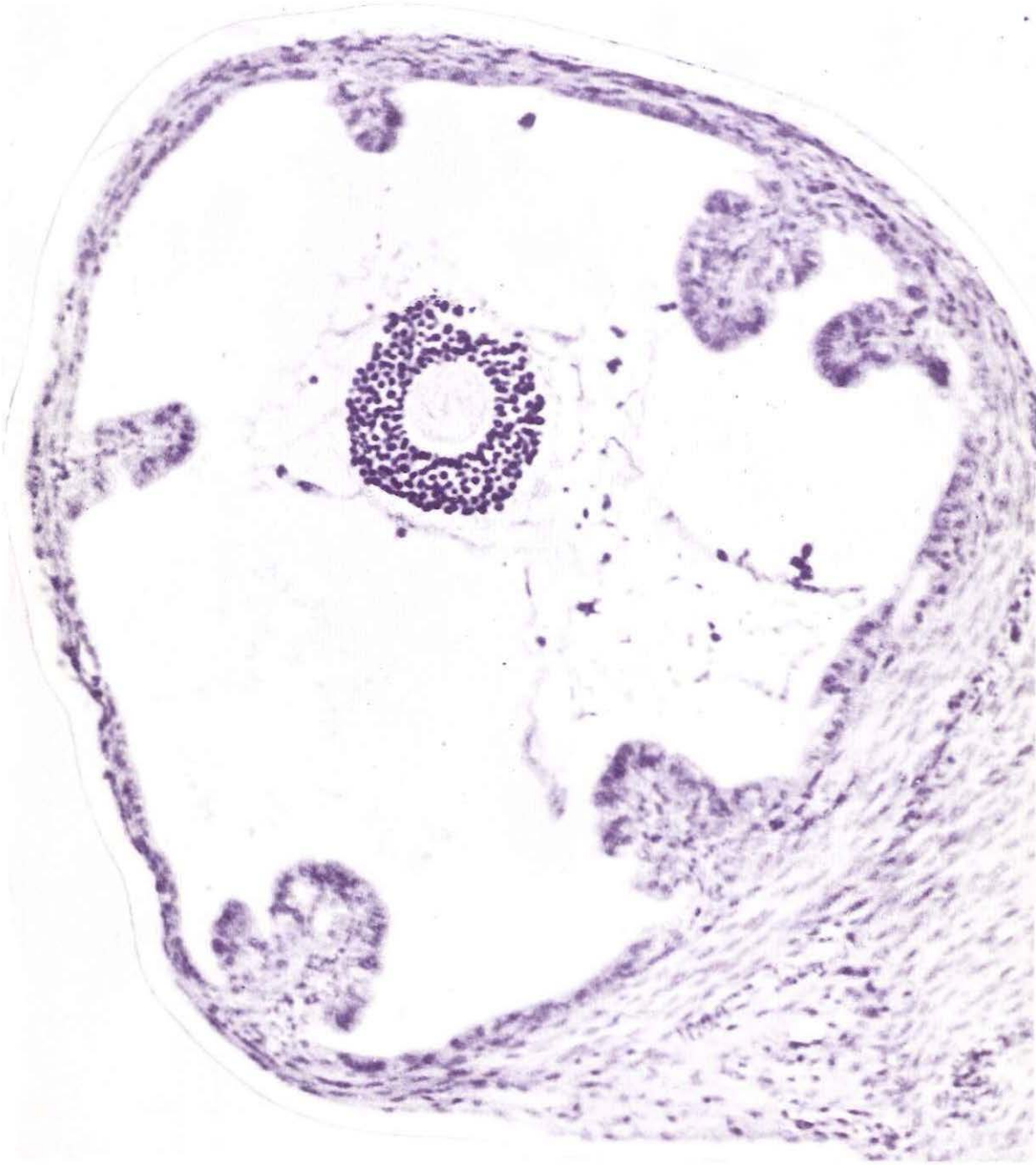

SEXUAL MATURATION IN THE FEMALE RAT

PROEFSCHRIFT

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AAN DE ERASMUS UNIVERSITEIT TE ROTTERDAM

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Abbreviations

AHA	: anterior hypothalamic area
b.w.	: body weight
CL	: corpus luteum; corpora lutea
CLA	: corpus luteum atreticum; corpora lutea atretica
CNS	: central nervous system
CO	: chiasma opticum
DC	: direct current
FSH	: follicle stimulating hormone
HCG	: human chorionic gonadotrophin
HF	: high frequency (current)
hpx	: hypophysectomy; hypophysectomized
i.u.	: international unit(s)
LH	: luteinizing hormone
ME	: median eminence
OAAD	: ovarian ascorbic acid depletion test
OB	: oestradiol benzoate
OVX	: ovariectomy; ovariectomized
P	: progesterone
PIF	: prolactin inhibiting factor
(m) POA	: (medial) preoptic area
RF	: releasing factor(s)
RIA	: radioimmunoassay
RU	: rat unit(s)
SC	: nucleus suprachiasmaticus
SS	: stainless steel
TP	: testosterone proprionate
VMH	: ventromedial hypothalamic nucleus
VO	: vaginal opening

GENERAL INTRODUCTION

Generally speaking, the onset of puberty is characterized by a chain of events through which the capacity of reproductive function develops. It marks the final phase of interacting maturational processes of the brain, the hypophysis and the gonads.
The processes leading to the onset of puberty are already highly interesting in themselves, but they are brought to our attention still more by clinical observations of " pubertas praecox ".

> " One of the most fascinating and least understood disorders of maturation is the syndrome of sexual precocity, the onset of puberal changes at an earlier age than normal " (Reiter & Kulin, 1972).

Some of these cases of " pubertas praecox " have been attributed to cerebral abnormalities, to thyroid or to adrenal disorders, with still other cases remaining unexplained (see Bauer, 1959; Donovan & van der Werff ten Bosch, 1965; Reiter & Kulin, 1972). Numerous investigations have been and are being performed to arrive at a better understanding of sexual maturation; studies of both the processes underlying normal puberty and studies of (induced) disturbances of the normal processes may contribute to this.

In nearly all species, the attainment of sexual maturity more or less coincides with the attainment of adult body size (Donovan & van der Werff ten Bosch, 1965), which means that certain age limits may normally be indicated for puberty to occur. However, it is well-known that a great number of environmental and endogenous factors may influence the age at which puberty occurs individually (review Donovan & van der Werff ten Bosch, 1965).
Environmental conditions such as light, season (Ramaley & Bunn, 1972), temperature, stress (Mandl & Zuckerman, 1952) and food-intake (Kennedy & Mitra, 1963) no doubt play a role. Transmaternal effects (Ellett, 1970), as well as the mode of functioning of the thyroid (Bakke et al., 1970), pineal (Osman et al., 1972) and thymus (Sakakura & Nishizuka, 1972; Nishizuka et al., 1973) are also of influence. Total bodily development,

apparently influenced by most of the factors mentioned above and expressed through skeletal maturation and body weight (" physiological " age, see Donovan & van der Werff ten Bosch, 1965),is of decisive importance. It is clear that the general mechanism underlying the onset of puberty may be best studied if all those factors are kept as constant as possible, since spontaneous puberty may then be expected to occur at a rather constant age and stage of bodily development. Under these circumstances experimentally induced changes in the onset of puberty may be attributed exclusively to the treatment given. Therefore the experiments to be reported have been performed in a highly inbred rat-strain kept under standardized environmental conditions.

PUBERTY - criteria

Different criteria and definitions of " the onset of puberty " have been used by endocrinologists. In general, behavioural or " psychological " changes have not received much attention. Attention has been focussed on purely physiological events.

Critchlow and Bar-Sela (1967) define puberty as " the stage at which the ability to reproduce sexually is achieved " and add that the term is often used to include events preceding this capability. In the female rat vaginal canalization is completed around the time of first ovulation and is therefore frequently taken as an index of puberty (Donovan & van der Werff ten Bosch, 1965). However, it has been recognized that, in general, vaginal opening singly is not a very precise index for puberty, since the relationship to first ovulation is somewhat variable (Weisz & Ferin, 1970), nor is it always a valid index of puberty in experimental situations. Several experimental treatments like hypothalamic lesioning (see Meijs-Roelofs & Moll, 1972), neonatal TP treatment (see Zarrow et al., 1969) or oestrogen treatment (Hagino et al., 1966) may lead to early vaginal opening without affecting the time of first oestrus and/or ovulation. In this thesis, where exclusively the rat is taken into consideration, the term " onset of puberty " is used for the occurrence of a sequence of endocrinological events that culminate in first ovulation, which is normally associated with opening of the vaginal membrane, followed by regular oestrous cycles. " Puberty " then stands for processes directly related to first ovulation, to be followed by oestrous cycles. " Precocious puberty "

will be used in the sense that the endocrinological events related to the normal onset of puberty occur at a significantly younger age and - and this is the more important criterion - at a stage of bodily development (as measured by body weight) that is significantly earlier than normally. As puberty marks the beginning of adult functioning of the hypothalamo-hypophysial-gonadal system, the developmental stage of the different parts of this system is of great importance. More specifically, for puberty to occur the hypothalamic centres for gonadotrophin release should have developed, gonadotrophin releasing factors should be present and be transported to the pituitary by way of the hypophysial portal system. In the hypophysis synthesis and release of gonadotrophins must take place and the ovaries should react to these gonadotrophins by (complete) follicular development and steroid-synthesis and -secretion.
Finally, the gonadal steroids and possibly other hormonal factors should exert a feed-back action on the hypothalamo-hypophysial system to establish an adult interaction between different parts of the system.
In the first part of this thesis a survey of literature on maturational changes that lead to puberty will be given, the developmental stages of the hypothalamo-hypophysial-ovarian system at various prepuberal ages will be discussed. The second part surveys data on experimentally induced changes in the onset of puberty. The third and last part reviews the bearing of the personal and of recent data in the literature on overall views and concepts of puberty. Detailed information on personal experimental results is given in the appendix.

PART I. MATURATIONAL PROCESSES LEADING TO PUBERTY

Data on the prepuberal development of parts of the hypothalamo-hypophysial-ovarian system have been long collected. Studies in the early thirties reported on pituitary gonadotrophin content in immature rats; furthermore ovarian transplantation and spaying experiments uncovered the existence of interaction between parts of the system in the immature rat. Further, a role of the central nervous system in sexual maturational processes was suggested (see reviews Donovan & van der Werff ten Bosch, 1965; Critchlow & Bar-Sela, 1967).

New experimental approaches, made possible by the introduction of new techniques, have greatly extended our knowledge of the developmental stages, functions and interactions of the hypothalamus, the hypophysis and the ovaries. A survey of data available now will follow here.
Data on development of the ovaries will be started with; hypophysial gonadotrophin content will be discussed thereafter, followed by a survey of recent measurements of gonadotrophin levels in the blood. Brain development will then be discussed and finally attention will be paid to the interactions taking place within the hypothalamo-hypophysial-ovarian system.

THE OVARY

On the basis of the changes in morphology, responsiveness to gonadotrophic hormones, and ovarian hormone synthesis, the developmental history of the ovary from birth to maturity can be subdivided in 3 periods (see review by Critchlow & Bar-Sela, 1967) : 1) THE INFANTILE PERIOD, 2) THE EARLY JUVENILE PERIOD, 3) THE LATE JUVENILE PERIOD. A short description of these periods will be given here.

1) THE INFANTILE PERIOD
During the infantile period (in the rat from birth till 10 days of age) the ovary passes through a phase of relative insensitivity to gonadotrophic hormones, which holds especially for the follicular part of the ovary : the interstitial tissue responds earlier to gonadotrophins than follicular elements (Price & Ortiz, 1944; Eckstein, 1962; Rowlands & Parkes, 1966; Critchlow & Bar-Sela, 1967). However, the ovary is not dormant during this period but continuous growth, differentiation and degeneration occurs (Peters, 1969). Primary follicles develop by way of attachment of follicle cells to the growing primary oocytes (see Pedersen & Peters, 1967; in mice), and the primary interstitial tissue develops (Dawson & McCabe, 1951; Rennels, 1951; Falck, 1953). Oocyte growth proceeds largely independently; but for follicle formation and cyto-differentiation of the interstitial cells gonadotrophins are needed already during infancy (Eshkol et al., 1970; Stegner et al., 1970, in mice). However, administration of even high amounts of exogenous gonadotrophins (review

Donovan & van der Werff ten Bosch, 1965; Critchlow & Bar-Sela, 1967) or unilateral ovariectomy (Peters & Braathen, 1973) causes neither accelerated follicular growth nor a greater number of follicles to grow. Apparently a maximal number of follicles starts growth at the end of the infantile period and the growth speed at this age seems higher than at any age thereafter (Pedersen, 1969, in mice). The interstitial tissue increases in volume at this time and, presumably, synthesis of ovarian steroids starts or becomes demonstrable (Critchlow & Bar-Sela, 1967). With the first appearance of (small) antral follicles, at the end of the infantile or rather during the early juvenile period, a clear dependency on gonadotrophins gradually develops.

2) THE EARLY JUVENILE PERIOD (about day 10 - day 20)
This period is characterized by rapid growth of great numbers of follicles, though the larger type of antral (Graafian) follicles does not appear yet. This is in line with the findings of Pedersen (1969; 1970; in mice) that the time required for a small follicle to grow to a large one is more than 14 days, even at early ages where speed of follicular growth is presumably maximal. During the early juvenile period an increasing volume of primary interstitial tissue develops and ovarian steroid-synthesis increases (see page 14). Reactivity to exogenous gonadotrophins is still very much limited in this period : the interstitial tissue does react by increase in volume and in steroid synthesis (see Critchlow & Bar-Sela, 1967) but follicular responses do not lead to capacity to ovulate yet.

3) THE LATE JUVENILE PERIOD (about day 20 - puberty)
The late juvenile period is characterized by the occurrence of large (Graafian) antral follicles that may respond to experimental stimuli with complete ovulatory processes. In this period continuing growth and atresia of follicles is found. The hypertrophied theca interna of large follicles becomes the secondary interstitial tissue, which increases in volume. Great numbers of large antral follicles were reported to be present at about day 21, which seems to be due to the numerous follicles starting growth in the infantile period (Pedersen, 1969; in mice). A massive degeneration of these gonadotrophin-dependent follicles takes place,

presumably due to absence of adequate hormonal interplay to support full development (Peters, 1969).
Morphologically the late juvenile ovary seems ready for adult function but no spontaneous ovulation takes place yet and thus corpora lutea are lacking. The ovulatory response to exogenous stimuli (see pp. 48 and 51) increases from about day 20 till about day 28 (Zarrow & Wilson, 1961; Zarrow & Quinn, 1963; Sugawara et al., 1969).

For comparison with the situation in the adult, it seemed of interest to study, in the prepuberal ovary, the presence of follicles of those size-ranges which have been found to play an important role during the cycle in the adult rat (Welschen & Rutte, 1971; Welschen, 1973). It has been found by these authors that during the cycle day-to-day changes occur in numbers of follicles of certain size-ranges; these changes finally lead to ovulation of a rather constant (about 10) number of ova at the end of the cycle. Presence of follicles of comparable sizes in the prepuberal ovary might give more detailed information on the ovarian capacity to produce mature follicles. Therefore, in our studies on morphological ovarian changes (Meijs-Roelofs et al., 1973), attention has been focussed on follicles exceeding a volume of 100×10^5 μm^3, which means that all follicles studied were antral follicles. Because of the absence of such follicles at early ages, the studies have been restricted to the transitory period from early juvenile to late juvenile and to the late juvenile period. Follicles $\geqslant 100 \times 10^5$ μm^3 were found to be absent before day 19, and could not be induced by PMS treatment over 24 hr before this age. A sharp increase in number of small antral follicles ($\leqslant 350 \times 10^5$ μm^3) was found on the following days. After days 22 - 24 the number of these follicles remained rather constant. The larger antral follicles appeared a few days later and in smaller numbers. The occurrence of follicles $\geqslant 500 \times 10^5$ μm^3 is of special interest, since, during the normal adult cycle, follicles exceeding this volume are always capable of ovulating after an ovulatory stimulus (Welschen, 1973). These large antral follicles were only occasionally present between days 21 and 28; after day 28 small numbers of follicles $\geqslant 500 \times 10^5$ μm^3 were always present and their number increased shortly before the first expected oestrus. In an acute experiment on immature rats of different ages, an ovulatory stimulus (injection of 15 i.u. HCG) did not induce ovulation of

these large follicles indicating a qualitative difference between " adult " and " immature " follicles of a volume $\geq 500 \times 10^5$ μm^3 (For details see appendix, Meijs-Roelofs et al., 1973). Summarizing, it seems that the ovary clearly represents a limiting factor for puberty to occur till about 20 days of age, the age at which the ovulatory process may be induced. Thereafter, in the late juvenile period, the ovary contains large follicles that may reach the capacity to ovulate and do ovulate under suitable experimental conditions, though spontaneous ovulation and capacity to ovulate are absent in these follicles.

OVARIAN STEROID PRODUCTION

Though indirect evidence has been presented for ovarian steroidal activity early in life, little is known about the capacity of the immature ovary to produce and secrete steroids. Histochemical studies indicate the steroidogenic capacity of the prepuberal ovarian interstitial tissue : presence and storage of cholesterol in the interstitial gland has been reported from the 7th - 10th day of life onwards (Dawson & McCabe, 1951; Rennels, 1951; Falck, 1953), presence of 3β-hydroxy-steroid dehydrogenase was first demonstrated between 7 and 11 days of age (Presl et al., 1965; Küppers, 1967). Quattropani & Weisz (1973) showed by in vitro experiments at 5 days of age the presence of the ovarian capability to convert progesterone into oestrogens.

Using a bio-assay, Cierciorowska & Russfield (1968) found that on the 14th day of life the rat ovary not only contains but also releases considerable amounts of oestrogen. This is in agreement with recent results, obtained by RIA (Meijs-Roelofs et al., in press). In these latter studies it was found that oestradiol-17β is detectable in the blood of 5-day old rats and reaches maximal values (55 - 60 pg/ml) in the period from 10 - 15 days of age. Thereafter, a decrease in oestradiol concentration results in low to undetectable values after day 25 (For details see appendix, Meijs-Roelofs et al., in press).

Progesterone

Progesterone secretion has been suggested to occur in the immature rat from at least the 20th day of life (Alden, 1947; Dawson & McCabe, 1951).

Taylor (1961), using a histochemical staining technique, demonstrated the production of progesterone in the thecal and interstitial tissue of rats of 2 and 4 weeks old. Determination of plasma progesterone indicated that progesterone is present in the blood of animals of 2 and 4 weeks old, but no complete data on progesterone concentrations in the blood of immature rats are available.

THE HYPOPHYSIS

LH and FSH

In the hypophysis gonadotrophin-producing basophil cells, containing glycoprotein granules, have been demonstrated during or shortly after the first week of life (Siperstein et al., 1954; Shiino & Rennels, 1967). Siperstein et al. (1954) report that the (future) gonadotrophin cells themselves are recognizable from the first day of life.

The gonadotrophin content of the immature pituitary has frequently been studied (see table 1). Initial work was concerned with total gonadotrophin content (McQueen-Williams, 1935; Lauson et al., 1939, see table 1); in later investigations bio-assay methods for separate evaluation of either LH or FSH content have been used. Although great variations in time of puberty exist among different strains of rats, and even within one strain of rats under different environmental conditions, a strikingly comparable developmental pattern of hypophysial gonadotrophin content has been found by various investigators.

An extremely high content of both LH and FSH, that even exceeds adult values, occurs in the immature hypophysis between 20 and 30 days of age (Lauson et al., 1939 - total gonadotrophins, Matsuyama et al., 1966; Weisz & Ferin, 1970 - LH; Hoogstra & Paesi, 1955; Kragt & Ganong, 1968; Labhsetwar, 1970 - FSH).

Pituitary FSH-stores especially, show a very constant developmental pattern : peak values are consistently found in the period from 20 - 25 days of age, a period during which also the so-called " sex-zones " of the anterior pituitary were found to be crowded with basophils (Siperstein et al., 1954). The FSH peak precedes the, more variable, LH peak; the latter seems to be more closely related to the time of VO (Lisk, 1968) and has been reported to occur between days 26 - 33 (Moore, 1965/1966), at day 30 (Lisk, 1968) or day 40 (Suzuki et al., 1971). Weisz & Ferin (1970,

Table 1

Pituitary gonadotrophin and prolactin content in immature female rats.

Technique used	Pituitary gonadotrophin content	Rat strain used	Age at puberty	Authors
FSH/LH				
Pituitary implantation; ovarian weight of recipient	Total gonadotrophin content: very high: day 18-23 high: day 27-30 prepuberal drop day 35-38	Long Evans	± day 42	McQueen Williams, 1935
Uterine weight method	Total gonadotrophins: 1) Immature hypophysis (14-35 days) considerably more potent than adult; Maximal potency day 21 2) Average prepuberal pituitary content at least twice that of puberal content	Sprague-Dawley	49-52 days	Lauson et al., 1939
Ventral prostate weight method	LH content in immature (± 30 days) hypophysis lower than adult,	Wistar	-	Hoogstra & Paesi, 1955
Testis weight method, augmentation with HCG	FSH content higher than adult			
OAAD-test	LH content in immature hypophysis comparable to adult	Sherman	-	Ramirez & McCann 1963
OAAD-test	LH content high day 26-33; lower, but still high day 34-37; low after day 38	Holtzman	38-41 days	Moore 1965/1966
Steelman-Pohley assay	FSH content increases from day 1-25; maximal at day 25, remains high till day 33; sharp drop immediately prior to puberty	Sprague-Dawley	39 days (average)	Corbin & Daniels, 1967
Steelman-Pohley assay	FSH content increases from day 16 to a peak at day 20, decreases to low values from day 35 - day 40, no acute change at VO	Sprague-Dawley	day 30-day 40 and later	Kragt & Ganong, 1968

OAAD-test	LH content increases from day 10 to a peak at day 30; significant decrease at VO	Sprague-Dawley	38.4 days (average)	Lisk, 1968
Steelman- Pohley assay (modified)	FSH content high at day 25-28, significant decrease at puberal changes (uterine ballooning)	Holtzman	day 35-day 40	Watanabe & McCann, 1969
Mouse Uterine weight method	Total gonadotrophins: increase from day 15-21; peak at day 21	Wistar-Sprague Dawley hybrids	42-44 days	Matsuyama et al., 1966
OAAD-test	LH content: increase from day 15-21; maximal day 21-28			
	Both total gonadotrophins and LH show precipitate decrease at VO			
Steelman-Pohley assay	FSH content: increase from day 10-21, (parallels LH pattern), decrease around VO	Sprague-Dawley	35-42 days	Weisz & Ferin, 1970
Steelman-Pohley assay (modified)	FSH content high from day 22-26, gradual decrease till day 36, rapid fall thereafter (approaching sexual maturity)	Porton	-	Fawke & Brown, 1970
Steelman-Pohley assay	FSH content of immature hypophysis is much higher than adult; FSH high at day 25 and declining towards puberty	Holtzman	± day 38	Labhsetwar, 1970
Steelman-Pohley assay (modified)	FSH content: peak value at day 25 gradual decrease thereafter			
OAAD-test	LH content increases from day 20-40 peak value day 40 (time of VO), sudden decrease day 45	Wistar	35-45 days	Suzuki et al., 1971
	Prolactin			
Pigeon Crop mucosa dry weight method	Prolactin content day 20-35, increase thereafter, sudden rise at VO and maximal value after VO (day 45).			
Intradermal pigeon Crop sac method	Prolactin content low and constant from day 21-31, marked increase shortly after puberty	Sprague-Dawley	± 37,5 days (average)	Minaguchi et al., 1968
RIA	Prolactin content low from day 21-33 (prior to VO), significant increase shortly after puberty	Sprague-Dawley	37.0 days (average)	Voogt, et al., 1970

postscript) indicated that the prepuberal LH peak might be biphasic; a first peak in LH potency was found around day 14; the second (higher) LH peak at day 21.

During late prepuberal stages, both LH and FSH potency show a marked decrease, which has been correlated with puberal changes such as uterine ballooning, VO and first ovulation. Siperstein et al., (1954) report massive degranulation of gonadotrophic cells between days 35 and 42, " almost certainly due to release of gonadotrophic hormones, coinciding with the first oestrus ". Degranulation of FSH cells seems to precede degranulation of LH cells. Some authors report a direct, acute time-relationship between puberal events and the decrease in pituitary gonadotrophin content (Corbin & Daniels, 1967; Ramirez & Sawyer, 1965), others report a more gradual decrease (Kragt & Ganong, 1968; Watanabe & McCann, 1969). Weisz & Ferin (1970), who also failed to show dramatic changes in hypophysial gonadotrophin content at the onset of puberty, attritubed this failure to their method of sampling and use of VO singly, as the basis of grouping the animals.

In immature rats, where precocious ovulation was induced by oestrogen treatment (Ramirez & Sawyer, 1965; Corbin & Daniels, 1969) or PMS treatment (Rennels & O'Steen, 1967; Klausing & Meyer, 1968; Zarrow & Dinius, 1971), a depletion of pituitary LH and/or FSH has been demonstrated in direct relationship with the first (induced) ovulation. Though this suggests a relationship between first ovulation and pituitary changes in general, one should bear in mind that a release of gonadotrophins into the blood is not necessarily accompanied by a decrease in pituitary gonadotrophin content since hypothalamic releasing factors may stimulate both release and synthesis of gonadotrophins (McCann, 1970).

PROLACTIN

Relatively few studies report on pituitary prolactin content in immature rats (see table 1). During the prepuberal period prolactin content is low (Minaguchi et al., 1968; Suzuki et al., 1971); a significant increase is seen after the onset of puberty. Minaguchi et al. (1968) demonstrated that daily oestradiol injections, given from day 25 - 30, increased pituitary prolactin levels markedly; these authors attribute the significant increase in pituitary prolactin levels after puberty to an increased oestrogen

secretion by the ovaries. The reported data are in agreement with those obtained by RIA by Voogt et al., 1970.

BLOODLEVELS OF GONADOTROPHINS

Until the development of the RIA technique data on the presence of gonadotrophins in the blood of immature female rats were based upon indirect observations. The regressive effects of hypophysectomy on ovarian interstitial tissue suggest LH secretion early in life (review Critchlow & Bar-Sela, 1967); with regard to FSH, absence of secretion early in life (before day 19) has been suggested (Shiino & Rennels, 1967), on the basis of the finding that ovarian follicles could not be stimulated by HCG injection before day 19. However, it has frequently been reported that in immature rats follicular development cannot be stimulated by FSH until around 16 to 21 days of age (reviews Critchlow & Bar-Sela, 1967; Donovan & van der Werff ten Bosch, 1965; see also page 12), the period during which the first antral follicles appear in the ovaries. This may account for the results of Shiino & Rennels.

A number of recent studies, in which gonadotrophins were measured by way of RIA technique, leave no doubt about the presence of both LH and FSH in the blood of immature female rats (see table 2). In different strains of rat it was found that FSH is circulating at very early ages, reaching maximal values at about 15 days of age (Kragt et al., 1971; Kragt & Dahlgren, 1972; Ojeda & Ramirez, 1972; Meijs-Roelofs et al., 1973; Weisz & Gunsalus, 1973). Moreover, these maximal values are extremely high in comparison with blood levels of FSH during the adult cycle. After the peak a decrease to dioestrous-like, adult values is reported by different authors (Kragt & Dahlgren, 1972; Ojeda & Ramirez, 1972; Weisz & Gunsalus, 1973). These relatively low values were mostly found in the period from about day 20 - day 35. Meijs-Roelofs et al. (1973), in measuring day-to-day changes in this period, found the decrease in FSH level to be a very steep one, taking place from day 21 to day 22 (see appendix). Important modifications in FSH level, directly connected with the onset of puberty have not been reported, presumably due to lack of adequate studies. On the basis of data on puberal changes in pituitary gonadotrophin content increased blood levels of FSH might be expected (see pp 16-18).

It may be seen from different studies that also LH is present in the blood of

Table 2 Gonadotrophin levels in the blood of immature female rats, estimated by RIA.

Age period studied (days)	Data on LH	Data on FSH	Rat strain used	Age at puberty (days)	Remarks	Author(s)
5 - 28; around time of VO	LH detectable at 7 days of age, maximal level: day 14, still high level: day 18, low levels: day 24-28. No clear changes around VO	Steep rise in FSH level from day 5 to a peak at ± day 14. Decrease to "adult" levels at about day 21; maintained thereafter	Sprague-Dawley	35 - 42	Large individual variation in LH; FSH peak level ± 800-1000 ng/ml Rat-FSH RP-I	Weisz & Ferin, 1970 Weisz & Gunsalus, 1973
1 - 12	LH present, level highly variable, often higher (2-4x) than dioestrous, adult values	FSH present neonatally levels much higher than dioestrous, adult values	Holtzman	-	prolactin levels measured; low in comparison with adults	Goldman et al., 1971 Goldman & Gorski, 1971
20 - 35	-	FSH present, values comparable to adult; no distinct pattern; large decrease on day 33	Holtzman	34 - 36	all FSH levels below 300 ng/ml Rat-FSH RP-I	Johnson, 1971
5 - 80	LH level high at day 15; marked decrease from day 15 till day 35-40	FSH level high at day 15; marked decrease from day 15 till day 35-40	Sprague-Dawley	-	-	Kragt et al., 1971
5 - 38	-	3-fold increase in FSH from day 5 to day 15; decrease to adult-like values thereafter	Sprague-Dawley	35 - 40	FSH level day 15 ± 1300 ng/ml Rat-FSH RP-I	Kragt & Dahlgren, 1972
5 - 45	LH level high at 10-12 days of age, then decrease to low level, low level maintained from 20-35 days of age, abrupt elevation at day 40	Rising FSH levels from day 5 to a peak at day 15, decrease thereafter	Inbred Wistar Holtzman mixture	± 40	FSH level day 15 ± 1600 ng/ml Rat-FSH RP-I	Ojeda & Ramirez, 1972
10 - 28	LH levels at 10-15 days of age higher than at later ages			± 32		Caligaris et al., 1972
5 - 35 (FSH) 7 - 35 (LH)	LH levels generally dioestrus-like; higher mean levels at ± day 14 and day 34	FSH levels rise to a peak at day 15; sharp decrease from day 21 to day 22 to dioestrus-like values, these values maintained till day 35	Wistar (R-Amsterdam)	± day 40	Large individual variation in LH FSH level day 15 ± 1200 ng/ml Rat-FSH RP-I	Meijs-Roelofs et al., 1973

very young female rats (see table 2). Generally, maximal values are found in the period from day 10 - 15, but these values generally remain far below the adult prooestrous LH values (Kragt et al., 1971; Meijs-Roelofs et al., 1973). A decrease to low values is generally found thereafter and these low values seem to be present till shortly before the onset of puberty (Weisz & Ferin, 1970; Kragt et al., 1971; Ojeda & Ramirez, 1972; Meijs-Roelofs et al., 1973). With the exception of Ojeda & Ramirez (1972), who report an abrupt elevation in plasma LH at day 40, - the usual age of puberty in their rats -, no RIA data are available on the acute LH changes at the onset of puberty. However, an LH peak preceding first ovulation has often been suggested and was demonstrated by bio-assay (Ramirez & Sawyer, 1965; see also Ramirez, 1971). Studies on acute, puberal changes in blood levels of both LH and FSH, measured by RIA, are in progress at our laboratory. Summarized data on hypophysial gonadotrophin content and blood levels of gonadotrophins are given in fig. 4 (page 57).
Data on prolactin levels in the blood of immature rats (Voogt et al., 1970) indicate that low levels are present prior to VO with a slight increase on the day before VO. A sharp increase in prolactin level is found on the day of VO (at first oestrus) and again at the next oestrus.

INFLUENCE OF HYPOPHYSECTOMY AND WEANING ON SERUM FSH LEVELS

Measurements of gonadotrophic hormones in the blood of immature, female rats showed very high levels at about 15 days of age, which holds especially for FSH levels (see Meijs-Roelofs et al., 1973). An abrupt decrease in serum FSH levels was observed on day 22, the age at which the animals are weaned. Because of this coincidence and in view of the fact that certain maternal hormones may reach the suckling rat by way of the mother milk (Price, 1947), the question arises whether a correlation exists between changes in gonadotrophin levels and weaning.
Bearing in mind that the small intestine of the rat readily absorbs maternal antibodies and certain other macromolecules up to the 18th - 20th day of age (Daniels et al., 1972), it seemed of interest to investigate whether the gonadotrophins present in the blood of immature rats originate purely from their own pituitary.

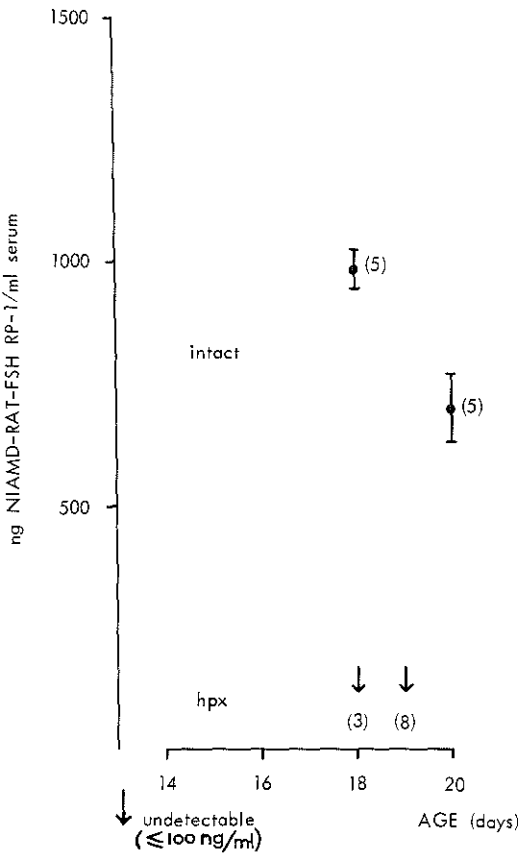

FIG. 1. Serum FSH levels 1 or 2 days after hypophysectomy.
( ) number of animals

Therefore the following studies were undertaken:

1) Rats were hypophysectomized on day 17 and returned to their mothers, serum FSH was measured 1 or 2 days after hpx. Intact rats served as controls.
2) a. Intact rats were weaned at 18 days of age and serum FSH was measured at day 20 or day 22.
   b. Rats were weaned at 25 days of age and (starting on day 22) special care was taken that the immature rats could not reach the food pellets, to ensure that they depended exclusively on the mother milk.

Rats weaned at the normal age of 22 days served as controls; for assay procedures see Meijs-Roelofs et al., 1973
Results are shown in figures 1 and 2.
It was found that hpx resulted in undetectable FSH levels, as measured

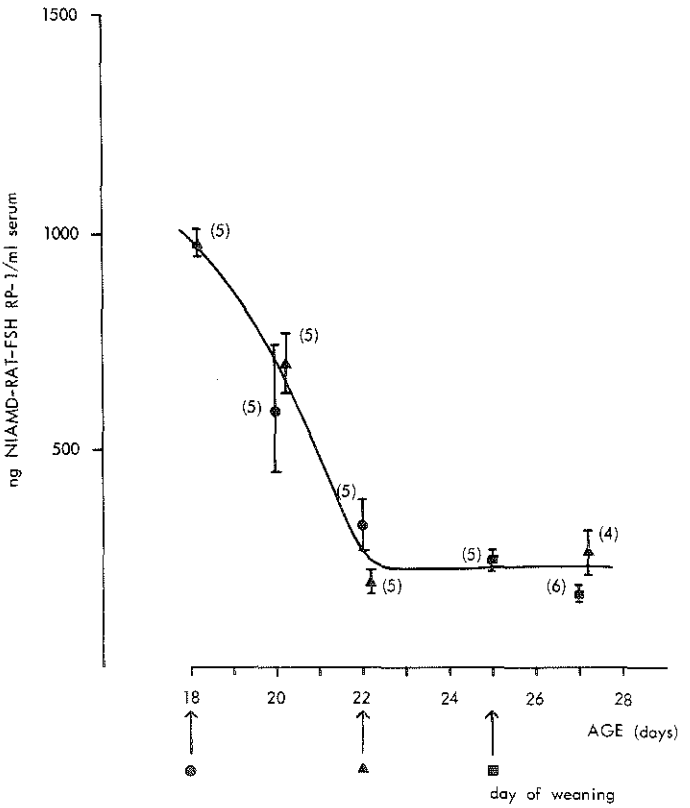

FIG. 2. Influence of weaning age on serum FSH levels.
( ) number of animals

1 or 2 days later (Fig. 1).
Neither weaning at an earlier age nor weaning at a later age than normally resulted in significant changes in serum FSH pattern (Fig. 2).
It therefore may be concluded that the FSH present in the blood of immature female rats originates exclusively from the animals own pituitary.

THE BRAIN

Maturational processes in the brain of the rat take place till about 4 weeks after birth, as demonstrated by studies on morphological development (Watson, 1903; Hyyppä, 1969a), enzyme activity and susceptibility to seizures (see Millichap, 1957; Heim & Timiras, 1963; see also Donovan & van der Werff ten Bosch, 1965). For effective hypothalamic control of pituitary gonadotrophic function, the presence of the hypothalamic gonadotrophin releasing factor and the development of the neurovascular connection between the hypothalamus and anterior pituitary seem of vital importance.

Florsheim & Rudko (1968) demonstrated that in the rat the hypothalamo-pituitary portal system is present and functional by the fourth postnatal day and possibly even earlier (see also Glydon, 1957; Campbell, 1966; Fink & Smith, 1971). Campbell & Gallardo (1966) reported that g onadotrophin-releasing activity has been detected in extracts of the median eminence of the new-born rat.

A complete and uniform picture of the amounts of gonadotrophin-RF present at different ages can not be given yet, but data are accumulating. Corbin & Daniels (1967) reported hardly detectable FSH-RF activity in hypothalamic extracts from foetal life till 15 days of age and maximal content, comparable with adult values, in the period from day 25 to day 40; a clearly decreased potency was found in rats just after vaginal opening. The later studies of Watanabe & McCann (1969) are in agreement with these findings, though the magnitude of the decline in FSH-RF at puberty was much smaller in their studies. Suzuki et al. (1971) also reported maximal FSH-RF activity in 20-day old rats. In contrast, Kragt & Dahlgren (1972) reported that hypothalamic FSH-RF reached a maximal level at 10 days of age.

The presence of adult-like LH-RF potency in stalk median eminence preparations of immature rats was reported by Ramirez & McCann (1963). In a later study (Ramirez & Sawyer, 1966) it was reported that the LH-RF potency in the period of 28 - 31 days of age was lower than from 33 till 39 days. This is in agreement with Suzuki et al. (1971), who found maximal LH-RF activity in 35-day old rats. Shortly before puberty a marked increase in LH-RF potency has been observed, followed by a sharp decrease, correlated with puberal events like uterine ballooning and VO. These puberal changes in LH-RF activity could be advanced by puberty-inducing

oestrogen treatment (Ramirez & Sawyer, 1966); the same holds for puberal FSH-RF changes (Corbin & Daniels, 1969).
PIF activity was reported to be low in immature rats, a rise was found at 45-days of age, i.e. after vaginal opening (Suzuki et al., 1971).
The presence and role of catecholamines in the immature rat brain is beyond the scope of this thesis (see Hyyppä, 1969b a.o.); so is sexual differentiation of the central nervous regulation of gonadotrophin secretion (see review Donovan & van der Werff ten Bosch, 1965).
In conclusion : from the available data it seems reasonable to assume that the gonadotrophin-release mechanism is present at least at the age of 10 days. Moreover, though no consistency has been reached about the exact time of occurrence, maximal FSH-RF potency seems to precede the maximal LH-RF potency. A similar time relationship has been deduced for the hypophysial content of FSH and LH (page 15).
PIF activity reaches maximal values after the onset of puberty, as was found for maximal pituitary prolactin content.

INTERACTIONS WITHIN THE HYPOTHALAMO-HYPOPHYSIAL-OVARIAN SYSTEM

A functional interrelationship between gonads and hypophysis in immature rats had been demonstrated in the early thirties. Development of hypophysial castration cells after OVX, increase in hypophysial gonadotrophic potency after OVX, precocious signs of puberty after temporary absence of the ovaries and various other experimental results (see reviews Donovan & van der Werff ten Bosch, 1965; Critchlow & Bar-Sela, 1967) clearly indicate gonadal inhibition on hypothalamo-hypophysial function. However, the age at which the feedback mechanism is established was not studied in detail in these studies. Using a comparable method Presl et al. (1963) reported ovarian influence on pituitary function at about 21 days of age; results from studies in which unilateral ovariectomy was performed indicate an inhibitory ovarian feedback mechanism to be operative at 25 days of age (Baker & Kragt, 1969; Ojeda & Ramirez, 1972) or already at 10 days of age (Gerall & Dunlap, 1971). Some recent reports clearly demonstrate the

presence of an inhibitory ovarian feedback mechanism still earlier. Chang & Nikotovitch-Winer (1970), on the basis of a histological study, concluded that an inhibitory ovarian feedback on gonadotrophin secretion was in operation as early as the first week of life. Neonatal ovariectomy resulted in accelerated differentiation and maturation of gonadotrophin secreting cells in the hypophysis.

A specific inhibitory influence of the ovaries on LH secretion early in life was convincingly shown by Caligaris et al. (1972), who reported a significant rise in serum LH concentration on day 10 after neonatal ovariectomy. These authors demonstrated also that a stimulatory feedback effect following administration of ovarian steroids on LH secretion develops in the period from 22 to 28 days of age. They conclude that the mechanisms regulating tonic LH release are ready to function at the time of birth or shortly thereafter, while mechanisms involved in phasic LH release mature around day 22. The latter conclusion is in agreement with effects obtained by oestrogen treatment, PMS treatment or by hypothalamic stimulation. These treatments, after which the ovulatory LH release is presumably triggered by endogenous oestrogens, will only cause ovulation when the treatment is given at about 22 days of age or later (see pp. 41, 48 and 29).

Early presence of an inhibitory ovarian feedback effect on FSH secretion has been suggested by the studies of Goldman & Gorski (1971), who demonstrated a decrease in serum FSH concentration after OB injection in 5 - 10 days old rats. However, their studies were performed in intact female rats and therefore no conclusions can be drawn about the feedback action of physiologically present amounts of oestrogen. That the ovary and, more precisely, the endogenous ovarian oestrogens exert under physiological conditions an inhibitory feedback action on FSH secretion in female rats of 13 - 15 days of age has been shown by Meijs-Roelofs et al. (in press). Ovariectomy performed on day 13 of age resulted in a significantly increased serum FSH concentration 2 days later, and this increase could be prevented by daily injections of physiological amounts of oestrogens (for details see appendix, Meijs-Roelofs et al., in press). It may be concluded from the available data that an inhibitory feedback mechanism regulating tonic release of both LH and FSH is operative very early in life; a positive feedback mechanism for phasic LH release matures in the 4th week of life. A stimulatory feedback action of oestrogen on FSH

release has also been reported during the 4th week of life (Naqvi & Johnson, 1969; Johnson & Naqvi, 1969; Corbin & Daniels, 1969, Ying et al., 1971, see also page 46).

An interesting hypothesis has been put forward that the sensitivity of gonadotrophin regulating mechanisms to the inhibitory feedback action of oestrogen is much higher in immature than in adult rats and that a shift in this sensitivity occurs before puberty. This hypothesis, which has no bearing on own experimental work, will be dealt with in the general discussion.

PART II. EXPERIMENTALLY INDUCED CHANGES IN THE ONSET OF PUBERTY

MODIFICATION OF BRAIN FUNCTION

HYPOTHALAMIC LESIONS

The role of the brain in mechanisms controlling sexual maturation has frequently been studied by way of the lesion technique (see Meijs-Roelofs & Moll, 1972). Lesions of different hypothalamic areas have been reported to induce advancement of puberty (see table 3). However, the interpretation of the results is far from simple. Comparison of the various studies is hindered by differences in lesion sites and lesion sizes. Moreover, the mode of action is uncertain (review Critchlow & Bar-Sela, 1967). For the interpretation of the results the destructive effect of the lesions, causing a release from inhibition, has frequently been emphasized (Donovan & van der Werff ten Bosch, 1959; Horowitz & van der Werff ten Bosch, 1962; Gellert & Ganong, 1960). However, it seems noteworthy that in these studies stainless steel electrodes and a direct current were often used, producing a type of lesion that in adult rats is mostly considered as " stimulatory " because of an irritative focus produced by metallic deposits (Everett & Radford, 1961). It has been demonstrated that the effect of hypothalamic lesions on the timing of VO is independent of the age at which the lesions were made (Horowitz & van der Werff ten Bosch, 1962), suggesting a chronic effect of destruction rather than an acute stimulatory effect. However, it has until now not been excluded that the acute effect of a direct current lesion in immature rats is stimulatory as in adult rats (see also page 35 , Velasco, 1972). In order to exclude stimulatory effects, Schiavi (1964) used a high frequency current. He confirmed that both anterior and middle hypothalamic lesions resulted in accelerated sexual maturation, as judged by early canalization of the vagina.

Apart from type of current and electrodes used, the importance of lesion

size should be mentioned : Bogdanove & Schoen (1959) demonstrated that extensive damage of the arcuate nucleus region results in gonad atrophy and Corbin & Schottelius (1960; 1961), who extensively destroyed the area from mammilary body to ventromedial nucleus, also observed an inhibition of reproductive organ development, a delay in canalization of the vagina and the absence of oestrous cycles.

Our own studies dealt with the influence of lesion size on VO, first ovulation and subsequent oestrous cycles. Anterior and middle hypothalamic lesions were made, using a high frequency current. It was found that in the anterior hypothalamus increase in lesion size resulted in increasing advancement of VO and a parallel tendency towards abnormal cyclicity, i.e. prolonged or persistent oestrus. Increasing the size of middle hypothalamic lesions resulted first in a decrease in advancement of VO, while further increase in size caused again a more pronounced advancement. In contrast to the effects of anterior hypothalamic lesions, the occurrence of ovulation and cycles were about normal in rats with middle hypothalamic lesions (for details see appendix : Meijs-Roelofs & Moll, 1972).

ELECTRICAL STIMULATION OF THE BRAIN

The use of a high frequency current has not provided a conclusive answer to the question about the mode of action of hypothalamic lesions (destructive or a-specifically stimulative (Meijs-Roelofs & Moll, 1972). Moreover, an effect inherent in anterior hypothalamic lesions seems to be disturbance of cycles to a more or less serious extent, in extreme cases comparable to the persistent-oestrous syndrome in adult female rats. This phenomenon was also observed after complete or anterior hypothalamic deafferentation in immature rats (Ramaley & Gorski, 1967). Therefore, other techniques seem necessary for further elucidation of cerebral control of sexual maturation. In personal studies, the technique of purely electrical stimulation of the hypothalamus was chosen. This technique has rarely been used in studies on sexual maturation (see appendix, Meijs-Roelofs, 1972, for results of others with the stimulation technique). Stimulation of the arcuate nucleus region performed at 27 days of age or later advanced puberty in all animals used, as did stimulation of the anterior hypothalamus at 29 days of age or later. If stimulation was performed at earlier ages, but not before 22 days of age, only a percentage of animals reacted with

Table 3 Effects of hypothalamic lesions on puberty.

Age at treatment (days)	Type of current and electrode	Lesion site	lesion size	Effects observed	Author(s)
14–15	DC	just behind or above CO;	variable	Advanced VO; advanced first oestrus (5/8); prolonged oestrus (4/7)	Donovan & van der Werff ten Bosch, 1956; 1959
10	platinum	behind CO or infundibular region	(small)	Advanced (?) VO	
3 – 4	electrode	variable: anterior hypothalamus	variable	Advanced VO and first oestrus; normal cycles ($^{3}/_{6}$), constant dioestrus ($^{2}/_{6}$) or constant oestrus ($^{1}/_{6}$)	Horowitz & van der Werff ten Bosch, 1962
18–19	DC; (electrode ?)	arcuate region anterior hypothalamus	small small	Increased uterine weight: presence of CLA and CL Increased uterine weight;	Bogdanove & Schoen, 1959
		arcuate region	large	Ovarian atrophy	
18–20	DC; insect-pin (SS) electrode	mostly anterior hypothalamus; arcuate region and VMH	highly variable	Increased ovarian and uterine weights, advanced VO ($^{5}/_{9}$) and first oestrus ($^{4}/_{5}$)	Krejci & Critchlow, 1959 Elwers & Critchlow, 1960
24	DC; (electrode ?)	arcuate region anterior hypothalamus	moderate to fairly large	Advanced VO and first oestrus, cycles irregular VO and first oestrus not effected	Gellert & Ganong, 1960
21	DC; wire electrode (SS)	arcuate region (from mammilary body to VMH)	large	Delayed VO, cycles with prolonged dioestrus ($^{5}/_{11}$) or constant oestrus ($^{1}/_{11}$)	Corbin & Schottelius, 1960
20	–	arcuate region	large	Delayed VO, no cycles (anoestrus)	Corbin & Schottelius, 1961
25–26	HF SS electrode	anterior hypothalamus		Increased uterine weight; Advanced VO; CL present in $^{3}/_{7}$ rats, cycles: persistent oestrus ($^{5}/_{12}$) or persistent dioestrus	Schiavi, 1964
		arcuate region	rather constant	Increased ovarian and uterine weights Advanced VO; CL present in $^{5}/_{9}$ rats cycles: persistent dioestrus in $^{1}/_{12}$ rats	

17; 25	(HF ?) (electrode ?)	arcuate region (basal)	-	Advanced VO; fairly regular cycles			Martinovitch et al., 1968
4	DC; (electrode, SS ?)	anterior hypothalamus	-	Advanced VO; CL present in $^{15}/_{21}$ rats			Relkin, 1968; 1971
21-23	DC; platinum electrode	supra optic area arcuate region	ø 0,7 - 1,3 mm	Advanced VO Advanced VO			Bloch & Ganong, 1971
22	HF; SS electrode	anterior hypothalamus	small (0,1 mm^2) moderate (0,5 mm^2) large (1,3 mm^2)	VO uneffected, CL present ($^{19}/_{19}$) Advanced VO, CL present ($^{3}/_{5}$) Advanced VO, CL only in $^{2}/_{15}$		cycles with slightly prolonged to persistent oestrus	Meijs-Roelofs & Moll, 1972
		arcuate region	small (0,2 mm^2) moderate (0,5 mm^2) large (1,2 mm^2)	Advanced VO Advanced VO Advanced VO	CL present; cycles slightly prolonged		

advancement of puberty. This percentage increased with increasing age. The advanced first ovulation was always followed by regular oestrous cycles. By giving an overdose of HCG (2 x 25 i.u. ; first injection 24 hr after stimulation)to the experimental animals and measuring ovarian weight (bio-assay for endogenous FSH) it appeared that the observed advancement of puberty after electrical hypothalamic stimulation was mediated via an increased FSH release (for details see appendix , Meijs-Roelofs, 1972). These experiments indicated that electrical stimulation of the hypothalamus is a convenient means of inducing early onset of puberty, presumably by increasing FSH release. Some doubts arose, however, about the usefulness of the bio-assay for endogenous FSH as used in these studies : the first HCG injection was given 24 hr after stimulation to make sure that the total of the acute stimulation-effect would be present. Johnson (1971) assumed that the FSH present at the time of first HCG injection accounts for most of the increase in ovarian weight and that, therefore FSH release can reliably be estimated quantitatively. However, after stimulation of the arcuate nucleus region not only FSH but also LH might have been released and the increase of ovarian weight might have been caused by both these hormones within the 24 hr period. Therefore, the possiblity is clearly present that, at the moment of first HCG injection, an estimation of total gonadotrophins rather than of FSH alone was made with this technique. It therefore seemed of interest to measure LH release as well as, in a more specific way, FSH release after hypothalamic stimulation in immature rats. This was done in our next studies using the now available RIA technique. It was found that electrical stimulation of the arcuate nucleus region, performed in 28-day old rats, resulted in a marked rise in serum LH concentration during the first hour after stimulation. The serum FSH concentrations, though significantly higher than control values at 3 and 4 hr after stimulation, showed very little change. Follicular development was also studied after stimulation; within 3 days large follicles, capable of ovulating after injection with HCG, were present in numbers that are normal for the adult cycle. In control rats no such follicles were generally found.
For details and discussion of results see appendix , Meijs-Roelofs & Uilenbroek, 1973.
Apart from these personal data, only one recent report (Kawakami & Terasawa, 1972) deals with the effect of electrical stimulation of the brain on gonadotrophin release in the immature rat. The authors report an

increase in both serum LH and serum FSH immediately, but not at 30 min., after stimulation of the arcuate nucleus region in 27-day old rats. Moreover 30 min. after amygdaloid stimulation and immediately or 30 min. after hippocampal stimulation an increase of serum FSH was found. Pituitary content of LH was increased immediately after arcuate, amygdaloid and hippocampal stimulation and also 30 min. after stimulation of these areas. Pituitary FSH was increased immediately after arcuate and hippocampal, and 30 min. after amygdaloid and hippocampal stimulation. Although the data for LH after stimulation of the arcuate nucleus region seem to be in agreement with our own,comparison of results is very difficult because of differences in the technique applied : these authors used a monophasic current for 30 min. only; moreover the implantation of electrodes (stainless steel) one day before stimulation already severely influenced the FSH release. In our work electrodes were not implanted and a biphasic current was applied during 60 min. (see Meijs-Roelofs, 1972).
The results of extra-hypothalamic stimulation seem highly interesting in comparison with results from extra-hypothalamic lesions (see pp. 36-37), but are still inconclusive. Some additional data on the effects of extra-hypothalamic stimulation are discussed on page 38 . Personal experiments in which medial pre-optic or amygdaloid stimulation is performed are in progress.

PREGNANCY AFTER STIMULATION OF THE HYPOTHALAMUS

Bearing in mind the definition of puberty: "the stage at which the ability to reproduce sexually is achieved" (Critchlow & Bar Sela, 1967), the question arises whether precocious puberty, i.e. precocious VO, ovulation and oestrous cycles, includes the capacity to sustain a precocious pregnancy.
Some recent studies indicate that after gonadotrophin treatment (Schuetz, 1971; Ying & Greep, 1973) or oestrogen treatment (Ying & Greep, 1971c) precocious, but otherwise normal, pregnancies may occur. It was tested whether this would also be the case in rats with advanced puberty after hypothalamic stimulation. First electrical stimulation of the arcuate nucleus region was performed with our standard procedure in nine 28-day old rats; then, on the day prior to the expected ovulation as judged from the state of the vagina the stimulated females were placed with a male of proven fertility. Nine litter-mate females served as controls and were placed with a male one day prior to the expected spontaneous first ovulation. The following morning mated animals were detected by the presence of vaginal plugs and/or the presence of sperm in the vaginal smears. Age and body weight of the mothers were noted at first oestrus and at the end of pregnancy, just after birth of the litter. It was found that 6/9 of the stimulated rats and 7/9 of the control rats mated successfully at first oestrus and delivered healthy young. Data are given in table 4.
It may be concluded that electrical stimulation of the hypothalamus, not only triggers a fully normal precocious first oestrus, but may also lead to a precocious but otherwise normal pregnancy.

Table 4

Pregnancy and litter-size in stimulated females with advanced puberty.

		Stimulated (6)	Control (7)
At first oestrus (mother)	Age	32.5 ± 0.4*	38.9 ± 0.5
	Body weight	61.7 ± 1.9*	89.9 ± 2.0
At delivery (mother)	Age	54.7 ± 0.3*	60.9 ± 0.6
	Body weight	142.4 ± 4.6*	159.4 ± 2.1
At birth (litter)	Total weight	41.4 ± 2.2	45.7 ± 3.1
	Number of individuals	7.8 ± 0.6	9.3 ± 0.5
Mean body weight of (young) individuals at weaning		28.7 ± 1.1 (45)	26.0 ± 0.6 (60)

*significantly different from controls

( ) number of rats

EXTRA-HYPOTHALAMIC LESIONS

Various investigators report maturational changes in the amygdala which may be related to the onset of puberty. These changes include increased protein synthesis (in mice, MacKinnon, 1970) and a decrease in local seizure thresholds in the amygdala (Terasawa & Timiras, 1968). A role of the limbic system in the regulation of the onset of puberty has been suggested by a great number of studies.

That the limbic system might be an important link in the chain of events leading to sexual maturation is indicated by the statement : " the limbic system seems to act as a funnel for information from the olfactory system and from many other cortical regions (and hence other sensory systems) down into the hypothalamus " (Raisman, 1970). Two tracts of the system are indicated as " of possible importance for hypothalamic control ", the stria terminalis * and the medial cortico hypothalamic tract **. Lesion

studies in which amygdaloid or hippocampal structures were damaged yielded rather contradictory results in either advancing or causing a delay in sexual maturation (see table 5); some authors attributed the differences found to the age at which lesions were made (Bloch & Ganong, 1971; Relkin, 1971). In alternative explanations differences in fiber systems damaged were suggested : Relkin (1971), in contrast to the earlier findings of Elwers & Critchlow (1960), found a delay in onset of puberty after amygdaloid lesions. He pointed at the possibility that other fiber systems than the stria terminalis, the " effective " area in the studies of Elwers & Critchlow, might be involved and modify the lesion effects.

Another suggestion has been made by Riss et al. (1963), namely that the influence of limbic structures on endocrine functions might not be of a rigid nature, but dependent on factors like environmental conditions. This view may find support in the observation of Kling (1964) that damage to the olfactory structures, presumably interfering with environmental stimuli necessary for hypothalamic function, retards the onset of puberty.

A most interesting finding is that of Velasco (1972) who compared the

* Stria terminalis fibers, passing from the amygdala to the ventromedial hypothalamic nucleus, spread out medially into the preoptic area, below the anterior commissure, just lateral to the medial-cortico hypothalamic tract, and descend gradually through the anterior hypothalamus to form a capsule of terminals around the ventromedial nucleus, terminating almost exclusively upon dendritic spines. According to Heimer & Nauta (1969) a postcommissurial, minor, part of the stria terminalis terminates close to the preoptic-supraoptic area.

** The medial-cortico hypothalamic tract, passing from the hippocampus to the arcuate nucleus, leaves the fornix column just behind the anterior commissure and streams medially towards the wall of the third ventricle, then descending as far as the dorsal border of the suprachiasmatic nuclei and turning backwards to the extreme anterior end of the arcuate nucleus. Terminals come into immediate contact with the dendrites of cells whose axons run directly into the median eminence and therefore seem of special interest.

Table 5 Influence of limbic structures on hypothalamic (gonadotrophic) function.

Age of Treatment (days)	Technique used; type of current and electrode	Area of limbic system involved	Effects observed	Action (of involved structure suggested	Author(s)
18-20	DC; insect-pin (SS)	Baso-medial region of amygdala, containing fibers of stria terminalis	Increased uterine weight. Advanced VO and CL present in 3/6 rats with VO	Inhibitory on gonadotrophin secretion	Elwers & Critchlow, 1960
18-20	DC; insect-pin (SS)	Stria terminalis transected	Increased uterine weight, advanced VO and CL present in 3/5 rats with VO.	Inhibitory on gonadotrophin secretion	Elwers & Critchlow, 1961
7	Aspiration of brain tissue	Hippocampus	Decreased ovarian and adrenal weight, reduced running activity	Influence of limbic structures on endocrine functions variable	Riss et al., 1963
3 - 19	Mechanical lesions	Olfactory stalk medial olfactory area hippocampus	Decreased ovarian and adrenal weight; delayed VO delayed VO VO not influenced	Primary olfactory structures interfere with hypothalamic function	Kling, 1964
21	DC; platinum electrode	Ventrolateral hippocampus	Delayed first oestrus in normal and TP treated rats; cycles undisturbed	Hippocampus must be intact for central action of TP in advancing puberty	Zarrow et al., 1969
4	DC; electrode?	Basal, medial and cortical amygdaloid nuclei; including converging fibers of stria terminalis	Delayed puberty (VO; luteinization)	Not clear	Relkin, 1971
21-23	DC platinum electrode	Corticomedial and basolateral nuclei of amygdala and stria terminalis within amygdala	VO and cycles not influenced	Absence of effect possibly by spontaneously advanced onset of puberty	Bloch & Ganong, 1971

24	DC 1) SS electrode	Medial amygdaloid nucleus	Increased uterine weight; Advanced VO	Stimulatory on gonadotrophin release	Velasco, 1972
	2) platinum electrode		Atrophy of ovary and uterus, (delayed VO)		
24	Knife-cuts; radiofrequency lesions or suction	Hippocampus (fimbria) stria terminalis	Advanced VO and advanced first ovulation	Inhibitory on gonadotrophin secretion Not clear whether destruction of stria terminalis or of fimbria is responsible for effect observed	Brown-Grant & Raisman, 1972

effects of amygdaloid lesions produced with a direct current and either stainless steel or platinum electrodes. Advancement of puberty was only observed after lesions made with stainless steel electrodes; his interpretation of the results is therefore that the effect observed is due to the stimulatory action, by deposition of metallic ions (Everett and Radford, 1961), of the technique used. The authors conclusion is a stimulatory influence of the amygdala on gonadotrophin release, in contrast with the conclusions drawn from lesion studies by Elwers & Critchlow (1960, 1961). However, the latter authors also reported a delay in onset of puberty after electrical stimulation of the amygdala (Bar-Sela & Critchlow, 1966). In these studies electrodes were implanted in the corticomedial amygdaloid region and in the hippocampal formation and stimulation was performed daily, starting at day 25-27, for 6 hr/day. A delay in onset of puberty was observed following stimulation of the amygdaloid region but not after stimulation of the hippocampus. It may be recognized from the data however, that the delay of puberty observed after amygdaloid stimulation holds only for the age and not for the body weight at which VO (the sign of puberty) was observed. Since body weights were clearly much lower at comparable ages in delayed, stimulated rats in comparison with both non-delayed, stimulated rats and with non-stimulated rats, and since puberty is more closely related to body weight than to age (Kennedy, 1957), the effect observed may be due to changes in body weight only.

The recent study of Brown-Grant & Raisman (1972, see table 5), supports the idea that the influence of the limbic system on gonadotrophic function in the immature rat is an inhibitory one. Whether this inhibitory influence must be attributed to amygdaloid or hippocampal structures is not clear. Experimental results in the adult rat, the induction of ovulation after electrical (Bunn & Everett, 1957) or electrochemical (Velasco & Taleisnik, 1969) stimulation, suggest a facilitatory influence of the amygdala on gonadotrophin-release, especially LH. Sawyer (1972) pointed to the fact that the induction of gonadotrophin release by electrical stimulation of the amygdala failed unless the titer of natural or exogenous oestrogen is adequate : oestrogen-primed, pentobarbital-blocked prooestrous or persistent oestrous animals were used in studies with positive results. On the basis of oestrogen implantation experiments (Lawton & Sawyer, 1970) and the biochemical demonstration of oestrogen-binding macro-molecules in the nuclei of cells in the amygdala (McEwen & Pfaff, 1970; Pfaff &

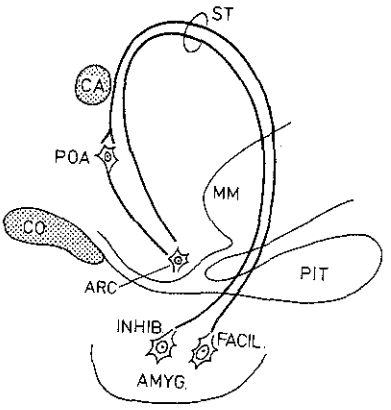

FIG. 3. Diagram of hypothetical stria terminalis (ST) projections of inhibitory (INHIB.) and facilitatory (FACIL.) neurons from the cortico-medial amygdala (AMYG.). ARC=arcuate nucleus region; CA=commissura anterior; CO=chiasma opticum; MM=mammillary body; PIT=pituitary. (after Sawyer, 1972).

Keiner, 1972; Stumpf 1970, 1972), a role of the limbic system in the oestrogen feed-back circuit has been assumed (see also Wildschut, 1972; Zolovick, 1972). Lawton & Sawyer (1970) concluded that the amygdala represent a stimulatory site of action for oestrogen in the feedback mechanism for LH. Surveying available data on amygdaloid influence on gonadotrophic function, Sawyer (1972) concludes : " ..... the cortico-medial amygdala of the female rat appears to contain two functional groups of neurons : (1) cells inhibitory to gonadotrophic function in general and (2) cells facilitatory to the ovulatory surge of pituitary LH release. Both appear to project to the hypothalamus via the stria terminalis ". (See fig. 3).

The author further suggests that in the immature rat, because of a relative lack of oestrogen, only the inhibitory cells may be activated.

Döcke (1972) reported that either amygdaloid lesions or oestrogen treatment advance sexual maturation, whereas the combined procedure does not, thus providing new evidence for an interaction between amygdala and oestrogens in their respective roles in sexual maturation (compare Zarrow et al., 1969, see table 5).

The total of available data points to a role of the limbic system in the

regulation of gonadotrophic function, and therefore in the regulation of sexual maturation. Further studies will be needed to clarify this role.

HORMONAL TREATMENTS

OVARIAN STEROIDS

Effects of oestrogen treatment on the onset of puberty (table 6 a)

Since the work of Hohlweg (1934), it has been known that oestrogen treatment in immature rats gives rise to ovarian changes, as normally seen in the adult rat : formation of corpora lutea occurred following a single injection with a high dose of oestrogen. This effect could be abolished by well-timed hypophysectomy (Hohlweg & Chamorro, 1937). Westman & Jacobsohn (1938) showed the involvement of the central nervous system in the " Hohlweg effect " since they found the effect to be absent if hypophysial stalk section was performed within $2\frac{1}{2}$ days after oestrogen treatment. Döcke & Dörner (1965) reported that precocious corpus luteum formation after oestrogen treatment could be prevented by lesions of the suprachiasmatic nuclei and the medial preoptic area, indicating that for this oestrogen action to take place the preoptic hypothalamo-hypophysial pathways should be intact.

That stimulatory effects of a different type of oestrogen treatment, a series of low doses, could lead to a precocious but otherwise normal onset of puberty was demonstrated by Ramirez (1964) and Ramirez & Sawyer (1965). With near physiological doses of oestrogen (0.05 µg/100 g b.w. / day) precocious canalization of the vagina was observed, due to a direct local oestrogen action, followed by first ovulation and normal oestrous cycles. Start of treatment before the age of 26 days appeared to be ineffective. Pituitary LH content dropped abruptly on the day of VO and a rise in plasma LH was found, phenomena also observed at natural onset of puberty. The authors conclude to a central action of oestrogen on the brain pituitary complex, thus influencing LH secretion. Corbin & Daniels (1969) demonstrated that also the FSH secretion was activated by similar oestrogen

treatment, though the exact timing of FSH secretion in relation to first ovulation is not clear. Hagino et al. (1966) showed that daily injections of a higher dose of oestrogen (4.0 µg/rat/day) exerted more pronounced direct effects on the vagina but suppressed ovulation, presumably due to inhibition of gonadotrophin release. Besides, these authors showed that precocious ovulation induced by oestrogen could be prevented by pentobarbital injections. Low dose oestrogen treatment was reported to be also effective when a single injection was given (Ying & Greep, 1971b; Ramirez, 1971). Ying & Greep (1971b) reported that the effectiveness of treatment increased when the age at which the single oestrogen injection was given increased from 22 to 28 days. Progesterone given 2 days after OB treatment increased the ovulatory response and this facilitatory action of progesterone was already operative at 24 days of age. Facilitation of OB-induced ovulation by progesterone was also reported by Döcke & Dörner (1966). Various mechanisms may be involved in the advancement of puberty by oestrogen treatment. Direct effects of oestrogen on the ovary will be discussed first, followed by a discussion of oestrogen effects on gonadotrophin release.

Direct effects of oestrogen on the ovary

Direct action of oestrogen upon the ovaries has also been reported. Croes-Buth et al. (1959) showed that in immature, hypophysectomized rats small and medium-sized follicles were clearly stimulated by daily low dose (0.5 µg) oestrogen treatment. According to these authors the absence of stimulation of the larger (antral-) follicles was presumably due to the absence of gonadotrophins in the hypophysectomized rats. Smith (1961) also demonstrated growth and maintenance of medium-sized follicles by oestrogen. Bradbury (1961), who implanted oestradiol directly on one ovary, reported a significant increase in ovarian weight and formation of corpora lutea, as direct oestrogen effects on the ovarian level. This author considered that there was an increased responsiveness of the ovary to gonadotrophins after oestrogen treatment, as well as an effect of oestrogen via the pituitary (see also Ying & Greep, 1971b). Paesi (1952, see also Byrnes & Meyer, 1951) showed the effects of oestrogen injection to be clearly dose-dependent: repeated injection with the lower doses resulted in decreased ovarian weight and atrophy of interstitial tissue, while with

Table 6a Effects of oestrogen treatment in immature female rats.

Age at start of treatment (days)	OB dose used	Treatment schedule	Effects observed	Oestrogen action suggested	Remarks	Author(s)
? (b.w. ± 50 g)	50–100 RU	single OB injection	Formation of CL; effect abolished by hpx; effect abolished by hypophysial stalk section within 2½ days after injection	Influence on gonadotrophin (LH) secretion within 4 days after injection. Role of CNS is important in oestrogen action	Massive OB dose	Hohlweg, 1934; Hohlweg & Chamorro, 1937; Westman & Jacobsohn, 1938
? (b.w. 35–40 m. poA	30 µg	single OB injection in intact rats or rats with hypothalamic lesions	75% of intact rats with CL formation; effect abolished by lesions of SC; m. poA m. poA	"Hohlweg" effect only with intact hypothalamus	High OB dose	Döcke & Dörner, 1965
23–26	15 µg	single OB injection	20% of rats with CL. formation? P administered 3 days (not earlier!) after OB facilitates ovulation-inducing effect	Oestrogen-induced gonadotrophin release facilitated by well-timed P-administration		Döcke & Dörner, 1966
30	range 0.002 – 0.05 µg	10 (daily) injections	Dose 0.002–0.009 µg OB causes a decrease in ovarian weight; a relative increase occurs with 0.012–0.02 µg OB and a second decrease with higher doses	1) Oestrogen influences gonadotrophin secretion 2) Inhibitory influence on FSH release at lower OB dose than stimulatory influence on LH release 3) Highest doses inhibits LH release	Long term treatment in intact rats; OB-dose not related to b.w.	Byrnes & Meyer, 1951
? (b.w. 40–60)	range 0.002 – 100 µg	7 times 2 daily OB injections	Low dose (0.01–0.05 µg) decrease in ovarian weight. Moderate dose (0.1–0.5 µg) rise in ovarian weight. High dose (0,5 µg) no further rise in ovarian weight	Oestrogen effects both LH and FSH release (judged from respectively appearance of interstitial tissue and follicular diameters); Low dose OB treatment inhibits LH and not yet FSH. Moderate and higher doses have dose dependent stimulatory actions	$^1/_5$ rats treated with 0.5 µg showed corpora lutea. Ovarian changes may be due to increased effectiveness of LH and FSH	Paesi, 1952
5, 14 or 26	0.01 to 0.05 µg per 100 g b.w.	daily OB injections (4 or more in intact or OVX rats)	VO advanced by 8 days, ovulation and oestrous cycles; VO also in OVX-rats. short and long term treatment equally effective. Drop in Pituitary LH / Rise in plasma LH in intact, not in OVX rats } on day of VO	VO by direct oestrogen action. Oestrogen induces LH secretion necessary for puberty (by central action on brain pituitary complex	Near-physiological OB-dose treatment not effective before 26 days of age	Ramirez, 1964; Ramirez & Sawyer, 1965

24	0.05 µg or 4.0 µg	daily OB injections (4) Pentobarbital (daily)	Advanced VO, ovulation and cycles to be prevented by pentobarbital injection OB dose of 4.0 µg suppresses ovulation; VO advanced	VO by direct oestrogen action Induction (0.05 µg OB) and suppression (4.0 µg OB) of ovulation presumably via CNS; pentobarbital postpones ovulatory LH release	Possibility exists that pentobarbital causes accelerated oestrogen inactivation	Ragino et al., 1966
26	0.05 µg per 100 g b.w.	daily OB injection (4–6) (OB + P; P or TP injection)	Advanced VO; presence of CL, normal cycles OB induces drop in pituitary FSH drop in hypothalamic FSH-RF Combined OB + P treatment not effective	Oestrogen plays a role in activation of FSH-RF/FSH mechanism, leading to normal puberty	Exact timing of FSH-RF/FSH changes in relation to 1st ovulation unknown	Corbin & Daniels, 1969
range 22–30	range 0.1 – 8.0 µg	single OB injection (followed by 2 days later FSH 0–2 days later	OB dose of 0.25–0.5 µg on day 28–30 induced 50–80% of rats to ovulate. P given 2 days after OB facilitates ovulation; FSH 1–2 days after OB accelerates induced ovulation. Phenobarbital 2 days after OB blocks ovulation	OB increases follicular sensitivity to exogenous gonadotrophins; this effect is more marked in older rats	% Ovulation induction after OB injection increases from day 22–28 P facilitation ready operative at 24 days of age	Ying & Greep, 1971b
range 26–34	0.05 µg – 1.0	single injection	Optimally effective on day 26: 100% ovulation 3 days later, normal number of ova (dose 0.05 µg) Decrement in ovulatory response thereafter; lowest response (% of rats ovulating; number of ova) day 37	OB treatment less effective after 26 days of age by diminished response of the ovary LH (demonstrated by HCG treatment)	Ovarian refractory period suggested from 27–29 days till 35–37 days of age	Ramirez, 1971

moderate and higher doses reversed effects were obtained. The author suggested that oestrogen could be effective via an influence on both LH and FSH release, whereas LH could be inhibited (interstitial atrophy) at an oestrogen dose that was too low to affect FSH release (as seen from follicular diameters) and that with the higher OB dose LH and FSH release were stimulated, as judged from effects on interstitial tissue and diameter of largest follicles. The possibility that the effects observed could also be caused by changes in effectiveness of LH and FSH instead of by their increased release was also suggested.
A recent report by Goldenberg et al. (1972) indicated such a role of oestrogen in follicular growth; increased incorporation of FSH in the ovary, favouring follicular growth, was found by these authors.

Effects of oestrogen treatment on gonadotrophin release (table 6b)

A number of studies have been concerned with the direct effect of oestrogen treatment on gonadotrophin release in immature rats. An increase in FSH release, as measured by a possibly less specific endogenous bio-assay, after a single or repeated OB injection(s) has been reported in 22-day old rats (Naqvi & Johnson, 1969; Johnson & Naqvi, 1969).
In 26-day old rats Ying et al. (1971) demonstrated that an ovulation-inducing oestrogen dose resulted in an increase in serum FSH 2 hr after injection, while serum LH remained low up to 48 hr after injection. Peak values of both serum LH and serum FSH were found at 56 hr after oestrogen injection. This indicates that the primary effect of low-dose oestrogen injection is first an increase in FSH release, resulting in ovarian follicular development followed by induction of ovulatory gonadotrophin release.
In rats of 28 days or older Caligaris et al. (1972), using a dose of 10 µg OB, reported a stimulatory action of oestrogen on LH release. Pretreatment with OB elicited this positive response already at the age of 22 days, progesterone injected 3 days after pretreatment with OB was even more effective.
On the basis of their data these authors conclude that the mechanism for phasic LH release is not mature before the age of 20 - 22 days, possibly due to incomplete development of some extra-hypothalamic structures.
An inhibitory action of oestrogen treatment on gonadotrophin release in

immature rats has also been demonstrated. Apart from the studies of Paesi (1952) and Byrnes & Meyer (1951) in intact rats, Ramirez & McCann (1963) and Parlow (1964) reported inhibitory effects of oestrogens on gonadotrophin release in gonadectomized rats. In a more recent study Goldman & Gorski (1971) demonstrated that in intact rats already at 5 - 10 days of age a single OB injection acutely inhibits the FSH release. The existence of an oestrogen-feedback mechanism operative physiologically in immature rats has already been discussed on pp. 25-27.

Effects of oestrogen implantation

The effects of oestrogen implantation experiments (see table 6b) are currently rather conflicting.
Döcke & Dörner (1965) reported induction of corpora lutea after implantation of oestradiol in the medio-basal area of the anterior hypothalamus, whereas implantation in the anterior pituitary was most effective.
In contrast, Motta et al. (1968) found implantation of oestrogen into the pituitary to be completely ineffective; implantation in the median eminence region induced precocious VO, corpus luteum formation and increase in plasma LH. Smith & Davidson (1968) reported that intrapituitary implantation of cholesterol, as well as implantation of oestrogen, was effective in advancing VO; for intrahypothalamic implantation effects were different for chronic and acute experiments.
In conclusion it can be said that prepuberal oestrogen treatment at the appropriate dose and at the appropriate age (i.e. not before 26 days of age) may result in precocious onset of puberty as indicated by early VO, first ovulation and oestrous cycles. Whereas VO is caused by a direct effect of oestrogen, ovulation is brought about by oestrogen-induced changes in both LH and FSH release. The primary effect of oestrogen treatment seems to be an increase in FSH release while ovulatory release of both LH and FSH is induced indirectly, via ovarian changes. A positive, direct action of oestrogen on the hypothalamo-hypophysial system for phasic LH release does not seem to be normally present before the age of 28 days, though oestrogen-priming may advance it. The fact that pentobarbital administration blocks the oestrogen-induced ovulation, together with the facilitatory action of progesterone, indicate that the oestrogen-induced

Table 6b Effects of oestrogen treatment on gonadotrophin release; effects of oestrogen implantation

Age at start of OB treatment (days)	OB dose used	Treatment Schedule	Effects observed	Oestrogen action suggested	Remarks	Author(s)
22-25	1 µg	single OB, P or TP injection	Increased ovarian weight after OB or TP treatment; no increase after P treatment	OB and TP treatment stimulate FSH release; presumably also FSH synthesis	Endogenous bio-assay for FSH	Naqvi & Johnson, 1969
22	1 µg	single OB or TP injection	FSH increase at 24-60 hr after OB: TP induces lower but cyclic FSH release	OB induces FSH release; effect within 84 hr Pituitary FSH store decreases	Endogenous bio-assay for FSH suitable ??	Johnson & Naqvi, 1969
22	1 µg	single or 2 OB injection(s)	Second OB injection at 60 hr after first injection induces FSH release within 24 hr , max. reached at 48 hr after first injection	Repeated OB-induced FSH release is possible	Endogenous bio-assay for FSH Production and release of FSH not related to pituitary FSH store	Johnson, 1971
26	0,5 µg	single OB injection	Increase in serum FSH 2 hr after injection	Direct effect on FSH release causing follicular changes leading to ovulatory gonadotrophin release	-	Ying et al., 1971
18-45	10 µg	single OB injection (second OB injection 2 days later,or P injections 3 days later)	OB treatment induced LH release on the next day in rats of 28 days or older. After OB-priming, effect in 22-day old rats. P given 3 days after OB, causes (acute) LH rise	Stimulatory oestrogen feedback for phasic LH release immature till 20-22 days	RIA for LH Compare Ying & Greep, 1971b for facilitatory action of P	Caligaris et al., 1972
5, 6, 7, 8, 10	0,02-0,2 µg	single OB injection	OB lowers serum FSH levels within 8-9 hr ; effect on LH not clear	In neonatal rats already inhibitory influence of OB treatment on gonadotrophin release	Intact females used	Goldman & Gorski, 1971

Oestrogen implantation experiments						
b.w. 35–40g	0.05–1.0 µg	OB pellet in hypothalamus or pituitary, †5 days later	Dose of 1.0 or 0.4 µg OB in mediobasal, anterior hypothalamus induces CL formation; pellets in contact with cerebrospinal fluid more effective; most effective: pellets in anterior pituitary	Oestrogen increases hypophysial sensitivity to gonadotrophin-RF		Döcke & Dörner, 1965
26 9	± 0.1 µg	implantation in ME, habenular region or pituitary, †13 days later	Implantation in ME: precocious VO and CL, drop in pituitary LH, rise in plasma LH. Implantation in habenular region: retarded VO, rise in pituitary LH. No changes after implantation of OB in pituitary	OB in ME stimulates LH release; OB in habenular region reduces LH release. Intrapituitary OB implants no effect on LH release	OAAD test for LH Habenular region is receptor area for oestrogen	Motta et al., 1968
26	2 different (?) doses	chronic implantation (for 5 weeks)	Implantation in ME, AHA-POA, cortex or midbrain: advanced VO, first period of cycles disrupted. Decrease in ovarian and uterine weight after ME implantation	Greater amount of oestradiol needed for inhibitory feed-back (ovarian, uterine weight decrease) than for stimulatory feedback (advancement of puberty)		Smith & Davidson, 1968
		acute implantation (for 48 hr)	Implantation in AHA-POA advanced VO; Implantation in ME ineffective Intrapituitary implantation of both oestradiol and cholesterol advances VO	Effect of precocious VO after OB implantation is not local		

processes are highly comparable to events occurring during the adult cycle (see Everett & Sawyer, 1950; Zeilmaker, 1966; Wildschut, 1972).
A direct effect of oestrogen on the ovary, notably a stimulation of follicular growth, has also been demonstrated, whereas follicular sensitivity to gonadotrophins seems to increase. A direct action of oestrogen on the hypophysial level, causing an increased sensitivity to gonadotrophin releasing factors may also play a role (Döcke & Dörner, 1965; see also Arimura & Schally, 1971; Weick et al., 1971).

GONADOTROPHINS

PMS

Induction of precocious ovulation in immature female rats by PMS or combined PMS-HCG treatment has been the subject of many studies since the initial finding by Cole (1936). With some exceptions (McCormack & Meyer, 1964; Grayburn & Brown-Grant, 1968; Ying & Meyer, 1969; Ying & Greep, 1971a), rather high doses of PMS ($\geqslant$ 20 i.u.) were generally used and often superovulation, i.e. ovulation of a supra-normal number of ova, was induced. In most cases, events following the first induced ovulation were not studied, so that it remains unclear whether a real precocious puberty was induced. The effect of PMS treatment was shown to be age-dependent. The initial response has been found in the period from 17 - 20 days of age (Zarrow & Wilson, 1961; Zarrow & Quinn, 1963; McCormack & Meyer, 1964), generally spoken the age at which antral follicles have developed in the ovaries. The initial ovulatory response to PMS treatment yielded a sub-normal number of ova, notwithstanding the high dose (30 i.u.) of PMS used (Zarrow & Quinn, 1963). The ovulatory response to PMS treatment of the immature rat at a certain age is clearly dose dependent; this holds for the percentage of rats reacting as well as for the number of ova released (Williams, 1945; McCormack & Meyer, 1964; Ying & Meyer, 1969).
A maximal response to PMS treatment was obtained at the age of 28 days if PMS was given alone and at 22 days of age if PMS injection was followed by HCG injection 56 hr later (Zarrow & Quinn, 1963).
Events occurring immediately after PMS injection and leading to ovulation have been studied and clarified in great detail and have proven highly comparable to events during the adult cycle. Involvement of the central

nervous system (Hagino, 1969) and endogenous hypophysial gonadotrophin release (Rowlands & Williams, 1941; Rowlands, 1944) are beyond doubt.
A considerable number of studies indicate that the endogenous ovulatory LH release takes place 52 - 56 hr after PMS injection (Strauss & Meyer, 1962; Quinn & Zarrow, 1964; Wagner & Brown-Grant, 1965; Klausing & Meyer, 1968; Sorrentino et al., 1972). This release may be blocked and delayed for 24 hr by well-timed pentobarbital administration (McCormack & Meyer, 1962; Klausing & Meyer, 1968; Hagino, 1969; Umezu, 1970).
A number of other phenomena resemble the condition in the adult rat. The time of the " critical period " for the LH release was found to be between 2 and 4 - 5 P. M., comparable to the critical period in the adult rat (Strauss & Meyer, 1962; Hagino, 1969, and others).
An endogenous circadian rhythm for LH release seems to be present already in the immature rat (Klausing & Meyer, 1968; McCormack & Bennin, 1970). Exposure to continuous light causes a delay in PMS-induced ovulation and varying the time of PMS injection alters the day but not the time of ovulation (McCormack & Bennin, 1970). An increase in pituitary LH content followed by a clear decrease at 56 hr after PMS treatment has been reported (Zarrow & Dinius, 1971); Klausing & Meyer (1968) also found a decrease in pituitary LH during the critical period after PMS treatment. However, Rennels & O'Steen (1967) could not detect changes in pituitary LH preceding PMS-induced ovulation. They did find a moderate decrease in pituitary FSH content.
Oestrogens play a role in PMS-induced ovulation by exerting a stimulatory feedback action on LH release. It has been shown that for the PMS-induced changes in pituitary LH the presence of the ovaries is necessary; in ovariectomized PMS treated rats pituitary LH changes could be restored by oestrogen treatment (Zarrow & Dinius, 1971). Grayburn & Brown-Grant (1968) demonstrated that, in contrast to PMS treatment, FSH treatment did not result in spontaneous ovulation, presumably due to lack of oestrogen secretion. After FSH treatment ovulation could be induced by combined oestrogen-progesterone treatment, but not by oestrogen alone. A role of oestrogen in PMS-induced ovulation was also shown by Ferin et al. (1969), who found that injection of anti-sera against oestradiol-17β , administered up to 15 hr prior to the expected LH release, blocked PMS-induced ovulation; ovulation could be restored by HCG administration.
A facilitatory action of well-timed oestrogen injection on PMS-induced

ovulation was shown by Hagino & Goldzieher (1970), who found that oestrogen injection on the morning of the second day after PMS injection was most effective. A clear dose dependency was also noted. Ying & Greep (1971a) confirmed the importance of both treatment schedule and dosage of oestrogen in reporting both stimulatory effects on ovulatory gonadotrophin release and inhibitory effects on tonic gonadotrophin release.
Progesterone may also be involved in PMS-induced ovulation in the immature rat. McCormack & Meyer (1963) demonstrated that progesterone given before 5 P.M. on the second day after PMS treatment increased the incidence of ovulation. This progesterone effect was found to be effective already in very young rats, receiving PMS on day 18. The facilitatory progesterone effect was found to be mediated by a neurally controlled release of pituitary LH (McCormack & Meyer, 1964; Gallo & Zarrow, 1970). Results from different reports suggest that the progesterone effect on PMS-induced ovulation may be a biphasic one and comparable to the progesterone effects during the adult cycle (see Everett, 1965; Zeilmaker, 1966; Caligaris et al., 1968). Zarrow & Hurlbut (1967), who systematically studied the effect of progesterone administered at the time of, or at different times after, PMS treatment found that progesterone given 48 hr after PMS injection facilitated ovulation, whereas progesterone, given simultaneously with or 24 hr after PMS injection suppressed the ovulation expected at 72 hr after PMS injection. However, Zarrow & Gallo (1969) demonstrated that progesterone given 24 hr after PMS treatment induced premature ovulation in a high percentage of rats, which may account for the absence of ova at 72 hr after PMS injection in the previous study. In a later study (Zarrow & Dinius, 1971) it was shown that progesterone injected 24 hr after PMS administration induced a drop in pituitary LH a few hours later. This indicates that also at 24 hr after PMS treatment progesterone exerts a stimulatory feed-back action on LH release.

Summarizing, it can be said that a single PMS injection may cause (super-) ovulation in the immature rat. The spontaneous ovulatory LH surge occurs the second day after PMS treatment and, under usual lightning conditions, this release occurs between 2 P.M. and 5 P.M., the so-called " critical period ". The ovulatory LH surge is triggered by a neural mechanism in which both oestrogen and probably progesterone are indispensable.
The PMS-induced ovulation, occurring 3 days after PMS injection may be

facilitated by administration of HCG (56 hr after PMS), oestrogen (on the morning of the second day at 9 A. M.) or progesterone (on the second day after PMS (48 - 56 hr).
Events subsequent to PMS-induced ovulation remain obscure.

FSH

Pure FSH preparations have been reported to induce precocious ovulation in immature rats. Zarrow & Gallo (1966) reported induction of ovulation in 28-day old rats after administration of a single FSH injection, provided FSH was suspended in a vehicle permitting delayed absorption from the site of injection. Injection of FSH dissolved in saline failed to induce ovulation, which is in agreement with results of Grayburn & Brown-Grant (1968). Absence of ovulation in these cases was presumably due to lack of adequate ovarian stimulation for steroid production to occur, and, therefore, lack of adequate triggering of ovulatory gonadotrophin release. Daily FSH injections were also able to induce ovulation in immature rats. This ovulation could be prevented by phenobarbital. Progesterone, given 2 days after the first FSH injection, facilitated the FSH induced ovulation, suggesting a similar mode of action as that of PMS treatment (Meyer & McCormack, 1967).

HCG

A single injection of HCG may induce ovulation in the immature rat. The initial response was found at 19 - 22 days of age (Sugawara et al., 1969; Sugawara & Takeuchi, 1970).
Induced ovulations were found to take place within 24 hr - 120 hr after HCG injection. A subnormal number of ova was found following HCG injection before 24 days of age; thereafter (near-) normal numbers of ova were observed. The effects of HCG treatment were clearly dose dependent : increasing the HCG dose from 2.5 to 20 i.u. led to an increase in both incidence of ovulation and number of ova; a decrease in number of ova and ovulation rate was found with higher (50 and 100 i.u.) doses of HCG.

PART III. GENERAL DISCUSSION

Since the reviews on puberty of Donovan & van der Werff ten Bosch (1965) and of Critchlow & Bar-Sela (1967) a great number of experimental data have changed and extended our insight in the mechanisms regulating the onset of puberty. An attempt will be made to summarize these new data and special attention will be paid to those findings which are not concordant with ideas or hypotheses put forward in the reviews mentioned above. On the basis of information now available, a concept of mechanisms regulating the onset of puberty will be given.

GONADOTROPHINS

Our knowledge on hormonal events in the immature rat has been greatly extended by the development of sensitive RIA methods. Direct measurements of gonadotrophin concentrations in the blood became possible and revealed a developmental pattern of circulating gonadotrophins which is rather different from the pattern, expected on the basis of indirect observations. Absence of FSH in the blood of immature rats before ± 21 days of age (Shiino & Rennels, 1967) followed by a gradual increase in circulating FSH during the 4th week of life (Critchlow & Bar-Sela, 1967) has been assumed. In contrast, recent measurements of FSH concentrations by RIA have shown high FSH concentrations before day 21, with peak values around day 15, and constant relatively low FSH concentrations during the fourth week of life (see pages 19-20 and Meijs-Roelofs et al., 1973). For effective release of gonadotrophins the demonstration of a functional hypophysial portal system (Florsheim & Rudko, 1968) and presence of gonadotrophin releasing factors (Campbell & Gallardo, 1966) during the first week of life (see also pp. 24-25) are of importance. The structural identification of gonadotrophin releasing factors (Schally et al., 1971), their synthesis (Schally et al., 1971; Sievertsson et al., 1971; Monahan et al., 1971) and localization in discrete hypothalamic regions (see McCann, 1970) constitute an important contribution to the knowledge of the gonadotrophin release mechanism. Administration of synthetic gonadotrophin RF has been reported to cause a

release of gonadotrophins also in the immature rat and it may result in accelerated sexual maturation (Schröder et al., 1972). A pituitary responsiveness to LH-releasing hormone that changes with age (Debeljuk et al., 1972), and which may be partially determined by amounts of oestrogen present (Arimura & Schally, 1971), may be part of the mechanisms controlling the onset of puberty.

OVARIAN STEROIDS

Ovarian oestrogen secretion has been thought to be low during most of the prepuberal period (see Critchlow & Bar-Sela, 1967). Measurement of oestradiol concentrations in the blood of immature rats by RIA has shown very high concentrations of oestradiol to be present around day 15 of life (Meijs-Roelofs et al., in press, see appendix).

For the possible interaction of ovarian steroids and the hypothalamo-hypophysial gonadotrophin release mechanism the presence of specific steroid binding sites in hypothalamus and hypophysis is important. It has been reported that various parts of the brain incorporate tritiated oestradiol in high amounts in 4 - 5 day old rats (Woolley et al., 1969; Presl et al., 1970) with no clear preferential up-take in hypothalamic areas. After a sharp decrease in total oestrogen up-take at about 10 days of age, the age at which the female or male type of gonadotrophic function of the hypothalamus is determined, preferential uptake of the median eminence region and the anterior hypothalamus develops and is established at approximately 3 weeks of age.

These data seem in agreement with recent findings of Kato (1972) who studied the maturation of isolable hypothalamic oestradiol receptors. Presence of a rudimentary receptor at 7 days of age and a rapid formation of oestradiol receptors between 14 and 21 days was found.

Preferential oestradiol uptake by the pituitary gland and uterus seems to exist already at 2 - 5 days of age (Woolley et al., 1969; Presl et al., 1970; Tuohimaa & Niemi, 1972).

INHIBITORY STEROIDAL FEEDBACK

The existence of a gonadal inhibitory feedback on gonadotrophin secretion in immature rats has often been discussed. Early studies indicated such a

feedback mechanism to be functioning early in life, though in later reports it has been concluded that a negative feedback mechanism is absent before the age of about 25 days (see page 25). In contrast again, ovarian inhibition on gonadotrophin secretion early in life, presumably within the first week, has recently been shown to exist and this holds for both LH and FSH secretion (Caligaris et al., 1972; Meijs-Roelofs et al., in press). It may be concluded that the tonic release mechanism for both LH and FSH is functioning very early in life.

It has been concluded from a considerable number of experiments (see reviews Donovan & van der Werff ten Bosch, 1965; Critchlow & Bar-Sela, 1967) that the sensitivity of the gonadotrophin regulating mechanism to the inhibitory oestrogen feedback action is much higher in the immature than in the adult rat. Initial studies involved the prevention of cytological castration changes in the pituitary by oestrogen injections (Hohlweg & Dohrn, 1932; Hoogstra & de Jongh, 1955), when lower amounts of oestrogen were needed in immature than in adult rats. In later studies it was indicated that the hypersensitivity for oestrogen involved primarily the LH releasing mechanism (Ramirez & McCann, 1963; McCann & Ramirez, 1964), though Byrnes & Meyer (1951), on the basis of ovarian and uterine changes after oestrogen administration in intact rats, concluded to hypersensitivity of the FSH releasing mechanism. Recent experiments in which blood LH levels were measured by RIA seem to confirm the hypersensitivity of the LH releasing mechanism for oestrogen. Steele & Weisz (1973) and Uilenbroek (unpublished results) found that a postcastration rise in blood LH-levels could be prevented with significantly lower amounts of oestrogen in the immature rat than in the postpuberal rat. A clear decrease in sensitivity for oestrogen was found to occur at approximately the time of puberty.

The hypothesis of a shift in hypothalamic sensitivity to oestrogen as an important factor for initiation of puberty has been used as an explanation for results of lesion-studies (Donovan & van der Werff ten Bosch, 1965) and oestrogen implantation studies (Smith & Davidson, 1968).

However, it should be realized that at the time this hypothesis was put forward the presence of oestrogens in the blood of immature rats was not known. The high amounts of oestrogen present around the 15th day of age are clearly not very effective in inhibiting the FSH release at this age, since very high FSH concentrations and to a lesser extent LH concentrations are found in the blood concommitantly (Meijs-Roelofs et al., in press), but

in our opinion it still is possible that the biological activity of circulating oestrogens at this early age is highly limited by the presence of specific binding proteins which disappear at later ages. These findings are therefore not necessarily contradictory to the hypothesis.
However, the findings that the (absolute) metabolic clearance rate of oestrogen is considerably lower in prepuberal than in adult rats (De Hertogh et al., 1970), together with the earlier findings that the function of the liver in inactivating ovarian hormones (Donovan & O'Keeffe, 1966) and the spectrum of steroids released before puberty (Donovan et al., 1967) may be different for the immature and the adult rat, clearly complicates the comparison between immature and adult rats. It therefore should be stressed that, though the hypothesis of a changing hypothalamic sensitivity provides an explanation for a number of unsolved problems concerning the onset of puberty, no conclusive evidence has been reached for the existence or significance of such mechanism. Though it has also been reported in the human (Kulin et al., 1969), the existence of a prepuberal hypersensitivity of the FSH-releasing mechanism to oestrogen certainly requests additional proof; for the LH release mechanism the hypersensitivity seems more convincingly proven, but alternative explanation of results via differences in oestrogen metabolism can not be excluded. The role of the developing specific oestradiol receptors in the hypothalamus in relation to feedback sensitivity warrants investigation.

STIMULATORY STEROIDAL FEEDBACK

Whereas an inhibitory gonadal feedback on tonic gonadotrophin secretion is present and functional early in life, a stimulatory steroidal feedback for cyclic gonadotrophin release only develops after the third week of life, as indicated by a great number of experiments.
Caligaris et al. (1972) demonstrated stimulatory effects of both oestrogen and progesterone on LH release during the fourth week of life which may explain that, from that age on, precocious ovulation may be induced by gonadotrophin treatment, oestrogen treatment, lesioning or stimulation of the hypothalamus. It is of interest that the development of the positive steroidal feedback mechanism seems to follow immediately, once the ovary has attained the capacity to ovulate, which happens at an age where large antral follicles appear, an event related to speed of follicular growth (see

pages 12-15).

FOLLICULAR DEVELOPMENT

During the infantile and early juvenile period the ovary has often been considered to be " refractory " to gonadotrophins since no large antral follicles could be experimentally induced during these periods. Eshkol et al. (1970) however, showed that for normal follicular growth during the infantile and early juvenile period the presence of gonadotrophins was indispensable and concluded that FSH presumably is responsible for follicular cell proliferation and organization, whereas LH promotes the FSH action and induces antrum formation. The studies of Pedersen (1969, 1970) on follicular growth speed may explain the lack of ovulation after gonadotrophic stimulation during the infantile and early juvenile period. The high amounts of FSH present during the late infantile and early juvenile period presumably are of major importance for follicular growth, while the concommitantly present high amounts of oestrogen may also exert their influence on the ovarian level by enhancing incorporation of FSH in the ovaries (Goldenberg et al., 1972) and in this way favouring follicular growth. It is incorrect to state that the ovary is " refractory " to gonadotrophins during the first period of life. It is the ovarian mode of reponse to gonadotrophins which is still limited during this period.

CONCEPT OF MECHANISMS REGULATING SEXUAL MATURATION

In the following it has been tried to outline an overall concept of sexual maturation, taking into account recent data obtained by others and ourselves. In the period from birth till 10 days of age, the so-called infantile period, developmental processes take place in the brain, the hypophysis and in the ovary. In the brain releasing factors are synthesized and a hypophysial portal system is present and functioning so that effective control of hypophysial function is established before the end of the first week of life. Hypophysial gonadotrophin content is increasing during this period. Gonadotrophins are present in the blood neonatally and reach high values by the tenth day of life (see fig. 4), at the end of the infantile period. In the ovary primary follicles

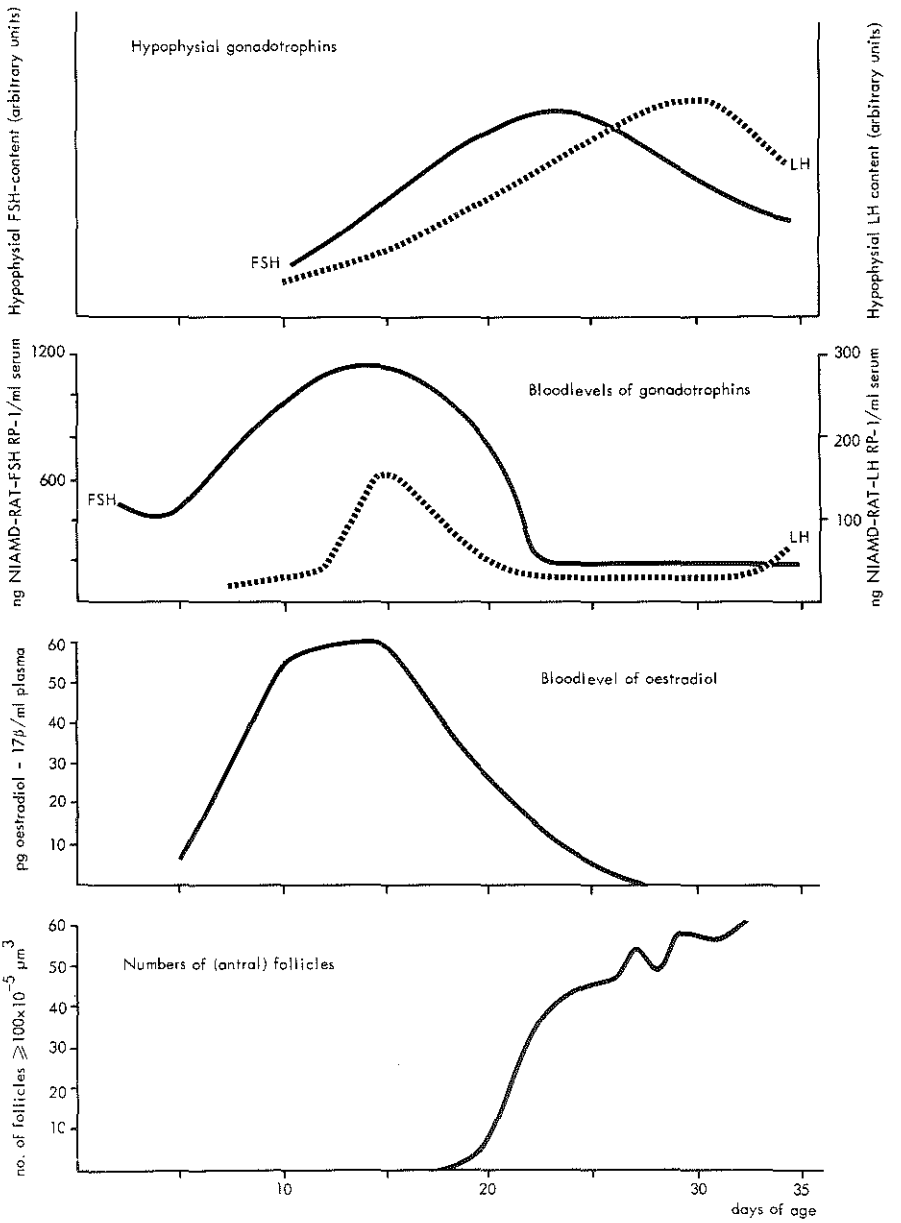

FIG. 4. Hypophysial gonadotrophin content, hormone levels in the blood and follicular development in the immature rat.

develop, oocyte growth proceeds largely independently, but for normal follicular growth gonadotrophins are required. Follicular growth speed is higher than at any later age, a maximal number of follicles starts growth in this period. Differentiation and degeneration occurs, the primary interstitial tissue is formed, reacts already to gonadotrophins and starts steroid production. Oestrogen is detectable in the blood at 5 days of age and reaches high values at 10 days of age (see fig. 4). At the ovarian level this high oestrogen concentration may be of importance for incorporation of FSH in the ovary which stimulates granulosa cell proliferation. An inhibitory feedback mechanism of steroids on LH release and presumably on FSH release is already functional, though this feedback action may be limited by the presence of specific oestradiol binding proteins in the blood.
In the next period, the early juvenile period, from the 10th to about the 20th day of age, small antral follicles appear for the first time. The interstitial tissue, the sole steroid producing tissue hitherto, increases in volume. Blood concentrations of both gonadotrophins and oestradiol reach peak values in this period, around day 15 (see fig. 4). These high concentrations are thought to be of major importance for intense follicular growth processes, taking place in the ovaries. Progesterone seems also to be produced and secreted already at about 2 weeks of age. Steroidal feedback mechanisms for both LH and FSH release are functional and their influence probably increases by the gradual disappearance of specific oestradiol binding proteïns. This may be the explanation for the simultaneous decrease in both gonadotrophin and oestradiol concentrations in the blood at the end of the early juvenile period. Ovarian follicles with antra are formed in increasing numbers but they do not reach adult properties: neither size nor capacity to ovulate can be induced at this stage by experimental treatment. Till the end of the early juvenile period the ovary clearly represents a limiting factor for ovulation to occur.
In the late juvenile period, from about the 20th day of life till the onset of puberty, a number of developmental processes seem to coïncide. At the beginning of the late juvenile period the ovary contains small numbers of antral follicles and induction of ovulation becomes possible; the number of ovulated ova increases with age. Preferential uptake of oestradiol in anterior hypothalamus and median eminence region develops and specific oestradiol receptors can be isolated from the hypothalamus. Moreover, a stimulatory steroidal feedback mechanism for phasic gonadotrophin release develops

between 22 and 28 days of age, which indicates readiness for cyclic, adult function. In the late juvenile period hypothalamic releasing factor content is high and presumably maximal. Hypophysial gonadotrophin content reaches maximal values, followed by a decrease in potency before puberty. Gonadotrophin concentrations in the blood are low, comparable to adult dioestrous values. The same holds for blood oestradiol concentrations during this period. No distinct pattern in fluctuation of hormonal concentrations has been distinguished till shortly before the first ovulation. In the period from 20 - 30 days of age an increasing (ovulatory) response to different experimental treatments is found. This holds for gonadotrophin treatment, steroid treatment and hypothalamic stimulation. These experimental treatments hardly ever result in ovulation 24 hrs later, indicating that the follicles present at the moment of treatment are not yet capable of ovulating. Mostly 3 or 4 days are needed in between the start of treatment (PMS, oestrogen, electrical stimulation) and the first, induced, ovulation. This indicates that either the antral follicles present had to attain the capacity to ovulate or new follicles had to develop for ovulation to occur; the capacity to ovulate does not yet develop spontaneously. It therefore seems that, though the total mechanism for cyclic function is present in the late juvenile period, at about the 28th day of age, the adjustment of interacting events on hypothalamo-hypophysial and ovarian level still has to take place. The hypothalamo-hypophysial axis has the potency for adult gonadotrophin release, tonic gonadotrophin release mechanisms are functioning and cyclic release may occur. However, the latter does not occur spontaneously, presumably due to lack of adequate stimulation of the cerebral centre(s) involved in ovulatory gonadotrophin release. In the ovary large follicles develop but the hormonal interplay presumably is not adequate to support full development of qualitatively adult-like follicles that will finally ovulate. This in itself may account for the lack of stimulation of cyclic gonadotrophin release, due to inadequate steroidal production. It is generally accepted that for stimulation of an ovulatory gonadotrophin release a threshold oestrogen concentration in the blood is of importance. It therefore is an attractive hypothesis that hypersensitivity of the hypothalamo-hypophysial system to oestrogen prevents this threshold oestrogen concentration to be reached, in which case a shift in sensitivity to oestrogen could be the major event in processes leading to the onset of puberty. In the late juvenile period relatively small experimental changes in hormonal

conditions (gonadotrophin treatment, oestrogen treatment, hypothalamic stimulation) are able to induce a follicular growth pattern that leads to first ovulation. Which event is crucial for spontaneous first ovulation and puberty to occur remains obscure. The question whether the key-limiting factor for puberty to occur is an increase in FSH release, as proposed by Critchlow & Bar-Sela (1967), or an increase in oestrogen concentration in the blood for stimulation of gonadotrophin release as suggested by several experimental situations, remains unanswered. Precise measurements of both gonadotrophin and steroid concentrations in the blood in the period preceding puberty might clarify the situation. However, the change leading to either increased gonadotrophin release or increase in steroid concentrations in the blood would still have to be elucidated. A change in general cerebral responsiveness to environmental stimuli may be crucial and may influence the hypothalamic gonadotrophic function via the limbic system. A role of the limbic system is suggested by a number of studies but further investigations will be needed.

Finally, events immediately preceding the first ovulation have been suggested to be highly comparable to those preceding ovulation in the adult cycle, but direct measurements of hormones are still needed to confirm this.

SUMMARY

The availability of a considerable number of new experimental data has greatly extended our knowledge of the maturational changes in the hypothalamo-hypophysial-ovarian system which lead to the onset of puberty. These data necessitate revision of hypotheses put forward earlier. Apart from new information on hypothalamic releasing factor content (pp. 24-25), hypophysial gonadotrophic potency (pp. 16-17), morphological ovarian development (pp. 11-14) and new and more precise data on the induction of precocious puberty (part II), the following findings, in part from personal studies (see appendix), may be summarized here :

1) The hormonal situation in the immature female rat has been revealed by radioimmunoassay studies. It has been found that gonadotrophins are not only present but even reach high concentrations, as compared to adult values, in the blood of the female rat before the 20th day of age. This is especially true for the FSH concentrations which reach peak values exceeding adult prooestrous values, around day 15 of age. The LH concentrations follow a less definite pattern and are comparatively low. After the 20th day of life gonadotrophin concentrations in the blood decrease and seem to remain at a relatively low level till the onset of puberty (pp. 12-14).
   Ovarian oestrogens also are already present in the blood of female rats before the 20th day of life. The general pattern is similar to the FSH pattern; a peak around day 15 reaching values higher than the adult value on the morning of prooestrus. Presence of specific oestrogen-binding proteins in the blood presumably limits the biological activity of oestrogens in the immature rat.
2) Interaction between ovarian steroids and gonadotrophin release exists early in life. An inhibitory influence of ovarian oestrogens on gonadotrophin secretion is present at the 10th day of life and presumably even earlier (pp. 25-26). A stimulatory influence of ovarian steroids on the hypothalamo-hypophysial system, controlling cyclic gonadotrophin release, develops after the 20th day of life (page 26). Such a development is also indicated by a gradual increase in effectiveness with age of various puberty advancing experimental treatments.
3) In the ovary of the immature rat, follicles of size ranges which play a

crucial role during the adult cycle, are absent before 19 days of age, thereafter they develop in increasing numbers and sizes, but normally they do not reach the capacity to ovulate till (shortly before) the first spontaneous ovulation (pp. 13-14).

4) Various methods (gonadotrophin or oestrogen treatment) are known to accelerate sexual maturation and to induce precocious first ovulation. Events after treatment often are highly comparable to those occurring during the adult cycle. Electrical stimulation of the hypothalamus has been found to be a useful tool for this purpose; the method is preferable to the lesion technique. Hypothalamic stimulation may cause gonadotrophin release resulting in maturation of follicles towards stages that are capable of ovulating. Normal ovulation occurs and normal mating and pregnancy is possible (pp. 29-33).

5) In conclusion the period from birth to puberty may roughly be divided in two phases, during the first of which the ovary responds already to gonadotrophic stimuli but is not yet competent to develop follicles capable of ovulating; in this period high concentrations of both gonadotrophins and oestrogens occur in the blood. During the second phase, after day 20, the ovary becomes competent to develop follicles capable of ovulating as shown by various experimental treatments, but does not yet spontaneously do so. Apparently adequate hormonal stimuli are lacking during some time since complete follicular development followed by spontaneous ovulation does not occur till about 40 days of age. Concentrations of both gonadotrophins and oestrogens in the blood are relatively low till shortly before first ovulation. The endogenous event initiating complete follicular development and evoking the first spontaneous ovulation remains unknown (p. 56-60).

SAMENVATTING

Onze kennis van de rijpingsprocessen welke in het hypothalamus-hypofyse-ovarium systeem plaatsvinden en leiden tot het optreden van puberteit, is door een aantal recente onderzoekingen aanzienlijk uitgebreid. Dit maakt het noodzakelijk een aantal bestaande hypothesen te herzien. Nieuwe gegevens zijn ter beschikking gekomen over het gehalte aan " releasing factor " voor gonadotropinen in de hypothalamus (zie blz. 24), over de morphologische ontwikkeling van het ovarium (blz. 11) en over mogelijkheid en mechanisme van induktie van vervroegd optreden van puberteit. Hieraan kunnen een aantal, deels uit persoonlijke proeven afkomstige (zie appendix), resultaten worden toegevoegd:

1) Door middel van de radioimmunoassay techniek konden hormoonspiegels in het bloed van prepuberale, vrouwelijke ratten voor het eerst direkt worden gemeten. Gevonden werd dat gonadotrope hormonen niet alleen aanwezig zijn in het bloed van zeer jonge vrouwelijke ratten, maar zelfs zeer hoge concentraties bereiken, en dit reeds vóór een leeftijd van 20 dagen. Dit geldt vooral voor de FSH-spiegels, welke op een leeftijd van ongeveer 15 dagen maximale waarden bereiken en zelfs hoger zijn dan de prooestrus waarde in volwassen dieren. De LH-spiegels zijn relatief laag en vertonen grote individuele variaties. Na dag 20 nemen de gonadotropinen-spiegels in het bloed af en blijven op een relatief laag niveau (zie blz. 12-14). Ook oestrogenen, afkomstig van het ovarium, zijn al vóór dag 20 aantoonbaar in het bloed; ook deze bereiken maximale concentraties op een leeftijd van ongeveer 15 dagen en zijn dan eveneens hoger dan de volwassen prooestrus piek. Het algemene verloop van de oestrogeen-spiegel met de leeftijd vertoont een sterke overeenkomst met het verloop van de gonadotropinen-spiegels (blz. 14). In jonge prepuberale dieren wordt de biologische werking van de circulerende oestrogenen vermoedelijk sterk beperkt door de aanwezigheid in het bloed van specifieke, oestrogeen-bindende eiwitten.
2) Reeds op zeer jonge leeftijd is er een wisselwerking tussen geslachtssteroiden en de gonadotropine-afgifte. Oestrogenen oefenen reeds op een leeftijd van 10 dagen, en mogelijk nog vroeger, een remmende invloed uit op de gonadotropine-afgifte. Een stimulerende werking van geslachtssteroiden op componenten van het hypothalamus-hypofyse systeem, ver-

antwoordelijk voor de cyclische afgifte van gonadotropinen, is aantoonbaar vanaf een leeftijd van ongeveer 22 dagen (zie blz. 26). De ontwikkeling van deze stimulerende terugkoppeling komt o.m. tot uitdrukking in het feit dat diverse experimentele behandelingen, toegepast voor het induceren van vervroegd optreden van puberteit, met toenemende leeftijd geleidelijk aan effektiever worden.

3) Het prepuberale ovarium bevat vóór een leeftijd van 19 dagen geen follikels van de typen, welke een rol spelen tijdens de volwassen cyclus. Na dag 19 komen dergelijke follikels in toenemende aantallen voor en bereiken grotere afmetingen. Normaliter worden er geen ovuleerbare follikels gevormd tot (kort voor) de eerste spontane ovulatie (blz. 13-14).

4) Elektrische prikkeling van de hypothalamus blijkt een bruikbare methode om puberteit te vervroegen welke te verkiezen is boven de lesie techniek. Hypothalamus prikkeling kan resulteren in een gonadotropine-afgifte, welke leidt tot follikelrijping, gevolgd door normale ovulatie. Normale paring en zwangerschap is mogelijk bij deze vervroegde ovulatie (blz. 29-33).

5) Gekonkludeerd kan worden dat de periode vanaf de geboorte tot puberteit te verdelen is in twee fasen. Gedurende de eerste fase is het ovarium reeds gevoelig voor gonadotrope hormonen, doch het kan nog geen ovuleerbare follikels voortbrengen.
Gedurende deze fase worden hoge concentraties van zowel gonadotropinen als van oestrogenen in het bloed aangetroffen.
Tijdens de tweede fase, na dag 20, is het ovarium in staat ovuleerbare follikels voort te brengen hetgeen met diverse experimentele behandelingen kan worden aangetoond. Spontaan zullen zich in het ovarium echter nog geen ovuleerbare follikels ontwikkelen.
Blijkbaar is de hormonale situatie gedurende het grootste deel van deze tweede fase nog ongeschikt voor volledige follikelrijping, leidend tot eerste ovulatie. Eerst op ongeveer dag 40 treedt spontaan de eerste ovulatie op. Gedurende deze tweede fase zijn, tot kort voor eerste ovulatie, lage gonadotropinen- en oestrogeen-concentraties aanwezig in het bloed. Welke gebeurtenis een volledig rijpen der follikels en uiteindelijk de eerste spontane ovulatie oproept is nog niet duidelijk (blz. 56-60).

REFERENCES

ALDEN, R.H. (1947). Implantation of the rat egg. II Alteration in osmiophilic epithelial lipids of the rat uterus under normal and experimental conditions. Anat. Record., 97: 1-19.

ARIMURA, A. & SCHALLY, A. (1971). Augmentation of pituitary responsiveness to LH-releasing hormone (LH-RH) by estrogen. Proc.Soc. exp. Biol. Med., 136: 290-293.

BAKER, F.D. & KRAGT, C.L. (1969). Maturation of the hypothalamic pituitary gonadal negative feedback system. Endocrinology, 85: 522-527.

BAKKE, J.L., GELLERT, R.J. & LAWRENCE, N.L. (1970). The persistent effects of perinatal hypothyroidism on pituitary, thyroidal and gonadal functions. J. Lab. clin. Med., 76: 25-33.

BAR-SELA, M.E. & CRITCHLOW, V. (1966). Delayed puberty following electrical stimulation of the amygdalae in female rats. Am. J. Physiol., 211: 1103-1107.

BAUER, H.G. (1959). Endocrine and metabolic conditions related to pathology in the hypothalamus: a review. J. Nervous Mental Disease, 128: 323-338.

BLOCH, G.J. & GANONG, W.F. (1971). Lesions of the brain and the onset of puberty in the female rat. Endocrinology, 89: 898-901.

BOGDANOVE, E.M. & SCHOEN, H.C. (1959). Precocious sexual development in female rats with hypothalamic lesions. Proc. Soc. exp. Biol. Med., 100: 664-669.

BRADBURY, J.T. (1961). Direct action of estrogen on the ovary of the immature rat. Endocrinology 68: 115-120.

BROWN-GRANT, K. & RAISMAN, G. (1972). Reproductive function in the rat following selective destruction of afferent fibres to the hypothalamus from the limbic system. Brain Research, 46: 23-42.

BUNN, J.P. & EVERETT, J.W. (1957). Ovulation in persistent estrous rats after electrical stimulation of the brain. Proc.Soc. exp. Biol. Med., 96: 369-371.

BYRNES, W.W. & MEYER, R.K. (1951). Effect of physiological amounts of estrogen on the secretion of follicle stimulating and luteinizing hormones. Endocrinology, 49: 449-460.

CALIGARIS, L., ASTRADA, J.J. & TALEISNIK, S. (1968). Stimulating and inhibiting effects of progesterone on the release of luteinizing hormone. Acta endocr., Copenh. 59: 177-185.

CALIGARIS, L., ASTRADA, J.J. & TALEISNIK, S. (1972). Influence of age on the release of luteinizing hormone induced by oestrogen and progesterone in immature rats. J. Endocr., 55: 97-103.

CAMPBELL, H.J. (1966). The development of the primary portal plexus in the median eminence of the rabbit. J. Anat., 100: 381-387.

CAMPBELL, H.J. & GALLARDO, E. (1966). Gonadotrophin releasing activity of the median eminence at different ages. J. Physiol., 186: 689-697.

CHANG, N. & NIKITOVITCH-WINER, M.B. (1970). The role of the ovarian feedback in the maturation of the pituitary gonadotrophic functions. Anat. Rec., 166: 290.

CIERCIOROWSKA, A. & RUSSFIELD, A.B. (1968). Determination of estrogenic activity of the immature rat ovary. Arch. Path., 85: 658-662.

COLE, H.H. (1936). On the biological properties of mare gonadotrophic hormone. Am. J. Anat., 59: 299-331.

CORBIN, A. & DANIELS, E.L. (1967). Changes in concentration of female rat pituitary FSH and stalk-median eminence follicle stimulating hormone releasing factor with age. Neuroendocrinology, 2: 304-314.

CORBIN, A. & DANIELS, E.L. (1969). Induction of puberty in immature female rat: Effect of estrogen on pituitary FSH and stalk-median eminence FSH releasing factor. Neuroendocrinology, 4: 65-74.

CORBIN, A. & SCHOTTELIUS, B.A. (1960). Effects of posterior hypothalamic lesions on sexual maturation of immature female albino rats. Proc. Soc. exp. Biol. Med., 103: 208-210.

CORBIN, A. & SCHOTTELIUS, B.A. (1961). Hypothalamic neurohormonal agents and sexual maturation of immature female rats. Am. J. Physiol., 201: 1176-1180.

CRITCHLOW, V. & BAR-SELA, M.E. (1967). Control of the onset of puberty. In Neuroendocrinology vol. 2, pp. 101-162 Eds. L. Martini & W.F. Ganong. New York, London: Academic Press.

CROES-BUTH, S., PAESI, F.J.A. & de JONGH, S.E. (1959). Stimulation of ovarian follicles in hypophysectomized rats by low dosages of oestradiol benzoate. Acta endocr., Copenh. 32: 399-410.

DANIELS, V.G., HARDY, R.N., MALINOWSKA, K.W. & NATHANIELSZ, P.W. (1972). Adreno-cortical hormones and absorption of macromolecules by the small intestine of the young rat. J. Endocr., 52: 405-406.

DAWSON, A.B. & McCABE, M. (1951). The interstitial tissue of the ovary in infantile and juvenile rats. J. Morphol., 88: 543-571.

DEBELJUK, L., ARIMURA, A. & SCHALLY, A.V. (1972). Pituitary responsiveness to LH releasing hormone in intact female rats of different ages. Endocrinology, 90: 1499-1502.

DE HERTOGH, R., EKKA, E., VAN DER HEIJDEN, I. & HOET, J.J. (1970). Metabolic clearance rates and the interconversion factors of estrone and estradiol-17β in the immature and adult female rat. Endocrinology, 87: 874-880.

DÖCKE, F. (1972). Neuro-hormonal control of puberty in female rats; presented at the "Symposium on differentiation and neuroendocrine regulation in the hypothalamo-hypophysial gonadal system", sept. 20-23, Berlin D.D.R.

DÖCKE, F. & DÖRNER, G. (1965). The mechanism of the induction of ovulation by oestrogens. J. Endocr., 33: 491-499.

DÖCKE, F. & DÖRNER, G. (1966). Facilitative action of progesterone in the induction of ovulation by oestrogen. J. Endocr., 36: 209-210.

DONOVAN, B.T. & O'KEEFFE, M.C. (1966). The liver and the feedback action of ovarian hormones in the immature rat. J. Endocr., 34: 469-478.

DONOVAN, B.T. & O'KEEFFE, M.C. & O'KEEFFE, H.T. (1967). Intrasplenic implants of oestradiol and uterine growth in infantile and pubertal rats. J. Endocr., 37: 93-98.

DONOVAN, B.T. & VAN DER WERFF TEN BOSCH, J.J. (1956). Precocious puberty in rats with hypothalamic lesions. Nature, 178: 745.

DONOVAN, B.T. & VAN DER WERFF TEN BOSCH, J.J. (1959). The hypothalamus and sexual maturation in the rat. J. Physiol., 147: 78-92.

DONOVAN, B.T. & VAN DER WERFF TEN BOSCH, J.J. (1965). Physiology of puberty. Monographs of the physiological society, number 15, pp. 1-216. Eds. H. Barcroft, H. Davson, W.D.M. Paton; London.

ECKSTEIN, P. (1962). Ovarian physiology in the non-pregnant female. In: The Ovary, vol. I, pp. 311-359. Ed. Zuckermann, Academic Press, London.

ELLETT, P. (1970). Transmaternal influence of the environment in determining age of vaginal canalization of female offspring in the rat. J. Endocr., 48: 291-292.

ELWERS, M. & CRITCHLOW, V. (1960). Precocious ovarian stimulation following hypothalamic and amygdaloid lesions in rats. Am. J. Physiol., 198: 381-385.

ELWERS, M. & CRITCHLOW, V. (1961). Precocious ovarian stimulation following interruption of stria terminalis. Am. J. Physiol., 201: 281-284.

ESHKOL, A., LUNENFELD, B. & PETERS, H. (1970). Ovarian development in infant mice. Dependence on gonadotrophic hormones. In: Gonadotropins and ovarian development. pp. 249-258. Ed. W.R. Butt, A.C. Crooke & M. Ryle. Edinburgh: E. and S. Livingstone.

EVERETT, J.W. (1965). The mammalian female reproductive cycle and its controlling mechanisms. In "Sex and Internal Secretions" vol. I pp. 497-555. Ed. W.C. Young. Williams & Wilkins, Baltimore.

EVERETT, J.W. & RADFORD, H.M. (1961). Irritative deposits from stainless steel electrodes in the preoptic rat brain causing release of pituitary gonadotropin. Proc. Soc. exp. Biol. Med., 108: 604-609.

EVERETT, J.W. & SAWYER, C.H. (1950). A 24-hour periodicity in the 'LH-release apparatus' of female rats, disclosed by barbiturate sedation. Endocrinology, 47: 198-218.

FALCK, B. (1953). Occurrence of cholesterol and formation of oestrogen in the infantile rat ovary. Acta endocr., Copenh. 12: 115-122.

FAWKE, L. & BROWN, P.S. (1970). Pituitary content of follicle stimulating hormone in the female rat. J. Reprod. Fert., 21: 303-312.

FINK, G. & SMITH, G.C. (1971). Ultrastructural features of the developing hypothalamo hypophyseal axis in the rat. Z. Zellforsch., 119: 208-226.

FERIN, M., ZIMMERING, P.E. & VAN DE WIELE, R.L. (1969). Effects of antibodies to estradiol-17β on PMS induced ovulation in immature rats. Endocrinology, 84: 893-900.

FLORSHEIM, W.H. & RUDKO, P. (1968). The development of portal system function in the rat. Neuroendocrinology, 3: 89-98.

GALLO, R.V. & ZARROW, M.X. (1970). Effect of progesterone and other steroids on PMS-induced ovulation in the immature rat. Endocrinology, 86: 296-304.

GELLERT, R.J. & GANONG, W.F. (1960). Precocious puberty in rats with hypothalamic lesions. Acta endocr., Copenh. 33: 569-576.

GERALL, A.A. & DUNLAP, J.L. (1971). Evidence that the ovaries of the neonatal rat secrete active substances. J. Endocr., 50: 529-530.

GLYDON, R.S. (1957). The development of the blood supply of the pituitary in the albino rat, with special reference to the portal vessels. J. Anat., 91: 237-244.

GOLDENBERG, R.L., VAITUKAITIS, J.L. & ROSS, G.T. (1972). Estrogen and follicle stimulating hormone interactions on follicle growth in rats. Endocrinology, 90: 1492-1498.

GOLDMAN, B.D. & GORSKI, R.A. (1971). Effects of gonadal steroids on the secretion of LH and FSH in neonatal rats. Endocrinology, 89: 112-115.

GOLDMAN, B.D., GRAZIA, Y.R., KAMBERI, I.A. & PORTER, J.C. (1971). Serum gonadotrophin concentrations in intact and castrated neonatal rats. Endocrinology, 88: 771-776.

GRAYBURN, J.A. & BROWN-GRANT, K. (1968). The role of oestrogen in the induction of ovulation in the gonadotrophin-treated immature rat. J. Endocr., 42: 409-416.

HAGINO, N. (1969). The hypothalamic time sequence controlling gonadotrophin release in the immature female rat. Neuroendocrinology, 5: 1-9.

HAGINO, N. & GOLDZIEHER, J.W. (1970). Effect of timing and quantity of estrogen on gonadotrophin induced ovulation in immature rats. Endocrinology, 86: 29-33.

HAGINO, N., RAMALEY, J.A. & GORSKI, R.A. (1966). Inhibition of estrogen-induced precocious ovulation by pentobarbital in the rat. Endocrinology, 79: 451-454.

HEIM, L.M. & TIMIRAS, P.S. (1963). Gonad-brain relationship: Precocious brain maturation after estradiol in rats. Endocrinology, 72: 598-605.

HEIMER, L. & NAUTA, W.J.H. (1969). The hypothalamic distribution of the stria terminalis in the rat. Brain Research, 13: 284-297.

HOHLWEG, W. (1934). Veränderungen des Hypophysenvorderlappens und des Ovariums nach Behandlung mit grossen Dosen von Follikelhormon. Klin. Wschr., 13: 92-95.

HOHLWEG, W. & CHAMORRO, A. (1937). Über die luteinisierende Wirkung des Follikelhormons durch Beeinflussung der luteogenen Hypophysen-vorderlappen sekretion, Klin. Wschr., 16: 196-197.

HOHLWEG, W. & DORHN, M. (1932). Uber die Beziehungen zwischen Hypophysenvorderlappen und Keimdrüsen. Klin. Wschr., 11: 233-235.

HOOGSTRA, M.J. & DE JONGH, S.E. (1955). The problem of the occurrence of castration cells in the hypophysis in very young animals. Acta physiol. pharm. neerl., 4: 145-152.

HOOGSTRA, M.J. & PAESI, F.J.A. (1955). A comparison between the F.S.H. and I.C.S.H. contents of the hypophysis of adult and immature rats. Acta physiol. pharm. neerl., 4: 395-406.

HOROWITZ, S. & VAN DER WERFF TEN BOSCH, J. J. (1962). Hypothalamic sexual precocity in female rats operated shortly after birth. Acta endocr., Copenh., 41: 301-313.

HYYPPÄ, M. (1969a). Differentiation of the hypothalamic nuclei during ontogenetic development in the rat. Z. Anat. Entwickl. gesch. 129: 41-52.

HYYPPÄ, M. (1969b). A histochemical study of primary catecholamines in the hypothalamic neurons of the rat in relation to the ontogenetic and sexual differentiation. Z. Zellforsch., 98: 550-560.

JOHNSON, D. C. (1971). Temporal aspects of oestrogen stimulated increases in plasma and pituitary follicle stimulating hormone. Acta endocr., Copenh. 66: 89-98.

JOHNSON, D. C. & NAQVI, R. H. (1969). A positive feedback action of androgen on pituitary follicle stimulating hormone: Induction of a cyclic phenomenon. Endocrinology 85: 881-885.

KATO, J. (1972). Sex hormone receptors in the hypothalamus and hypophysis; presented at the "Symposium on differentiation and neuroendocrine regulation in the hypothalamo-hypophysial gonadal system", sept. 20-23, Berlin, D. D. R.

KAWAKAMI, M. & TERASAWA, E. (1972). Electrical stimulation of the brain on gonadotropin secretion in the female prepuberal rat. Endocrinol. Japon., 19: 335-347.

KENNEDY, G. C. (1957). The development with age of hypothalamic restraint upon the appetite of the rat. J. Endocr., 16: 9-17.

KENNEDY, G. C. & MITRA, J. (1963). Body weight and food intake as initiating factors for puberty in the rat. J. Physiol., 166: 408-418.

KLAUSING, M. N. & MEYER, R. K. (1968). Anterior pituitary ovulating capacity in the PMSG-primed immature female rat. J. Reprod. Fert., 17: 41-48.

KLING, A. (1964). Effects of rhinencephalic lesions on endocrine and somatic development in the rat. Am. J. Physiol., 206: 1395-1400.

KRAGT, C. L. & DAHLGREN, J. (1972). Development of neural regulation of follicle stimulating hormone (FSH) secretion. Neuroendocrinology, 9: 30-40.

KRAGT, C. L. & GANONG, W. F. (1968). Pituitary FSH content in female rats at various ages. Endocrinology, 82: 1241-1245.

KRAGT, C.L., MASKEN, J.F. & BLOCH, G.J. (1971). Similarities and differences in plasma FSH and LH concentrations in developing male and female rats. Abstr. XXV int. Congr. Physiol. Sci. Munich, Proc. int. Un. Physiol. Sci., 9: 323.

KREJCI, M.E. & CRITCHLOW, V. (1959). Precocious uterine stimulation following hypothalamic and amygdaloid lesions in female rats. Anat. Rec., 133: 300.

KULIN, H.E., GRUMBACH, M.M. & KAPLAN, S.L. (1969). Changing sensitivity of the puberal gonadal hypothalamic feedback mechanism in man. Science. 166: 1012-1013.

KÜPPERS, S. (1967). Zur Fermenthistochemie des Rattenovariums während der postnatalen Entwicklung. Acta Histochem., 27: 267-284.

LABHSETWAR, A.P. (1970). Age dependent changes in the pituitary gonadal relationship IX Sex difference in rat pituitary FSH content. J. Reprod. Fert., 22: 165-167.

LAUSON, H.D., GOLDEN, J.B. & SEVRINGHAUS, E.L. (1939). The gonadotropic content of the hypophysis throughout the life cycle of the normal female rat. Am. J. Physiol., 125: 396-404.

LAWTON, I.E. & SAWYER, C.H. (1970). Role of amygdala in regulating LH secretion in the adult female rat. Am. J. Physiol., 218: 622-626.

LISK, R.D. (1968). Luteinizing hormone in the pituitary gland of the albino rat : Concentration and content as a function of sex and age. Neuroendocrinology 3: 18-24.

MacKINNON, P.C.B. (1970). A comparison of protein synthesis in the brain of mice before and after puberty. J. Physiol., 210: 10p-11p.

MANDL, A.M. & ZUCKERMAN, S. (1952). Factors influencing the onset of puberty in albino rats. J. Endocr., 8: 357-364.

MARTINOVITCH, P.N., IVANISEVIC, O.K. & MARTINOVIC, J.V. (1968). The effect on the onset of puberty on whole body irradiation of infant female rats with and without hypothalamic lesions. Experientia, 24: 835-840.

MATSUYAMA, E., WEISZ, J. & LLOYD, C.W. (1966). Gonadotrophin content of pituitary glands of testerone sterilized rats. Endocrinology, 79: 261-267.

McCANN, S.M. (1970). Neurohormonal correlates of ovulation. Fed. Proc., 29: 1888-1894.

McCANN, S.M. & RAMIREZ, V.D. (1964). The neuroendocrine regulation of hypophyseal luteinizing hormone secretion. Rec. Progr. Hormone Res., 20: 131-170.

McCORMACK, C.E. & BENNIN, B. (1970). Delay of ovulation caused by exposure to continuous light in immature rats treated with pregnant mare's serum gonadotrophin. Endocrinology 86: 611-619.

McCORMACK, C.E. & MEYER, R.K. (1962). Ovulating hormone release in gonadotrophin treated immature rats. Proc. Soc. exp. Biol., Med., 110: 343-346.

McCORMACK, C.E. & MEYER, R.K. (1963). Ovulation induced by progesterone in immature rats pretreated with pregnant mare serum gonadotropin. Gen. and Compar. Endocr., 3: 300-307.

McCORMACK, C.E. & MEYER, R.K. (1964). Minimal age for induction of ovulation with progesterone in rats: evidence for neural control. Endocrinology, 74: 793-799.

McEWEN, B.S. & PFAFF, D.W. (1970). Factors influencing sex hormone uptake by rat brain regions. I. Effect of neonatal treatment, hypophysectomy and competing steroid on estradiol uptake. Brain Research, 21: 1-16.

McQUEEN-WILLIAMS, M. (1935). Sex comparison of gonadotropic content of anterior hypophyses from rats before and after puberty. Proc. Soc. exp. Biol. Med., 32: 1051-1052.

MEYER, R.K. & McCORMACK, C.E. (1967). Ovulation in immature rats treated with ovine follicle stimulating hormone: facilitation by progesterone and inhibition by continuous light. J. Endocr., 38: 187-194.

MEIJS-ROELOFS, H.M.A. (1972). Effect of electrical stimulation of the hypothalamus on gonadotrophin release and the onset of puberty. J. Endocr., 54: 277-284.

MEIJS-ROELOFS, H.M.A. & MOLL, J. (1972). Differential effects of anterior and middle hypothalamic lesions on vaginal opening and cyclicity. Neuroendocrinology 9: 297-303.

MEIJS-ROELOFS, H.M.A. & UILENBROEK, J.Th.J. (1973). Gonadotropin release and follicular development after electrical stimulation of the hypothalamus in the immature female rat. In: The development and maturation of the ovary and its functions. Ed. H. Peters. Exc. Med. ICS, 267: 117-123.

MEIJS-ROELOFS, H.M.A., UILENBROEK, J.Th.J., de JONG, F.H. & WELSCHEN, R. Plasma oestradiol-17β and its relation to serum follicle-stimulating hormone in immature female rats. J. Endocr., (in press)

MEIJS-ROELOFS, H.M.A., UILENBROEK, J.Th.J., OSMAN, P. & WELSCHEN, R. (1973). Serum levels of gonadotropins and follicular growth in prepuberal rats. In: The development and maturation of the ovary and its functions. Ed. H. Peters. Exc. Med. ICS, 267: 3-11.

MILLICHAP, J.G. (1957). Development of seizure patterns in newborn animals. Significance of brain carbonic anhydrase. Proc. Soc. exp. Biol. Med. 96: 125-129.

MINAGUCHI, H., CLEMENS, J.A., MEITES, J. (1968). Changes in pituitary prolactin levels in rats from weaning to adulthood. Endocrinology, 82: 555-558.

MONAHAN, M., RIVIER, J., BURGUS, R., AMOSS, M., BLACKWELL, R., VALE, W. & GUILLEMIN, R. (1971). Synthèse totale par phase solide d'un décapeptide qui stimule la sécrétion des gonadotropines hypophysaires LH et FSH. C.R. Acad. Sc. Paris, t 273, série D: 508-510.

MOORE, W.W. (1965/1966). Changes in pituitary LH concentration in prepubertal and postpubertal rats. Neuroendocrinology, 1: 18-24.

MOTTA, M., FRASCHINI, F., GIULIANI, G. & MARTINI, L. (1968). The central nervous system, estrogen and puberty. Endocrinology, 83: 1101-1107.

NAQVI, R.H. & JOHNSON, D.C. (1969). Increased production of follicle-stimulating hormone in immature female rats by a single injection of androgen or oestrogen. J. Endocr., 45: 29-36.

NISHIZUKA, Y., SAKAKURA, T., TANAKA, Y. & KOJIMA, A. (1973). Disturbance in female reproductive function in neonatally thymectomized mice. In: The development and maturation of the ovary and its functions. Ed. H. Peters Exc. Med. ICS, 267: 171-180.

OJEDA, S.R. & RAMIREZ, V.D. (1970). Failure of estrogen to block compensatory ovarian hypertrophy in prepuberal rats bearing medial basal hypothalamic FSH implants. Endocrinology, 86: 50-56.

OJEDA, S.R. & RAMIREZ, V.D. (1972). Plasma level of LH and FSH in maturing rats: response to hemigonadectomy. Endocrinology, 90: 466-472.

OSMAN, P., WELSCHEN, R.W. & MOLL, J. (1972). Anti-gonadotropic and anti-growth effects of the pineal gland in immature female rats. Neuroendocrinology, 10: 121-128.

PAESI, F.J.A. (1952). The effect of small doses of oestrogenon the ovary of the immature rat. Acta endocr., Copenh. 11: 251-268.

PARLOW, A.F. (1964). Differential action of small doses of estradiol on gonadotrophins in the rat. Endocrinology, 75: 1-8.

PEDERSEN, T. (1969). Follicle growth in the immature mouse ovary. Acta endocr., Copenh. 62: 117-132.

PEDERSEN, T. (1970). Follicle kinetics in the ovary of the cyclic mouse. Acta endocr., Copenh. 64: 304-323.

PETERS, H. (1969). The development of the mouse ovary from birth to maturity Acta endocr., Copenh. 62: 98-116.

PETERS, H. & BRAATHEN, B. (1973). The effect of unilateral ovariectomy in the neonatal mouse on follicular development. J. Endocr., 56: 85-89.

PETERS, H. & PEDERSEN, T. (1967). Origin of follicle cells in the infant mouse ovary. Fert. Steril. 18: 309-314.

PFAFF, D.W. & KEINER, M. (1972). Estradiol-concentrating cells in the rat amygdala as part of a limbic-hypothalamic hormone-sensitive system. In: The neurobiology of the amygdala. Advances in Behavioral Biology, vol. 2. pp. 775-785. Ed. B.E. Eleftheriou, Plenum press, New York-London.

PRESL, J., HORSKY, J., HENZL, M. & JIRASEK, J. (1963). The development of a feedback mechanism between the ovary and the diencephalic hypophysial system in the rat. J. Endocr., 26: 287-294.

PRESL, J., JIRASEK, J., HORSKY, J. & HENZL, M. (1965). Observations on steroid-3β-ol dehydrogenase activity in the ovary during early postnatal development in the rat. J. Endocr., 31: 293-294.

PRESL, J., ROHLING, S., HORSKY, J. & HERZMANN, J. (1970). Changes in uptake of ^{3}H-estradiol by the female rat brain and pituitary from birth to sexual maturity. Endocrinology, 86: 899-902.

PRICE, D. (1947). The influence of maternal hormones on the reproductive organs of suckling rats. Anat. Rec., 97: 519-549.

PRICE, D. & ORTIZ, E. (1944). The relation of age to reactivity in the reproductive system of the rat. Endocrinology, 34: 215-239.

QUATTROPANI, S. & WEISZ, J. (1973). In vitro steroidogenesis by the infantile rat ovary. In: The development and maturation of the ovary and its functions. Ed. H. Peters. Exc. Med., 267: 49-50.

QUINN, D.L. & ZARROW, M.X. (1964). Inhibition of pregnant mare's serum-induced ovulation in the immature rat. Endocrinology, 74: 309-313.

RAISMAN, G. (1970). An evaluation of the basic pattern of connections between the limbic system and the hypothalamus. Am. J. Anat., 129: 197-202.

RAMALEY, J.A. & BUNN, E.L. (1972). Seasonal variations in the onset of puberty in rats. Endocrinology, 91: 611-613.

RAMALEY, J.A. & GORSKI, R.A. (1967). The effect of hypothalamic de-afferentation upon puberty in the female rat. Acta endocr., Copenh. 56: 661-674.

RAMIREZ, V.D. (1964). Advancement of puberty in the rat by estrogen. Anat. Rec., 148: 325.

RAMIREZ, V.D. (1971). Steroid hormones and brain function. UCLA Forum, Med. Sci., 15: 301-310.

RAMIREZ, V.D. & McCANN (1963). Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology, 72: 452-464.

RAMIREZ, V.D. & SAWYER, C.H. (1965). Advancement of puberty in the female rat by estrogen. Endocrinology, 76: 1158-1168.

RAMIREZ, V.D. & SAWYER, C.H. (1966). Changes in hypothalamic luteinizing hormone releasing factor (LHRF) in the female rat during puberty. Endocrinology, 78: 958-964.

REITER, E.O., GOLDENBERG, R.L., VAITUKAITIS, J.L. & ROSS, G.T. (1972). A role for endogenous estrogen in normal ovarian development in the neonatal rat. Endocrinology, 91: 1537-1539.

REITER, E.O. & KULIN, M.E. (1972). Sexual maturation in the female. Normal development and precocious puberty. In: Pediatric Clinics of North America, 19: 581-603. Ed. N.M. Nelson.

RELKIN, R. (1968). Combined effects of hypothalamic lesioning and light in the advancement of puberty. Endocrinology, 82: 865-868.

RELKIN, R. (1971). Relative efficacy of pinealectomy, hypothalamic and amygdaloid lesions in advancing puberty. Endocrinology, 88: 415-418.

RENNELS, E.G. (1951). Influence of hormones on the histochemistry of ovarian interstitial tissue in the immature rat. Am. J. Anat, 88: 63-107.

RENNELS, E.G. & O'STEEN, W.K. (1967). Alterations in LH and FSH content and weight of the anterior pituitary gland of immature female rats treated with PMS. Endocrinology, 80: 82-88.

RISS, W., BURSTEIN, S.D. & JOHNSON, R.W. (1963). Hippocampal or pyriform lobe damage in infancy and endocrine development of rats. Am. J. Physiol., 204: 861-866.

ROWLANDS, I.W. (1944). The production of ovulation in the immature rat. J. Endocr., 3: 384-391.

ROWLANDS, I.W. & PARKIS, A.S. (1966). Hypophysectomy and the gonadotrophins. In Marshall s physiology of reproduction vol. III pp. 26-47. Ed. A.S. Parkes, University of Cambridge.

ROWLANDS, I.W. & WILLIAMS, P.C. (1941). Comparative activity of the gonadotrophin in horse pituitary glands and in pregnant mare's serum. J. Endocr., 2: 380.

ROWLANDS, I.W. & WILLIAMS, P.C. (1943). Production of ovulation in hypophysectomized rats. J. Endocr., 3: 310-315.

SAKAKURA, T. & NISHIZUKA, Y. (1972). Thymic control mechanism in ovarian development: Reconstitution of ovarian dysgenesis in thymectomized mice by replacement with thymic and other lymphoid tissues. Endocrinology, 90: 431-437.

SAWYER, C.H. (1972). Functions of the amygdala related to the feedback actions of gonadal steroid hormones. In: The neurobiology of the amygdala. Advances in Behavioral Biology, vol. 2pp. 745-762. Ed. B.E. Eleftheriou, Plenum press, New York, London.

SCHALLY, A.V., ARIMURA, A., KASTIN, A.J., MATSUO, H., BABA, Y., REDDING, T.W., NAIR, R.M.G. & DEBELJUK, L., (1971). Gonadotropin-releasing hormone: One polypeptide regulates secretion of luteinizing and follicle stimulating hormones. Science, 173: 1036-1038.

SCHIAVI, R.C. (1964). Effect of anterior and posterior hypothalamic lesions on precocious sexual maturation. Am. J. Physiol., 206: 805-810.

SCHRÖDER, H.G., ENGELBART, K., SANDOW, J. & HEPTNER, W. (1972). The effect of synthetic LH/FSH-RH on the maturation of infantile female rats. Acta endocr., Copenh. Suppl. 159: abstract no. 56.

SCHUETZ, A.W. (1971). Effect of precocious ovulation and mating on pregnancy in prepuberal rats. Endocrinology, 89: 1325-1328.

SHIINO, M. & RENNELS, E.G. (1967). Pituitary follicle stimulating hormone in vivo and ovarian development in the infant rat. Endocrinology, 81: 1379-1386.

SIEVERTSSON, H., CHANG, J.K., BOGENTOFT, C., CURRIE, B.L., FOLKERS, K. & BOWERS, C.Y. (1971). Synthesis of the luteinizing releasing hormone of the hypothalamus and its hormonal activity. Biochem. and Biophys. Res. Communic., 44: 1566-1571.

SIPERSTEIN, E., NICHOLS, C.W., GRIESBACH, W.E. & CHAIKOFF, I.L. (1954). Cytological changes in the rat anterior pituitary from birth to maturity. Anat. Rec., 118: 593-619.

SMITH, B.D. (1961). The effect of diethylstillbestrol on the immature rat ovary. Endocrinology, 69: 238-245.

SMITH, E.R. & DAVIDSON, J.M. (1968). Role of estrogen in the cerebral control of puberty in female rats. Endocrinology, 82: 100-108.

SORRENTINO, S., REITER, R.J., LEE, L.A. & SCHALCH, D.S. (1972). Timing and neural pathways involved in release of luteinizing hormone after pregnant mare serum administration. Neuroendocrinology, 9: 341-348.

STEELE, R.E. & WEISZ, J. (1973). Changes in sensitivity of the oestradiol-LH feedback system with puberty in the female rat. In: The development and maturation of the ovary and its functions. Ed. H. Peters. Exc. Med. ICS, 267: 154-155.

STEGNER, H.E., LUNENFELD, B. & ESHKOL, A. (1970). Ultrastructural ovarian changes after deprivation of endogenous gonadotrophins and the effect of FSH and HMG in the deprived immature animals. In: Gonadotrophins and Ovarian development, pp. 249-257. Ed. W.R. Butt, A.C. Crooke & M. Ryle. Edinburgh: E. and S. Livingstone.

STRAUSS, W.F. & MEYER, R.K. (1962). Neural timing of ovulation in immature rats treated with gonadotrophin. Science. 137: 860-861.

STUMPF, W.E. (1970). Estrogen-neurons and estrogen-neuron systems in the periventricular brain. Am. J. Anat., 129: 207-218.

STUMPF, W.E. (1972). Estrogen, androgen and glucocorticosteroid concentrating neurons in the amygdala, studies by dry autoradiography. In: The neurobiology of the amygdala. Advances in Behavioral Biology, vol. 2 pp. 763-744. Ed. B.E. Eleftheriou, Plenum press, New York-London.

SUGAWARA, S. & TAKEUCHI, S. (1970). Ovulatory response to a single dose of human chorionic gonadotrophin in the immature rat. Endocrinology, 86: 965-969.

SUGAWARA, S., UMEZU, M. & TAKEUCHI, S. (1969). Effect of a single dose of human chorionic gonadotrophin on the ovulatory response of the immature rat. J. Reprod. Fert., 20: 333-335.

SUZUKI, M., KAWAGOE, S. & HIROI, M. (1971). Mechanism of the onset of puberty, with special reference to the dynamics of the hypothalamic gonadotropin releasing factor and pituitary gonadotropin. Acta Med. et Biol., 19: 1-25.

TAYLOR, F.B. (1961). Histochemical changes in the ovaries of normal and experimentally treated rats. Acta endocr., Copenh, 36: 361-374.

TERASAWA, E. & TIMIRAS, P.S. (1968). Electrophysiological study of the limbic system in the rat at the onset of puberty. Am. J. Physiol., 215: 1462-1467.

TUOHIMAA, P. & NIEMI, M. (1972). Uptake of sex steroids by the hypothalamus and anterior pituitary of pre- and neonatal rats. Acta endocr., Copenh. 71: 37-44.

UMEZU, M. (1970). The influence of PMS treatment on pituitary ovulating hormone in female immature rats. Jap. J. of Anim. Reprod., 15: 120-125.

VELASCO, M.E. (1972). Opposite effects of platinum and stainless steel lesions of the amygdala on gonadotropin secretion. Neuroendocrinology, 10: 301-308.

VELASCO, M.E. & TALEISNIK, S. (1969). Release of gonadotropins induced by amygdaloid stimulation in the rat. Endocrinology, 84: 132-139.

VOOGT, J.L., CHEN, C.L. & MEITES, J. (1970). Serum and pituitary prolactin levels before, during and after puberty in female rats. Am. J. Physiol., 218: 396-399.

WAGNER, J.W. & BROWN-GRANT, K. (1965). Studies on the time of luteinizing hormone release in gonadotrophin treated immature rats. Endocrinology, 76: 958-965.

WATANABE, S. & McCANN, S.M. (1969). Alterations in pituitary follicle-stimulating hormone (FSH) and hypothalamic FSH releasing factor (FSH-RF) during puberty. Proc. Soc. exp. Biol. Med., 132: 195-201.

WATSON, J.B. (1903). Animal education; an experimental study on the pschychical development of the white rat correlated with the growth of its nervous system, University of Chicago.

WEICK, R.F., SMITH, E.R., DOMINGUEZ, R., DHARIWAL, A.P.S., DAVIDSON, J.M. (1971). Mechanism of stimulatory feedback. Effect of estradiol benzoate on the pituitary. Endocrinology, 88: 293-301.

WEISZ, J. & FERIN, M. (1970). Pituitary gonadotrophins and circulating LH in immature rats. A comparison between normal females and males and females treated with testosterone in neonatal life. In: Gonadotrophins and Ovarian Development pp. 339-350. Ed. W.R. Butt, A.C. Crooke & M. Ryle. Edinburgh. E and S Livingstone.

WEISZ, J. & GUNSALUS, P. (1973). Estrogen levels in immature female rats: true or spurious-ovarian or adrenal? In: The development and maturation of the ovary and its functions. Ed. H. Peters. Exc. Med. ICS. 267: 158-159.

WELSCHEN, R. (1973). Amounts of gonadotrophins required for normal follicular growth in hypophysectomized adult rats. Acta endocr., Copenh. 72: 137-155.

WELSCHEN, R. & RUTTE, M. (1971). Ovulation in adult rats after treatment with pregnant mare serum gonadotrophin during oestrus. Acta endocr., Copenh. 68: 41-49.

WESTMAN, A. & JACOBSOHN, D. (1938). Endokrinologische Untersuchungen an des Follikelhormons. Acta obstet. gynecol. scand., 18: 115-123.

WILDSCHUT, J. (1972). Oestradiol en de ovulatie bij de rat. Academisch proefschrift Leiden.

WILLIAMS, P.C. (1945). Studies on the biological action of serum gonadotrophin. 1. Decline in ovarian response after hypofysectomy. J. Endocr., 4: 127-130.

WOOLLEY, D.E., HOLINKA, C.F. & TIMIRAS, P.S. (1969). Changes in ^{3}H-estradiol distribution with development in the rat. Endocrinology, 84: 157-161.

YING, S.Y., FANG, V.S. & GREEP, R.O. (1971). Estradiol benzoate (EB)-induced changes in serum-luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in immature female rats. Fert. Steril., 22: 799-801.

YING, S.Y. & GREEP, R.O. (1971a). Effect of estradiol on ovulation in immature rats primed with different doses of pregnant mare's serum (PMS). Endocrinology, 89: 213-218.

YING, S.Y. & GREEP, R.O. (1971b). Effect of age of rat and dose of a single injection of estradiol benzoate (EB) on ovulation and the facilitation of ovulation by progesterone (P). Endocrinology, 89: 785-790.

YING, S.Y. & GREEP, R.O. (1971c). Effect of a single low dose of estrogen on ovulation, pregnancy and lactation in immature rats. Fert. Steril., 22: 165-169.

YING, S.Y. & GREEP, R.O. (1973). Effect of gonadotropins and steroids on the maturation of the female reproductive function. In: The development and maturation of the ovary and its functions. Ed. H. Peters. Exc. Med. ICS, 267: 181-184.

YING, S.Y. & MEYER, R.K. (1969). Dose-dependent pregnant mare's serum-induced ovulation in immature rats. Proc. Soc. exp. Biol. Med., 130: 40-43.

ZARROW, M.X. & DINIUS, J. (1971). Regulation of pituitary ovulating hormone concentration in the immature rat treated with pregnant mare serum J. Endocr., 49: 387-392.

ZARROW, M.X. & GALLO, R.V. (1966). FSH-induced ovulation in the immature rat. Endocrinology, 79: 445-447.

ZARROW, M.X. & GALLO, R.V. (1969). Action of progesterone on PMS-induced ovulation in the immature rat. Endocrinology, 84: 1274-1276.

ZARROW, M.X. & HURLBUT, E.C. (1967). Inhibition and facilitation of PMS-induced ovulation in the immature rat following treatment with progesterone. Endocrinology, 80: 735-740.

ZARROW, M.X. & QUINN, D.L. (1963). Superovulation in the immature rat following treatment with PMS alone and inhibition of PMS-induced ovulation. J. Endocr., 26: 181-188.

ZARROW, M.X. & WILSON, E.D. (1961). The influence of age on superovulation in the immature rat and mouse. Endocrinology, 69: 851-855.

ZARROW, M.X., NAQVI, R.H. & DENENBERG, V.H.C. (1969). Androgen-induced precocious puberty in the female rat and its inhibition by hippocampal lesions. Endocrinology, 84: 14-19.

ZEILMAKER, G.H. (1966). The biphasic effect of progesterone on ovulation in the rat. Acta endocr., Copenh. 51: 461-468.

ZOLOVICK, A.J. (1972). Effects of lesions and electrical stimulation of the amygdala on hypothalamic hypophysial regulation. In: The Neurobiology of the amygdala. Advances in Behavioral Biology, vol. 2 pp. 775-785. Ed. B.E. Eleftheriou, Plenum press, New York-London.

CURRICULUM VITAE

Schrijfster van dit proefschrift, geboren 8 december 1943 te 's-Hertogenbosch behaalde in 1961 cum laude het diploma HBS-B aan de Rijks HBS aldaar.
Aansluitend begon zij haar studie in de biologie aan de Universiteit van Amsterdam; in januari 1965 werd het kandidaatsexamen K afgelegd. Voor het doktoraalexamen werd een hoofdvak in de systematische dierkunde, over de ontwikkeling en levenswijze van Sphaeromicola dudichi Klie, een commensale ostracode van houtborende kreeftachtigen, bewerkt aan het Zoölogisch Museum te Amsterdam, en voor een deel aan het "Station Marine d'Endoume" te Marseille. Verder werden onderwerpen bewerkt in de parasitologie (Instituut voor Tropische Hygiëne) en in de zoölogie aan het zoölogisch laboratorium. Aan laatstgenoemd instituut werd in het kader van een studenten-/kandidaatsassistentschap van 1963-1968 geassisteerd bij het prakticum zoölogie. In april 1968 werd het doktoraalexamen biologie cum laude afgelegd. Vanaf september 1968 is zij werkzaam als wetenschappelijk medewerkster aan de afdeling Anatomie van de fakulteit der geneeskunde waar een taak in het embryologie onderwijs en het onderzoek beschreven in dit proefschrift werden verricht.

APPENDIX

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EXCERPTA MEDICA

Serum levels of gonadotropins and follicular growth in prepuberal rats

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A number of reports have been published dealing separately with either follicular development (Umezu, 1970), pituitary contents of FSH (Kragt and Ganong, 1968; Watanabe and McCann, 1969) and of LH (Moore, 1966) or with levels of circulating FSH (Goldman *et al.*, 1971; Johnson, 1971) and LH (Weisz and Ferin, 1970) in prepuberal female rats. From these studies no accurate correlation can be made between blood levels of gonadotropins and patterns of follicular development at certain ages, since large differences exist between the experimental animals. This holds especially for the age at which puberty – first ovulation, followed by cyclic function of the ovary – occurs. Therefore we studied both follicular development and levels of circulating gonadotropins in one strain of rats, a highly inbred Wistar substrain, R-Amsterdam, which under the housing conditions in our laboratory shows puberty occurring regularly on about day 40 (Meijs-Roelofs, 1972). In addition, the ovarian responsiveness to exogenous gonadotropin was studied at various ages after hypophysectomy and subsequent treatment with PMS.

Follicular development

Throughout the prepuberal period differential counts of follicles were made. Attention was focussed on follicles with volumes exceeding 100×10^5 μm^3 (largest diameter about 275 μ; type 6, 7 and 8 of Pedersen and Peters, 1968). Groups of 4 to 8 rats were killed at different ages and after routine histological procedures follicular volumes were measured as described elsewhere (Welschen, 1973). The data are given in Figure 1.

It was found that follicles with a volume $\geqslant 100 \times 10^5$ μm^3 appear for the first time at day 19. The number of these follicles increased sharply until day 23 and then more slowly till day 27. After that age no significant changes in the number of follicles exceeding this volume were found until about 2 days before first oestrus, when the number sharply decreased. Follicles of a volume range of $200–500 \times 10^5$ μm^3 appeared at day 20. Their number also increased till day 27, then remained constant until about 2 days before first oestrus, when it decreased sharply. Follicles in a volume range of $\geqslant 500 \times 10^5$ μm^3 were only incidentally found between days 21 and 28. They were always present in low numbers after day 29 until about 2 days before first oestrus. Then their number increased to values corresponding to the

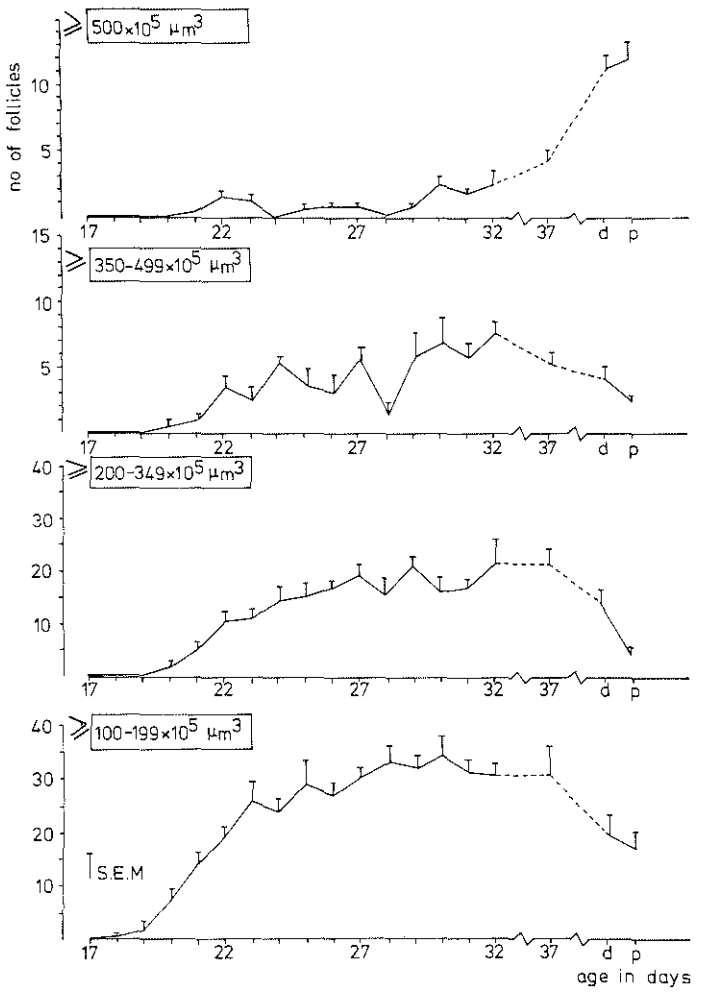

Fig. 1 *The number of follicles present in various volume classes during the prepuberal period.* d = *dioestrus*; p = *prooestrus.*

normal number of ovulations at first oestrus.

The increase of the number of follicles ($\geq 500 \times 10^5$ μm^3) explains only 20% of the decrease of the number of smaller follicles observed during the same period. During the days preceding first oestrus a wave of atresia apparently occurs in follicles $< 500 \times 10^5$ μm^3.

The capacity of follicles to ovulate in response to 15 I.U. HCG was tested in groups of 4–8 rats at 18, 22, 25, 28, 30, 31, 32 or 35 days of age. In none of these rats was ovulation observed, in spite of the presence of follicles $\geq 500 \times 10^5$ μm^3 which are capable of ovulating during the normal cycle in adult rats (Welschen and Rutte, 1971) and probably also in immature rats shortly before first oestrus.

Body weights, ovarian and uterine weights of the rats in which the follicular population was studied are given in Figure 2. Both body weight and ovarian weight increased steadily

during the period studied. In contrast, uterine weight did not change significantly between days 17 and 28. On the following days a sharp increase was observed.

The data obtained in the present study differ considerably from similar data on Wistar rats reported by Umezu (1970), especially with regard to diameters and numbers of the largest follicles.

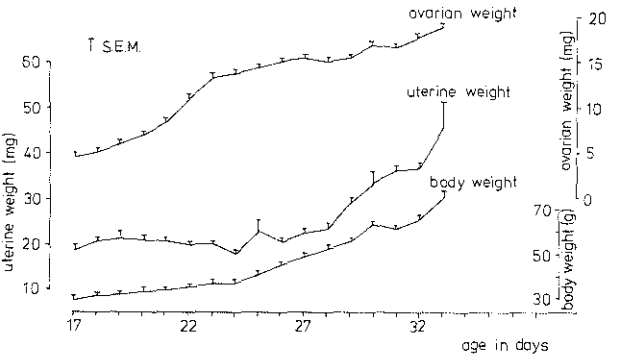

Fig. 2 *Body weight, ovarian weight and uterine weight during the prepuberal period.*

Radioimmunoassay of serum LH and FSH

LH and FSH were measured in the serum of peripheral blood from immature rats ranging in age from 8 to 35 days. The animals were bled once. Blood was taken by puncture of the ophthalmic venous plexus under light ether anaesthesia at about 15.00 h and allowed to clot in a refrigerator overnight prior to centrifugation. Estimations were made in blood samples from individual animals if older than 12 days. In younger rats blood from 2 to 4 animals was pooled.

The LH determination was carried out in a double-antibody radioimmunoassay as described by Niswender *et al.* (1968), using anti-ov-LH (GDN-15) as antiserum and ovine LHI^{125} (LER 1056C2) as tracer (OO RAT LH RIA*). All serum values are expressed in ng NIAMD-rat-LH RP-1. The sensitivity of this OO RAT LH system is 10 ng/ml serum. Serum samples were assayed in duplicate in 50 μl and 100 μl or in 100 μl and 200 μl aliquots depending on their potency.

Rat FSH was assayed with the RR RAT FSH RIA supplied by the NIAMD. Serum values are expressed in ng NIAMD-rat-FSH RP-1. The sensitivity of this assay system is 100 to 200 ng/ml serum when measured with a volume of 200 and 100 μl serum respectively.

LH concentrations in immature female rats showed in general rather constant values (Fig. 3). They are comparable to those measured in the same system in dioestrous adult rats. Higher mean levels were found around day 15 and day 34. However, levels were always consider-

* According to Niswender *et al.* (1968) the first capital letter refers to the species from which LH is used to obtain the antiserum. The second capital letter refers to the species from which LH is purified to be used as a tracer.

ably lower than prooestrous levels in adult rats (up to 1000 ng NIAMD-rat-LH RP-1/ml serum, Daane and Parlow, 1971) and also lower than levels one month after castration (about 400 ng/ml serum, Uilenbroek, unpublished observations).

A similar pattern of LH values in immature female rats was found by Weisz and Ferin (1970). Their data also suggest an LH peak around day 14. Their absolute values tend to be higher than those found in the present study, but in general absolute values are difficult to compare because of factors such as the differences between the standards used.

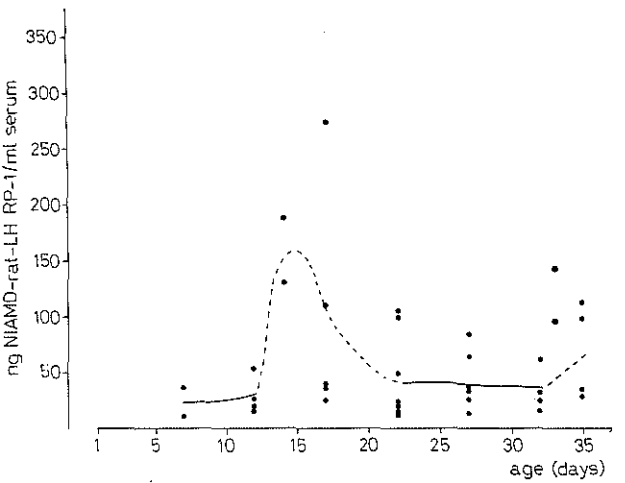

Fig. 3 *Serum LH concentrations in immature female rats measured by OO RAT LH radioimmunoassay and expressed in ng NIAMD-rat-LH RP-1. Each point represents one individual animal. Only on day 7 and day 12 were pools of 2 to 4 animals used.*

Data on FSH concentrations during the same period are given in Figure 4. Between days 8 and 21, high levels (800–1400 ng/ml serum) were found with a peak around day 15. A sharp decrease to a level of 200–300 ng FSH/ml serum, values hardly detectable in our assay system, can be seen on day 22. This level is maintained until day 35. FSH concentrations from days 22 to 35 are comparable to FSH concentrations found during dioestrus in adult rats. The levels found around day 15 reach values twice as high as those occurring during late prooestrus and early oestrus (about 500 ng NIAMD-rat-FSH RP-1/ml, Daane and Parlow, 1971; see also Gay *et al.*, 1970). They are in the range of post-castration values (about 1500 ng/ml, Uilenbroek, unpublished observations).

Our data are essentially in agreement with those of Goldman *et al.* (1971), Kragt and Dahlgren (1972) and Ojeda and Ramirez (1972). They also observed peak levels of FSH around day 15. However, the acute decrease on day 22 observed in the present study has not been reported by these workers.

Effects of hypophysectomy and subsequent treatment with PMS at various ages on follicular growth, ovarian weight and uterine weight

Rats were hypophysectomized at the age of 17, 22, 27 or 32 days between 9.00 and 10.00 h. They were injected immediately afterwards with 0.9 % NaCl or 2, 4 or 8 I.U. PMS (Gestyl,

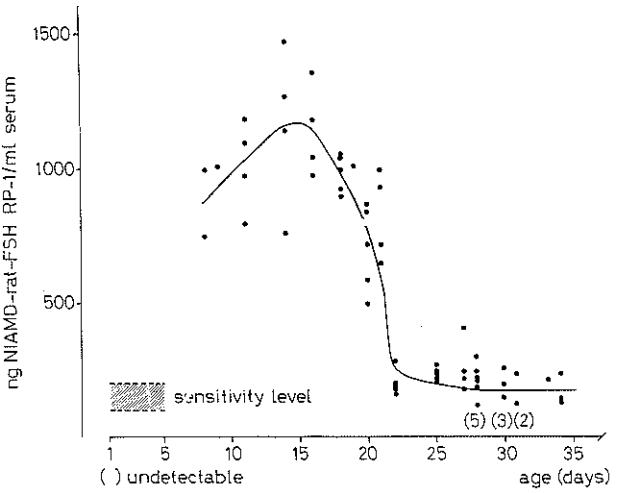

Fig. 4 *Serum FSH concentrations in immature female rats measured by RR RAT FSH radioimmunoassay and expressed in ng NIAMD-rat-FSH RP-1. Each point represents one individual animal. Only on day 8 and day 11 were pools of 2 to 4 animals used. On the left is indicated the sensitivity level of this assay system. Numbers in parentheses represent the number of animals with values below 100 or 200 ng/ml.*

Organon, dissolved in 0.1 ml 0.9 % NaCl) per 100 g body weight. The rats received a second injection of half the initial dose 8 hours later. We will refer to the different treatments mentioning the first dose only. Twenty-four hours after the operation the rats were killed. Ovarian, uterine and body weights were recorded and the follicles $\geqslant 100 \times 10^5\ \mu m^3$ were differentially counted.

The data on follicular growth are given in Figure 5. The statistical significance of the differences recorded is indicated in Table 2. On day 18 no follicles $\geqslant 100 \times 10^5\ \mu m^3$ were normally present. After hypophysectomy and treatment with even a relatively large dose of PMS*, follicles of this volume range were also absent. After hypophysectomy on day 22, the number of follicles $\geqslant 100 \times 10^5\ \mu m^3$ decreased significantly. Normal follicular growth could only be maintained with 8 I.U. PMS. Hypophysectomy on day 27 or day 32 also caused a decrease in the number of follicles $\geqslant 100 \times 10^5\ \mu m^3$. In both cases 4 and 8 I.U. PMS maintained normal follicular growth.

Data on ovarian weight (Tables 1 and 2) show that hypophysectomy with or without subsequent treatment with PMS on day 17 exerted no effect on this parameter. In contrast hypophysectomy on day 22, 27 or 32 caused an acute decrease of the weights of the ovaries. In these cases 2 I.U. PMS maintained normal ovarian weight after hypophysectomy whereas 4 and 8 I.U. caused an increase to values significantly above those in intact rats.

Data on uterine weight (Tables 1 and 2) show a lack of effect of hypophysectomy and

* In earlier studies we found that in adult rats 8 I.U. was required to maintain normal follicular growth from prooestrus to oestrus, whereas only 4 I.U. was required during the other days of the cycle (Welschen, 1973).

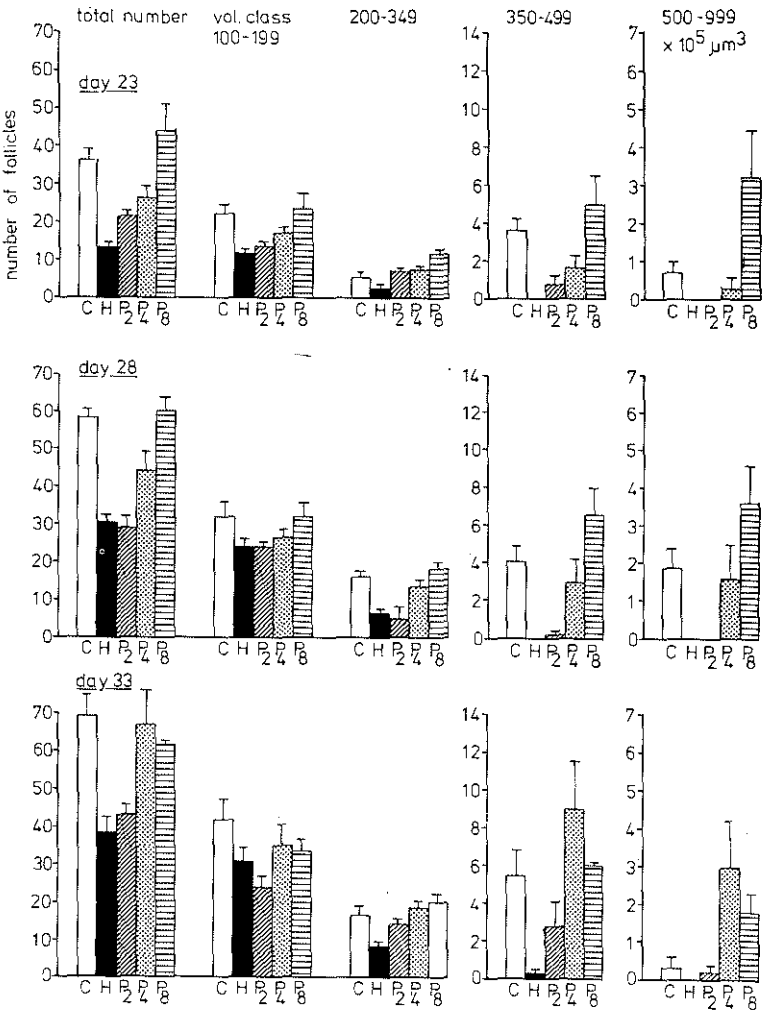

Fig. 5 *The number of follicles present in various volume classes at different ages in intact rats and in hypophysectomized rats, treated with 0.9 % NaCl or PMS 24 hr prior to autopsy. c = intact control; h = after hypophysectomy; P_2, P_4, P_8 = hypophysectomy followed by 2, 4, 8 I.U. PMS per 100 g body weight; bar = S.E.M.*

subsequent PMS treatment during day 17. Hypophysectomy on day 22 was also ineffective whereas it caused an acute decrease of uterine weight if performed on day 27 or 32. PMS treatment was very effective after hypophysectomy on day 22, 27 or 32. In most cases even 2 I.U. PMS caused a significant increase of uterine weight over control values.

Discussion

The FSH measurements presented show a clear picture which is in good agreement with the data of Kragt and Dahlgren (1972) and Ojeda and Ramirez (1972). However, the results of

Table 1 *Ovarian, uterine and body weights at different ages in control animals and in animals that were hypophysectomized 24 hr before and treated with saline or regimens of 2, 4 or 8 I.U. PMS per 100 g body weight*

Day of autopsy		Intact	Hypophysectomized and treated with			
			Saline	2 I.U. PMS	4 I.U. PMS	8 I.U. PMS
18	ovaries (mg)	5.5 ±0.1	4.0 ±0.3	4.8 ±0.3	5.5 ±0.4	6.1 ±0.3
	uterus (mg)	19.7 ±0.8	16.9 ±0.5	17.9 ±0.4	18.8 ±0.6	19.9 ±0.6
	body (g)*	30.3 ±0.4	25.0 ±1.0	26.4 ±0.7	27.5 ±1.5	24.8 ±0.7
	no. of animals	(6)	(3)	(6)	(6)	(6)
23	ovaries (mg)	10.8 ±0.5	7.5 ±0.4	10.0 ±0.2	12.4 ±0.5	14.2 ±0.9
	uterus (mg)	18.9 ±0.3	19.0 ±0.6	24.0 ±1.3	26.6 ±0.8	31.5 ±1.3
	body (g)*	33.9 ±0.8	29.0 ±0.8	33.5 ±1.4	33.0 ±0.9	30.7 ±1.3
	no. of animals	(13)	(5)	(5)	(11)	(5)
28	ovaries (mg)	15.2 ±0.3	11.8 ±0.2	13.2 ±0.3	17.5 ±0.8	20.4 ±0.5
	uterus (mg)	25.9 ±1.5	20.9 ±0.9	28.4 ±2.3	41.0 ±2.7	44.6 ±1.7
	body (g)*	47.7 ±1.1	43.8 ±0.9	38.9 ±1.2	40.1 ±0.8	42.5 ±0.9
	no. of animals	(9)	(6)	(4)	(9)	(11)
33	ovaries (mg)	17.1 ±0.2	13.7 ±0.1	17.7 ±1.2	23.9 ±0.5	25.8 ±0.7
	uterus (mg)	35.7 ±1.5	27.8 ±0.4	53.2 ±3.8	62.9 ±2.4	65.5 ±4.7
	body (g)*	58.1 ±1.1	57.9 ±1.7	55.8 ±1.5	54.8 ±2.2	55.9 ±3.2
	no. of animals	(6)	(4)	(5)	(5)	(4)

* In all operated rats body weight decreased about 15 % during the postoperative period.

Table 2 *Summary of the effects of hypophysectomy and subsequent treatment with regimens of 2, 4 or 8 I.U. PMS on follicular growth, ovarian weight and uterine weight*

Day of autopsy	Follicular growth				Ovarian weight				Uterine weight			
	0	2	4	8	0	2	4	8	0	2	4	8 I.U. PMS
18					=	=	=	=	=	=	=	=
23	−	−	−	=	−	=	+	+	=	+	+	+
28	−	−	=	=	−	=	+	+	−	=	+	+
33	−	−	=	=	−	=	+	+	−	+	+	+

The data on intact and operated rats given in Fig. 5 and Table 1 are compared. For statistical analysis, Wilcoxon's 2-sample test was used. A difference was considered as statistically significant if the double tail probability was <0.05. Significant decreases or increases are given as − and + respectively, whereas the absence of significant differences is indicated as =. Of the organ weights, both absolute and relative values are compared. In these cases − and + mean that both absolute and relative values were significantly different.

the LH measurements are less clear because of the large variations in individual values and the relatively small numbers of samples used. Nevertheless, the results are in agreement with those of other authors (Weisz and Ferin, 1970; Ojeda and Ramirez, 1972). From the studies referred to and the results presented, it may be concluded that FSH levels are extremely high

until about day 21, and in the range of adult dioestrous values between day 22 and first prooestrus. LH levels probably are elevated about day 15, and in the range of adult dioestrous levels from day 17 to day 35, when a second elevation may occur.

Our data on the composition of the population of follicles $\geqslant 100 \times 10^5$ μm^3 differ markedly from those reported for Wistar rats by Umezu (1970). This may be due to the differences in substrain and housing conditions of the rats used.

The effects of hypophysectomy indicate the age after which, in intact rats, the growth of the large follicles and of the other ovarian structures (as indicated by ovarian weight) and their steroid secretion (as indicated by uterine weight) is stimulated by endogenous gonadotropins. Moreover, the effects of hypophysectomy and subsequent treatment with PMS provide information on the amounts of gonadotropins required to maintain normal follicular growth, growth of other ovarian structures and normal steroid production. On the basis of the data obtained we would like to suggest the following:

1. Until day 18 neither the extremely high FSH levels present in intact rats nor the highest PMS dose injected in hypophysectomized rats were capable of inducing follicles $\geqslant 100 \times 10^5$ μm^3, i.e. follicles which show cyclic changes in number, in the adult rat. Assuming that the high FSH levels present do have a biological significance, it may be suggested that ovarian follicles, under these conditions of strong stimulation, still require a period of about 3 weeks to reach this volume. Probably a similar line of reasoning may be followed for LH since Shiino and Rennels (1967) found that HCG injections do not result in further maturation of follicles during this period.

2. The sharp decrease of plasma FSH levels about day 21 occurs simultaneously with a sharp increase of the number of follicles $\geqslant 100 \times 10^5$ μm^3. Both phenomena may be causally related. Possibly, the high FSH levels first induce the sharp increase in the number of large follicles – the amount of PMS required to maintain normal follicular growth after hypophysectomy is very high at that time – following which, the increasing number of follicles produce sufficiently large amounts of steroids to inhibit FSH release strongly.

3. During the period from about day 20 until a few days before first oestrus, plasma gonadotropin levels seem to be relatively ineffective, especially the LH levels, as is indicated by a number of findings: firstly, few follicles reach large volumes; secondly, follicles $\geqslant 500 \times 10^5$ μm^3 are incapable of ovulating during this period, in contrast to similar follicles in adult rats (Welschen, 1973) and, thirdly, the uterus is not stimulated (until day 28) or is only weakly stimulated (after day 28), suggesting rather low steroid levels. These three phenomena are also observed in hypophysectomized rats treated with FSH preparations in the absence of LH (Lohstroh and Johnson, 1966).

4. Shortly before first oestrus considerable changes occur in the follicular population. These changes might be induced when LH and/or steroid levels reach 'threshold' values. The well known direct action of oestrogens causing an increase of the responsiveness of follicles to gonadotropins (Bradbury, 1961) may be part of this mechanism.

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References

BRADBURY, J. T. (1961): Direct action of estrogen on the ovary of the immature rat. *Endocrinology, 68*, 115.

DAANE, T. A. and PARLOW, A. F. (1971): Periovulatory patterns of rat serum follicle stimulating hormone and luteinizing hormone during the normal estrous cycle: effects of pentobarbital. *Endocrinology, 88*, 653.

GAY, V. L., MIDGLEY Jr., A. R. and NISWENDER, G. D. (1970): Patterns of gonadotrophin secretion associated with ovulation. *Fed. Proc., 29*, 1880.

GOLDMAN, B. D., GRAZIA, Y. R., KAMBERI, I. A. and PORTER, J. C. (1971): Serum gonadotropin concentrations in intact and castrated neonatal rats. *Endocrinology, 88*, 771.

JOHNSON, D. C. (1971): Serum follicle-stimulating hormone (FSH) in normal and androgenized male and female rats. *Proc. Soc. exp. Biol. (N.Y.), 138*, 140.

KRAGT, C. L. and DAHLGREN, J. (1972): Development of neural regulation of follicle stimulating hormone (FSH) secretion. *Neuroendocrinology, 9*, 30.

KRAGT, C. L. and GANONG, W. F. (1968): Pituitary FSH content in female rats at various ages. *Endocrinology, 82*, 1241.

LOHSTROH, A. J. and JOHNSON, R. E. (1966): Amounts of ICSH and FSH required for follicular development, uterine growth and ovulation in the hypophysectomized rats. *Endocrinology, 79*, 991.

MEIJS-ROELOFS, H. M. A. (1972): Effect of electrical stimulation of the hypothalamus on gonadotrophin release and the onset of puberty. *J. Endocr., 54*, 277.

MOORE, W. W. (1966): Changes in pituitary LH concentration in prepubertal and postpubertal rats. *Neuroendocrinology, 1*, 333.

NISWENDER, G. D., MIDGLEY, A. R., MONROE, S. E. and REICHERT, L. E. (1968): Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131}I. *Proc. Soc. exp. Biol. (N.Y.), 128*, 807.

NISWENDER, G. D., MIDGLEY Jr., A. R. and REICHERT Jr., L. E. (1968): Radioimmunologic studies with murine, bovine, ovine and porcine luteinizing hormone. In: *Gonadotropins 1968*, pp. 299–306. Editor: E. Rosemberg. Geron-X Inc., Los Altos.

OJEDA, S. R. and RAMIREZ, V. D. (1972): Plasma level of LH and FSH in maturing rats: response to hemigonadectomy. *Endocrinology, 90*, 466.

PEDERSEN, T. and PETERS, H. (1968): Proposal for a classification of oocytes and follicles in the mouse ovary. *J. Reprod. Fertil., 17*, 555.

SHIINO, M. and RENNELS, E. G. (1967): Pituitary follicle stimulating hormone in vivo and ovarian development in the infant rat. *Endocrinology, 81*, 1379.

UMEZU, M. (1970): The basal observations on puberty in female rats. The changes in weight of ovaries and uterus and the development of ovarian follicles. *Jap. J. Anim. Reprod., 16*, 71.

WATANABE, S. and MCCANN, S. M. (1969): Alterations in pituitary follicle stimulating hormone (FSH) and hypothalamic FSH-releasing factor (FSHRF) during puberty. *Proc. Soc. exp. Biol. (N.Y.), 132*, 195.

WEISZ, J. and FERIN, M. (1970): Pituitary gonadotrophins and circulating LH in immature rats – a comparison between normal females and males and females treated with testosterone in neonatal life. In: *Gonadotrophins and Ovarian Development*, pp. 339–350. Editors: W. R. Butt, A. C. Crooke and M. Ryle. E. and S. Livingstone, Edinburgh.

WELSCHEN, R. (1973): Amounts of gonadotrophins required for normal follicular growth in hypophysectomized adult rats. *Acta endocr. (Kbh.), 72*, 137.

WELSCHEN, R. and RUTTE, M. (1971): Ovulation in adult rats after treatment with pregnant mare serum gonadotrophin during oestrus. *Acta endocr. (Kbh.), 68*, 41.

J. Endocr. (1973), 59,
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PLASMA - OESTRADIOL -17β AND ITS RELATION TO SERUM FOLLICLE - STIMULATING HORMONE IN IMMATURE FEMALE RATS*

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*) Presented in part at the " Colloque sur l'ovogenèse et la folliculogenèse ", 14 - 15 December 1972, Nouzilly, France

SUMMARY

Oestradiol-17β (E_2) was measured by radioimmunoassay in plasma of immature female rats. Maximal E_2 levels of 55 - 60 pg/ml were found at 10 - 15 days of age; from day 25 to day 35 E_2 levels were low to undetectable. The E_2 measured appeared to be of ovarian origin : ovariectomy (OVX) performed on day 13 resulted in a decreased E_2 level 2 days later (13 pg/ml) as compared with the value from the control litter-mates (46 pg/ml); following adrenalectomy (ADRX) the level of circulating E_2 remained normal (54 pg/ml). The effects of OVX and ADRX on E_2 levels were paralleled by effects on uterine weights.
In the rat strain used, levels of follicle-stimulating hormone (FSH) in the serum, measured by radioimmunoassay, were high from day 10 to day 20 and showed a steep decrease on day 21. After OVX on day 15 this decrease in serum FSH was not observed.
The influence of circulating E_2 on serum levels of FSH was further studied after OVX followed by treatment with varying doses of oestradiol-benzoate. Ovariectomy on day 13 resulted in a significantly increased FSH level 2 days later (1770 ng NIAMD-rat-FSH RP-1/ml) as compared with the value obtained from control animals (1033 ng/ml). This increase was not observed after daily injections of 0.1 μg oestradiol benzoate/100 g body weight.
The results indicate that E_2 and FSH levels show a similar pattern between 5 and 35 days of age. Furthermore, an inhibitory feedback mechanism between oestrogens and FSH concentrations was found to be operative. The implications of these findings are discussed.

INTRODUCTION

In rats secretion of oestrogens from the ovaries seems to take place early in life. The observation of subnormal uterine weights at 10 days of age (Baker & Kragt, 1969) or at day 14 (Price, 1947) after neonatal ovariectomy, indicates that ovarian oestrogen secretion occurs in the second week of life.
Measurements of circulating oestrogens by fluorimetric methods indicated low levels on day 5 and rather high and constant levels from day 10 onwards (Presl, Horský, Herzmann, Mikuláš & Henzl, 1967; Presl, Herzmann & Horský, 1969). Using a radioimmunoassay method, Weisz & Gunsalus (1973) observed extremely high oestrone levels around day 10, but could not obtain reliable results for oestradiol-17β (E_2). Moreover, the source of the oestrogens measured appeared to be mainly the adrenal gland.
The data mentioned above suggest the presence of circulating oestrogens from day 5 to day 10 onwards, but the exact levels and the source of these oestrogens remain to be elucidated. Furthermore, the question arises, whether at these early ages oestrogens are already involved in the regulation of gonadotrophin secretion. Extremely high gonadotrophin levels, suggesting a rather uninhibited gonadotrophin release, are found between 10 and 20 days of age (Weisz & Ferin, 1970; Ojeda & Ramirez, 1972; Meijs-Roelofs, Uilenbroek, Osman & Welschen, 1973; Weisz & Gunsalus, 1973). For the subsequent period, i.e. after day 20 available data based on unilateral ovariectomy experiments all suggest that an inhibitory feedback

mechanism is operative. Observations concerning a similar mechanism at earlier ages seem inconclusive (Baker & Kragt, 1969; Gerall & Dunlap, 1971; Ojeda & Ramirez, 1972).
Therefore, it was the aim of the present study to 1) measure the concentration of E_2 in peripheral plasma throughout the period from birth to sexual maturity in the female rat, 2) investigate whether the E_2 measured was of ovarian or of adrenal origin, 3) investigate the possible influence of circulating E_2 on the levels of follicle stimulating hormone (FSH) in serum.

MATERIALS AND METHODS

Immature female rats of an inbred Wistar substrain, the R-Amsterdam strain, were used. They were kept in a controlled temperature of 22-25° C, under controlled light conditions : 14 h light; 10 h darkness, with the middle of the light period at 12.00 h. Weaning of the animals was carried out on day 22 of life, whereafter the rats received standard dry pellets and tap water ad libitum. Litter-mates were divided between experimental and control groups.
Animals that were operated on before weaning were generally kept with their own mothers, with the exception of those used in experiments in which oestradiol benzoate (17β -hydroxy-1,3,5 (10)-oestratien-3-yl-benzoate; OB) was injected. In this case non-injected, oil-injected and OB-injected groups were kept with separate mothers. Ovariectomy (OVX) and adrenalectomy (ADRX) were performed by bilateral approach under ether anaesthesia. Blood for serum FSH determination was obtained under light ether anaesthesia by puncture of the ophthalmic venous plexus and was allowed to clot overnight in a refrigerator before centrifugation.
Blood for plasma E_2 determination was obtained from non-anaesthesized rats by decapitation. In the latter case the blood of 7 - 28 rats was pooled after addition of a few drops of heparin to prevent clotting. Serum and plasma samples were stored at -20° C.
Serum FSH was estimed, as described previously (Meijs-Roelofs et al., 1973), by a double-antibody radioimmunoassay with the kit provided by the National Institute of Arthritis and Metabolic Diseases. Serum FSH was expressed in ng NIAMD-rat-FSH RP-1/ml serum. Duplicate volumes of 100 and 200 µl serum were assayed. Concentrations of FSH calculated from the 200 µl volumes were consistently lower than those from 100 µl volumes. A similar systematic difference was described by Seki, Seki, Yoshihara & Maeda (1971) and was attributed to an unknown serum component inhibiting displacement of labelled rat-FSH by unlabelled rat-FSH. Therefore, the FSH concentrations were calculated on the basis of the results from 100 µl volumes.
Plasma E_2 was estimated by radioimmunoassay. The antibody used was raised against an oestradiol-17β -6-(0-carboxy methyl)oxime-BSA complex. Its properties with regard to cross-reactivity with steroids different from E_2 have been described by Exley, Johnson & Dean (1971). Procedures used for extraction of E_2 from plasma and for subsequent purification, as well as accuracy, precision and blank values of the method have been reported previously by de Jong, Hey & van der Molen (1973). All samples were assayed in two unequal volumes of plasma. Values measured with this assay system in adult rats with a 5-day cycle ranged from a mean of 1.75 pg/ml at metoestrus to a mean of 33.1 pg/ml at prooestrus, both measured at 11.00 h. (unpublished results).

The statistical analysis of results was carried out using Wilcoxon's two sample test. A difference was considered as significant if the double tail probability was ≤ 0.05.

DESCRIPTION OF EXPERIMENTS

Experiment 1. Plasma levels of E_2 in intact female rats, influence of OVX and ADRX.

Oestradiol-17β levels were measured in pools of plasma from female rats ranging in age from 5 to 35 days. The rats were killed at 15.00 h. In addition, E_2 levels were measured in plasma of rats in which OVX, ADRX or the combined operation was performed on day 13 and from which blood was collected on either day 15 or day 17 at 15.00 h. In a pilot experiment the effect of sham operation on E_2 levels was studied; as no difference in E_2 levels was found between intact rats (41 pg/ml) and sham operated rats (39 pg/ml), sham operation was not used in further experiments.

Experiment 2. Effect of steroids on FSH levels in serum.

Series A Groups of rats were ovariectomized on day 13 between 09.00 h and 11.00 h and were injected with either OB dissolved in 0.1-0.2 ml peanut oil or with 0.1-0.2 ml peanut oil only. Daily doses of 0.05, 0.1, 0.25 and 0.5 µg OB/100 g body weight were used. Three injections were given, the first on day 13 at 15.00 h, the second on day 14 at 12.00 h and the third on day 15 at 9.00 h. Intact oil-injected rats served as additional controls. Blood for radioimmunoassay of serum FSH concentration was collected on day 15 at 15.00 h. In most cases uterine weights were recorded.
In addition, plasma E_2 levels were estimated in rats treated as described above with a daily dose of either 0.1 or 0.5 µg OB/100 g body weight. Blood was collected from these rats on day 15 at 15.00 h for radioimmunoassay of plasma E_2 concentration and uterine weights were recorded.

Series B Groups of rats were ovariectomized on day 15 between 09.00 and 11.00 h. From day 25 onwards the rats were injected with either OB dissolved in 0.1-0.2 ml peanut oil or with 0.1-0.2 ml peanut oil only. Daily doses of 0.05, 0.1, 0.25 and 0.5 µg OB/100 g body weight were used. Three injections were given, the first on day 25 at 15.00 h, the second on day 26 at 12.00 h and the third on day 27 at 09.00 h. Intact oil-injected rats served as additional controls. Blood for radioimmunoassay of serum FSH concentration was collected on day 27 at 15.00 h and in some groups uterine weights were recorded.

Series C Groups of rats underwent ADRX, OVX or both ADRX and OVX operations on day 13 between 09.00 and 11.00 h. Blood was collected for radioimmunoassay of serum FSH concentrations on day 15 at 15.00 h. Uterine weights were recorded.

RESULTS

Experiment 1. Plasma levels of E_2.

Plasma E_2 levels in immature female rats showed maximal values (55-60 pg/ml) from day 10 to day 15, preceded by a low value on day 5. A gradual decrease in E_2 levels was observed between day 15 and day 25; from day 25 to day 35 very low and mostly undetectable E_2 levels were found (see Table 1 and Fig. 1).

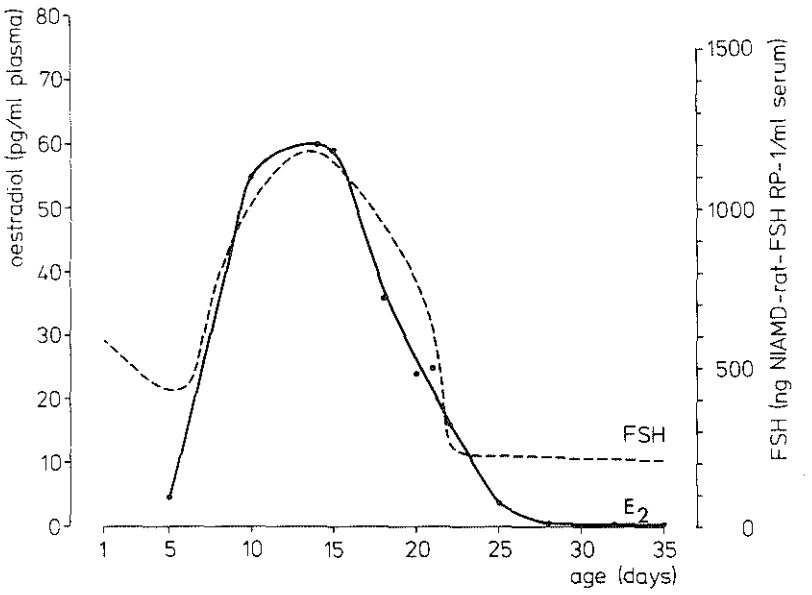

Fig. 1. Plasma oestradiol-17β (E_2) concentrations (——) in immature female rats are shown. For comparison serum follicle stimulating hormone (FSH) concentrations (– – –) as described earlier by Meijs-Roelofs et al. (1973) have been included in the figure, with the addition of data from day 1 to day 10.

Table 1. Plasma oestradiol-17β concentrations in immature female rats

Age in days	Number of animals/pool	Oestradiol-17β pg/ml mean	Age in days	Number of animals/pool	Oestradiol-17β pg/ml mean
5	28	5 (4.4;5.3)*	21	15	25 (22.8;26.6)
10	14	55 (58.2;52.0)	22	15	16 (15.0;16.5)
14	26	60	25	19	4 (2.2;4.9)
15	24	58	28	11	undetectable (0.0;1.2)
18	20	36 (34.8;37.3)	32	12	undetectable (0.7;1.0)
20	20	24 (21.1;26.3)	35	7	undetect able (0.0;0.0)

* figures in parentheses: concentrations measured in the two volumes of plasma used.

Influence of OVX and ADRX on E_2 levels

The effect of removal of two potential sources of E_2, the ovaries and the adrenal glands on levels of circulating E_2, is shown in Table 2. Ovariectomy on day 13 resulted within 2 days in considerably lowered plasma E_2 levels; after 4 days E_2 was hardly detectable. Adrenalectomy on day 13 did not result in a decreased E_2 level within 2 days and induced little or no decrease within 4 days. After the combined operation the E_2 levels showed a more rapid fall than after OVX alone: within 2 days E_2 was hardly detectable. The effects of OVX, ADRX or the combined operation on plasma E_2 levels were closely paralleled by the effects of these operations on uterine weights. The weight of the uteri decreased after OVX, more rapidly if OVX was combined with ADRX, but the weight was not influenced by ADRX alone.

Experiment 2. Effect of steroids on serum FSH levels

For reference purposes data on serum FSH levels in normal immature rats measured earlier (Meijs-Roelofs et al., 1973), are combined with the present data on plasma E_2 levels, and are given in Fig. 1. Serum FSH levels after OVX alone or after OVX followed by OB-treatment are

Table 2. Effect of ovariectomy (OVX), adrenalectomy (ADRX) or OVX combined with ADRX on day 13 post partum on the plasma oestradiol-17β concentrations and uterine weights of immature female rats on days 15 and 17 (mean $\pm$ S.E.M.).

Treatment on day 13	Age at autopsy	Number of animals	Uterine wt mg/100 b.wt	Statistics * (uterine wt)	Oestradiol-17β pg/ml plasma
Untreated	15	3	81.6 $\pm$ 3.4		46 (52.0;39.0)
OVX	15	9	63.6 $\pm$ 2.4	UTD	13 (15.0;11.2)
ADRX	15	14	75.2 $\pm$ 2.9	OVX	54 (58.0;50.1)
OVX & ADRX	15	12	39.6 $\pm$ 1.2	UTD, OVX, ADRX	0 (0; 0)
Untreated	17	5	76.6 $\pm$ 1.7		29 (31.9;25.6)
OVX	17	8	49.9 $\pm$ 1.3	UTD	4 (0.4;7.8)
ADRX	17	9	73.2 $\pm$ 1.5	OVX	22 (30.6;12.5)
OVX & ADRX	17	7	43.5 $\pm$ 1.6	UTD, ADRX	4 (0; 7.3)

* significant difference ($P \leq 0.05$) between untreated (UTD), OVX or ADRX groups as indicated.

Concentrations measured in the two different volumes of plasma used are given in parentheses.

summarized in Table 3 and Fig. 2a-2b.

Series A Ovariectomy on day 13 resulted in a significantly increased FSH level on day 15 (1770 ng/ml) as compared to the level in intact rats (1033 ng/ml). A daily dose of 0.1 µg OB/100 g body weight prevented this rise (700 ng/ml). Higher doses of OB decreased the FSH level still more (Fig. 2a).

Measurements of E_2 in pooled plasma of similarly treated rats indicated that a daily dose of 0.1 µg OB/100 g body weight resulted in an E_2 level of 50 pg/ml on day 15, a value comparable to that in intact 15-day-old rats. A daily dose of 0.5 µg OB/100 g body weight resulted in an E_2 level exceeding 100 pg/ml. Uterine weights in the ovariectomized rats treated

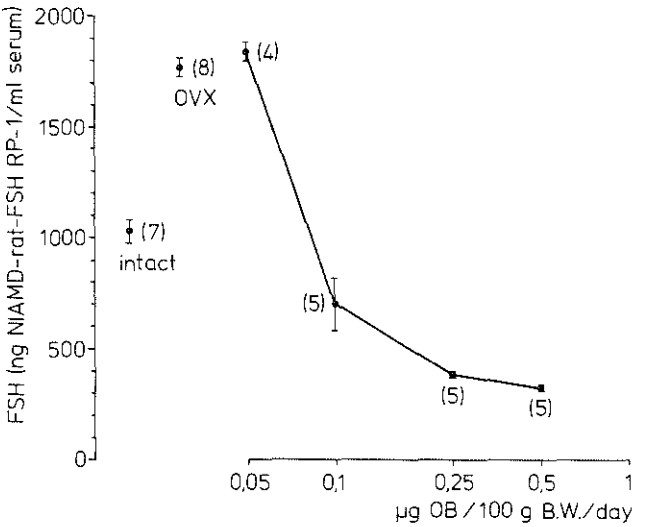

Fig. 2a. Serum follicle stimulating hormone (FSH) concentrations (mean ± S.E.M.) in 15-day old female rats, ovariectomized (OVX) on day 13 and injected from day 13 to day 15 with a daily dose of oestradiol benzoate. Values for intact and OVX control rats are shown. Numbers in parentheses are numbers of pooled samples (each of 1-2 animals).

Table 3. Effect of oestradiol benzoate on serum follicle stimulating hormone (FSH) levels and uterine weights in immature female rats ovariectomized (OVX) on day 13 or day 15 post partum. (mean ± S. E. M.)

Group	OVX on day 13	Treatment from day 13-day 15 (dosage / 100 g b.wt / day)	Autopsy on day 15			
			Uterine wt mg/100 g b.wt	Statistics * (uterine wt)	FSH concn ** ng/ml serum	Statistics * (FSH concn)
A	-	oil	72.6 ± 2.3 (6)		1033 ± 56 (7)	
B	+	oil	63.0 ± 3.8 (11)	A	1770 ± 42 (8)	A
C	+	0.05 µg OB	91.0 ± 5.9 (4)	B	1842 ± 42 (4)	A
D	+	0.1 µg OB	84.6 ± 4.5 (8)	B	700 ± 122 (5)	B
E	+	0.25 µg OB	108.6 ± 3.4 (8)	A, B	384 ± 12 (5)	A, B
F	+	0.5 µg OB	137.7 ± 6.7 (4)	A, B, C, D	324 ± 9 (5)	A, B
Group	OVX on day 15	Treatment from day 25-day 27	Autopsy on day 27			
A	-	oil	49.0 ± 3.2 (4)		267 ± 39 (8)	
B	+	oil	33.4 ± 2.6 (3)		1787 ± 64 (9)	A
C	+	0.05 µg OB	-		1812 ± 37 (4)	A
D	+	0.1 µg OB	96.5 ± 5.2 (7)	A, B	1508 ± 41 (7)	A, B, C
E	+	0.25 µg OB	-		1010 ± 25 (6)	A, B, C, D
F	+	0.5 µg OB	159.4 ± 4.9 (7)	A, B, D	995 ± 29 (7)	A, B, C, D

number of animals or number of pools (each of 1-2 animals) is given in parentheses

* significant difference ($P \leq 0.05$) between group A, group B, group C, group D as indicated

** expressed in NIAMD-rat-FSH RP-1

with 0.1 µg OB were not significantly different from those in untreated rats. Treatment with 0.5 µg OB nearly doubled uterine weights.

Series B Ovariectomy on day 15 induced an increase in FSH concentration on day 27 (from 1033 ng/ml on day 15 to 1787 ng/ml on day 27), whereas in intact rats the FSH levels decreased over the same period from 1033 ng/ml to 267 ng/ml. Oestradiol benzoate treatment from day 25 to 27 resulted in a decrease in FSH level, if doses ≥0.1 µg OB/100 g body weight were used. With the highest dosage (0.5 µg OB) the FSH level was reduced from 1787

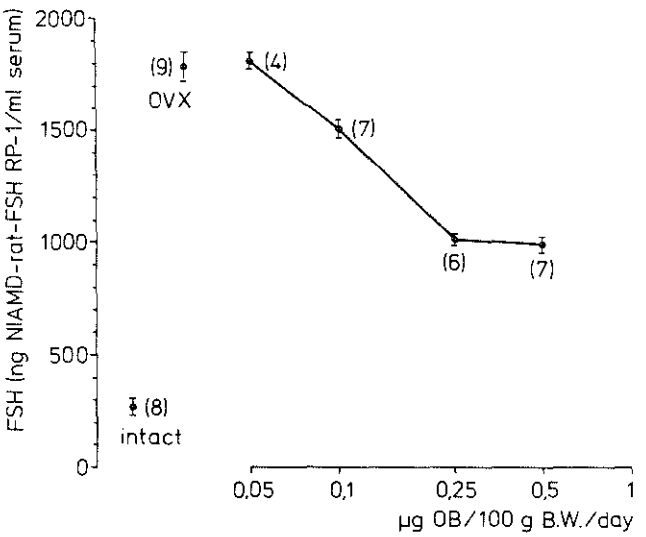

Fig. 2b. Serum follicle stimulating hormone (FSH) concentrations (mean ± S.E.M.) in 27-day old female rats, ovariectomized (OVX) on day 15 and injected from day 25 to day 27 with a daily dose of oestradiol benzoate. Values for intact and OVX control rats are shown. Numbers in parentheses are numbers of pooled samples (each of 1-2 animals).

Table 4. Effect of ovariectomy (OVX), adrenalectomy (ADRX) or OVX combined with ADRX on day 13 post partum on serum follicle stimulating hormone (FSH) concentrations and uterine weights of immature female rats on day 15 (mean ± S.E.M.)

Treatment on day 13	Age at autopsy	Uterine wt mg/100 g b.wt	Statistics * (uterine wt)	FSH concn ** ng/ml serum
untreated	15	61.7 ± 2.7 (4) ***		1225 ± 55 (2)
OVX	15	45.8 ± 1.2 (6)	UTD	2133 ± 67 (3)
ADRX	15	57.6 ± 2.2 (7)	OVX	1214 ± 53 (5)
OVX & ADRX	15	40.9 ± 1.4 (6)	UTD, OVX, ADRX	2020 ± 78 (7)

* significant difference ($P \leq 0.05$) between untreated UTD, OVX or ADRX as indicated

** expressed in ng NIAMD-rat-FSH RP-1

*** number of animals or number of pools (each of 1-2 animals) is given in parentheses

ng/ml (day 27, no OB) to 995 ng/ml (day 27, OB treatment) (Fig. 2b).

Series C In contrast to the effect of OVX, ADRX did not influence the level of serum FSH within 2 days (see table 4). Again (see experiment 1) uterine weights were decreased after OVX, more markedly if OVX was combined with ADRX, but were not influenced by ADRX alone.

DISCUSSION

The present study indicates that in the immature female rat high plasma E_2 levels exist during the period 10 - 20 days post partum. The maximal values found (60 pg/ml) exceed the preovulatory E_2 surge in peripheral plasma of adult rats, averaging 19 pg/ml, as measured by Naftolin, Brown-Grant & Corker (1972) using a competitive-protein-binding technique and 33.1 pg/ml as measured by radioimmunoassay in our laboratory (unpublished results). This agreement between the results obtained with two different techniques, together with the close correlation between E_2 level and uterine weights observed in both intact and in ovariectomized rats treated with OB, provide additional evidence for the specificity of the E_2 measurements.

The origin of E_2 appears to be the ovary and not the adrenal gland since OVX lowered plasma E_2 levels whereas ADRX did not. However, the more rapid decrease in E_2 concentration after combined OVX and ADRX suggests an influence of the adrenal gland.

The finding of high plasma E_2 levels at about 15 days post partum, concomitant with the serum FSH peak as measured earlier in the same strain of rats (Meijs-Roelofs et al., 1973), raises the question whether at this early age oestrogens already exert an inhibitory feedback on gonadotrophin release. In the present studies a rise in serum FSH level after OVX was shown to occur in the period from day 13 to day 15. Moreover, this rise in serum FSH could be prevented by injection of physiological doses of oestrogens. Goldman & Gorski (1971) demonstrated that in intact female rats 5- to 10- days old, serum FSH concentration could be decreased by injection of OB or testosterone propionate. These findings are in agreement with the present data although the experiments cannot be fully compared because of differences in age and dosages.

However, in an earlier experiment (Goldman, Grazia, Kamberi & Porter,

1971) this group did not find a rise in serum FSH concentration after OVX, probably because of too brief an experimental period; this was discussed by Dunlap, Preis & Gerall (1972).

On the basis of the observation of increased FSH levels after OVX, which can be reversed by administration of physiological doses of OB, the existence of an inhibitory feedback on FSH by ovarian steroids in the female rat before 20-days of age seems to be beyond doubt. This is in contrast to the conclusions drawn from most of the results of compensatory ovarian hypertrophy experiments (Baker & Kragt, 1969; Ojeda & Ramirez, 1972). However, before day 20 this type of experiment seems to be inadequate for detection of changes in gonadotrophin levels since in this early period of life no large follicles can be induced and therefore only minor changes in ovarian weight might be expected.

The present results also demonstrate that the decrease in serum FSH normally occurring on day 21, as shown in Fig. 1, takes place only in the presence of the ovaries. In an earlier report (Meijs-Roelofs et al., 1973) it was suggested that this fall in FSH levels could be attributed to increased oestrogen levels, caused by the sharp increase in the number of antrum follicles at that time. In the light of the present findings, the simultaneous decrease of E_2 and FSH levels around day 21, this view seems highly unlikely. Possible explanations are: first, an increasing sensitivity of the hypothalamo-hypophysial system for E_2. However, in the present experiments it was found that the doses of oestradiol benzoate used resulted in lower serum FSH concentrations after injection from day 13 - day 15 than after injection from 25 - 27 days of age. Though this may suggest a decrease of hypothalamo-hypophysial sensitivity for oestrogen with age it should be realized that results may not be compared since an essential difference exists between the two experimental groups: In the younger rats oestrogen injections were started on the day of OVX, whereas in the older ones oestrogen injections started only on the 10th postoperative day. The second possibility to explain the simultaneous decrease of E_2 and FSH levels around day 21 is an increase in biological activity of the E_2 present. This last view is supported by the findings of Nunez, Engelmann, Benassayag, Savu, Crepy & Jayle (1971) and Raynaud, Mercier-Bodard & Baulieu (1971), who demonstrated a decrease in the amounts of E_2-binding protein in the blood of immature rats. Nunez et al., (1971) showed this decrease to be most

marked in the period from day 15 to day 21.
Similarly, the high amounts of E_2-binding protein present during the period from birth to day 15 may account for the simultaneous rise in both FSH and E_2 concentration from day 5 to day 15 of life.
Speculating on the biological significance of the high amounts of oestradiol present concomitantly with the high FSH concentrations, the interaction between oestrogen and FSH in the ovary may be of importance. Goldenberg, Vaitukaitis & Ross (1972) demonstrated that oestrogen increases both granulosa proliferation and incorporation of FSH in the follicles, in this way stimulating follicular growth. This hypothesis is supported by the findings of Reiter, Goldenberg, Vaitukaitis & Ross (1972) namely a significant decrease in follicular growth in the neonatal rat after treatment with specific anti-oestradiol serum.

The authors gratefully acknowledge the gift of antibodies raised against the oestradiol-17β-6-(0-carboxymethyl)oxime-BSA complex from Dr D. Exley (London), and the gift of the FSH kit from the National Institute of Arthritis and Metabolic Diseases. They are indebted to Drs J. Moll and H.J. van der Molen for their valuable criticism during the preparation of the manuscript. The technical assistance of Mrs L.A Gribling-Hegge, Mrs M. Loonen-Rutte, Miss A.H. Hey and Mr P. Kramer is highly appreciated.

REFERENCES

BAKER, F.D. & KRAGT, C.L. (1969). Maturation of the hypothalamic-pituitary-gonadal negative feedback system. Endocrinology 85: 522-527.

DUNLAP, J.L., PREIS, L.K. & GERALL, A.A. (1972). Compensatory ovarian hypertrophy as a function of age and neonatal androgenization. Endocrinology 90: 1309-1314.

EXLEY, D., JOHNSON, M.W. & DEAN, P.D.G. (1971). Antisera highly specific for 17β-oestradiol. Steroids 18: 605-620.

GERALL, A.A. & DUNLAP, J.L. (1971). Evidence that the ovaries of the neonatal rat secrete active substances. J. Endocr., 50: 529-530.

GOLDENBERG, R.L., VAITUKAITIS, J.L. & ROSS, G.T. (1972). Estrogen and follicle stimulating hormone interactions on follicle growth in rats. Endocrinology 90: 1492-1498.

GOLDMAN, B.D. & GORSKI, R.A. (1971). Effects of gonadal steroids on the secretion of LH and FSH in neonatal rats. Endocrinology 89: 112-115.

GOLDMAN, B.D., GRAZIA, Y.R., KAMBERI, I.A. & PORTER, J.C. (1971). Serum gonadotrophin concentrations in intact and castrated neonatal rats. Endocrinology 88: 771-776.

de JONG, F.H., HEY, A.H. & VAN DER MOLEN, H.J. (1973). Effect of gonadotrophins on the secretion of oestradiol-17β and testosterone by the rat testis. J. Endocr., 57: 277-284.

MEIJS-ROELOFS, H.M.A., UILENBROEK, J.Th.J., OSMAN, P. & WELSCHEN, R. (1973). Serum levels of gonadotrophins and follicular growth in prepuberal rats. In: The development and maturation of the ovary and its functions. Ed. H. Peters. Excerpta Medica ICS, 267: pp. 3-11 Amsterdam.

NAFTOLIN, F., BROWN-GRANT, K. & CORKER, C.S. (1972). Plasma and pituitary luteinizing hormone and peripheral plasma oestradiol concentrations in the normal oestrous cycle of the rat and after experimental manipulation of the cycle. J. Endocr., 53: 17-30.

NUNEZ, E., ENGELMANN, F., BENASSAYAG, C., SAVU, L., CREPY, O & JAYLE, M.F. (1971). Mise en évidence d'une fraction protéique liant les oestrogènes dans le sérum de rats impubères. C.r. hebd. Séanc. Acad. Sci., Paris 272: 2396-2399.

OJEDA, S.R. & RAMIREZ, V.D. (1972). Plasma level of LH and FSH in maturing rats: Response to hemigonadectomy. Endocrinology 90: 466-472.

PRESL, J., HERZMANN, J. & HORSKÝ, J. (1969). Oestrogen concentrations in blood of developing rats. J. Endocr., 45: 611-612.

PRESL, J., HORSKÝ, J., HERZMANN, J., MIKULÁŠ, I. & HENZL, M. (1967). Fluorimetric estimation of oestrogen in the blood of infant female rats. J. Endocr., 38: 201-202.

PRICE, D. (1947). The influence of maternal hormones on the reproductive organs of suckling rats. Anat. Rec. 97: 519-549.

RAYNAUD, J.P., MERCIER-BODARD, C. & BAULIEU, E.E. (1971). Rat estradiol binding plasma protein. (EBP). Steroids 18: 767-784.

REITER, E.O., GOLDENBERG, R.L., VAITUKAITIS, J.L. & ROSS, G.T. (1972). A role for endogenous estrogen in normal ovarian development in the neonatal rat. Endocrinology 91: 1537-1539.

SEKI, K., SEKI, M., YOSHIHARA, T. & MAEDA, H. (1971). Radioimmunoassays for rat follicle stimulating and luteinizing hormones. Endocr. jap. 18: 477-485.

WEISZ, J. & FERIN, M. (1970). Pituitary gonadotrophins and circulating LH in immature rats - a comparison between normal females and males and females treated with testosterone in neonatal life. In: Gonadotrophins and ovarian development. pp 339-350. Eds. W.R. Butt, A.C. Crooke & M. Ryle. Edinburgh: E. and S. Livingstone.

WEISZ, J. & GUNSALUS, P. (1973). Estrogen levels in immature female rats: true or spurious - ovarian or adrenal? In: The development and maturation of the ovary and its functions. Ed. H. Peters. Excerpta Medica ICS, 267: pp. 158-159.

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Differential Effects of Anterior and Middle Hypothalamic Lesions on Vaginal Opening and Cyclicity

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Abstract

Lesions of different sizes were placed in the anterior hypothalamus or in the middle hypothalamus (level of arcuate nuclei) of 22-day-old female rats. Animals with anterior lesions showed a distinct parallelism between lesion size, advancement of vaginal opening (VO), and tendency towards prolonged and persistent oestrus. The largest lesions advanced VO by 13 days and caused persistent oestrus in nearly all rats. Lesions in the middle hypothalamus had no marked influence on cyclicity, but a dependence of VO on the size and localization of the lesion was clearly present. Small lesions destroying parts of the arcuate nuclei and large lesions destroying the arcuate nuclei nearly completely and extending into the area of the paraventricular nuclei caused an advancement of VO by 8 days or more. However, lesions of intermediate size, similar to those of the previous category but not reaching the paraventricular nuclei, advanced VO by 3–4 days only. The bearing of these findings on the control of sexual maturation is discussed.

Key words

Hypothalamic lesions
Puberty
Vaginal opening
Ovulation
Sexual maturation

Experimentally induced lesions in the hypothalamus may advance puberty. This was first demonstrated by DONOVAN and VAN DER WERFF TEN BOSCH [1956] and was confirmed and investigated in greater detail in a number of later studies [see review by CRITCHLOW and BAR-SELA, 1967]. In the rat, early puberty has been observed following lesions in the anterior hypothalamus as well as in the middle hypothalamus at the level of the arcuate nuclei. Furthermore, an association of early puberty and prolonged or persistent oestrus has been encountered with varying frequency, especially following lesions in the anterior hypothalamus.

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The present report deals with two aspects of early puberty in rats with hypothalamic lesions: (1) the dependence of the effect of the lesions on their size and localization; (2) the association of advanced puberty and prolonged or constant oestrus.

Materials and Methods

The experiments were carried out with female rats of the R-Amsterdam strain, a Wistar substrain, which regularly shows 5-day cycles. Litter size was adjusted to 8–10 animals. The animals were weaned and subjected to surgery on the 22nd day of life, when the body weight was 32–40 g. Litters were always equally divided into experimental and control groups; operated and control litter mates were caged together, without males. Lesions were made stereotaxically and aimed at either the anterior hypothalamus at the level of the suprachiasmatic nuclei or the middle hypothalamus at the level of the arcuate nuclei. A high frequency current and 2 electrodes (⌀ 0.2 mm), 1.0 mm apart, were employed for this purpose. The current was varied in order to obtain lesions of different sizes. Intact animals served as controls. The use of intact control animals was based on the results of a separate experiment in which age and body weight at vaginal opening (VO) and at first oestrus were compared in 30 sham-operated and 32 intacts rats. The mean age values of the 2 groups differed by less than 1 day and the mean weight values by less than 4 g.

The animals were weighed and examined for VO daily. Advancement of VO in groups of operated animals was accepted when both age and body weight at VO were significantly different from those in control animals. Smears were taken daily from animals with open vaginae, and were classified as oestrous when nearly all elements were cornified epithelial cells and when nucleated and cornified epithelial cells were present in equal numbers with complete or nearly complete absence of leucocytes. The rats were killed 20–30 days after VO. Brains and ovaries were prepared for histological examination to verify the localization of the lesions and to obtain information on the presence or absence of corpora lutea. The brains were sectioned coronally.

While the experiments were in progress, it was necessary to move the colony to new animals quarters. This influenced the age and weight at VO and also the percentage of oestrous smears after VO. The results obtained under the two different housing conditions have been calculated separately and have been marked A and B in table I.

On the basis of size and localization of the lesions, animals with anterior as well as those with middle hypothalamic lesions were subdivided in three classes: animals with small, moderate, and large lesions. This classification was based on the maximal cross-sectional area of the lesions determined in drawings of the histological material, made at a magnification of ×123. The mean values of the maximal cross-sectional areas of the lesions were 0.1, 0.5, and 1.3 mm^2 in the anterior hypothalamus, and 0.2, 0.5, and 1.2 mm^2 in the middle hypothalamus. Data on animals with lesions that were outside the anatomically defined range of these 6 groups were discarded. The description of the localization of the lesions is based on the atlas of DE GROOT [1959].

Observations

Lesions in the anterior hypothalamus (table I). Lesions falling in the category of small lesions always destroyed parts of the suprachiasmatic

nuclei; in most cases, they touched the dorsal surface of the optic chiasm and had a symmetrical or slightly asymmetrical localization as depicted in figure 1a. In a few cases, the position was completely unilateral (1 rat) or clearly asymmetrical (3 rats). No functional effects of these lesions on VO were found, though cycling was somewhat disturbed. Animals with symmetrically placed lesions showed a tendency towards prolonged oestrus, but corpora lutea were present in all instances. Lesions of moderate size comprised a rather small group of 5 animals. They were, with 1 exception, symmetrically localized and caused a more extensive destruction of the area of the suprachiasmatic nuclei than did lesions of the previous category (fig. 1b). These lesions advanced VO by 4 days and caused clearly prolonged oestrous phases in 3 out of 5 animals. In the ovaries of all such animals, cystic follicles were found; in 2 out of the 3 rats with prolonged oestrous phases, corpora lutea were completely absent.

In the group with large lesions, the greater part of the anterior hypothalamic area, regularly including the suprachiasmatic nuclei, was destroyed. Figure 1c illustrates a case with an unusually large lesion that did not completely destroy the suprachiasmatic nuclei. The position of some lesions was asymmetrical, but unilateral lesions were not encountered. In this group, VO was advanced by 13 days and, out of the total number of 15 animals, 13 of this group showed persistent oestrus with complete absence of corpora lutea.

Lesions in the middle hypothalamus (table I). The small-sized lesions were always restricted to the arcuate nuclei; of the total area of these nuclei, at least 50% remained intact. In 3 cases, the lesions were clearly asymmetrical or unilateral. The small-sized lesions caused a definite advancement of VO, which averaged 13 days in the A experiments and 8 days in the B experiments. Cycles were less regular in lesioned than in control animals, showing prolonged periods of di-oestrus as well as of oestrus. Figure 1d gives an example of a slightly asymmetrical lesion of this group.

Lesions of moderate size destroyed the area of the arcuate nuclei nearly completely. They were predominantly symmetrically localized and extended farther into the hypothalamus than those in the previous category, causing partial destruction of the ventromedial nuclei (fig. 1e). These lesions advanced VO by 3–4 days and influenced cycling as in the previous group.

The largest middle hypothalamic lesions were similar to the moderate lesions but extended into the area of the paraventricular

nuclei (fig. 1f). VO in these animals was significantly earlier than in the group of moderate lesions, but did not differ significantly from VO following small lesions. Both oestrous and dioestrous periods were slightly prolonged. In 1 animal, not included in the material of table I, an exceptional lesion was seen, the area of the arcuate nuclei, including

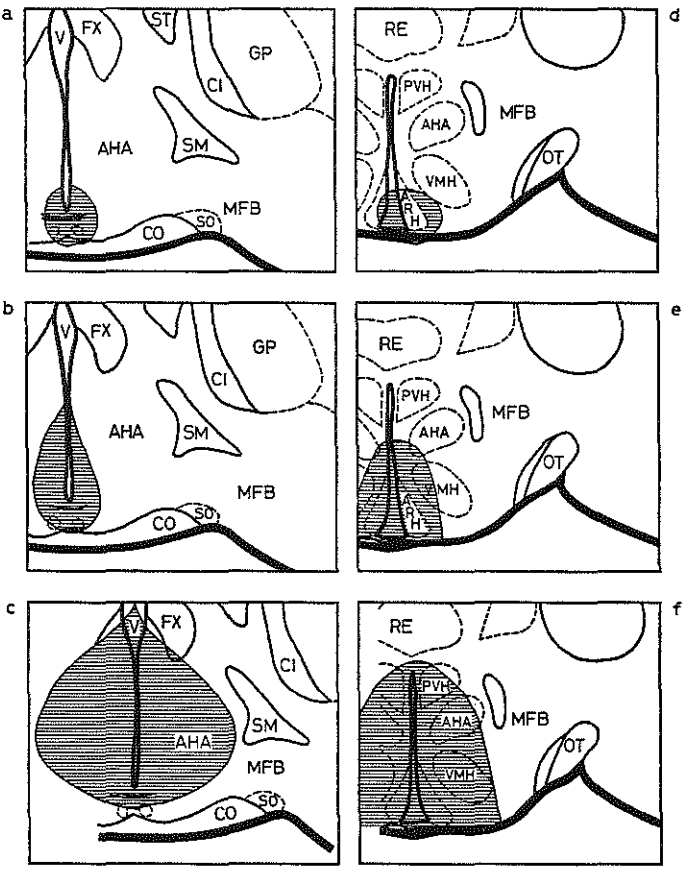

Fig. 1. Illustrations of individual cases of lesioned animals. Anterior hypothalamic lesions: *a* small; *b* moderate; *c* large. Middle hypothalamic lesions: *d* small; *e* moderate; *f* large. AHA = anterior hypothalamic area; ARH = arcuate nucleus; CI = internal capsule; CO = optic chiasm; FX = fornix; GP = globus pallidus; MFB = medial forebrain bundle; OT = optic tract; PVH = paraventricular nucleus; RE = nucleus reuniens thalami; SM = stria medullaris thalami; SO = supraoptic nucleus; ST = stria terminalis; V = ventricle; VMH = ventromedial nucleus.

Table I. Effect of anterior and middle hypothalamic lesions on VO and cyclicity

	Housing[a]	Small lesions	Moderate lesions	Large lesions	Intact controls
Anterior hypothalamus					
Age at VO (days)	A	** 40.0±0.6[b] (5)[c]	* 37.6±0.5 (5)	* 28.8±0.5 (15)	42.2±0.5 (26)
	B	** 38.5±0.5 (14)			38.5±0.4 (26)
Body weight at VO (g)	A	* 79.4±2.9 (5)	* 73.4±4.2 (5)	* 45.8±1.9 (15)	99.1±1.3 (26)
	B	** 87.6±1.6 (14)			88.5±1.4 (26)
Oestrous smears[d]	A	** 54 (5)	** 63 (5)	* 83 (15)	48 (26)
	B	* 48 (14)			35 (26)
Middle hypothalamus					
Age at VO (days)	A	* 29.0±1.4 (5)	* 38.0±0.6 (4)	* 33.7±1.2 (3)	42.2±0.5 (22)
	B	* 29.8±1.1 (4)	* 34.9±0.5 (8)	* 29.7±0.5 (7)	38.0±0.3 (36)
Body weight at VO (g)	A	* 53.9±5.5 (5)	* 72.8±1.5 (4)	* 58.8±5.1 (3)	96.5±1.5 (22)
	B	* 54.3±2.8 (4)	* 67.4±3.0 (8)	* 56.3±3.2 (7)	87.1±1.0 (36)
Oestrous smears	A	** 52 (5)	** 58 (4)	** 57 (3)	48 (22)
	B	** 42 (4)	** 38 (8)	** 39 (7)	40 (36)

[a] A and B indicate the 2 housing conditions (see text).
[b] Standard error of mean.
[c] Number of animals.
[d] Days with oestrous smears/total number of smears ×100 (1 smear each day over 20–30 days).
*Significantly different from controls ($p \leqslant 0.05$); ** not significantly different from controls ($p > 0.05$).

the transitional area between median eminence and pituitary stalk, being completely destroyed. In this animal, retarded VO and atrophic ovaries were found.

Housing conditions apparently influenced cyclicity, in that the controls of the A series showed a greater percentage of oestrous smears than did those of the B series. This finding was due to the occurrence of 4-day cycles or to prolonged oestrous periods (3 days per 5-day cycle).

Discussion

The present data confirm previous observations demonstrating early VO in the rat, following lesions in the anterior as well as in the middle hypothalamus [Critchlow and Bar-Sela, 1967]. They also confirm that, especially after anterior hypothalamic lesions, early VO may be accompanied by persistent oestrus. However, the use of lesions of varying size extends our knowledge of the influence of hypothalamic lesions on sexual maturation in two respects.

I. Prolonged and constant oestrus, which may accompany early VO in animals with anterior hypothalamic lesions, has usually been regarded as an incidental side-effect of the lesions. The parallelism between lesion size, advancement of VO, and a tendency towards prolonged and persistent oestrus, which is indicated by our findings, suggests that induction of prolonged oestrous periods and of persistent oestrus may be an intrinsic effect of anterior hypothalamic lesions causing early VO. This is of importance, since acceptance of a strict definition of puberty, e.g., 'the stage of development at which the ability to reproduce is achieved' [CRITCHLOW and BAR-SELA, 1967], would then raise doubt regarding the question whether rats with such lesions do, in fact, show accelerated true puberty. It cannot be excluded that the lesions cause the persistent-oestrus syndrome, with early VO as an incidental side-effect. The identical localization of lesions causing persistent oestrus in the adult female [MCCANN and RAMIREZ, 1964] supports this contention.

For the interpretation defended above, it is of little importance whether only the differences in size of our lesions are emphasized or the differences in localization also, which cannot be separated from the differences in size. Possibly, the size of the lesions was of primary importance in the case of these lesions, since strict anatomical localization of effective lesion sites seems to be absent in the anterior hypothalamus [CRITCHLOW and BAR-SELA, 1967]. It may be added that, while the occurrence of prolonged and constant oestrus after early VO in animals with anterior hypothalamic lesions has not received much attention, it can be clearly recognized in a number of studies [DONOVAN and VAN DER WERFF TEN BOSCH, 1959; BOGDANOVE and SCHOEN, 1959; SCHIAVI, 1964].

II. Lesions in the middle hypothalamus possibly influence sexual maturation via mechanisms that differ from those involved in the case of anterior lesions. This is indicated by the finding that middle hypothalamic lesions do not cause any tendency towards the development of the persistent-oestrus syndrome. The absence of distinct influences on cyclicity following middle hypothalamic lesions, in contrast with the presence of such influences in animals bearing anterior lesions, has been mentioned by BOGDANOVE and SCHOEN [1959] and by CRITCHLOW and BAR-SELA [1967]; but the data of GELLERT and GANONG [1960] and of SCHIAVI [1964] also show this differential effect. Moreover, we found a different relationship between the type of lesion and its influence on VO for anterior and middle hypothalamic lesions. In the anterior hypo-

thalamus, advancement of VO seemed to increase with lesion size. In contrast, in the middle hypothalamus, even the smallest lesions induced an advancement of VO that was not surpassed by any of the other categories. Moreover, lesions of the moderate-size class were less effective than were both the less extensive and more extensive lesions. The area of the arcuate nuclei was damaged in all three groups. Assuming exclusively stimulatory or inhibitory effects of the lesions on sexual maturation, this erratic relationship between the size and the functional effect of the lesions cannot be explained. The hypothesis that the region dorsal to the arcuate nuclei contains systems with stimulatory influences and other systems with inhibitory influences on sexual maturation provides an acceptable explanation for these data.

References

Bogdanove, E.M. and Schoen, H.C.: Precocious sexual development in female rats with hypothalamic lesions. Proc. Soc. exp. Biol. Med. *100:* 664–669 (1959).

Critchlow, V. and Bar-Sela, M.E.: Control of the onset of puberty; in Martini and Ganong Neuroendocrinology, vol. 2, pp. 101–162 (Academic Press, New York 1967).

Donovan, B.T. and Werff ten Bosch, J.J. van der: Precocious puberty in rats with hypothalamic lesions. Nature, Lond. *178:* 745 (1956).

Donovan, B.T. and Werff ten Bosch, J.J. van der: The hypothalamus and sexual maturation in the rat. J. Physiol. *147:* 78–92 (1959).

Gellert, R.J. and Ganong, W.F.: Precocious puberty in rats with hypothalamic lesions. Acta endocrin., Kbh. *33:* 569–576 (1960).

Groot, J. de: The rat forebrain in stereotaxic coordinates (North Holland Publishers Co., Amsterdam 1959).

McCann, S.M. and Ramirez, V.D.: The neuroendocrine regulation of hypophyseal luteinizing hormone. Recent Progr. Hormone Res. *20:* 131–181 (1964).

Schiavi, R.: Effect of anterior and posterior hypothalamic lesions on precocious sexual maturation. Amer. J. Physiol. *206:* 805–810 (1964).

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EFFECT OF ELECTRICAL STIMULATION OF THE HYPOTHALAMUS ON GONADOTROPHIN RELEASE AND THE ONSET OF PUBERTY

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SUMMARY

Electrical stimulation of the hypothalamus with biphasic pulses was performed in immature female rats. When performed at 27 days of age or later, electrical stimulation in the arcuate nucleus region advanced puberty in all animals, as did stimulation of the anterior hypothalamus at 29 days of age or later. Stimulation in younger rats did not uniformly advance puberty. The responsiveness to electrical stimulation thus seems to develop a few days earlier in the arcuate nucleus region than in the anterior hypothalamus.

In a second experiment the possible involvement of follicle-stimulating hormone (FSH) in the advancement of puberty was investigated: the simplified augmented ovarian weight assay for endogenous FSH was performed in rats stimulated in the arcuate nucleus region as well as in controls. A marked increase in ovarian weight, indicating increased FSH levels, was demonstrated in all animals stimulated on day 27 or later; at earlier ages only a percentage of the stimulated animals responded. This percentage paralleled the percentage of animals that showed advancement of puberty.

It is concluded that electrical stimulation in both the arcuate nucleus region and the anterior hypothalamus advances the onset of puberty. It is suggested that electrical stimulation causes increased plasma FSH levels and, in consequence, precocious puberty.

INTRODUCTION

In the female rat puberty generally occurs at about day 40 (Critchlow & Bar-Sela, 1967). Well before the onset of puberty, at 3–4 weeks of age, both pituitary follicle-stimulating hormone (FSH) and hypothalamic FSH-releasing factor content have been shown to be high, even higher than in adults (Corbin & Daniels, 1967), so that puberty might be advanced by precocious activation of the hypothalamic centres involved in FSH release. Since in adult rats gonadotrophin release can be induced by electrical stimulation of the hypothalamus (Critchlow, 1958; Everett, 1965) with minimal damage to the brain (Rowland, 1966), this procedure was applied to infant animals. The augmented ovarian weight assay for endogenous FSH (Naqvi & Johnson, 1969; Johnson & Naqvi, 1970) was performed to investigate whether the effects obtained were indeed mediated through increased FSH secretion.

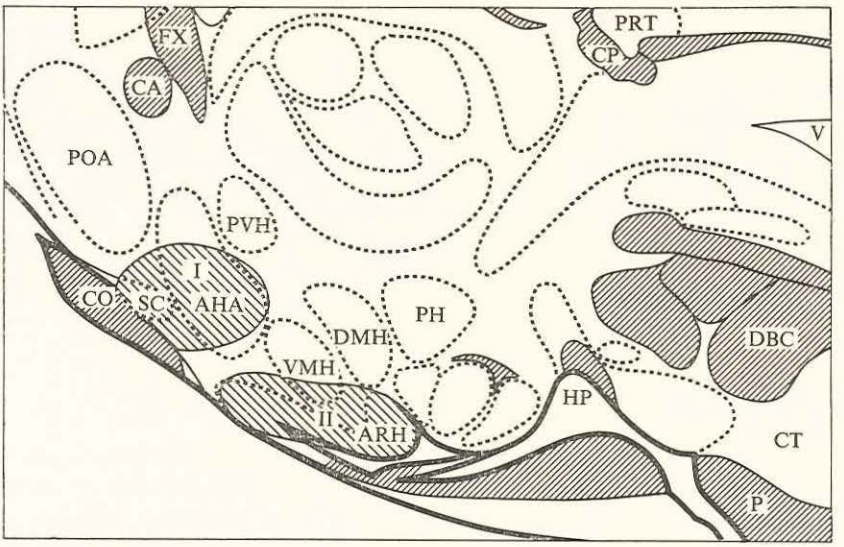

Fig. 1. Sagittal section of the brain of the rat showing the areas within which stimulation was performed. I = anterior area; II = arcuate nucleus region; AHA = anterior hypothalamic area; ARH = arcuate nucleus; CA = commissura anterior; CO = optic chiasma; CP = commissura posterior; CT = nucleus centralis tegmenti; DBC = decussatio brachiorum conjunctivorum; DMH = dorsomedial nucleus; FX = fornix; HP = habenulo-interpeduncular tract; P = pons; PH = posterior nucleus; POA = preoptic area; PRT = area pretectalis; PVH = paraventricular nucleus; SC = suprachiasmatic nucleus; V = ventricle; VMH = ventromedial nucleus.

MATERIALS AND METHODS

Immature female rats of the R-Amsterdam strain, a Wistar substrain with a 5-day cycle, were used. At the age of 22 days the rats were weaned and selected for a body weight of 32–40 g. The animals were kept in a controlled temperature of 22–25 °C and under controlled light conditions: 14 h light, 10 h darkness, with the middle of the dark period at 12.00 h. The rats received standard dry pellets and tap water *ad libitum*. In each experiment litters were divided equally into experimental and control groups; 4–7 litter-mates were kept per cage. All animals were weighed daily and checked for vaginal opening (VO).

From the day of VO, vaginal smears were taken daily till the animals were killed. Some animals were killed after first oestrus (after the first fully cornified smear); others were allowed to live for some time in order to compare the cycles of operated and untreated animals.

Under the conditions mentioned the untreated rats (114) showed regular growth. Vaginal opening occurred at $39{\cdot}9 \pm 0{\cdot}2$ days of age and at a body weight of $93{\cdot}9 \pm 0{\cdot}6$ g.

Experiment I. *Effect of electrical stimulation of the hypothalamus on the onset of puberty*

Electrical stimulation was performed stereotaxically in the arcuate nucleus region or in the anterior hypothalamus (Fig. 1). Two bipolar stainless steel electrodes (Rhodes Medical Instruments, California, model SNE-100) were used; the diameter of the central wire was 75 μm, and of the shaft 250 μm. The centre lead protruded

Table 1. *Effect of sham operation in the arcuate nucleus region of female rats at 25–30 days of age* (*means* ± S.E.M.)

Type of rat	No. of animals	Age at operation (days)	Body weight at operation (g)	Vaginal opening at	
				Age (days)	Body weight (g)
Sham-operated	7	25–26	43·0 ± 1·0	39·6 ± 1·1	93·3 ± 2·2
Intact	9		41·1 ± 1·1	39·8 ± 0·9	91·6 ± 2·5
Sham-operated	4	27–28	51·0 ± 1·5	37·0 ± 0·7	82·2 ± 1·7
Intact	9		57·1 ± 1·6	38·3 ± 0·4	95·8 ± 1·7
Sham-operated	5	29–30	52·6 ± 1·6	41·8 ± 1·0	90·1 ± 2·2
Intact	11		53·9 ± 2·5	40·2 ± 0·9	90·7 ± 2·6

750 μm and was exposed for 250 μm; the shaft was exposed for 250 μm. Each electrode was inserted 0·5 mm from the mid-sagittal plane.

Electrical stimulation consisted of trains of pulses applied for a period of 60 min at different times of day between 09.00 h and 17.00 h. The pulses had a rectangular biphasic form with equal positive and negative phases of 1 ms, and were applied at a frequency of 50 biphasic pulses/s. The electrodes were connected in parallel and the stimuli were applied through two Grass stimulus isolation units, linked in opposite polarity to obtain the biphasic form of the pulses. Each train of pulses, delivered by a Grass S-8 stimulator, lasted 10 s and was followed by a 10 s pause. The peak to peak current of the biphasic pulses, 300–350 μA, was monitored on an oscilloscope, and a large ballast resistance, approximately 200 times greater than the electrode resistance, was placed in series with the central wires to minimize overall variations in applied current. Sham-operated animals had electrodes inserted but did not receive electrical stimulation. Since no significant difference in age at VO was found between 16 sham-operated rats and their 29 untreated litter-mates (Table 1), untreated rats served as controls in most experiments.

When the animals were killed all ovaries and the brains of the operated animals were prepared for histological study of the sites of stimulation and for determination of the presence of corpora lutea.

When the stimulation sites were found not to be in the anterior hypothalamus, nor in the arcuate nucleus region, the data on the paired operated and control animals were discarded.

Experiment II. *Effect of electrical stimulation of the hypothalamus on FSH release*

Electrical stimulation was performed as described above. Since it was not expected that the mechanism by which advancement of puberty was obtained would be different for the anterior hypothalamus and the arcuate nucleus region, the assay for endogenous FSH was only performed in animals stimulated in the arcuate nucleus region. Endogenous FSH was measured by the increase of ovarian weight after human chorionic gonadotrophin (HCG) injection (procedure based on a combination of methods: Naqvi & Johnson (1969); Johnson & Naqvi (1970)). Stimulated, sham-operated and untreated animals received two HCG injections (Pregnyl, Organon) of 25 i.u. each, dissolved in 0·5 ml 0·9 % NaCl soln, with the first injection 24 h after stimulation and the second 7·5 h later. Controls were treated simultaneously.

Table 2. *Effect of electrical stimulation of the arcuate nucleus region in female rats at 22–33 days of age (means ± S.E.M.)*

Type of rat	Stimulation effect*	No. of animals	Age at stimulation (days)	Body weight at stimulation (g)	Vaginal opening at: Age (days)	Vaginal opening at: Body weight (g)
Stimulated	+	1	22	40	36	78
	−	13		34·2 ± 0·6	39·5 ± 0·5	90·2 ± 1·5
Intact		14		35·1 ± 1·1	40·8 ± 0·4	93·0 ± 1·6
Stimulated	+	4	23–24	33·8 ± 1·0	33·5 ± 1·8	66·8 ± 7·1
	−	5		38·2 ± 1·8	39·8 ± 1·2	88·0 ± 4·3
Intact		9		36·0 ± 1·1	39·9 ± 0·5	94·6 ± 1·4
Stimulated	+	6	25–26	43·8 ± 0·8	31·7 ± 0·6	61·3 ± 3·0
	−	3		42·3 ± 3·2	38·7 ± 1·9	82·0 ± 5·0
Intact		9		41·4 ± 1·1	39·8 ± 0·9	91·6 ± 2·5
Stimulated	+	9	27–28	57·1 ± 1·3	32·1 ± 0·1	68·0 ± 1·6
	−	0		—	—	—
Intact		9		57·1 ± 0·6	38·3 ± 0·4	95·8 ± 1·7
Stimulated	+	11	29–30	56·9 ± 1·7	34·2 ± 0·3	71·5 ± 1·1
	−	0		—	—	—
Intact		11		53·9 ± 2·5	40·2 ± 0·9	90·7 ± 2·6
Stimulated	+	12	31–32	70·4 ± 1·2	35·3 ± 1·9	75·8 ± 1·3
	−	0		—	—	—
Intact		12		66·3 ± 1·5	39·2 ± 0·5	93·1 ± 1·2
Stimulated	+	3	33	70·0 ± 5·0	36·3 ± 0·3	76·3 ± 3·2
	−	0		—	—	—
Intact		3		77·7 ± 3·2	38·0 ± 0·0	95·0 ± 2·1

* + = puberty advanced; − = no effect.

The animals were killed with ether 54 h after the first injection. The ovaries were dissected out by removing the bursae with adhering fat under a dissection microscope, they were then blotted on paper towelling and weighed on a torsion balance. Brains of operated animals were prepared for histological study. In some experiments sham operation was performed but in most cases unoperated rats served as controls (see Table 4).

RESULTS

Experiment I. Effects of electrical stimulation of the hypothalamus on the onset of puberty.

The effect of electrical stimulation in the arcuate nucleus region on the onset of puberty is shown in Table 2.

The criteria used for advancement of puberty were based in part on comparison with untreated litter-mate controls since litter-mates tend to show VO at approximately the same age (Bar-Sela & Critchlow, 1966). In addition the stimulated animals were compared with the 114 pooled controls. The combined criteria were: (1) both age and body weight of a stimulated rat at VO should be lower than those of each of the untreated litter-mates; (2*a*) age at VO should be at least 3 days less than the mean age of the 114 pooled controls; (2*b*) body weight at VO should be at least 15 g lower than the mean body weight of the 114 pooled controls. Advancement of puberty was accepted if both criterion 1 and either 2*a*, 2*b* or both, were fulfilled.

Table 3. *Effect of electrical stimulation of the anterior hypothalamus of female rats at 22–33 days of age (means* ± S.E.M.)

Type of rat	Stimulation effect*	No. of animals	Age at stimulation (days)	Body weight at stimulation (g)	Vaginal opening at: Age (days)	Vaginal opening at: Body weight (g)
Stimulated	+	0	22–24	—	—	—
	−	8		36·8 ± 1·7	41·6 ± 0·5	81·4 ± 3·0
Intact		8		35·7 ± 1·4	40·4 ± 1·4	96·9 ± 2·2
Stimulated	+	1	25–26	46	31	62
	−	9		40·9 ± 1·6	39·7 ± 0·7	91·9 ± 3·2
Intact		10		42·3 ± 1·2	39·6 ± 0·6	91·3 ± 2·3
Stimulated	+	11	27–28	53·1 ± 1·9	32·8 ± 0·2	67·5 ± 2·6
	−	4		47·8 ± 3·3	40·8 ± 1·0	95·5 ± 3·2
Intact		15		51·5 ± 1·3	40·8 ± 0·7	97·5 ± 1·9
Stimulated	+	6	29–30	61·8 ± 3·1	33·5 ± 0·2	70·5 ± 3·0
	−	0		—	—	—
Intact		6		66·7 ± 5·0	37·8 ± 1·1	90·2 ± 3·9
Stimulated	+	6	31–32	69·2 ± 3·8	36·0 ± 0·4	77·2 ± 3·3
	−	0		—	—	—
Intact		6		68·7 ± 2·1	43·2 ± 1·5	105·2 ± 2·0
Stimulated	+	2	33	72·0 ± 2·0	36·5 ± 0·5	79·0 ± 1·0
	−	0		—	—	—
Intact		2		74·0 ± 0·0	39·0 ± 0·0	96·5 ± 1·5

* + = puberty advanced; − = no effect.

Stimulation of the arcuate nucleus region on day 22 resulted in advancement of puberty in 1 out of 14 rats; if stimulation was performed on days 23–24, puberty was advanced in 4 out of 9 rats; on days 25–26, 6 out of 9 rats responded and from day 27 until day 33 all stimulated animals responded. The most pronounced advancement was found after stimulation on days 25–26: 8·1 days with a weight difference of 30·3 g. The interval between the day of stimulation and VO decreased rapidly with increasing age. It was about 10 days after stimulation on days 23–24, but only about 4 days after stimulation on day 27 and later.

The results of stimulation of the anterior hypothalamus are shown in Table 3. On days 22–24 stimulation of the anterior hypothalamus was followed by advancement of puberty in 0 out of 8 rats, on days 25–26 in 1 out of 10 rats, and on days 27–28 in 11 out of 15 rats; from day 29 till day 33 all animals responded. Maximal advancement was found after stimulation on days 25–28. Comparison of Tables 2 and 3 indicates that the initial response as well as the age where all rats responded was later for the anterior hypothalamus than for the arcuate nucleus region. Also the age at which 50 % of the animals responded seemed to be later for the anterior hypothalamus.

In nearly all animals the day of ovulation coincided with that of VO: in 114 untreated control rats first vaginal oestrus was found 0·09 ± 0·03 days after VO, this interval was 0·03 ± 0·02 days in stimulated animals showing precocious puberty (n = 72) and 0·12 ± 0·06 days in ineffectively stimulated animals (n = 42). All rats killed after first ovulation showed normal numbers of ova/corpora lutea (9–12).

Both stimulated and untreated rats showed regular cycles. In 40 untreated rats vaginal smears were taken during 1–4 cycles after VO: the mean length of 78 cycles

Table 4. *Ovarian weight in stimulated rats after administration of human gonadotrophin* (*means* ± S.E.M.)

Type of rat*	No. of animals	Age at stimulation (days)	Body weight (g) at Stimulation	Body weight (g) at Killing	Ovarian weight (mg)
Stimulated +	4	23–24	38·5 ± 2·0	45·0 ± 2·6	37·2 ± 1·4
Stimulated −	5	23–24	39·0 ± 1·1	45·0 ± 1·5	29·2 ± 1·3
Sham-operated	9	23–24	38·0 ± 1·1	43·4 ± 1·5	26·0 ± 1·1
Intact	9	23–24	38·2 ± 1·4	47·0 ± 1·7	29·1 ± 0·7
Stimulated +	5	25–26	44·8 ± 2·2	50·2 ± 2·0	38·2 ± 1·0
Stimulated −	4	25–26	47·3 ± 2·3	50·5 ± 2·9	31·3 ± 2·2
Intact	9	25–26	45·2 ± 1·7	54·7 ± 1·7	30·3 ± 1·3
Stimulated +	11	27–28	54·1 ± 1·4	58·9 ± 2·0	43·3 ± 1·1
Stimulated −	0	27–28	—	—	—
Sham-operated	4	28	53·5 ± 1·3	59·3 ± 1·4	36·4 ± 3·3
Intact	11	27–28	53·0 ± 1·5	63·0 ± 1·2	32·6 ± 1·7
Stimulated +	5	29	62·2 ± 4·0	68·6 ± 4·2	43·1 ± 3·0
Stimulated −	0	29	—	—	—
Intact	5	29	62·0 ± 3·3	70·2 ± 4·1	33·9 ± 2·1

* + = ovarian wt increased; − = no effect.

was 5·9 ± 0·2 days. The comparable result for 52 cycles of 28 rats stimulated in the arcuate nucleus region was a mean cycle length of 5·9 ± 0·2 days. Twenty-four cycles of 15 rats stimulated in the anterior hypothalamus indicated a mean cycle length of 6·4 ± 0·3 days. These data include both effectively and ineffectively stimulated rats.

Histological preparations of the brains of stimulated animals showed minor damage in the arcuate nucleus region or in the anterior hypothalamus; no differences in size and localization of stimulation sites were found between responding and non-responding animals.

Experiment II. *Effect of electrical stimulation of the hypothalamus on FSH release*

The results of the endogenous FSH assay in animals stimulated in the arcuate nucleus region are shown in Table 4. Endogenous FSH levels in stimulated animals were considered to be increased (1) if the ovarian weight was higher than that of all simultaneously treated litter-mate controls, and (2) if the difference between the ovarian weight of an individual stimulated rat and the mean ovarian weight of all intact controls of that age-group was $\geqslant$ 4 mg ($\sim$ about 10%). Sham-operated animals showed ovarian weights not different from those of intact rats (Table 4).

Using the criteria mentioned it was found that after stimulation, increased ovarian weights occurred in 4 out of 9 rats stimulated on days 23–24 and in 5 out of 9 rats stimulated on days 25–26. On days 27–29 all stimulated animals showed a significant increase in ovarian weight. Comparison of these results with those of the first experiment indicates a parallelism between both types of data: at various ages the percentage of stimulated animals which showed advanced puberty paralleled that which showed increased FSH release.

DISCUSSION

It is well-known that hormonal treatment and hypothalamic lesions may result in advancement of puberty. The present experiments indicate that electrical stimulation of the hypothalamus may also cause precocious puberty.

Since histological sections of the brain of stimulated animals showed only minor damage, it seems reasonable to assume that the results obtained were due to a purely stimulatory effect. With increasing age the percentage of animals showing precocious puberty increased to 100%. This is of interest since precocious puberty induced by lesions has been shown to be independent of the age at which the lesions were made (Horowitz & van der Werff ten Bosch, 1962). This indicates an essential difference between the mechanisms by which lesions and stimulation, as used here, affect the neural control of puberty. To our knowledge purely electrical stimulation in otherwise untreated prepuberal female rats has only been performed by Bar-Sela & Critchlow (1966). Their studies, however, included only one animal with a stimulation site that we used, i.e. the anterior hypothalamus. In this animal stimulation over several days did not result in advancement of sexual maturation. Hagino, Watanabe & Goldzieher (1969) stimulated the brains of 30-day-old female rats in which ovulation induced by exogenous gonadotrophin was blocked by Nembutal. They found that the blocked ovulatory luteinizing hormone (LH) release could be restored by stimulation, especially of the arcuate nucleus region. Our results also indicate that stimulation at day 30 may be effective in inducing gonadotrophin release. The observation that responsiveness to stimulation develops later in the anterior hypothalamus than in the arcuate nucleus region cannot yet be explained.

It was to be expected that the effect we obtained, precocious puberty, would be mediated by increased gonadotrophin secretion. As far as FSH is concerned, our data support this assumption: the results of the assay for endogenous FSH did indeed indicate increased FSH levels after stimulation of the arcuate nucleus region. After stimulation at varying ages increased FSH levels were found in about the same percentage of animals as precocious puberty.

Comparison of our results with prepuberal oestrogen treatment is interesting: Ramirez & Sawyer (1965) showed that prepuberal oestrogen treatment with nearly physiological doses resulted in advancement of puberty of more than a week – also the maximal advancement obtained by us – provided treatment started at day 26; treatment started earlier was not more effective. They conclude that endogenous oestrogen secretion may constitute a final key limiting factor in the onset of puberty, because of its stimulatory function in the mechanism controlling LH secretion.

Corbin & Daniels (1969) demonstrated that a similar prepuberal oestrogen treatment had a clear influence on the FSH release just preceding puberty. Our results, and those after oestrogen treatment, may involve the same final mediator: electrical stimulation induces increased FSH release, this then causes ovarian stimulation and presumably oestrogen secretion; the latter may be mimicked by exogenous oestrogen. In both types of experiments the increased oestrogen level may then have its stimulatory effect on the LH releasing mechanism and in this way on the onset of puberty.

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REFERENCES

Bar-Sela, M. E. & Critchlow, V. (1966). Delayed puberty following electrical stimulation of the amygdalae in female rats. *Am. J. Physiol.* **211**, 1103–1107.

Corbin, A. & Daniels, E. L. (1967). Changes in concentration of female rat pituitary FSH and stalk median eminence follicle stimulating hormone releasing factor with age. *Neuroendocrinology* **2**, 304–314.

Corbin, A. & Daniels, E. L. (1969). Induction of puberty in immature female rats: effect of oestrogen on pituitary FSH and stalk-median-eminence FSH-releasing factor. *Neuroendocrinology* **4**, 65–74.

Critchlow, V. (1958). Ovulation induced by hypothalamic stimulation in the anesthetized rat. *Am. J. Physiol.* **195**, 171–174.

Critchlow, V. & Bar-Sela, M. E. (1967). Control of the onset of puberty. In *Neuroendocrinology* vol. 2, pp. 101–162. Eds L. Martini & W. F. Ganong. New York, London: Academic Press.

Everett, J. W. (1965). Ovulation in rats from preoptic stimulation through platinum electrodes. Importance of duration and spread of stimulus. *Endocrinology* **76**, 1195–1201.

Hagino, N., Watanabe, M. & Goldzieher, J. W. (1969). Inhibition by adrenocorticotrophin of gonadotrophin induced ovulation in immature female rats. *Endocrinology* **84**, 308–314.

Horowitz, S. & van der Werff ten Bosch, J. J. (1962). Hypothalamic sexual precocity in female rats operated shortly after birth. *Acta endocr., Copenh.* **41**, 301–313.

Johnson, D. C. & Naqvi, R. H. (1970). A simplified augmented ovarian weight assay for follicle stimulating hormone. *Proc. Soc. exp. Biol. Med.* **133**, 536–539.

Naqvi, R. H. & Johnson, D. C. (1969). Increased production of follicle-stimulating hormone in immature female rats by a single injection of androgen or oestrogen. *J. Endocr.* **45**, 29–36.

Ramirez, D. V. & Sawyer, C. H. (1965). Advancement of puberty in the female rat by estrogen. *Endocrinology* **76**, 1158–1168.

Rowland, V. (1966). Stereotaxic techniques and the production of lesions. In *Neuroendocrinology* vol. 1, pp. 107–128. Eds L. Martini & W. F. Ganong. New York, London: Academic Press.

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Gonadotropin release and follicular development after electrical stimulation of the hypothalamus in the immature female rat

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A number of data indicate that the gonadotropin release mechanism is already present at early ages in prepuberal female rats. From day 5 to about day 20 high gonadotropin levels, especially of FSH, are found in the blood (Goldman *et al.*, 1971; Kragt and Dahlgren, 1972; Ojeda and Ramirez, 1972; Meijs-Roelofs *et al.*, *this Volume*, pp. 3-11), although no large (antrum) follicles are formed in the ovaries. At the age of about 20 days, when marked follicular growth and presumably ovarian steroid secretion develops, a significant reduction of circulating gonadotropins takes place (Ojeda and Ramirez, 1972; Meijs-Roelofs *et al.*, *this Volume*, pp. 3-11). This relatively low level of gonadotropins is maintained until shortly before the first oestrus. Pituitary content of gonadotropins reaches maximal values during this period (Moore, 1966; Corbin and Daniels, 1967; Kragt and Ganong, 1968; Weisz and Ferin, 1970; Fawke and Brown, 1970). This suggests strong inhibition of gonadotropin release, starting at about day 20, which is presumably due to steroid secretion concomitant with the commencing follicular development. The prepuberal follicular development is characterized by continuous growth as well as degeneration of follicles (Peters, 1969) but a prooestrous pattern of follicles does not appear till the onset of puberty. However, relatively small amounts of *exogenous* gonadotropins may induce a follicular development leading to first ovulation. This holds for a single dose of PMS (Rowlands, 1944; Ying and Meyer, 1969), of HCG (Sugawara and Takeuchi, 1970), and of FSH (Zarrow and Gallo, 1966).

An advancement of puberty can also be induced at the hypothalamic level by electrical stimulation of the centres involved in gonadotropin release, so by increasing the *endogenous* gonadotropins (Meijs-Roelofs, 1972). However, no detailed information is available about the chain of events leading to puberty after such stimulation. Accordingly, the present study deals with gonadotropin release and follicular development in rats with advanced puberty, induced by electrical stimulation of the hypothalamus.

Electrical stimulation of the hypothalamus

Electrical stimulation was performed bilaterally in prepuberal rats either in the arcuate nucleus region or in the anterior hypothalamus. Bipolar stainless steel electrodes were used and stimulation consisted of biphasic matched pairs of 1 msec rectangular pulses, adminis-

tered at a frequency of 50 biphasic pulses/sec; 10 sec trains of pulses were followed by a 10 sec pause, total stimulus duration was 60 min; peak to peak current was 300–350 μA.

In previous experiments it was found that both stimulation of the arcuate nucleus region and of the anterior hypothalamus causes precocious puberty (Meijs-Roelofs, 1972). However, the effect was clearly age-dependent. After stimulation of the arcuate nucleus region the percentage of animals with advanced puberty increased gradually to 100 % (Table 1), when the age at stimulation was increased from 22 to 28 days.

Table 1 *Effect of electrical stimulation of the arcuate nucleus region*

Type of rat	Stimulation effect	No. of animals	Age at stimulation (days)	Vaginal opening at	
				age (days)	body weight (g)
Stimulated	+	1	22	36	78
	—	13		39.5 ± 0.5*	90.2 ± 1.5
Intact		14		40.8 ± 0.4	93.0 ± 1.6
Stimulated	+	6	25–26	31.7 ± 0.6	61.3 ± 3.0
	—	3		38.7 ± 1.9	82.0 ± 5.0
Intact		9		39.8 ± 0.9	91.6 ± 2.5
Stimulated	+	9	27–28	32.1 ± 0.1	68.0 ± 1.6
	—	0			
Intact		9		38.3 ± 0.4	95.8 ± 1.7

* ± S.E.M.

The response was rather constant following stimulation of the arcuate nucleus region on day 28: in nearly all cases vaginal opening, and the first ovulation, occurred on day 32.

Serum gonadotropins after stimulation on day 28

Female rats were stimulated on day 28 and from each animal a single sample of blood was taken. Blood was collected by puncture of the ophthalmic venous plexus under light ether anaesthesia at different intervals after stimulation. The animals were kept alive and checked daily for vaginal opening (VO) and first oestrus, as indicated by a totally cornified vaginal smear. Mortality after bleeding was rather high: 17 out of 63 rats died. In the remaining 46 rats VO and first oestrus occurred at a mean age of 32.7 ± 0.1 days. Brains of all animals were histologically examined and only those animals in which the stimulation sites were located in the arcuate nucleus region were used. Serum levels of FSH were estimated by the RR RAT FSH radioimmunoassay kit supplied by the NIAMD*. Serum LH was measured

* For abbreviations see Meijs-Roelofs *et al.*, *this Volume*, pp. 3–11.

by the OO RAT LH radioimmunoassay developed by Niswender *et al.* (1968). The results are expressed in terms of the standards supplied by the NIAMD. Serum samples were assayed in duplicate and at two dose levels: 25 μl and 100 μl or 100 μl and 200 μl for LH, depending on their potency, and 100 μl and 200 μl for FSH. The sensitivity of the assay systems used is described in a previous paper (Meijs-Roelofs *et al.*, *this Volume*, pp. 3-11).

Electrical stimulation in the arcuate nucleus region on day 28 resulted in a significant rise of serum LH (Fig. 1). The highest level was observed when the animals were taken out of the

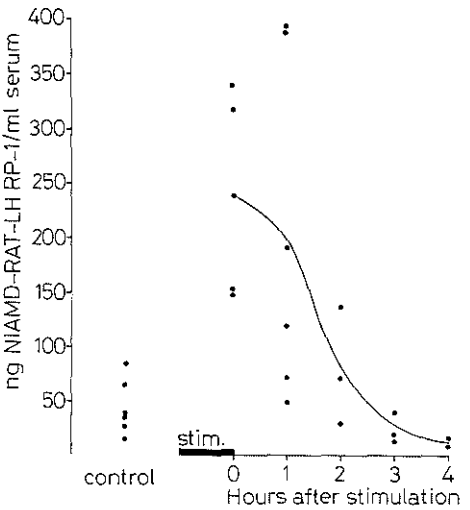

Fig. 1 *Serum LH concentrations in 28-day-old female rats at different times after electrical stimulation in the arcuate nucleus region, inducing precocious puberty. Each point represents one individual animal. Control animals on the left are not stimulated.*

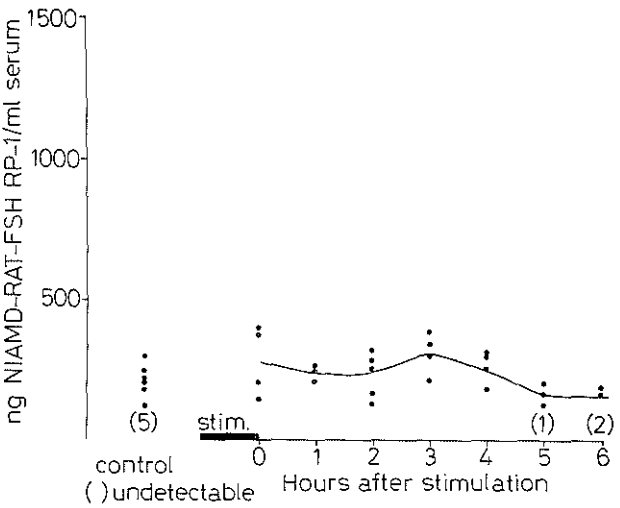

Fig. 2 *Serum FSH concentrations in 28-day-old female rats at different times after electrical stimulation in the arcuate nucleus region, inducing precocious puberty. Each point represents one individual animal. Control animals on the left are not stimulated.*

stereotactic apparatus (time 0) at the end of the stimulation period of 60 min. One hour after the end of the stimulation period large variations were seen. This probably means that whereas the LH levels of some animals were at that time at a high level, the LH levels of other animals were already declining. A further decrease was seen at 2 hr and 3 hr after stimulation.

The FSH levels after stimulation showed a less definite pattern (Fig. 2). In comparison with controls all values showed little change. However, FSH levels at 3 hr and 4 hr after stimulation were significantly higher than those of non-stimulated litter mate control animals (Wilcoxon test $P < 0.01$ and $P = 0.05$, respectively). A decline in FSH concentration was seen at 5 hr and 6 hr after stimulation. The maximal values found after stimulation were about 400 ng NIAMD-rat-FSH RP-1/ml. For comparison: FSH levels measured by Daane and Parlow (1971), using the same assay system and standard, in rats during the prooestrous-oestrous period were never higher than 500 ng/ml.

Follicular development in rats after stimulation on day 28

After hypothalamic stimulation the number of ovulated eggs at precocious first ovulation was always in the same range (9–12) as the numbers found both at spontaneous first ovulation and during the cycle of the adult rat. Therefore, follicular development after stimulation was studied. Female rats were stimulated in the arcuate nucleus region at 28 days of age and killed 1, 2 or 3 days later. Ovaries and brains were prepared for routine histological procedures. Ovaries were serially sectioned for determination of numbers of follicles and follicular volumes (Welschen, 1973). Only the ovaries of rats with stimulation sites in the arcuate nucleus region were used. Results are given in Table 2. The day after stimulation (day 29), stimulated and untreated control animals showed no differences in numbers of follicles of any volume class. However, during the next two days the number of large follicles ($\geqslant 500 \times 10^5\ \mu m^3$) increased significantly in the stimulated rats, whereas from day 30 to day 31 a significant decrease in numbers of follicles in the volume classes 100–199 and 200–499 $\times 10^5\ \mu m^3$ and a significant

Table 2 *Volumes and numbers of follicles in one ovary of immature rats, stimulated in the arcuate nucleus region on day 28*

Type of rat	Volume range of follicles $\times 10^5\ \mu m^3$	Mean no. of follicles ± S.E.M.		
		day 29	day 30	day 31
Stimulated	$\geqslant 1000$	– (8)*	0.2 ± 0.2(5)	0.3 ± 0.2(7)
	500–999	1.4 ± 0.6	4.8 ± 1.7	4.3 ± 0.5**
	200–499	13.8 ± 1.7	13.2 ± 1.2	3.1 ± 0.7***
	100–199	12.3 ± 1.2	7.2 ± 1.0	5.0 ± 1.6**
	total > 100	27.4 ± 1.2	25.4 ± 2.1	12.7 ± 1.8***
Untreated	$\geqslant 1000$	– (6)	– (5)	– (6)
	500–999	1.2 ± 0.3	1.0 ± 0.5	0.3 ± 0.2
	200–499	13.8 ± 1.2	10.2 ± 0.4	12.2 ± 0.8
	100–199	15.3 ± 1.5	16.8 ± 1.8	17.2 ± 1.0
	total > 100	30.3 ± 1.9	28.2 ± 2.2	29.7 ± 1.5

* No. of animals.
** Significantly different from numbers on day 29.
*** Significantly different from numbers on day 29 and day 30.

decrease in total number of follicles $\geqslant 100 \times 10^5\ \mu m^3$ were found. In untreated rats no changes were found in numbers of follicles of any volume class.

In adult rats all follicles $\geqslant 500 \times 10^5\ \mu m^3$ are normally capable of ovulating and may be induced to ovulate by HCG injection on dioestrus-2 (Welschen and Rutte, 1971). In our stimulated prepuberal rats follicles $\geqslant 500 \times 10^5\ \mu m^3$ were already present on day 30, and in numbers comparable to those during the cycle in adults. To test whether prepuberal follicles of this size can also be induced to ovulate on day 30, rats stimulated on day 28 were injected with a single dose of 15 I.U. HCG on day 30 at 3 p.m. and tubal eggs were counted the next morning. Non-stimulated rats injected with the same dose of HCG served as controls. Results are given in Table 3. All stimulated rats ovulated, whereas only 1 egg was found in 1 out of 6 untreated rats.

Table 3 *Ovulation in immature rats stimulated in the arcuate nucleus region on day 28 and treated with HCG on day 30*

Type of rat	No. of rats	HCG on day 30	No. of tubal eggs ± S.E.M.
Stimulated	6	15 I.U.	8.5 ± 0.7
Untreated	6	15 I.U.	0.2 ± 0.2

Discussion

In an earlier study it was found that electrical stimulation of the hypothalamus, performed at an appropriate age, advances the onset of puberty in female rats. By means of an endogenously performed bioassay it was found that the advancement of puberty observed was mediated via an increased FSH release (Meijs-Roelofs, 1972). However, FSH was estimated in a very indirect way and over a 24 hr period. With the availability of the more sensitive radioimmunoassay method it became possible to evaluate both LH and FSH release after stimulation in a more direct way. This showed that only a marginal increase in FSH occurred; the increase was significant 3 and 4 hr after stimulation of the arcuate nucleus region. Increase in LH, however, was much more pronounced and had already commenced during the stimulation period (60 min), as maximal blood levels were found immediately after stimulation. The initial peak was followed by a sharp fall to control levels 2 to 3 hr after the stimulation period.

The times of release of LH and FSH found in this study are in agreement with those observed in adult rats following electrical or electrochemical stimulation. Cramer and Barraclough (1971), using a similar type of electrical stimulation in the preoptic area, also observed a rise in LH occurring during the stimulation period (40 min).

Similarly, Kalra *et al.* (1971), who stimulated adult Nembutal blocked prooestrous rats electrochemically in the median eminence arcuate region, observed a rise in plasma LH with the highest level one hour after stimulation (compare end of stimulation period in our study). In their study the elevation of plasma FSH also reached its highest level 3 hr after stimulation, but the level they found was more elevated.

Clemens *et al.* (1971), after electrochemical stimulation in the median eminence arcuate complex, found the highest LH concentrations 0.5 hr after stimulation and the FSH elevation 3 hr after stimulation, but blood was sampled only 0.5 hr and 3 hr after stimulation. In accordance with results found in this study, all workers report a higher elevation for LH than for FSH.

However, the relatively low FSH elevation might be of importance in the advancement of the processes leading to puberty. In studies involving administration of exogenous gonadotropin both LH and FSH preparations, administered in a single small dose, are effective in inducing precocious puberty. This suggests that either the LH or the FSH elevation after stimulation might be sufficient to advance puberty, but the combination of LH and FSH release could also be of decisive importance. The reasoning for this view is as follows: A release pattern with a high LH peak preceding a relatively low rise in FSH is also seen during pro-oestrus in adult rats. In adult rats this pattern was shown to be of importance, not only to induce ovulation of the mature follicles present, but also to start the development of a new generation of follicles (Welschen, 1973). This latter aspect may be of crucial importance in inducing puberty. This view is supported by data on follicular development. Follicular development after stimulation on day 28, resulting in ovulation on day 32, remarkably resembled follicular changes taking place during the cycle in the adult. In both cases a gradual increase in numbers of follicles $\geqslant 500 \times 10^5$ μm^3 was seen first, followed by a significant decrease in follicles between 200–499 $\times 10^5$ μm^3. Moreover, follicles $\geqslant 500 \times 10^5$ μm^3, which are capable of ovulating during the cycle, proved to be capable of ovulating in stimulated immature rats too.

The numbers of follicles that ovulated in immature rats after stimulation were always within the normal range, and the numbers of ova after stimulation on day 28 and HCG treatment on day 30 were also normal. On the basis of the data presented it seems justified to conclude that electrical stimulation of the brain triggers a fully normal first oestrus with a normal number of ovulations.

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References

CLEMENS, J. A., SHAAR, C. J., KLEBER, J. W. and TANDY, W. A. (1971): Areas of the brain stimulatory to LH and FSH secretion. *Endocrinology*, *88*, 180.

CORBIN, A. and DANIELS, E. L. (1967): Changes in concentration of female rat pituitary FSH and stalk median eminence follicle stimulating hormone releasing factor with age. *Neuroendocrinology*, *2*, 304.

CRAMER, O. M. and BARRACLOUGH, C. A. (1971): Effect of electrical stimulation of the preoptic area on plasma LH concentrations in proestrous rats. *Endocrinology*, *88*, 1175.

FAWKE, L. and BROWN, P. S. (1970): Pituitary content of follicle stimulating hormone in the female rat. *J. Reprod. Fertil.*, *21*, 303.

GOLDMAN, B. D., GRAZIA, Y. R., KAMBERI, I. A. and PORTER, J. C. (1971): Serum gonadotrophin concentrations in intact and castrated neonatal rats. *Endocrinology*, *88*, 771.

KALRA, S. P., AJIKA, K., KRULICH, L., FAWCETT, C. P., QUIJADA, M. and MCCANN, M. (1971): Effects of hypothalamic and preoptic electrochemical stimulation on gonadotrophin and prolactin release in proestrous rats. *Endocrinology*, *88*, 1150.

KRAGT, C. L. and DAHLGREN, J. (1972): Development of neural regulation of follicle stimulating hormone (FSH) secretion. *Neuroendocrinology, 9*, 30.

KRAGT, C. L. and GANONG, W. F. (1968): Pituitary FSH content in female rats at various ages. *Endocrinology, 82*, 1241.

MEIJS-ROELOFS, H. M. A. (1972): Effect of electrical stimulation of the hypothalamus on gonadotrophin release and the onset of puberty. *J. Endocr., 54*, 277.

MOORE, W. W. (1966): Changes in pituitary LH concentration in prepubertal and postpubertal rats. *Neuroendocrinology, 1*, 333.

NISWENDER, G. D., MIDGLEY, A. R., MONROE, S. E. and REICHERT, L. E. (1968): Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131}I. *Proc. Soc. exp. Biol. (N.Y.), 128*, 807.

OJEDA, S. R. and RAMIREZ, V. D. (1972): Plasma level of LH and FSH in maturing rats: response to hemigonadectomy. *Endocrinology, 90*, 466.

PETERS, H. (1969): The development of the mouse ovary from birth to maturity. *Acta endocr. (Kbh.), 62*, 98.

ROWLANDS, I. (1944): The production of ovulation in the immature rat. *J. Endocr., 3*, 384.

SUGAWARA, S. and TAKEUCHI, S. (1970): Ovulatory response to a single dose of human chorionic gonadotrophin in the immature rat. *Endocrinology, 86*, 965.

WEISZ, J. and FERIN, M. (1970): Pituitary gonadotrophins and circulating LH in immature rats – a comparison between normal females and males and females treated with testosterone in neonatal life. In: *Gonadotrophins and Ovarian Development*, pp. 339–350. Editors: W. R. Butt, A. C. Crooke and M. Ryle. E. and S. Livingstone, Edinburgh.

WELSCHEN, R. (1973): Amounts of gonadotrophins required for normal follicular growth in hypophysectomized adult rats. *Acta endocr. (Kbh.), 72*, 137.

WELSCHEN, R. and RUTTE, M. (1971): Ovulation in adult rats after treatment with pregnant mare serum gonadotrophin during oestrus. *Acta endocr. (Kbh.), 68*, 41.

YING, S. Y. and MEYER, R. K. (1969): Dose dependent pregnant mare's serum induced ovulation in immature rats. *Proc. Soc. exp. Biol. (N.Y.), 130*, 40.

ZARROW, M. X. and GALLO, R. V. (1966): FSH-induced ovulation in the immature rat. *Endocrinology, 76*, 445.