

# Alterations in Pituitary Follicle-Stimulating Hormone (FSH) and Hypothalamic FSH-Releasing Factor (FSH-RF) during Puberty<sup>1</sup>

(34179)

S. WATANABE<sup>2</sup> AND S. M. McCANN<sup>3</sup>

Department of Physiology, University of Texas Southwestern Medical School, Dallas, Texas 75235

Puberty has long been thought to be initiated by increased gonadotropin secretion by the adenohypophysis (1, 2). Recently, this event was shown to be associated with a decline in pituitary luteinizing hormone (LH) concentration, a rise in plasma LH, and a fall in the hypothalamic content of LH-RF (3, 4). Apparently, increased release of LH-RF by the hypothalamus initiates the increased discharge of LH. The present investigation was undertaken to determine if parallel changes in the secretion of FSH take place at puberty as well.

**Methods.** Immature female albino rats of the Holtzman strain (Holtzman Co., Madison, Wisc.), 21–23 days of age upon arrival at the laboratory, were used both for observation of puberty and assay of FSH. Male rats of the Sprague-Dawley strain (Simonsen Co., California) weighing 180–240 g were employed as donors of anterior pituitaries for incubation to measure FSH-RF.

Immature rats were caged in groups of 6 in an air-conditioned room with controlled lighting (14 hr of light and 10 hr of darkness), and supplied with Purina laboratory chow and water *ad libitum*.

From 25 to 41 days of age, at intervals of several days, 3–10 rats from each group were weighed and killed by decapitation. Daily vaginal smears were determined in animals

with open vaginas. Near the time of expected puberty, immature rats were inspected at least twice daily for vaginal opening. At sacrifice experimental rats were assigned to 5 groups as follows: (i) Rats with closed vaginas and nonactivated uterus (anestrous type D). (ii) Rats with vaginas about to open, and ballooned uteri (proestrous type B). (iii) Rats with freshly opened vaginas and ballooned uteri (proestrous type B). (iv) Rats with freshly opened vaginas but uteri not ballooned (estrous or diestrous type NB). (v) Rats 1 day after vaginal opening with estrous vaginal smear. This classification of experimental animals in various phases of puberty is similar to that of Ramirez and Sawyer (4).

At sacrifice a portion of the hypothalamus, including mainly the pituitary stalk and median eminence (SME) was removed and frozen immediately on dry ice for measurement of FSH-RF. The anterior pituitary was removed and weighed to the nearest 0.01 mg on a torsion balance and also frozen on dry ice for determination of pituitary FSH. After inspection of uterine and vaginal state, hypothalami and pituitaries were pooled from each group of rats according to the above criteria. Weights of sex accessories were also determined after removal of the anterior pituitary and the stalk–median eminence.

The hypothalamic extracts were assayed for FSH-releasing activity *in vitro* at doses of 1/8 hypothalamic equivalent/pituitary. The method of incubation performed here has been described elsewhere (5). In order to compare the FSH-releasing activity of hypothalamic extracts of rats of different ages at the same time, the FSH-releasing activity in one control hypothalamic extract was com-

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<sup>2</sup> Population Council Fellow. Present address: 430 Yoshida-Machi, Nishikanbaragun, Niigata Prefecture, Japan.

<sup>3</sup> Please address all reprint requests to: Dr. S. M. McCann, Department of Physiology, Southwestern Medical School, Dallas, Texas 75235.

TABLE I. Changes in Pituitary FSH in Prepubertal Rats.

Exptl. group of animals	Assay no.	Age at sacrifice (days)	No. of donors	Pituitary FSH concentration ( $\mu\text{g}/\text{mg}$ )	Pituitary FSH content ( $\mu\text{g}/\text{pit.}$ )	$\lambda$
1	1	28	7	39.81 (33.73–46.99)	103.2 (87.4–121.7)	.067
		31	7	28.51 (24.49–33.19)	80.1 (68.8– 93.3)	.062
		34	7	23.77 (20.65–27.35)	80.6 (70.2– 92.8)	.057
		34	7	24.04 (20.99–27.54)	75.2 (65.7– 86.2)	.056
		35	7	21.63 (18.88–24.77)	75.3 (65.7– 86.2)	.055
2	3	25	10	38.10 (28.90–50.58)	84.2 (63.4–111.8)	.106
		28	10	21.32 (16.29–27.92)	60.8 (46.4– 79.6)	.102
		35	10	14.35 (11.09–18.58)	46.8 (36.2– 60.6)	.097

pared with that in several experimental samples.

The incubation media were assayed for their FSH concentration by the method of Steelman and Pohley (6) as modified by Parlow and Reichert (7). Three-point assays were performed with two doses (50 and 150  $\mu\text{g}/\text{rat}$ ) of NIH-FSH ovine S-3 standard<sup>3</sup> and 1 dose of unknown. Pituitaries of rats at various ages were similarly assayed. The results were calculated according to standard statistical procedures (8) and expressed as  $\mu\text{g}$  of FSH/mg of pituitary.

*Results. Changes in anterior pituitary FSH concentration and content prior to and at puberty.* Pituitary FSH was measured initially in 2 separate experiments in animals which were 25–35 days of age. There was a marked decline in both pituitary FSH concentration and content during this time interval (Table I, Fig. 1). For unexplained reasons the levels were consistently higher in Expt. 1 than in Expt. 2, but the decrease with time was similar in both.

Attempts were made in these experiments to measure any change in FSH at the time of puberty, but the dose of pituitary extract administered was too low (assay 1–3), and the values for ovarian weight were less than those obtained with the lower dose of FSH standard resulting in invalid assays. Consequently, the experiment was repeated and a higher dose (5 mg) of pituitary extract was given. In this experiment values for ovarian weight that lay between those obtained with the 2 doses of standard (Table II) were observed which made it possible to calculate

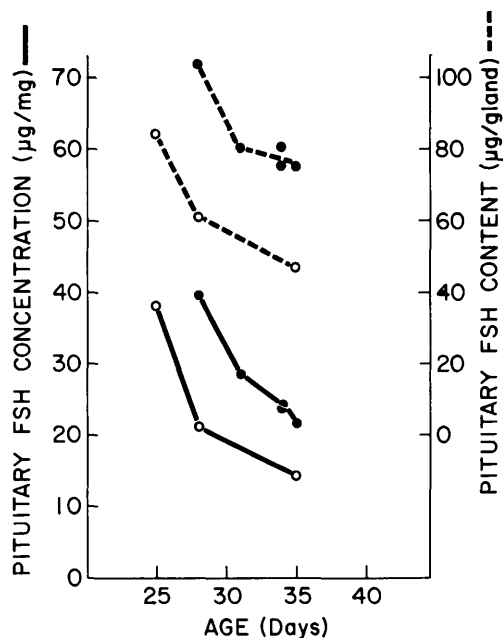


FIG. 1. Change in pituitary FSH concentration and content in prepubertal rats. Each point represents a separate assay.

the potency of the pituitaries. The rats were all 39 days of age and were divided into 5 groups. The first group of animals had closed vaginas and no evidence of uterine stimulation, and pituitary FSH concentration and content were similar to the values previously recorded in rats of 35 days of age in Expts. 1 and 2. In the second group the vaginas had not yet opened, but the uteri were ballooned with fluid. Pituitary FSH concentration was significantly lowered on comparison with the first group of animals that showed no sign of pubertal changes. In groups 3–5 vaginal

TABLE II. Changes in Pituitary FSH in Pubertal Rats.

Exptl. group of animals	Assay no.	Age at sacrifice (days)	No. of donors	Vaginal opening	Ovulation	Uterine state	Pituitary FSH concentration ( $\mu\text{g}/\text{mg}$ )	Pituitary FSH content ( $\mu\text{g}/\text{pit.}$ )	$\lambda$
4	3	39	10	- <sup>a</sup>	-	D <sup>c</sup>	13.00 (10.38-16.29)	39.1 (31.2-49.0)	.091
		39	7	-	-	B	9.04 ( 7.21-11.32) <sup>d</sup>	35.8 (28.6-44.8)	.092
		39	10	+	-	B	8.84 ( 7.02-11.13) <sup>d</sup>	40.9 (32.5-51.5)	.094
		39	3	+	+	N:B	7.57 ( 6.07- 9.44) <sup>d</sup>	30.0 (24.0-37.4) <sup>d</sup>	.091
		39	9	+	+	N:B	8.79 ( 7.02-11.02) <sup>d</sup>	33.3 (26.6-41.8)	.092

<sup>a</sup> - = vagina closed; + = vagina open.

<sup>b</sup> Vaginal opening was observed on day 38 (1 day after vaginal opening).

<sup>c</sup> D = diestrus (unstimulated), B = ballooned, N:B = not ballooned, estrous or diestrous type.

<sup>d</sup> Significantly less than group I.

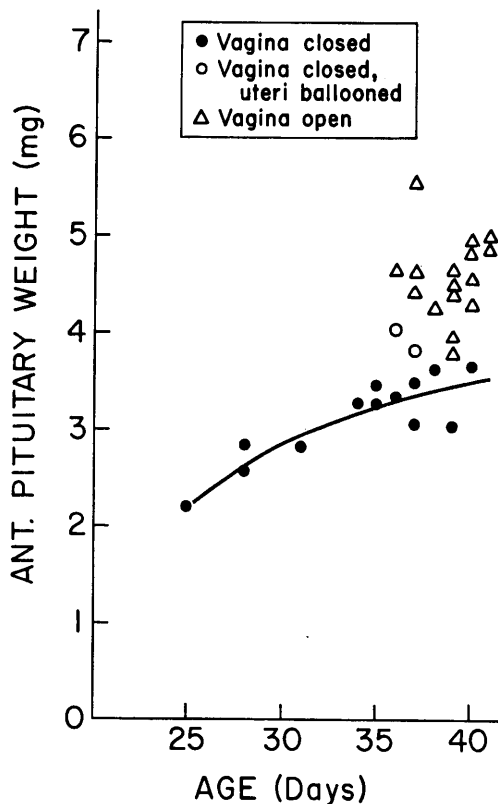


FIG. 2. Changes in anterior pituitary weight with age. In this and subsequent figures each point represents the mean of a separate group of 3-10 rats. (triangles), groups of rats with open vaginas; (circles), those with closed vaginas but ballooned uteri.

opening had already occurred, but there was no further decline in FSH concentration, regardless of the presence or absence of ovulation or continued ballooning of the uteri. These results indicate that there was a further fall in FSH concentration at the time of puberty.

By contrast, changes in pituitary FSH content were minimal at this time. There was a significant decline on comparison to the unstimulated controls only in the group of rats which had ovulated. The failure of FSH content to decline in the face of the decline in concentration at the time of puberty is explained by the fact that pituitary weight increased strikingly at or near puberty (Fig. 2, Table V). There was only a gradual increase in pituitary weight prior to this time. Pituitary weight was increased not only in

TABLE III. Hypothalamic FSH-RF Content in Prepubertal Rats.

Exptl. group of animals	Assay no.	No. of donors	Age at sacrifice (days)	Vaginal opening	Uterine state	FSH-Released ( $\mu\text{g}/\text{mg}$ of pit./6 hr)	$\lambda$	% Control released	
1	1	Control (cortex)					2.43 ( 1.39- 4.25)	.231	100
		7	28	—	D <sup>a</sup>	4.79 ( 2.71- 8.46)	.232	197	
		7	35	—	D	9.67 ( 4.48-20.84)	.334	398	
		7	36	—	D	5.56 ( 3.14- 9.87)	.249	228	
		5	36	—	B	20.38 (12.66-39.20)	.266	837	
2	3	Control (cortex)					4.45 ( 2.39- 5.83)	.105	100
		10	25	—	D	7.11 ( 5.49- 9.20)	.106	160	
		10	28	—	D	5.60 ( 4.19- 7.46)	.119	125	
		10	35	—	D	6.85 ( 5.23- 8.97)	.111	154	

<sup>a</sup> D = diestrus, B = ballooned.

the animals whose vaginas were already open, but also in those on the verge of vaginal opening as indicated by ballooning of the uteri.

*FSH-releasing content of hypothalami of female rats prior to and at puberty.* FSH-releasing activity, as determined by release of FSH by pituitaries incubated *in vitro*, was detectable at all ages studied, *i.e.*, from 25 days on (Tables III and IV). At the time just prior to vaginal opening, when the uteri

were ballooned, 2 estimates were made. In one there was a marked elevation in FSH-releasing activity (Assay 1), and, in the other (Assay 5), there was no detectable change on comparison to unstimulated control rats of the same age.

A number of comparisons were made of the FSH-releasing activity in hypothalami from rats of the same age which either showed no sign of entering puberty or had reached puberty as evidenced by vaginal opening

TABLE IV. Comparison of FSH-RF in Rats on Same Day with Vaginas Open or Closed.

Exptl. group of animals	Assay no.	Age at sacrifice (days)	Vaginal opening	Uterine state	FSH-Released ( $\mu\text{g}/\text{mg}$ of pit./6 hr)	% Depletion <sup>b</sup>
1	2	40	—	D <sup>a</sup>	13.67 ( 7.70-24.25)	0
		40	+	B	7.18 ( 4.05-12.75)	47.4
		41	+	N-B	5.06 ( 2.70- 9.30)	62.8
3	4	37	—	D	9.52 ( 7.28-12.40)	0
		37	+	B2; N-B3 <sup>c</sup>	10.97 ( 8.30-14.50)	-15.0
		37	+	N-B	7.31 ( 4.77-10.50)	23.4
3	4	38	—	D	14.34 (10.48-19.63)	0
		38	+	B	9.27 ( 7.20-11.94)	35.3
		40	+ opened 39 d	N-B	10.08 ( 7.86-12.92)	29.6
4	5	39	—	D	6.92 ( 5.19- 9.23)	0
		39	—	B	6.96 ( 5.50- 8.81)	(-5) <sup>d</sup>
		39	+	B	6.37 ( 4.97- 8.17)	8.2
		39	+	N-B	7.18 ( 5.55- 9.30)	-3.5
		39	+ opened 38 d	N-B	6.93 ( 5.30- 9.08)	0

<sup>a</sup> D = diestrus (unstimulated), B = ballooned, N-B (not ballooned, estrous or diestrus type).

<sup>b</sup> From control unstimulated animals in each experimental group.

<sup>c</sup> Two animals with ballooned uteri, 3 without.

<sup>d</sup> Not included in calculation of mean depletion (see text) since vaginas not open.

TABLE V. Organ Weights in Prepubertal and Pubertal Rats.

Exptl. group of animals	Age at sacrifice	No. of rats	Vaginal opening	Uterine state	Body wt (g)	Ant pit. wet wt (mg)	Ovary (mg)	Uterus (mg)
1	28	7	—	D	74.0 ± 1.6	2.59 ± 0.09		
	31	7	—	D	89.8 ± 1.9	2.81 ± 0.10		
	34	14	—	D	98.1 ± 2.8	3.26 ± 0.10		
	35	7	—	D	103.9 ± 4.0	3.48 ± 0.24		
	36	7	—	D	101.1 ± 1.8	3.33 ± 0.10	16.0 ± 0.8	50.1 ± 2.5
	36	5	—	B	112.2 ± 1.4	4.03 ± 0.80	21.2 ± 0.8	219.0 ± 9.1
	36	3	+	N-B	110.0 ± 3.5	4.63 ± 0.28	27.3 ± 4.6	156.7 ± 3.6
	37	6	—	D	108.0 ± 2.8	3.06 ± 0.10	17.6 ± 1.7	75.9 ± 7.5
	37	7	—	B	117.8 ± 1.4	3.83 ± 0.17	17.7 ± 1.3	142.3 ± 27.9
	37	4	+	N-B;B	116.8 ± 1.8	4.62 ± 0.17	23.6 ± 5.2	158.7 ± 22.4
	39	4	+	N-B	124.8 ± 0.5	4.39 ± 0.43	33.8 ± 1.4	166.6 ± 0.9
	39	4	+	N-B	124.5 ± 0.6	4.48 ± 0.31	18.1 ± 0.4	84.8 ± 5.9
	40	5	—	D	109.4 ± 7.8	3.67 ± 0.22	16.2 ± 0.1	83.9 ± 22.2
	40	8	+	B	129.6 ± 2.2	4.83 ± 0.14	25.0 ± 2.3	224.2 ± 9.7
	41	7	+	N-B	132.1 ± 2.5	4.87 ± 0.20	32.1 ± 1.5	133.7 ± 3.6
	41	3	+	N-B	129.3 ± 3.9	5.00 ± 0.14	26.9 ± 2.2	169.2 ± 5.0
	2	25	10	—	D	62.7 ± 0.1	2.21 ± 0.10	
28		10	—	D	81.4 ± 1.1	2.85 ± 0.10	15.6 ± 1.0	41.5 ± 2.7
35		10	—	D	108.6 ± 1.7	3.26 ± 0.14	17.9 ± 0.9	50.9 ± 3.7
40		6	+	B	127.1 ± 1.6	4.56 ± 0.10	26.1 ± 2.9	208.8 ± 13.8
40		8	+	N-B	131.5 ± 2.8	4.96 ± 0.17	24.4 ± 2.7	131.4 ± 9.0
3	37	5	—	D	103.2 ± 4.1	3.48 ± 0.14		
	37	5	—; +	B	110.4 ± 3.2	3.91 ± 0.17		
	37	5	+	B;N-B	118.8 ± 2.6	4.41 ± 0.14		
	37	5	+	N-B	112.0 ± 2.6	5.55 ± 0.24		
	38	5	—	D	109.6 ± 0.7	3.62 ± 0.17		
	38	4	+	B	122.5 ± 2.2	4.26 ± 0.14		
	40	4	+	N-B	121.3 ± 3.9	4.28 ± 0.20		
4	39	10	—	D	98.1 ± 2.7	3.01 ± 0.36		
	39	7	—	B	117.9 ± 2.3	3.96 ± 0.23		
	39	10	+	B	116.6 ± 1.1	4.63 ± 0.18		
	39	3	+	N-B	117.3 ± 2.4	3.96 ± 0.26		
	39	9	+	N-B	104.7 ± 4.0	3.79 ± 0.16		

(Table III). Comparisons were made in 4 experiments with a total of 4 unstimulated groups and 9 groups which had reached puberty. There was decreased FSH-releasing activity on comparison to the unstimulated group of the same age in 6 of the 9 pubertal groups. In four of these 6 individual comparisons this difference was significant. In the other 3 groups there was no detectable change in FSH-releasing activity in 2 cases and an insignificant rise in 1. When all results were tabulated, the hypothalami from the pubertal animals released  $20.9 \pm 8.9\%$  less FSH than those of the unstimulated ani-

mals of the same age ( $p < .05$ ). The mean FSH release by pituitaries incubated with hypothalami from the 4 groups of unstimulated rats was  $11.11 \pm 1.76 \mu\text{g}/\text{mg}$  of AP/6 hr, whereas the release by glands incubated with hypothalami from the 9 groups of pubertal rats was  $7.82 \pm 0.59 \mu\text{g}/\text{mg}$  AP/6 hr ( $p < .05$ ).

*Body and organ weight changes prior to and at puberty.* As pointed out earlier by others, larger animals tend to reach puberty at an earlier age than smaller ones (1, 9). The present data (Table V) support this finding, and, in fact, with one exception,

groups of pubertal rats with open vaginas or ballooned uteri indicative of incipient vaginal openings weighed 110 g or more (Fig. 3). In the exceptional case, the mean weight of the group was 105 g.

There was clearly a spurt in ovarian growth at or near the onset of puberty as indicated by the fact that ovarian weights of rats at or near puberty were always larger than those of rats of the same age which had not entered puberty (Fig. 4). A similar statement could also be made for uterine weight which clearly underwent a striking increase at the time of puberty (Fig. 5).

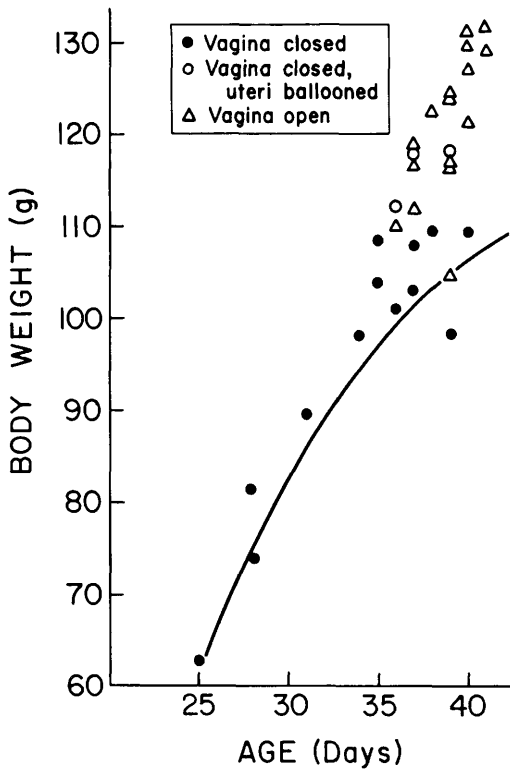


FIG. 3. Changes in body weight of female rats at puberty.

Lastly, anterior pituitary weight which only increased gradually prior to the initiation of puberty, increased markedly in groups of animals with ballooned uteri or vaginal opening which signified the onset of puberty as noted earlier (Fig. 2).

*Discussion.* These results provide further evidence for the rapid activation of the pitui-

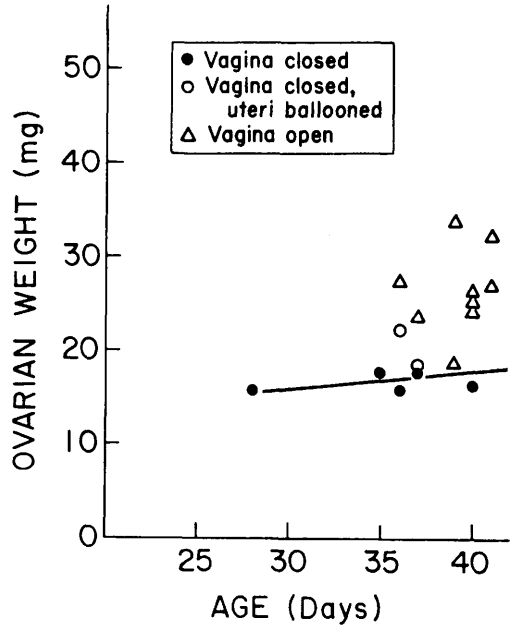


FIG. 4. Changes in ovarian weight at puberty.

tary-gonadal axis which occurs at the time of puberty and is reflected in the striking increases in anterior pituitary, ovarian, and uterine weights observed (1). That the ovarian and uterine enlargement is secondary to enhanced discharge of gonadotropins from the anterior pituitary is suggested by the falls

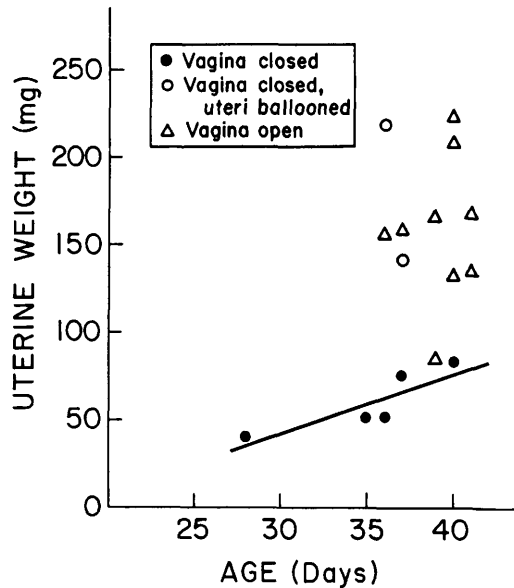


FIG. 5. Changes in uterine weight at puberty.

in pituitary FSH concentration which occurred prior to and at the time of puberty. Our data agrees in general with that reported recently by Kragt and Ganong (10). Although we believe that the decrease in FSH concentration reflects enhanced release of hormone, we realize that proof for this assumption requires determination of elevated plasma levels of the trophic.

An attempt was made to implicate FSH-RF in the pubertal changes in FSH by measuring the content of stored releasing factor in the hypothalamus just before and after puberty. The data indicate that a decline occurs, but the magnitude of the change was small. This finding is in agreement with the results recently reported by Corbin (11) since the completion of this study; however, the magnitude of the decline in FSH-RF at puberty which he observed appears to be greater. Corbin assayed for the hypothalamic FSH-RF content by the pituitary FSH depletion method, instead of the *in vitro* procedure employed in the present experiments. The decline in hypothalamic FSH-RF levels at puberty is consistent with the hypothesis that enhanced release of this factor may initiate FSH discharge from the pituitary at this time.

In view of the evidence for enhanced release of LH at the time of puberty (3, 4), it would appear that puberty is brought about by combined release of increased amounts of both FSH and LH, probably at least in part triggered by discharge of the gonadotropin-releasing factors, FSH-RF and LH-RF.

Our data reemphasize the role of growth in initiating puberty, for the onset of puberty was more related to the body weight of the rats than their chronological age.

*Summary.* A decline in pituitary FSH concentration and content was associated with

prepubertal development of female rats. A further decline in pituitary FSH concentration occurred at the time of vaginal opening, but pituitary FSH content fell only slightly at this time because of a concurrent increase in anterior pituitary weight. FSH-releasing activity was present in the hypothalamus from the time of first measurement at 25 days of age and exhibited a small but significant decline at the time of vaginal opening. Puberty was associated more closely with body weight of the animals than with their chronological age and was associated with increase in pituitary, ovarian and uterine weights. The data are consistent with the hypothesis that increased release of FSH triggered by a release of FSH-RF is involved in the induction of puberty.

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