurohypophysis sends numerous fiber bundles into this lobe. Seasonal gonadal function exists and oviposition is induced in the female by the presence of the male. It has been suggested that this may be due to a direct action of neurohypophyseal hormones on peripheral organs.

The secretion of gonadotropins by the amphibian pituitary is also dependent upon CNS contact. However, most of the neurosecretory neurons terminate in the pars intermedia and a clear understanding of neural regulation of reproduction is not known.

Avian species exhibit a wide variety of reproductive patterns (Farner, Wilson and Oksche, 1967). Seasonal gonadal development accompanied by migratory behavior is present in many species. A correlation exists between neurosecretory accumulation in the median eminence and gonadal function. The pituitary is dependent upon hypothalamic stimulation for gonadotropin secretion. In those species which secrete crop milk for their young the release of prolactin by the pituitary is also very much regulated by the CNS (Kragt and Meites, 1965). Ovulation in the chicken can be blocked by pentobarbital. Systemic and intrahypothalamic implants

---

1 Supported by USPHS Program Project Grant AM06704, NIH Training Grant #AM05613 of National Institutes of Arthritis and Metabolic Diseases, University of California Breon Fund and Simon Fund.

2 Associate Professor of Physiology, Colorado State University, Fort Collins 80521.
of progesterone can induce ovulation. Lesions of the preoptic and paraventricular nuclei cause cessation of egg laying in hens. In many of the above situations “Third order neuroendocrine reflexes” exist. Two endocrine organs exist after the CNS neurohumoral regulator, and the target organs produce in many instances a feedback signal regulating the function of some or all of the preceding steps in the reflex.

Numerous observations indicate that nutritional and environmental sensory cues influence the onset of reproduction in many domestic species. Increasing photoperiods result in early sexual maturation and increased egg production in chickens (Morris and Fox, 1958; Lert, Wilson and Hart, 1960; King, 1961). Reducing the length of clear and red photoperiods decreased gonadotropin production and release in cockerels (Harrison et al., 1970). White leghorn pullets exposed to green and blue light attain sexual maturity earlier than pullets exposed to red and clear light (Harrison et al., 1969).

Seasonal differences in the interval between birth and the subsequent onset of reproduction in the ewe have been stressed by Hafez (1952) and Watson and Gamble (1961). Spring farrowed gilts tend to reach puberty earlier and to weigh more at puberty than autumn farrowed gilts (Zimmerman et al., 1960; Sorensen, Thomas and Gossett, 1961). This interaction of season and nutrition has also been stressed by Short and Bellows (1971) in cattle. Wiltbank, Kasson and Ingalls (1969) demonstrated in heifers that factors other than growth rate and nutrition control the age of onset of puberty.

If the zoological concept that ontogeny recapitulates phylogeny holds for reproductive function, one might speculate that the mechanisms regulating the onset of puberty in mammals may include any of the following: (1) substances of neural origin which stimulate or inhibit the responsiveness of gonads to gonadotropic hormones; (2) both stimulatory and inhibitory neural controls for gonadotropin secretion; (3) a period in development during which feedback mechanisms are not functional because of a lack of final target organ (gonadal) secretions; (4) a later period in development during which these feedback components are functional.

Most of the experimental evidence utilized in this paper for drawing conclusions about neuroendocrine mechanisms in mammals has been drawn from the rat. However, it is possible to glean from the literature limited data supporting these concepts in other species as well.

The first concept listed above is probably the least well documented since it would appear very early in the development of mammals. Nonetheless, even in adult animals, observations have been made to support the idea. Hopkins and coworkers (1965, 1970) have demonstrated that factors which enhance the action of gonadotropins can be extracted from cerebral cortical tissue of rats. Previously, the same workers had demonstrated (Hopkins and Pincus, 1963) that reserpine decreased the ability of exogenous gonadotropin to stimulate ovulation in rats. The pineal has long been suspected to produce substances which delay reproductive development (Kitay and Altschule, 1954). Recently it has been shown that the pineal gland synthesizes melatonin (Lerner, Case and Takehashi, 1960). Wurtman, Axelson and Chu (1963) and others have shown that injections of melatonin retard vaginal opening and decrease ovarian weight in female rats. Serotonin, a compound structurally related to melatonin and also found in the pineal gland, retards sexual maturation in female mice (Robson and Botros, 1961). Serotonin also decreases the ovulation inducing effect of progesterone in immature rats, delays vaginal opening when given daily after 24 days of age and decreases ovarian weight (O'Steen, 1965). The possibility exists that these factors also exert central (CNS) effect (Wurtman, 1970; Martini, Fraschini and Motta, 1968). Many drugs which influence the indole amines can effect induced ovulation (Brown, 1968). However, the biphasic effects observed in response to the substances and to their inhibitors strongly suggests multiple sites of action and only further study will clarify sites and mechanisms of action.

From the classical studies of Harris and Jacobsohn (1951) and Nikitovitch-Winer and Everett (1959) on the effects of transplantation of pituitary tissue from immature to mature animals or to a site remote from the hypothalamus, the concept of neural stimulation of FSH and LH secretion and the tonic inhibition of prolactin release has been developed. Pharmacological agents known to act on the brain also markedly inhibit gonadotropin secretion (Markee, Sawyer and Hollinshead, 1948). Atropine administered at the beginning of estrus will inhibit ovulation in cattle (Hansel, 1958). Sensory input signals to the ovulatory release mechanism may involve inhibitory input since constant light prevents the ovulatory surge of LH and FSH release in the adult rat (Daane and Parlow, 1971). Evidence has been compiled to clearly demonstrate the existence of a prolactin inhibitory factor (Meites, 1970). Evidence also suggests the presence of prolactin stimulatory activity in hypothalamic extracts from rats.
(Nicoll et al., 1970). Further consideration of neural pathways which influence pituitary secretion will be presented in the discussion. However, it is readily evident that complex inhibitory and stimulatory mechanisms control the secretion of gonadotropins by the pituitary.

Follicle stimulating hormone releasing factor (FSHRF) and luteinizing hormone releasing factor (LRF) have been isolated from hypothalamic extracts of many species and the chemical structure of LRF was recently announced by Schally and coworkers (1971).

The data presented in this paper will contribute information on the latter two concepts related to ontogeny, and relevant literature will be cited in the discussion. Numerous investigators have suggested that the onset of puberty is related to a decrease in sensitivity of the estrogen negative feedback system (Byrnes and Meyer, 1951; Hoogstra and DeJongh, 1954; Ramirez and McCann, 1963). A series of experiments has been designed and undertaken to: (1) determine the normal ontogeny of pituitary secretion of FSH and LH in male and female rats; (2) to determine the effects of ovariectomy at two age periods on the rate of change of plasma FSH and LH in female rats; and (3) to determine the effects of numerous dosages of estradiol benzoate on circulating FSH and LH levels in these ovariectomized animals. Lastly, a series of pharmacological agents were administered prior to puberty to determine the effect of neuroactive substances on puberty onset.

Materials and Methods

Plasma Levels of FSH and LH during Development. Lactating mothers were obtained with pups 5 days of age (six per litter) from a commercial supply house (Bio-Science, Oakland, Calif.). Blood was collected in heparinized syringes from the abdominal aorta under ether anesthesia from female and male prepuberal animals at 5, 10, 15, 20, 25, 30 and 40 days of age. In all experiments, animals were sacrificed between 2 and 5 pm unless otherwise specified. Weaning took place at 20 days of age. After centrifugation the plasma was stored at -20°C until assayed. A similar protocol was utilized for collection of blood from selected adult females 75 to 80 days of age during each of the 4 days of the estrous cycle. Blood samples were drawn at 10 am on all days of the cycle and, in addition, samples were taken from animals at 2 to 4:30 pm and at 7 pm on the afternoon of proestrus.

Samples were obtained from postpuberal females killed at two day intervals between 40 and 60 days and also at 65, 70, 75 and 80 days of age.

In this and in all subsequent experiments FSH and LH were quantitated by radioimmunoassay procedures developed in our laboratory utilizing the NIAMD reagents. The techniques employed are slight modifications of those described earlier by Niswender et al., (1969) and as previously described in abstract form (Kragt, Bloch and Cons, 1970). After observing the marked changes that occur in plasma levels of hormones in intact female rats, three age periods were selected for intensive study, the first from birth to 25 days of age, the second from 20 to 40 days, and the third from 75 to 80 days of age.

Effect of Ovariectomy on Plasma FSH and LH. In an initial experiment, groups of animals were ovariectomized or sham operated either at birth, 5, 7, 10, 15 or 20 days of age and sacrificed at 5, 10, 15, 20 and 25 days of age after blood samples were collected for subsequent FSH and LH assay. In a second experiment, in this age range, animals were ovariectomized at 7 days of age and bled and sacrificed at 9, 11, 13 and 15 days of age. In the third experiment animals were either sham operated or ovariectomized at 20 days of age and bled and sacrificed daily until either 29 (sham) or 32 days of age (ovariectomized).

Effect of Various Doses of Estradiol on Plasma FSH and LH in Ovariectomized Animals. Females were ovariectomized at 8 days of age and were treated daily with 1 µg of estradiol benzoate. Blood samples were drawn and animals were sacrificed at 10, 12, 14 and 16 days of age. Untreated animals ovariectomized at 7 days of age and sacrificed at 9, 11, 13 and 15 days of age as well as intact animals sacrificed at 5 days and 15 days of age served as controls in this study.

In another experiment, ovariectomy was performed on the day of weaning (20 days of age) and either sesame oil, 0.05, 0.1, 1.0, 2.0, 5.0, or 10.0 µg of estradiol benzoate/100 g body weight was administered as a single dose subcutaneously at 27 days of age. Ovariectomy was also performed on another group of rats which were 75 days of age. After 1 week similar dosages of estradiol were administered. Blood samples were collected in both experiments 2 days after estrogen administration.

Effect of Various Hormones and Drugs on Puberty Onset. A number of substances have been administered chronically to immature female rats between 20 to 50 days of age. Both progesterone (1 mg/day) in oil and the anti-
estrogen clomiphene (1.0 or .1 mg/day) in saline were administered as single injections. Similarly, disulfiram, an inhibitor of the enzyme which converts dopamine to norepinephrine, and parachlorophenylalanine, an inhibitor of serotonin synthesis were administered to other groups of rats. All animals were observed for vaginal opening and subsequent ovulation as determined by the presence of corpora lutea. The time of sacrifice was at 50 days except in those animals treated with clomiphene which, because of very early vaginal opening, were sacrificed at 35 days of age.

Results and Discussion

Ontogeny of FSH and LH Synthesis, Storage and Release by the Pituitary. The cells of the anterior pituitaries of female rats possess all of the apparatus necessary for synthesizing and packaging the glycoproteins FSH and LH, as early as 5 to 10 days of age. This capacity is decreased in females at about 20 days of age for some reason. Male rats develop the storage capacity about 10 days later than the female, however, this persists throughout the age span studied. These data are illustrated in figures 1 and 2, which summarize data previously published on the pituitary storage of FSH and LH in the female and male, respectively (Kragt and Ganong, 1968; Matsuyama, Weisz and Lloyd, 1966). These data agree closely with morphological observations of Siperstein et al. (1954) who noted increasing granulation in the male pituitary as the animal matured and a decreasing glycoprotein content and degranulation as puberty approached in the female rat. Since it is possible to culture pituitary cell lines in vitro which secrete distinctly only FSH or LH, the source of these hormones physiologically (in vivo) is most probably from two separate cells (Steinberger and Chowdhury, 1971). The question arises as to the reason for an abrupt decline in storage of these hormones in the female after 20 days of age (especially FSH). The subsequent data on plasma levels will show that it is not due to an outpouring of hormone and depletion of content. A very rapid inhibition of release precedes the slower reduction in content. Two possibilities exist: synthesis of FSH may be totally inhibited and a slow release gradually decreases content to low storage levels, or both synthesis and release are inhibited and the biological activity is destroyed by an intracellular lysosomal mechanism similar to those previously described for prolactin cells (Smith and Farquhar, 1966). Estrogens have been shown to increase lysosomes and associated enzymes in the uterus (Smith and Henzl, 1969). It is probable that similar mechanisms may exist for the pituitary. Recent studies on the affect of ovariectomy on lysosomes in gonadotropin cells indicate that alkaline phosphatase is associated with the secretion granule formation (Smith, 1969). Estrogen administration
for 9 days to castrated females increased the number of dense-body type lysosomes. Proteolytic enzymes and gonadotropin inhibitors such as sialidases have been isolated from rat, ovine and bovine pituitaries (Talbert, DiPillo and Gordis, 1957; Woods and Simpson, 1961; Reichert, Gavin and Neill, 1971). The possibility that intermediates are involved in the negative feedback effect of estrogen on gonadotropin secretion is indicated by the fact that protein synthesis inhibitors (Schally et al., 1969) prevent this estrogen action. The 15-fold greater storage of FSH by the adult male pituitary as compared to that of the female is also of interest. Whatever testicular secretions are present at this time they do not destroy the packaging and storage processes. At this age, plasma levels of FSH in the male are also elevated so that the increased pituitary content is not due to suppression of release. Similar data has been published by Swerdloff et al. (1971).

In our female rats only minimal fluctuation in storage of FSH occurs throughout the estrous cycle. While LH may fluctuate three- to four-fold, FSH varies only by 50%. That these low levels of storage in the female are hormonally and not genetically regulated is indicated by the effect of ovariectomy at birth on pituitary FSH storage (Baker and Kragt, 1969).

The data presented in figure 3 indicate that concurrent with rapid synthesis and storage of FSH during the first 15 days of life of the female, rapid release of the hormone also takes place. This would suggest that hypothalamic

![Figure 3. FSH and LH in plasma from female rats.](image-url)
FRF which also decreases at this time (Kragt and Dahlgren, 1971) can stimulate both synthesis and release. This concept has also been suggested on the basis of reinitiation of synthesis of gonadotropins in pituitary transplants by the infusion of hypothalamic extract. The very rapid decrease in the release of the FSH which occurs at about 15 days is puzzling. No good explanation exists for this phenomenon. An inhibitory ovarian hormone is clearly probable. The gene responsible for synthesis of FSH is not repressed because synthesis continues and more hormone accumulates in the pituitary for a period of about 5 days. This separation of synthesis and release processes supports the existence of a follicle stimulating hormone synthesizing factor separate from the releasing factor (Corbin and Daniels, 1968). Circulatory levels of LH vary greatly in female animals at 15 days of age and it would appear that the optimal time for sacrifice may not have been chosen. The data do, however, allow one to conclude that FSH increases in the plasma prior to LH in prepuberal females.

By comparison, in the male (figure 4) FSH and LH are both slightly elevated between 10 and 20 days of age but never reach levels observed in the 15-day-old female (figure 3). As in the female, the period between 20 and 40 days of age is characterized by very low levels of both plasma FSH and LH. Again, FSH and LH decrease at the same rate and similar mechanisms seem to be functioning. However, this process is much slower than that observed in the female during the same time interval. At 40 days of age plasma FSH increases three-fold while LH increases less than two-fold. However, at 75 days LH increases much more sharply. In marked similarity to the female, circulating FSH increased prior to the increase in plasma LH although the interval differed between sexes.

Several workers have suggested that a shift in FSH/LH ratio may take place at the time of puberty in the rat and during the ovulatory surge in the adult female (Hoogstra and Paesi, 1955). This shift is evident in these data as well (figure 3). The FSH levels measured in the prepubertal animal were two times the LH values and 13 times those of basal 20-day values. In marked contrast, during the adult cycle LH values during proestrus were three times the FSH
values and 15 times those of basal 20-day values.

The elevation of plasma LH on the morning of diestrus has not been reported by other laboratories. However, all of those observations were made on 5-day or 4- to 5-day cycling animals or retired breeders. This difference may explain the mechanism for short 4-day cycles. Two peaks of progesterone and pregnenolone have also been observed in ovarian venous effluent (Hashimoto et al., 1968). Furthermore, it is possible to make 4-day cycling rats out of 5-day cycling rats by injections of estrogen on diestrus (Everett, 1961). This has been recently extended by Weick et al. (1971) who demonstrated that intrapituitary implants of estrogen could do the same. These effects are most probably associated with estrogen-induced LH release.

The greater gonadotropin content of prepuberal gilt pituitaries than those of adult females has been attributed to a greater amount of FSH (Hollandbeck et al., 1956; Parlow, Anderson and Melampy, 1964). Very comprehensive studies have also been carried out by Hafs and coworkers on the development of reproductive function in cattle. Macmillan and Hafs (1969) concluded that puberty was not associated with any marked change in body growth in bulls and suggest that puberty is dependent upon both FSH and LH. Desjardins and Hafs (1968) observed that FSH levels in the pituitary of the heifer peak at 1 month then decline and remain low. This was followed by a

---

### TABLE 1. EFFECT OF BILATERAL OVARIECTOMY ON PLASMA FSH AND LH LEVELS IN FEMALE RATS

<table>
<thead>
<tr>
<th>Age at ovarietomy</th>
<th>Age at sacrifice</th>
<th>No. of animals</th>
<th>Intact FSH (ng/ml)</th>
<th>LH (ng/ml)</th>
<th>No. of animals</th>
<th>Ovariectomized FSH (ng/ml)</th>
<th>LH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>5</td>
<td>8</td>
<td>452±36</td>
<td>143±11</td>
<td>4</td>
<td>432±25</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>9</td>
<td>868±81</td>
<td>234±47</td>
<td>9</td>
<td>1699±191</td>
<td>320±34</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>5</td>
<td>1233±68</td>
<td>792±92</td>
<td>5</td>
<td>1941±71</td>
<td>379±42</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>4</td>
<td>1388±8</td>
<td>188±8</td>
<td>4</td>
<td>530±137</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>5</td>
<td>561±66</td>
<td>222±7</td>
<td>10</td>
<td>2379±208</td>
<td>574±62</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>4</td>
<td>266±53</td>
<td>247±22</td>
<td>5</td>
<td>1871±114</td>
<td>414±48</td>
</tr>
</tbody>
</table>

---

**Figure 5.** FSH and LH in plasma from female rats ovariectomized at 7 days of age.
rapid increase in LH between 2 and 6 months of age just prior to puberty which in these animals extended from 7 to 10 months of age (Desjardins and Hafs, 1969). These data suggest that similar changes in pituitary gonadotropin storage occur in pigs, cattle and rats.

Effect of Ovariectomy at Different Ages on Plasma FSH and LH. Table 1 presents the data on the effects of ovariectomy at ages between birth and 20 days on plasma FSH and LH 5 to 10 days later. The following general statements can be made about the data: (1) no inhibitory feedback was demonstrated for either hormone between birth and 5 days of age; (2) FSH levels increase following ovariectomy to a greater degree and at an earlier age than do LH levels; (3) LH values at 15 days of age are highly variable in the intact animal; (4) ovariectomy on day 7 eliminated the LH peaks observed at 15 days and plasma LH levels stabilized at values about 2 to 3 times baseline; (5) plasma FSH values stabilized at about 8 to 10 times baseline values observed at 20 to 25 days of age. Figure 5 summarizes the effect of ovariectomy at 7 days of age on plasma FSH and LH at varying time intervals thereafter and compares it to values for intact animals and to animals ovariectomized at 8 days of age given estradiol benzoate each day. The conclusions discussed above are again evident and the additional values obtained by sacrificing animals at two day intervals rather than at 5- to 10-day intervals strengthens those conclusions.

Results from animals ovariectomized at 20 days of age are summarized in figure 6. Plasma FSH increased to a greater degree and at a faster rate after surgery at this age than at younger ages. LH reached levels about five times intact values a week after ovariectomy and declined thereafter. FSH exhibited a very pronounced rapid increase 1 day post-operation. This was followed by a decline to lower values and a second increase (days 31 to 32) to even higher levels. The mechanisms causing this multiphasic response of FSH are totally unknown. One can speculate that the initial rapid increase is due to withdrawal of steroid hormones having a negative feedback on the release mechanisms. The decline may be due to the postulated short-loop feedback regulatory mechanism and the secondary increase may reflect resumption of high levels of FSH synthesis in the pituitary after steroid withdrawal. All of this remains highly conjectural until tested further. It is interesting to note that the relatively elevated LH levels coincide with the relatively low FSH values at 29 days of age.

Comparison of these data with those obtained by Gay and Midgley (1969) and Blackwell and Amoss (1971) in adult females indicates that plasma LH also increases at a very slow rate post-ovariectomy in adult rats and that the rate is independent of the stage of the cycle at the time of surgery. FSH data from our laboratory indicate that plasma FSH is elevated 9 days post-ovariectomy to levels similar to those of the immature 20-day-old rat ovariectomized for the same period of time; however, daily sampling has not yet been undertaken.

From the data in the literature and the data presented, it appears that distinct differences exist in the rate of LH increase after ovariectomy at the many ages studied. Before 15 days, the ovaries contribute little negative feedback. Thereafter, such effects are demonstrable. The FSH data would suggest the same. The possibility of a biphasic response as observed in 20-day-old animals needs to be explored in adult females as well. Similarly, the rate of increase in circulating FSH after ovariectomy of the heifer was greater than that of LH (Swanson et al., 1971). Negative feedback has been demonstrated in prepuberal gilts (Dailey et al., 1970) and in the prepuberal ewe (Foster et al., 1971).

Effect of Ovariectomy and Estradiol Benzoate on Plasma FSH and LH. Chronic injections of 1 µg of estradiol benzoate per 100 g body weight of females ovariectomized at 8 days of age decreased plasma LH and FSH to below levels observed in intact females (figure 5). These data agree with those of the effects of ovariectomy which indicate that negative feedback from the ovary exists for FSH at that age and further indicate that the hypothalamic-pituitary system responsive to negative feed-
back of estradiol is also functional. However, the actual substance circulating at this age which is physiologically acting to suppress FSH secretion remains to be determined. The secretion from the ovary, which is responsible for the LH release at 15 days, is also unknown.

In animals 27 days of age a single injection of estradiol benzoate, if given in the proper dosage, can increase circulating levels of LH eight- to 10-fold 2 days later. In these experiments (table 2) only the 5 µg dosage was effective. FSH secretion was not affected to the same degree as that of LH. This change in secretion is reminiscent of the change seen at the time of the ovulatory surge in intact animals.

A marked contrast exists between the immature and adult ovariectomized female (table 3). It was not possible to demonstrate either a positive or a negative effect of a single dose of estradiol in the adult over the same range as that administered to immature females. However, the degree of uterine ballooning that was observed in animals treated with the highest dosage was not as great as that observed in intact females of this strain at proestrus. It is probable that higher dosages may demonstrate this positive effect.

Nonetheless, it is very evident that a change takes place in that mechanism which is responding "positively" to estrogen between 20 to 40 and 75 to 85 days of age or immediately prepuberally versus postpuberally. This mechanism relates more to the release of LH than to FSH. The possibility exists that this effect is due to either the withdrawal of steroid negative feedback or to the presence of a positive stimulatory action of the estrogen. Circulating levels of the estradiol have not been determined yet so therefore no definite conclusions can be drawn.

**TABLE 2. EFFECT OF ESTRADIOL BENZOATE (EB) ON PLASMA FSH AND LH**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage µg/100 g B.W.</th>
<th>FSH (ng/ml)</th>
<th>LH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>0</td>
<td>177±7</td>
<td>139±24</td>
</tr>
<tr>
<td></td>
<td>265±20</td>
<td>144±10</td>
<td></td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>0</td>
<td>1295±84</td>
<td>800±148</td>
</tr>
<tr>
<td></td>
<td>1509±112</td>
<td>617±50</td>
<td></td>
</tr>
<tr>
<td>Ovariectomy + EB</td>
<td>0.05</td>
<td>1493±40</td>
<td>471±65</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>1346±120</td>
<td>361±95</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1090±85</td>
<td>318±63</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1594±124</td>
<td>788±103</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>1659±195</td>
<td>1387±295</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>986±86</td>
<td>330±57</td>
</tr>
</tbody>
</table>

**Age at:**
- Ovariectomy: 20 days
- E.B. injection: 27 days
- Sacrifice: 29 days

However, Ramirez and McCann (1963) have administered low levels of estradiol chronically over this time period and ovulation has occurred. This would argue that the continued presence of a critical level of estrogen is necessary for the acute "dumping" of a large quantity of LH.

The question arises as to where this estrogen is acting. Abundant evidence exists indicating that labeled estradiol is taken up in large quantities by the pituitary and also to a lesser but yet significant extent by the hypothalamus (Alvarez and Ramirez, 1970; Woolley, Holinka and Timiras, 1969). Smith and Davidson (1968) have argued that this action is specifically exerted on the anterior hypothalamus since "estrodos" implanted into this region for short periods of time could induce precocious puberty. Furthermore, only this area has been shown to fluctuate in its affinity for estradiol during the estrous cycle (Kato and Villee, 1967). The stimulatory effect of estrogen on the release of LH has been demonstrated in the sheep (Goding et al., 1969), monkey (Yamaji et al., 1971) and rat. Similarly, a peak in plasma estradiol immediately precedes the ovulatory surge of LH in these species.

The age-related variation in estrogen's stimulatory effect on LH cannot be explained on the basis of differences in plasma binding of estradiol since Ramaley (1970) has shown this to be relatively constant throughout the ages studied in this series. The ability of the liver to catabolize estrogens does change with age, but the adult capacity is reached by 30 days of age. The argument that this can account for differential uptakes at about 5 to 10 days of age but not at 30 days of age could be strengthened if the plasma half-lives for estradiol were known. A primary sex difference in the rate of release of LH post-castration has been reported and is greater in males than in females (Blackwell and Amoss, 1971).
First ovulation could also be associated with increasing responsiveness of the estrogenized pituitary to LRF as well as to the increased neuronal activity due to estrogen action. Although Antunes-Rodrigues, Dhariwal and McCann (1966) have reported that the pituitary of the rat does not change in sensitivity to LRF during the estrous cycle, another laboratory has shown that the estrogen primed ovariectomized rat is more sensitive to LRF than the untreated ovariectomized rat (Arimura and Schally, 1971). The sheep pituitary has also been observed to be more responsive to LRF during estrus than during anestrus (Reeves, Arimura and Schally, 1971). In vitro incubation of pituitaries with estrogen increases LH release (Piacsek and Meites, 1966). D. T. Armstrong (personal communication) has recently observed that estrogen induces the production of 5-alpha-reductase in anterior pituitaries. This enzyme rapidly converts progesterone to the 5-alpha derivative and may alter biological activity of the progesterone.

Effect of Various Hormones and Drugs on Puberty Onset. In our studies, chronic progesterone prevented ovulation in a large percentage of the pubertal animals. The antiestrogen chlormiphene at both dosages markedly hastened vaginal opening, however, this event was not associated with ovulation in any of the animals.

Drugs which alter catecholamine storage in the hypothalamus do not affect the onset of puberty but all initiate pseudopregnancy immediately following first ovulation suggesting that catecholamines influence prolactin more than gonadotropins. The effects of these drugs in adult animals may be mediated through prolactin release followed by inhibition of gonadotropin secretion. Parachlorophenylalanine successfully inhibited the onset of vaginal opening and also dissociated vaginal opening and first ovulation by at least 4 days.

Evidence is accumulating from a great number of laboratories to implicate dopamine (DA) norepinephrine (NE) and serotonin in the release of the ovulatory surge of LH in rats. However, the present evidence does not allow one to determine the precise roles for each substance. Complete surgical deafferentiation of the hypothalamus leads to abolition of cyclicity, constant estrus, depletion of NE, retention of median eminence DA terminals, and tonic, possibly slightly elevated, secretion of FSH and LH (Fuxe and Hökfelt, 1970). The data of Schneider and McCann (1969) indicate that DA is a potent releaser of LRF and FRF and an inhibitor of PIF secretion. It will exert these actions when incubated in vitro with hypothalamic fragments, or if infused into the third ventricle, but will not release FSH and LH if infused directly into the pituitary (Kamberi, Schneider and McCann, 1970). Similarly, DA has no effect on the release of LH or FSH when incubated together with pituitaries in vitro. However, at very large concentrations, when polyamines are formed, the biological activity of FSH is destroyed (Van Loon and Kragt, 1970). This is of interest when one considers the observations that certain polyamines can deplete pituitary FSH in vivo but not induce a release in vitro. Since the pituitary can take up dopamine and metabolize it, polyamines may be a component of lysosomal systems for degradation of pituitary hormone stores.

It is also of interest to note that Björklund et al. (1970) have demonstrated the existence of DA neurons which enter the median eminence from each lateral side close to the brain surface.

### TABLE 4. EFFECT OF VARIOUS HORMONES AND DRUGS ON PUBERTY ONSET

<table>
<thead>
<tr>
<th>Drug</th>
<th>Age at vaginal opening</th>
<th>Ovulation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>progesterone (1mg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>39.6±1.3</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>clomiphene 1 mg</td>
<td>38.9±1.4</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>25.0±0.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>clomiphene .1 mg</td>
<td>25.0±0.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>38.5±1.2</td>
<td>100</td>
<td>pseudopregnant</td>
</tr>
<tr>
<td>reserpine (pair fed)</td>
<td>40.3± .7</td>
<td>100</td>
<td>pseudopregnant</td>
</tr>
<tr>
<td>control</td>
<td>41.3±1.3</td>
<td>100</td>
<td>pseudopregnant</td>
</tr>
<tr>
<td>disulfiram</td>
<td>39.1±1.3</td>
<td>100</td>
<td>pseudopregnant</td>
</tr>
<tr>
<td>control</td>
<td>35.9±0.6</td>
<td>100</td>
<td>pseudopregnant</td>
</tr>
<tr>
<td>alpha-methyl-p-tyrosine</td>
<td>35.1± .9</td>
<td>100</td>
<td>pseudopregnant</td>
</tr>
<tr>
<td>control</td>
<td>35.8± .6</td>
<td>100</td>
<td>pseudopregnant</td>
</tr>
<tr>
<td>p-chlorophenylalanine</td>
<td>43.4± .5</td>
<td>0</td>
<td>vaginal opening separated</td>
</tr>
<tr>
<td>control</td>
<td>36.0± .6</td>
<td>90</td>
<td>from ovulation</td>
</tr>
</tbody>
</table>
Lesions which destroy this area when placed in prepuberal animals induce precocious puberty (Donovan and Van der Werff Ten Bosch, 1965). It is possible that adrenergic (NE) fibers carrying the input from the optic tract act to partially inhibit the release of DA since constant light induces an intermediate amount of turnover (equivalent to diestrus) or activity of DA in the arcuate nucleus (Fuxe and Hökfelt, 1970) and an intermediate FSH and LH release (Daane and Parlow, 1971).

During pseudopregnancy, pregnancy, and lactation the anterior hypothalamic nucleus, also dopaminergic, has an increased DA turnover, suggesting that this neural component inhibits the release of FSH, LH, and prolactin since secretion of all three hormones is markedly decreased during pseudopregnancy (Voogt, Chen and Meites, 1970).

When considering the mechanisms controlling the onset of puberty, it is necessary to also consider the action of the pituitary hormones on the gonad. Both the male and female will be discussed with regard to the action of these hormones on gonadal steroidogenesis (see suggested summary in figure 6). Lostroh and Johnson (1966) have emphasized the need for both FSH and LH to initiate sexual maturity in male and female rats. It is not possible to initiate estrogen or testosterone release from the gonad of hypophysectomized female or male rats with either FSH or LH alone. After FSH treatment, LH can markedly stimulate steroidogenesis. It is interesting to note that in both the female and male, FSH release is markedly elevated just prior to LH release. In the male at 40 days of age the increase coincides with the secretion of large quantities of testosterone (Knorr, Vanha-Perttula and Lipsett, 1970). There is a shift in adrostenedione:testosterone secretion ratio at this time in the male rat (Inano, Hori and Tamaoki, 1967). A similar shift has been reported to occur in the young bull calf (Lindner and Mann, 1960). LH release immediately thereafter may also increase testosterone production by increasing cholesterol conversion to progesterone. It is also being suggested that prolactin may increase steroidogenesis along this pathway by inhibiting the conversion of progesterone to 20-alpha-hydroxyprogesterone, a step which does not lead to testosterone synthesis.

In the adult rat ovary progesterone may be converted to either dehydroepiandrosterone or 17-alpha-hydroxyprogesterone prior to androstenedione formation (Kalvert and Bloch, 1968). Addition in vitro of FSH to slices of rat ovaries promoted the release of estrogens into the medium as did the addition of androstenedione (Yuhara, Cohen and Frieden, 1963). Therefore, FSH may promote testosterone formation which can serve as precursor for estradiol formation in the female. Ovaries possessing only follicles may produce considerable amounts of estradiol and testosterone as demonstrated by Weisz and Lloyd (1965) in androgenized anovulatory females. In the immature female this conversion of testosterone to estrone may not occur since uterine development during the period between 15 and 35 days of age indicates only minimal estrogen secretion. However, androgen production may be rather high during this time period since the prostate gland in female rats of those strains which possess it show maximal development at this age. The only other times during reproduction when the prostate hypertrophies are during pregnancy and lactation. It is interesting to speculate that these periods of anovulation, as reviewed by Rothchild (1966), may relate to an androgen-induced inhibition of the pituitary. Skinner, Mann and Rowson (1968) have suggested that androstenedione may be a more potent inhibitor of LH release than testosterone. Polyanions containing amino sugars have been shown to influence enzyme activity in many systems (Bernfeld, 1966) and FSH may activate the enzyme necessary for converting androstenedione to testosterone and estradiol. Estradiol may act as that local mitotic stimulatory hormone to promote follicular development and granulosa cell proliferation to further augment steroidogenesis. It is probable that androgens secreted in response to the FSH act

![Figure 7. Hypothetical sites of action of FSH, LH and prolactin on steroidogenesis.](image-url)
to trigger some LH release since low doses of androgens can exert a positive feedback on LH secretion in the male rat (Bloch, Kragt and Masken, 1971). This feedback appears to be too minor to cause LH to reach ovulatory levels.

A critical time period within each day exists for administration of PMS in order to induce ovulation at 20 to 30 days of age suggesting that neural substrates conveying environmental signals for diurnal sensitivity are also functional. Johnson and Naqvi (1969) have suggested that androgens “unmask” a 24-hr. periodicity in FSH secretion at this age. However, the low plasma levels of both LH and FSH at this time do not support the concept. Several workers have suggested that estrogens at this age promote generalized brain development. Our data would suggest that whatever is being secreted at this time, it is inducing maturation of a very potent inhibitory mechanism. Androgens have been shown to markedly decrease prolactin content of the pituitary. It is probable that these two mechanisms are linked in some fashion.

The Role of Prolactin in Puberty Onset.

The influence of prolactin will be considered here to briefly illustrate its probable role in the generalized hypothesis for puberty onset. Marked increases in plasma prolactin occur the day prior to vaginal opening and on the afternoon of proestrus in adult animals. Prolactin induced marked uterine ballooning and ovulation in immature females treated systemically or with intrahypothalamic implants (Voogt et al., 1970; Lyons, Simpson and Evans, 1941). Prolactin may act to promote follicular estrogen secretion by preventing the conversion of progesterone to 20-alpha-hydroxysteroid; this may also account for the reported luteolytic actions of the hormone (Malven, 1969). Luteal cells of the rat do not produce estrogens and the luteotropic action of prolactin may then be to raise the progesterone levels sufficiently to inhibit LH and FSH release, by inhibiting the 20-alpha-OH SDH enzyme of the corpora lutea.

Considerable evidence exists indicating that the limbic system is involved in regulation of puberty onset via prolactin-FSH-LH release. The limbic system is very much involved in stress responses. It has long been known that many stresses could induce prolactin release. Pathways associated with olfactory pathways course through the limbic system and have been correlated with alterations in prolactin secretion. Exogenous prolactin hastens puberty in the rat. However, no data have been reported on the effect of amygdaloid or limbic lesions on prolactin secretion. It has, however, been reported that estrogen implants into the amygdala induce prolactin release (Tindal, 1966). Relkin (1971) has reported that amygdaloid lesions placed at 5 days of age delay the onset of puberty, while other labs report that similar lesions at 20 days of age or later stimulate the onset. Since deafferentiation decreases NE stores and not DA stores in the median eminence, it is probable that pathways (NE) from the limbic system are tonically inhibitory.

Summary

A Generalized Hypothesis for Puberty Onset. Puberty is characterized by a “cascade” or a long series of inductive events which culminate in the ability to reproduce. A series of positive stimulatory feedback loops appear to exist prepuberally and become progressively more functional with age. These feedback loops transfer and amplify the original input signal to reach maximum oscillation, finally culminated by a cataclysmic event (progesterone secretion) which interrupts all or most of the “positive” feedback loops (figure 8).

A neural signal, mediated by hypothalamic amines, initiates these events by increasing the release of FSHRF to induce the synthesis and release of FSH in both sexes. In both sexes, FSH acts to sensitize the gonad to LH by perhaps activating the enzyme responsible for conversion of androstenedione to testosterone. In males, LH potentiates the testosterone production by providing additional progesterone precursor. In females, significant estrogens are produced only after prolactin and LH release and this does not occur for some time (20 days). Since no corpora are present at this age, androgen production must be present and may be the reason for anovulation. Androgens produced act positively to release slightly more LH. LH acts to promote more estrogens. Estrogens increase the responsiveness of the ovary to LH and promote more FSH and prolactin re-

![Figure 8. Hypothetical diagram of positive (+) feedback loops.](image-url)
lease by acting on pituitary, hypothalamic and limbic systems. These hormones, in turn, promote further estrogen secretion which eventually leads to a "quantal" discharge of LRF and LH sufficient for ovulation and accessory organ development needed for reproduction.

**Literature Cited**


Hafez, E. S. E. 1952. Studies on the breeding season and reproduction of the ewe. I. The breeding season in different environments. II. The breeding season in one locality. J. Agr. Sci. 42:189.


Hoogstra, M. J. and F. J. A. Paesi. 1955. A comparison between the FSH- and ICSH-contents of the hy-


Kalvert, M. and E. Bloch. 1968. Conversion of 4'-14C-dehydroepiandrosterone to estrone and 17β-estradiol by the rat ovary with observations on variations during the estrous cycle. Endocrinol. 82:103-121.


role of dopamine as transmitter to promote dis­

function in the regulation of the secretory process


Talbert, G. B., F. DiPillo and L. Gordis. 1957. Antago-

nistic action by FSH on ovarian stimulation pro-

duced by rat pituitary gonadotrophin. Endocrinol. 61:611.


DISCUSSION

Question: P. V. Malven

Is this positive feedback system that you are proposing unique to the prepuberal animal?

C. L. Kragt: From the data that we have, a very conservative approach is to say we have only demonstrated it in the prepuberal animal. It has been demonstrated in other species postpuberally as well. However, we do have enough data to indicate that there is clearly a difference in responsiveness of the element prepuberally. Whether it is existent prepuberally and not postpuberally can not be stated in an absolute sense. That conclusion from the data that we have would be too broad. We can simply say, as Ramirez and others have said for the negative component, that there is a difference in responsiveness in prepuberal as compared to postpuberal animals and only further work can provide such specific answers.

Question: D. M. Nelson

Dr. Kragt, I certainly enjoyed your paper. My questions relate to the information on the slides that you gave us on clomiphene. What was the age of the female rat pup at the time you injected her?

C. L. Kragt: We started at 20 days of age as in all of the protocols that we showed on that particular slide. We carried all animals through 50 days. We were hoping to demonstrate effects on vaginal opening which usually occur around 37 to 38 days of age. In that particular experiment, because the animals did exhibit open vaginas at 25 days of age or immediately following the injection of clomiphene we laparotomized certain groups of animals the day that they showed vaginal opening. Therefore, the time duration for treatment was approximately 5 days in that series.

Question: D. M. Nelson

Do you have plasma levels of the gonadotropins on the females shortly after clomiphene injection?

C. L. Kragt: We do not. It would be a very interesting point and a very necessary study to undertake in order to draw conclusions about the actions of clomiphene. The actions of clomiphene are multiphasic in the rat as it relates to dosage and therefore we would definitely have to study this substance in a similar manner to that which we used for estradiol in order to pinpoint the mechanism of action of clomiphene. With the one or two dosages that we used, it's just insufficient data to draw a good conclusion. If we could give a spectrum of maybe 8 to 10 dosages, then I think I could say something more definite.

Question: D. M. Nelson

One further question. Some people feel that there may be an augmentive effect pertaining to release of LH with progesterone in addition to estrogen. Are you contemplating research in this particular area?

C. L. Kragt: Yes. Very definitely. Historically Dr. Everett and others have proposed that progesterone acts on a neural mechanism to initiate the ovulatory surge of LH. Currently, the bulk of the data places that hypothesis in a secondary position. We want to administer progesterone over dosage ranges of the magnitude that we have used for estrogen. We haven't done so. There is evidence in the literature relative to progesterone, however, from several laboratories. Weick and Davidson have recently published data on the influence of progesterone implants in the pituitary and hypothalamus on circulating levels of gonadotropins.

Question: W. C. Foote

I was interested in the plasma levels of FSH and LH shown in the prepuberal female rat. I was wondering if you have any information on the ovarian activity during this same period. With these high circulating levels of FSH and LH, one might expect an increase in follicular activity. At least with the species of animals I am most familiar with, treatment with exogenous gonadotropins induced increased follicular activity.

C. L. Kragt: The evidence in the literature for the rat indicates that the 15-day period is a very critical period in the ontogeny of the rat. If you administer exogenous gonadotropin to immature female rats before this period, they will not respond. After 15 days they respond as assessed by depletion of cholesterol from the ovary or by ovulation. Also, after 15 days of age you can induce ovulation with exogenous estrogen as a single dose, as demonstrated by Höllweg; and with HCG; or HCG-PMS combinations. We like to
use that evidence first of all to suggest that the positive element which we are observing in the 20 day old animal is the net result of this surge of FSH that is released into the plasma at around 15 days of age. We do know that LH at this time also influences those enzymes involved with steroidogenesis. It is possible to induce the production of certain dehydrogenases with exogenous LH. There are changes in the amounts of dehydrogenases present in the ovary before and after 15 days of age. So we feel that this surge of LH and FSH, predominantly FSH, serves as both a biochemical and anatomical differentiator leading to the final capability for the ovary to respond to a subsequent surge of LH for ovulation.

We like to suggest that this surge is the initiating component for puberty but we leave open the possibility that it is a mechanism related to sexual differentiation rather than puberty onset in the female.

I would like to add one comment as it relates predominantly to farm animals and domestic species. The work of Dr. Nalbandov and colleagues, and Parlow and Melampy, indicates that in swine the storage of FSH and LH before puberty onset is much greater than after puberty onset. The same can be said for cattle as shown by Dejardins and Hafs. The series of studies by Dr. Hafs is excellent. They also demonstrated the presence of gonadotropins in the pituitary of both the heifer and the bull calf before puberty. So I believe the precedent exists in many species that these hormones are present before puberty. I think that in the rat, with which I am most familiar, the surge at 15 days of age is an initiating differentiating mechanism. Does that answer your question, in part?

Question: W. C. Foote

Thank you. You reminded me of one other thing you might comment on. You mentioned the early differentiation effect in the female. There is considerable work I believe to show that the hypothalamus, particularly in the rat, does undergo differentiation into male and female function and that this is usually completed by day 10. You mentioned the similarity in your data between the male and the female and did not differentiate between them in the hypothesis that you are developing. Do you see any connection or any problem here with the differentiation into the male and female hypothalamus and the hypothesis that you have mentioned?

C. L. Kragt: Obviously, I haven't thought about all of these factors but what I can say is the following: I do think that clearly there are always problems in trying to tease apart endocrine mechanisms. I don't think the data are inconsistent thus far with either of the two proposals. At the latest meetings in Munich, Dr. Ladosky, who has done some work on the differentiation of brain, presented data on the role of serotonin in sexual differentiation. At that particular meeting last week he stepped up after my presentation and mentioned that he felt that there may be some relationship between the gonadotropins and serotonin. But clearly I don't see any inconsistency thus far between male and female sexual differentiation and the data that we have. I believe that conservatively one should say that there is clearly evidence to support the concept that FSH and LH at this time are acting in a sexual differentiation fashion in the female.

Question: T. G. Dunn

I was interested in your slides showing the effect of estradiol administration to the older rats at low dose levels. Have you examined the effects of these low dose levels immediately following injection? In other words, you waited two days and then quantitated LH and FSH. What effect does the low dose level have within say 4 or 5 hours?

C. L. Kragt: That's a very excellent question and clearly something we haven't done. You've touched upon a concept which I think is very important in the developing area of these studies. There appears to be multiple pools of releasable gonadotropins. The evidence is very strong as it relates to prolactin. There is a pool with a very rapid turnover rate and one with a very slow rate. One has a rate constant of minutes and the other a rate of hours or days. I think we can only say that the effects that we have observed relate to something that is very long-termed in nature and it may not relate at all to a very acute rapid release in response to circulating levels of the estrogen. We do believe that it is due to the presence of the estrogen and not to estrogen withdrawal. We have not actually measured the plasma levels of the estrogen over this time period. It's necessary, and we're trying to develop the assay for estrogen.

Question: J. Reeves

Almost similar to the last question. How did
you arrive at collecting your samples 48 hr. after estrogen treatment in contrast to a 12 to 20 hr. span?

**C. L. Kragt:** What we did was to conduct an initial pilot study to determine the effect of one dosage level of estradiol over several time periods after the administration of the substance to ovariectomized females. We have found that the maximal effect on release rate of LH in response to this particular series occurred at that time interval. Physiologically, the ovulatory surge of LH in the rat occurs about 2 days after the increase in plasma estradiol.

**Question: J. Reeves**

Would you expect any different results if you had collected at 20 hr. in contrast to 48 hours?

**C. L. Kragt:** I imagine there would have been, because this is a little bit like looking through a window at a dynamic event which is passing before you. We may see only one component of what may be a biphasic effect and we may be looking at one component. Experiments become very complex when trying to demonstrate what is happening dynamically.

**Question: I. Geschwind**

You may know that in the human fetus there's a peak of gonadotropin levels at just about 5 months, as shown by Selna Kaplan, and this level is comparable to that seen in a post-menopausal woman and is really quite large. I wonder whether the peak you see in the 15 day old rat is equivalent to this and really has a differentiative function of some sort rather than one concerned with vaginal opening or puberty.

**C. L. Kragt:** I think this is a very distinct possi-

bility. I am quite aware of the results of Dr. Kaplan and Dr. Grumbach. I think that we clearly must leave open the possibility that this is the primary function of that 15 day surge. I'd like to state it this way right at the moment; I believe the surge relates to the onset of the ability of the female to respond periodically with ovulatory surges of LH. I do think that it is very definitely related to the onset of reproduction in the female rat. Whether it relates very specifically to that acute event which takes place at the time of first ovulation I do not know. I think it may be more related to events long prior to first ovulation. The initiating component for the first ovulation I think very definitely is that ovulatory surge of LH. The shift that occurs in gonadotropin secretion between 15 days of age and 75 days of age in the female is interesting. A marked dominance of FSH over LH exists at 15 days of age. Then as you progress through time and the animal develops, the ovulatory surge results in an LH dominance of at least 10 fold over FSH. And I think this theme has been consistent throughout the literature for many years. That component related to first ovulation, the onset of puberty, may very well be the shift in dominance of LH over FSH.

**Question: V. L. Estergreen**

Do you have any direct evidence for the stimulation of the transformation of androstenedione to testosterone by FSH as you showed in your slides?

**C. L. Kragt:** No. I want to emphasize this point. I'm only using that slide for a bit of speculation to stimulate discussion. We have no data. I'm not a steroid biochemist but I like to read the literature and make suggestions but I currently don't have any data from our laboratory to back it up at all.