CHAPTER 6

Relation of Neuroendocrine System to Reproductive Decline in Female Rats

PIERRE ASCHHEIM

1. Introduction

The reproductive decline in female mammals is universal. Age-related sterility in rats is complete at about 15–17 months (Miller *et al.*, 1979), when approximately 50–60% of the lifetime is spent. This type of sterility is multifactorial, the most important factor being senescence of the uterus. Oocyte alterations also occur. Disregarding these reproductive aspects, we will focus on aging of the hypothalamic-hypophysial-ovarian axis.

When this work was started 20 years ago, the contrast soon became apparent between (1) the modified, but persistent ovarian function in rats until the end of their lifespan, and termination of ovarian function in women at menopause, (2) the predominance of aging of the neuroendocrine regulatory mechanisms in the rat, and the predominance of intrinsic ovarian aging in women. Since menopause is restricted to humans and some infrahuman primates, the rat improperly appeared to be a model for other mammalian reproductive aging, a model characterized by hypersecretion of prolactin (PRL) during aging, implicating age-related alterations in hypothalamic-pituitary sensitivity to control input, and changes in neurotransmitter activity.

At present, it is known (Parkening *et al.*, 1980a) that reproductive aging in other rodents is different than in rats. There is no age-related

PIERRE ASCHHEIM • Unité de Recherches Gérontologiques, INSERM U.118, 75016 Paris, France.

J. Meites (ed.), *Neuroendocrinology of Aging*

increase in plasma PRL concentration in C57BL6 mice, Chinese hamsters or Mongolian gerbils. In Syrian hamsters, PRL concentration even decreases with age. Moreover, CBA mice represent the closest rodent model known at present for human ovarian aging! (Aschheim, 1982). But three reasons remain for justifying research in the rat model: (1) it is necessary to understand age-related changes in sexual neuroendocrinology in the rat, since it is the most widely used laboratory animal in this field; (2) it is necessary to establish reference data for age controls in studies on chronic drug administration, and, (3) the early aging of the neuroendocrine control system of ovarian function in the rat is an interesting model *per se,* and may explain the plasticity of ovarian aging and the facility of its experimental manipulation.

This chapter will attempt to disclose new trends in research in reproductive aging. During the last three years (from mid-1978 to mid-1981), there appeared nearly as many publications on this subject as in the preceding 15-year period. This resulted, at least in part, from (1) the increased use of hormone radioimmunoassays (RIAs), especially by collection of serial blood samples, and (2) the fully justified interest and use of middle-aged rats, during cyclicity or in the transition period from cyclicity to senile disruptions of the estrous cycle. In addition to accumulation of new data which are sometimes contradictory, two tendencies can be observed: (1) a greater awareness of difficulties in interpreting age-related hormonal blood concentrations in terms of secretion rate, when other possibly age-related changes in parameters of hormonal kinetics remain uncontrolled, and (2) a justified reserve in attributing hormonal changes to aging *per se* when they may result only from previous differences in the hormonal milieu. In this respect, the influence of ovarian steroids on central (hypothalamic-hypophysial) aging has recently been reevaluated.

The chapter begins with comments on the above points. It then describes aging of the ovary and its neuroendocrine regulation in old rats displaying senile deviations of the cycle (SDC), and in aging rats during the period of cyclicity and the transition period to acyclicity. Consideration is given next to experimental modifications of hypothalamic-hypophysial-ovarian aging. Finally, extrinsic and intrinsic aging and their physiological significance will be discussed.

2. Preliminary Comments on Hormonal Data in Old Rats

The numerous hormonal data reported for old rats in recent years are difficult to present in tabulated form. This results not only from the

normal discrepancies found in animal husbandry, technical procedures, experimental designs, etc., but because aging itself is a complex and diverse process.

The activity of hormones is usually inferred from their concentrations in serum or plasma. In addition, age can modify hormone availability by altering vascular supply as well as the number and (or) quality of target cell receptors. But when hormone production rate is inferred from serum concentration alone, it is only justified if other parameters of hormonal kinetics are held constant. Table 1 presents some possible age-related changes which may alter the significance of blood hormone concentrations. A decrease in metabolic clearance rate (MCR) of luteinizing hormone (LH) is suggested by Fig. 1 (data from Riegle and Miller, 1978, by permission). By 75 min after injection of LH-releasing hormone (LHRH), its stimulatory effect disappears, but the residual LH level remains higher in old than in young rats, particularly after the second injection of LHRH. With a decrease of MCR, hormone concentration overestimates the production rate.

Both plasma volume and body weight increase with age. The expanded plasma volume in the larger body mass of old rats has been measured in males by Kaler and Neaves (1981), and in females by Aschheim and Fayein (unpublished). Whereas, in old recurrently pseudo-

Table 1. Parameters Whose Possible Age-Changes May Alter the Significance of Hormonal Blood Concentration in Terms of Secretion Rate in Old Rats

Metabolic clearance rate	Unknown. Decrease suggested for LH by Fig. 1[a]
Plasma volume	Increases with body weight which increases with age. Male rat,[b] female rat[c]
Hormonal transport	Unknown. No specific binding proteins for estrogen in adult rats.[d] Other binding proteins?
"Aged" hormones	Big LH molecule in male rats.[e] Discrepancy between LH concentration measured by RIA or RRA suggests also alteration of LH molecule in aged female mice[f]
Number and/or size of hormone producing cells	Increase in number of prolactin-producing pituitary cells.[g] Decrease in number and nuclear size of hypothalamic neurons[h,i]

[a] Riegle and Miller, 1978.
[b] Kaler and Neaves, 1981.
[c] Aschheim and Fayein (unpublished).
[d] Germain *et al.*, 1978.
[e] Conn *et al.*, 1980.
[f] Parkening *et al.*, 1980*b*.
[g] Kawashima, 1974.
[h] Hsu and Peng, 1978.
[i] Lin *et al.*, 1976.

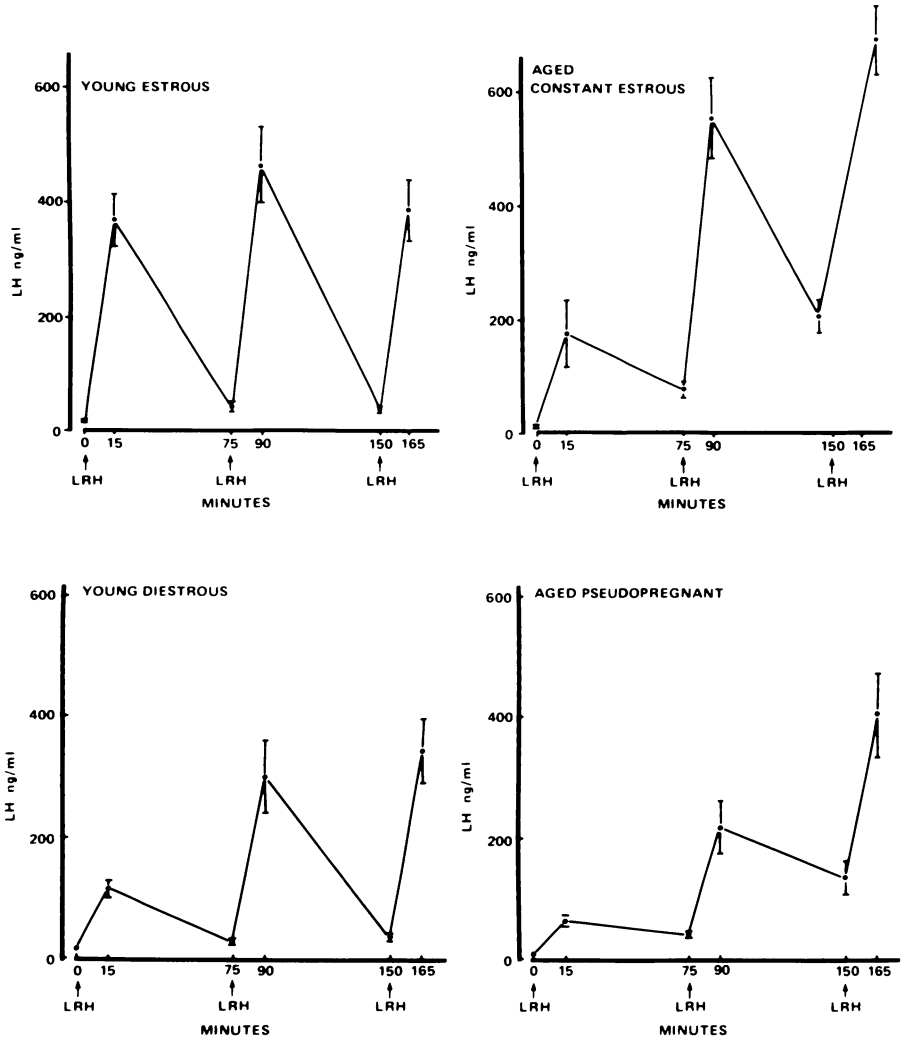

Fig. 1. Effect of three intravenous injections of 500 ng of LHRH on serum LH in young estrous and diestrous, and aged constant estrous and persistent diestrous, female rats. Serum LH is shown as the group mean with indicated SEM. Serial blood samples were taken before LHRH injection (0, 75 and 150 min) and 15 min after LHRH stimulation (15, 90, and 165 min). From G. D. Riegle and A. E. Miller, 1978, in: *The Aging Reproductive System* (E. L. Schneider, ed.), Raven Press, New York, with permission.

pregnant rats, progesterone concentration is elevated but significantly lower during the plateau period of luteal function than in young pseudopregnant controls (Fayein and Aschheim, 1980), the total amount of circulating progesterone is similar in both groups (which differ by 30% in their respective body weights). Due to this hormonal dilution in old rats, hormone concentrations in the blood underestimate secretion rate. This applies to progesterone hypoconcentration as well as to PRL-hyperconcentration.

In female rats, little is known about age-related changes in binding proteins and free or bound hormonal fractions. But "aged hormones" deserve comment. Conn *et al.* (1980) described a large molecular size of LH in aged male rats. Castration of young rats resulted in similar alterations, reversed by steroid treatment, suggesting a relation not to aging *per se* but to changed testosterone milieu. Moreover, the MCR of the large LH form was decreased. Discrepancies between immunological and biological activities of LH in aged female mice, as reported by Parkening *et al.* (1980b), may also be due to such a qualitative change.

For the gerontologist, age-related changes in hormonal concentration reflecting production rate, must ultimately be ascribed to the number and/or quality of hormone producing cells. There is a proliferation with age (leading to tumors) of pituitary PRL producing cells (Kawashima, 1974). The concentration of serum PRL increases significantly. The increase in PRL production by the pituitary of acyclic rats is also seen *in vitro*. But when expressed as per mg of pituitary weight, accounting for cell proliferation in old age, the unitary activity of these newly formed cells decreases significantly (Aschheim and Rasolonjanahary, 1979).

Hormonal data must also be related to age changes in hormonal rhythmicity, pulsatile secretory pattern of LH, PRL surges, etc., as will be seen later. Hormonal concentration results from intricate, multidirectional processes, and therefore contradictory age-related changes in numerous parameters can occur. By itself, hormone concentration cannot clarify an endocrinological condition in the aged rat. It must be completed by stimulation and inhibition tests, by *in vitro* studies, and by examination of target structures. This is not intended to discredit measurements of hormonal concentrations, but to indicate that their critical evaluation may involve many interesting new gerontological aspects of homeostatic regulation.

The second comment concerns the gerontological validity of hormonal comparisons, a point which I emphasized many years ago and which is now taken into consideration by most investigators. Young cyclic and old acyclic rats differ not only by age, but also by their hormonal

background, which in turn is "spontaneously" age related. Valid comparisons dealing only with age must also be made between young and middle-aged cyclic rats. Old, previously acyclic, but experimentally "recycled" rats can be usefully added to the study. Likewise, persistent estrous rats must be compared at different developmental stages of this condition, complemented by young rats made persistent-estrous, for example, by constant illumination. Stimulation and inhibition tests are frequently performed on ovariectomized or ovariectomized rats given hormone replacement. Here too, it is advisable to standardize the neuroendocrine status preceding ovariectomy and the time elapsed after ovariectomy, when hormonal changes due only to aging are investigated.

3. Relation of Neuroendocrine System to Ovarian Function after the End of Cyclicity

3.1. Senile Patterns of Ovarian Function

3.1.1. Senile Deviations of the Estrous Cycle

The incidence of regular 4- or 5-day estrous cycles decreases with age as indicated in Wistar rats by the first two lines in Table 2. At the end of the first year of life, estrous cycles are progressively replaced by two SDCs: persistent estrus (PE) and recurrent pseudopregnancies (RPP; Aschheim, 1961). PE predominates during the second and RPP during the third and last year of life. All PE rats were previously cyclic, as were most RPP rats. But some of the latter derive from PE, implicating resumption of ovulation. Another, rather unusual pattern, senile anestrus, has been described, but it could be pathological and will be disregarded here. Cyclic rats represent 5–10% of the population after the age of 18 months.

PE, characterized by a persistent cornified vaginal smear, is an anovulatory condition. The ovaries are devoid of corpora lutea and therefore are relatively small (weight = 40–60 mg). They contain numerous follicles of all sizes, healthy as well as atretic, and in addition cystic follicles, particularly in rats which aged during the PE condition. Estradiol levels are moderate, 18 pg/ml at 11–13 months, 30 pg/ml at 16 and 30 months of age (Lu *et al.*, 1979) and rather constant (Huang *et al.*, 1978). Low to medium levels were reported for estrone, testosterone, and androstenedione, and very low levels for progesterone and 20α-

Table 2. Evolution of Some Biological Parameters during Aging of Cyclic Rats.[a,b]

	Age in months								
	2–3	3–4	4–5	6–7	7–8	9–10	10–11	11–12	12–13
Cyclic rats	100	94	85	77		72	60	<60	>40
Regular Wistar cyclers at									
4 days	60	50	50	26		16			
5 days	20	30	40	60		70			
Persistence of cycles during the month following hemiovariectomy		100			65				40
Long-term persistence of ovulatory compensation after hemiovariectomy at 30 days of age		79			61		38		0
Persistence of the cycle after injection of 5 μg of estradiol benzoate at estrus		94	81		63	33			17
Increase of circulating FSH 1 mth after castration		510						380	
Increase of circulating LH 1 mth after castration		560						350	

[a] Values are given as percentages.
[b] From P. Aschheim (1979), with permission.

hydroxyprogesterone (Lu *et al.*, 1979). In PE rats, moderate amounts of estradiol are secreted continuously, unopposed by progestogens.

RPP are characterized by prolonged diestrous vaginal smears, lasting 15 days or more, interspaced by proestrus and/or estrus associated with ovulation of a normal number of oocytes, and then followed by a new diestrous period. The ovaries (weighing 80–110 mg) contain large corpora lutea and well-developed follicles. Traumatic deciduomata can readily be induced by irritation of the uterus at day (D) 4 of pseudopregnancy (Aschheim, 1961). As in young rats displaying RPP in the presence of vasectomized males, circulating progesterone concentration shows a rapid increase until D3, an elevated plateau level of 56 ng/ml until D9 (longer extension in old rats), and a decline afterwards (Fayein and Aschheim, 1980). As mentioned in Section 2, the plateau progesterone values are lower in old than in young rats, but the total amount of circulating progesterone is equal in the two groups. The 20α-hydroxyprogesterone values are also increased (Lu *et al.*, 1979). Estradiol levels are very low at diestrus (Lu *et al.*, 1979). In contrast to previous statements (Huang *et al.*, 1978), old RPP rats display a cyclic pattern in terms of luteal function.

3.1.2. Hormonal Data

Data on pituitary hormones in SDC can be summarized by an age-related hypersecretion of PRL associated with moderate or low levels of follicle-stimulating hormone (FSH) and LH, which clearly are not increased as after ovariectomy.

Pituitary PRL content is high in PE rats (Clemens and Meites, 1971), including nontumorous pituitary glands (Kawashima, 1974). Circulating PRL levels are also elevated in old PE rats. Kawashima (1974), Watkins *et al.* (1975a), Huang *et al.* (1976a), Lu *et al.* (1979) and Rasolonjanahary (1979) reported values of 160–400 and even 1200 ng/ml. But it is important to emphasize that this hormonal increase is age-related. Prolactin levels at the beginning of PE, in rats 11–16 months old (Lu *et al.*, 1979) or 8 months old (Rasolonjanahary, 1979), are significantly lower than at 18 months or afterwards, and are similar to basal PRL values of cycling rats of the same age. Damassa *et al.* (1980) studied the 24-hr pattern of PRL secretion in PE rats and demonstrated a diurnal surge at 1700 hr, whereas at 200 hr, a smaller nocturnal rise did not show statistical significance. Prolactin values were also elevated in RPP rats, but less than in PE, to 100–200 ng/ml (Watkins *et al.*, 1975a; Huang *et al.*, 1976a; Lu *et al.*, 1979; Rasolonjanahary, 1979). There is a moderate age-related increase in blood PRL values from 18 to 24 months (Fayein and Aschheim, 1980). The PRL surge is nocturnal in old spontaneous RPP rats (Damassa *et al.*, 1980), whereas in young rats with induced pseudopregnancy, daily diurnal and nocturnal surges are observed. The suppression of the former may be related to the low estradiol level in old RPP rats (Gilman *et al.*, 1981).

Pituitary content of FSH is high in PE (Clemens and Meites, 1971). LH content in PE is low, whereas in RPP it increases throughout the pseudopregnancy sequence to three-fold the PE level and then falls at ovulation (Aschheim, 1968). Variations in circulating FSH and LH concentrations in old PE and RPP rats do not differ markedly from those seen during the estrous cycle (excluding ovulatory surges) of adult rats (Huang *et al.*, 1978; Lu *et al.*, 1979). FSH and LH basal values are higher in PE than in RPP, as reported by Huang *et al.* (1978), although no major differences were noted by Lu *et al.* (1979). In these rats, there is no hypersecretion of FSH as after ovariectomy, and no significant hyposecretion of basal LH. But as discussed in Section 2, changes in plasma volume, MCR, pulsatile secretory pattern, or biological activity of gonadotropin molecules can alter the significance of assays of hormonal concentrations in the blood.

Hypothalamic LHRH content is not altered in aged rats (Steger *et*

al., 1979a). LHRH is present in sufficient amount to sustain basal LH secretion *in vitro* (Riegle and Miller, 1978). Meites *et al.* (1979) described a depressed catecholamine and an enhanced serotonin activity in old male rats as compared to young ones, and mentioned a similar difference between old and young females. Hypothalamic dopamine (DA) concentration was reduced in old RPP rats (Clemens, 1979). Walker (1981) reported the interesting finding that the circadian rhythm of hypothalamic serotonin turnover, seen in cyclic rats, is abolished in old PE (as well as in other conditions leading to PE). Reinitiation of cycling in old rats restored the rhythm of serotonin dynamics.

3.1.3. Structural Changes in the Ovary, Pituitary, and Hypothalamus

Ovary. No age-related regression of ovarian weight or of ovarian vascularization occurs in rats. As in all other mammals, there is an age-related decrease in the number of oocytes; but in rodents, except for the CBA mouse, a complete exhaustion of oocytes does not occur by the end of life. In mice, the proportion of oocytes lost per 100-day interval remains constant and is strain-specific. But in rats, it decreases contin uously with increasing age; between 100 and 200 days of age, the proportion lost is 0.281; between 500–600 days, it is only 0.082. During the first mentioned interval, a loss of 1140 oocytes results in 2924 residual oocytes, and during the second interval, a loss of 154 results in 1734 residual oocytes (Mandl and Zuckerman, 1951). An ultrastructural alteration in preovulatory oocyte morphology will be dealt with in Section 4.1, since it has been described in aging cyclic rats. Growing and mature follicles in acyclic old rats do not differ microscopically from those seen in young rats. Gonadotropin binding and aromatase activity in granulosa cells of mature Graafian follicles are similar in old PE and young proestrous rats (Erickson *et al.,* 1979). Corpora lutea of old RPP show the same binding capacity for LH and the same 3β-hydroxysteroid dehydrogenase (3β-HSD) activity as those of young rats (Steger *et al.,* 1976).

In about 25% of old acyclic rats, mostly in PE, 2–4 follicles per ovary can be observed which exhibit a striking densification of granulosa cells (Aschheim, unpublished). Follicles measure about 350 μm in diameter, and the external limits are regular (round or slightly waving). A small antrum is present, with or without an oocyte. Granulosa cells are numerous, small, densely packed, proliferating to the center, with few mitoses. In a few instances, I have observed a true intraovarian follicular rupture without any sign of luteinization, the oocyte lying in a nearby lymphatic space. All the granulosa cells show a very high 3β-HSD activity,

which usually is seen only in the external layer of granulosa cells of preovulatory follicles. I could show experimentally that these follicles are not precursor structures of granulosa-cell tumors, but they may represent abortive steps to ovulation occurring during PE.

Other structural changes occur in old ovaries, including thickening of the basement membrane of small follicles, proliferation of the surface ("germinal") epithelium and of the rete ovarii, and accumulation of numerous phagocytes filled with lipofuscin granules in the interstitial compartment. Interstitial cells of thecal origin are transformed into "deficiency" or "wheel" cells which are considered to result from decreased biological activity of LH. Testis-like tubes, a particular form of follicular atresia, are present, and epithelial cellular cords appear, proliferate, and may become tumorous. In electron microscopy, epithelial cord and deficiency cells show a low steroidogenic activity, which can be normalized and notably increased by treatment with exogenous human chorionic gonadotropin (hCG) (Crumeyrolle-Arias *et al.,* 1976). Few of these structural changes are due to intrinsic ovarian aging, as will be seen in Section 6, by comparison with ovarian aging in rats hypophysectomized when 26 days old.

Pituitary. The proportion of PRL-producing cells increases with age (Kawashima, 1974). Enlarged pituitaries, weighing more than 23 mg, are encountered in 12.5% of acyclic rats at 13–14 months of age; the proportion increases to almost 50% at 16–18 months (Aschheim, 1975). At 24 months, all rats show macroscopic adenomas or microscopic proliferation of PRL-producing cells (Aschheim and Pasteels, 1963). Their ultrastructure has been studied by Kawashima (1974) and by Kovacs *et al.* (1977), who also demonstrated by immunoperoxidase staining the presence of immunoreactive PRL and the absence of growth hormone (GH) and thyrotropin (TSH). Peng and Peng (1973) measured the uptake of tritiated estradiol in the pituitary of old rats in SDC, and reported that per mg of hypophysial tissue it was significantly lower than in young rats, but per pituitary it was the same.

Hypothalamus. Morphometric studies have been done in hypothalamic areas concerned with gonadotropin regulation (Lin *et al.,* 1976; Hsu and Peng, 1978). The preoptic area implicated in cyclic release of these hormones in adults shows a large decrease of the total number of neurons and of individual nuclear volume in 24–30-month-old rats. In the areas implicated in tonic release, the situation differs; the arcuate nucleus shows a large decrease in neuron numbers and a moderate one in nuclear volume, whereas in the ventromedial nucleus there is no cellular loss and a variable reduction of nuclear volume. Uptake of tritiated estradiol is also significantly lower in the anterior hypothalamus

of old rats compared with young rats (Peng and Peng, 1973). Analysis of its subcellular distribution reveals an impaired (decreased) translocation of estradiol from cytosol to nucleus in old PE rats (Jiang and Peng, 1981). Unfortunately, nothing is known about the evolution of these parameters between the ages of 4–24 months.

An interesting morphological change has been reported by Brawer (Brawer *et al.*, 1980; Schipper *et al.*, 1981). During aging of PE rats, an ovary-dependent degeneration occurs selectively in the arcuate nucleus. It is characterized by a significant increase in the numbers of astrocytic granules, starting during cyclicity, and principally in the numbers of reactive microglial cells, starting with PE. This "gliosis" within the arcuate nucleus is not related to neuronal degeneration of perikarya, but rather of axons, terminals, and sometimes dendrites connecting the medial preoptic area to the medial basal hypothalamus. This neurotoxic effect is absent in aging rats ovariectomized when young. At present, it is not known if morphological changes representing chemical deafferentation also occur in the hypothalamus of RPP rats.

Immunostaining demonstrated LHRH-positive perikarya in the medial preoptic and septal area (but not in the arcuate nucleus), of 16–20-month-old PE rats, in contrast to young rats. Staining intensity of LHRH-containing nerve elements, especially axon terminals in the median eminence, was also much higher in old than in young rats (Merchenthaler *et al.*, 1980). Age-related subneuronal distribution of LHRH has been studied by Barnea *et al.* (1980). With aging, an increase in the LHRH content of the hypothalamus occurs predominantly in subneuronal structures which give rise to fragile synaptosomes and free granules, suggesting alterations in the physicochemical properties of the plasma membranes of the neuronal terminals.

3.2. Aging of the Neuroendocrine Regulation of Ovarian Function

3.2.1. Physiological Aspects: Reinstatement of Cyclicity, Heterochronic Grafts of Ovary and Pituitary

SDC do not represent a fixed status. I have already mentioned the "spontaneous" transition from PE to RPP, indicating resumption of ovulation. RPP rats exposed to permanent light change to PE and resume RPP when returned to alternating photoperiods (Aschheim, 1961). Whereas these rats continue to show senile neuroendocrine regulation, they can secrete the ovarian hormones, progesterone and estrogen, previously lacking. Different agents have been used to reinstate ovarian

cycles. In PE rats, this is achieved in order of decreasing efficiency by administration of progesterone, adrenocorticotropic hormone (ACTH), ether stress, L-dopa and other drugs, correcting a hypothalamic deficiency of catecholamines (Huang *et al.*, 1976b, Linnoila and Cooper, 1976). Luteinizing hormone (LH) surges have been observed on proestrous days of induced cycles in old PE rats by use of progesterone and ACTH (Huang *et al.*, 1976b). The incidence, amplitude, and timing of LH surges are more irregular than in young rats. Epinephrine treatment, injection of LHRH, and electrical stimulation of the preoptic area, can also induce ovulation (Meites, *et al.*, 1978). RPP rats resume cycles during treatment with lergotrile mesylate, a DA agonist (Clemens, 1979).

SDC are reversible, since cyclicity can be reinstated by a unique "trigger" mechanism. The resultant sequence of ovarian cycles is self-sustained. A single injection of hCG or LH in old PE rats first induces a pseudopregnancy, followed by a succession of about 11 autonomous ovarian cycles (Aschheim, 1965). Middle-aged PE rats generally resume short cycles directly after the induced ovulation, without pseudopregnancy, because of their lower PRL values (Everett, 1980; for age-related PRL concentration in PE, see Section 3.1.2). A single modification of environmental conditions can also restore cyclicity in a certain percentage of SDC, e.g., exposure of PE rats to constant darkness (Aschheim, 1965), isolation of previously grouped RPP, or chronic exposure to cold (+5°C) (Aschheim and Latouche, 1975).

Reinstatement of cyclicity is more or less easy to realize and is generally of limited duration. But an efficient treatment like a single injection of hCG in PE produces cycling in a large number of old rats and allows for comparison with young rats. Moreover, reversibility of the PE condition is an argument against an intrinsic cellular deficiency in hormone production or release at any one of the sites implicated in regulation of cyclicity, or in related neurotransmitter activity. It points more to a deficiency in the control mechanisms of cellular activity, and to changes in the sensitivity of regulatory structures (Aschheim, 1965).

Studies of heterochronic ovarian grafts demonstrate that the ovary is not primarily responsible for SDC. Adult cycling rats, ovariectomized and grafted with old ovaries originating from rats in SDC, resume cycles. Old PE or RPP rats, ovariectomized and grafted with prepubertal ovaries, resume PE or RPP, respectively (Aschheim, 1964/5). Grafting of an old pituitary under the median eminence of a young previously hypophysectomized rat results in resumption of ovarian cycles, but less frequently than grafts of young pituitaries (Peng and Huang, 1972). The grafted old gland seems to be more difficult to revascularize than the young pituitary and/or its functional performance is impaired.

3.2.2. Changes in Steroidal Feedback Regulation of the Hypothalamus, Neurotransmitter Activity, and Pituitary Response to LHRH

A significant increase of pituitary LH stores was found after ovariectomy of PE or RPP old rats, but was significantly lower than in ovariectomized young or old formerly cyclic rats. Prolactin was implicated, since in PE rats "recycled" during the month before ovariectomy, the postcastration rise of LH stores was enhanced (Aschheim, 1970). A complete study of circulating FSH, LH, and PRL levels after ovariectomy or ovariectomy and estrogen treatment has been reported by Huang *et al.* (1976a). Postovariectomy levels of FSH and LH in PE and RPP old rats were significantly greater than in intact control rats, but lower than in young ovariectomized rats. The rise was nullified by estrogen treatment. After ovariectomy, PRL values decreased significantly in young cyclic and old PE rats (but residual PRL concentration remained higher in PE), but did not change in RPP. Estrogen treatment increased PRL values in all categories. Is the smaller increase of gonadotropins after ovariectomy in old rats directly related to age or is it due, at least partly, to the higher residual levels of circulating PRL, a hormone known to inhibit the postcastration rise of LH? (Grandison *et al.*, 1977). Clemens (1979) reported that in ovariectomized, formerly RPP rats, lergotrile mesylate inhibited PRL secretion, but did not allow serum LH levels to rise.

The positive feedback of injected estradiol or progesterone on the LH surge in old acyclic rats, ovariectomized and pretreated with estrogen, is also diminished (Howland, 1976; Huang *et al.*, 1980) or abolished (Gosden and Bancroft, 1976; Peluso *et al.*, 1977), when compared to results in young, formerly cyclic rats. Can the observed differences be ascribed, not to age *per se,* but to differences in the gonadal steroid background? In acutely ovariectomized old rats, the positive feedback of estradiol and progesterone on LH secretion was observed only in formerly RPP, not in formerly PE animals (Lu *et al.*, 1980). But in PE, the positive feedback effect was seen five weeks after ovariectomy (Lu *et al.*, 1981) and was significantly smaller than in young, formerly cyclic, controls. After chronic implantation of these two long-term ovariectomized groups with estradiol, it was abolished in both age groups (Lu *et al.*, 1981). Thus, estrogen is the discriminating factor in the divergent responses of RPP and PE rats of the same age, and can induce a similar deficiency of response in rats of different ages, possibly through its neurotoxic action on the hypothalamus. The smaller positive steroidal feedback effect in old PE than in young cycling rats five weeks after ovariectomy is ascribed by Lu *et al.* (1981) to an insufficient recovery period after the operation. But proof of a later restoration of the full

magnitude of the neuroendocrine response is presently lacking, particularly since a previous paper (Lu *et al.*, 1977) observed an age-related difference that persisted for 55 days after ovariectomy. This is another example of a condition where direct age effects and those due to a modified steroidal milieu are not yet entirely delineated.

Since there are differential age-dependent responses of LH to ovariectomy, it is not surprising that catecholamine responses also differed in the anterior hypothalamus (Huang *et al.*, 1979) and the median eminence (Wilkes and Yen, 1981). In the anterior hypothalamus, norepinephrine concentration and turnover were lower in old than in young ovariectomized rats, whereas DA concentration did not differ. In the median eminence, ovariectomy did not increase norepinephrine concentration in old rats and the increase in DA concentration was smaller than in young rats.

The amplitude of LH pulses is significantly reduced in old ovariectomized, formerly PE rats (Steger *et al.*, 1979b; Estes *et al.*, 1980). This difference between old and young rats could account for the smaller increase of LH after ovariectomy, possibly mediated by the deficiency of neurotransmitter activity after ovariectomy, or it could result from the higher basal levels of PRL.

In contrast to the above changes, contradictory results were reported on acute hypophysial stimulation by LHRH in old intact or ovariectomized rats, with or without steroidal pretreatment. LH release was similar in old and young ovariectomized rats pretreated with estrogen (Peluso *et al.*, 1977), and in intact old and young rats (McPherson *et al.*, 1977). The peak serum levels of LH were attained later in old rats. According to Lu *et al.* (1980), circulating LH increased similarly after a single LHRH pulse in old PE and RPP rats. In long-term ovariectomized old and young rats chronically implanted with estradiol, LHRH induced a similar rise in circulating LH (Lu *et al.*, 1981). Therefore, hypophysial sensitivity to LHRH was not impaired with aging. However, Watkins *et al.* (1975b) reported smaller LH release in intact old acyclic rats than in young cycling rats, as did Howland (1976) in ovariectomized old vs. young animals. Riegle and Miller (1978), using a single injection of LHRH, confirmed the results of Watkins *et al.* (1975b). The response to LHRH was modified after multiple injections (Fig. 1); the increase in LH was greater after the second and third LHRH injections in old PE and RPP rats, and reached levels similar to those obtained in young controls. Comparison of pituitary sensitivity to LHRH between young and old rats, respectively, in induced (young) and spontaneous (old) PE or RPP may clarify these discrepancies (see Section 2). Interpretation of results on age-related negative and positive steroidal feedback regulation, pul-

satile LH secretion, and pituitary sensitivity to LHRH, remains controversial, and will be considered further in Section 5.1.

4. Relation of Neuroendocrine System to Ovarian Function during Cyclicity and Transition Period

4.1. Aging of Ovarian Function during Cyclicity: Physiological and Structural Changes

The problem is to detect, measure, understand, and if possible, modify age-related changes in ovarian cycles which can be latent, but finally result in qualitative changes such as the SDC. Table 2 illustrates changes in some parameters observed in Wistar rats (Aschheim, 1979). The decrease in the percentage of cyclic rats is accentuated between 10–13 months. During that period, cyclers become the minority of a stable population whose mortality is insignificant. In this strain, there is an important shift between 3–10 months from regular four-day cyclers to regular five-day cyclers. Rats with longer cycles are rather unusual, even in older animals.

In 12-month-old Holtzman rats, six-day cycles appear, characterized by delayed ovulation (Fugo and Butcher, 1971). Preovulatory overripeness of ova results in an increased incidence of developmental anomalies, similar to those seen after experimentally delayed ovulation of young rats. The important hormonal difference with normal cyclers is the earlier increase of plasma estradiol levels (Butcher and Page, 1981). These authors showed that in experimental six-day cyclers, this particular pattern of estrogen secretion was responsible for the detrimental effects on the ova and uterine environment.

In 1976, Van der Schoot described a delayed LH surge with a reduced steepness in 10-month-old–five-day cyclers as compared to 5-month-old controls, but without any significant difference in the numbers of ruptured follicles. Gray *et al.* (1980) confirmed that in four-day cyclers the preovulatory LH surge was significantly lower in rats aged 11 months than in controls aged three months. Cooper *et al.* (1980) showed a relationship between the time of the LH surge and its amplitude. Later surges, as in 10.5-month-old rats, were consistently lower than the normally timed LH surges, as in 4.5-month-old controls.

Other age-related neurohormonal changes have been described at other times of the ovarian cycle. In a study of 13 such parameters at proestrous morning, Wilkes *et al.* (1979) found three modifications: norepinephrine concentration in the median eminence, circulating FSH,

and androstenedione levels were increased in 12- as compared to 6-month-old rats. The significance of these apparently linked changes, which did not persist after transition to PE, is not entirely clear. In our laboratory, Rasolonjanahary (1979) found a three- to four-fold increase in circulating PRL concentration on diestrous morning in five-day cyclers 2–16 months of age, with a first significant rise between 2–3 months of age, a plateau level until after nine months, and another sharp rise around 12 months of age. Prolactin is the only hormone in rats displaying an age-related increase during cyclicity as well as in SDC. The increase of PRL in old cyclic rats may result from the slightly higher estradiol levels reported by Lu and Kledzik (1981), rising earlier and declining later than in young controls. Moreover, estradiol-binding activities in both the hypothalamus and pituitary are greater in old than in young females throughout their cycles (Lu and Kledzik, 1981). Peluso *et al.* (1979) reported that estradiol concentration within the ovary was higher in old irregular than in young regular cyclers during proestrus and estrus.

Changes of cycle length, of estradiol, and of LH secretion patterns and levels may be related to some structural modifications occurring in aging ovaries. Peluso and England-Charlesworth (1981) examined, at the ultrastructural level, the transformation of preovulatory follicles into ovarian cysts which often result from ovulatory delay in old irregularly cycling rats. Peluso *et al.* (1981) also described several interesting ultrastructural alterations which occurred in 50% of the preovulatory oocytes. The most striking anomaly was the deterioration of the stacking arrangements of the cytoplasmic rays, which are composed of RNA. These oocytes could not resume meiosis *in vitro* and showed a reduced incorporation of [^{3}H]uridine into RNA. Are these changes due entirely or partly to intrinsic oocyte aging and/or to the age-related changes in hormonal milieu? Intrinsic oocyte aging may be detected in similar studies on long-term hypophysectomized rats after gonadotropic stimulation. The transformation of interstitial cells into "wheel" cells, reported in Section 3.1.3, already occurs in 13-month-old cyclic rats (Aschheim, 1976) and may be related to the decreased LH surge.

A synthetic view on neurohormonal modifications during cycles in middle-aged rats is not yet possible. It is my belief that the decreased and delayed LH surge and the oocyte alterations are the best established facts, and it is important that they occur only in part of the middle-aged population. The progressive character of aging changes during cyclicity and the latent heterogeneity of apparently homogenous cyclic age groups are better illustrated after adaptation tests (Aschheim, 1979; Table 2). Hemiovariectomy performed at increasing ages initiates an increasing

proportion of SDC (Table 2, third line, represents the inverse expression of the same condition, the persistence of cycles during the month following the operation). After hemiovariectomy performed at 30 days of age, the number of rats displaying ovulatory compensation of the remaining ovary decreases with age (Table 2, fourth line). This forms part of an advanced aging complex (see Section 5.3). The decreasing persistence with age of the estrous cycle after injection of estradiol benzoate at estrus (Table 2, fifth line) is the basis for a biological aging test of ovarian cyclicity (Aschheim, 1974), as seen in the next section. All these adaptation tests demonstrate an age-dependent decreasing stability of ovarian cycles. They raise problems about the individual selection which they induce.

4.2. Aging of the Neuroendocrine Regulation of Cyclic Ovarian Function

4.2.1. An Aging Test of Cyclic Ovarian Function: Mechanism, Applications

The aging test (Aschheim, 1974) is based on the fact that the ability to respond by becoming pseudopregnant due to estradiol benzoate injection at estrus increases with age in cyclic rats. The dose–response curves are homologous for a wide range of injected estrogen. This age-related facility to release PRL in response to estrogen results in an increasing percentage of induced pseudopregnancies (Table 2, fifth line, represents the inverse expression of this condition, the decreasing persistence of cycles after the injection of 5 μg of estradiol benzoate at estrus). A maintained cycle represents a "young" response to the injection, pseudopregnancy an "old" one. After the experimental sequence, normal cycles recur. The aging test measures (a) in the same age group, the latent heterogeneity (the "biological" age) of an apparently homogeneous cyclic population, and (b) a possible modification of biological age by comparison between an experimental and a control group of the same chronological age.

The increased sensitivity to estrogen may act through the increased ability of this hormone to inhibit the production and/or the activity of the hypothalamic PRL-inhibiting factor, by its direct stimulation of the pituitary, or through both mechanisms. The hypophysial response also was investigated *in vitro* (Aschheim and Rasolonjanahary, 1979). Prolactin release was measured by RIA after incubation with or without addition of estradiol to the medium from pituitaries from cycling rats aged 3–14 months. They all released similar amounts of PRL, whereas *in vivo,*

injected estrogen induced 6% pseudopregnancy at 3–4 months and 83% at 12–13 months of age. The aging test thus appeared to act at the hypothalamic and not at the hypophysial level.

Applications of the Test. In addition to retarded aging induced by contraceptive steroids (Section 5.2) and advanced aging due to early hemiovarietomy (Section 5.3), the following experimental conditions have been tested (Aschheim, unpublished results). Four-month-old rats were exposed for 4 months to either constant light, which induced persistent estrus, or the presence of vasectomized males, which induced repetitive pseudopregnancies. After the end of treatment and reinstatement of cycles, the aging test did not show any significant difference in biological age when these 9- to 10-month-old rats were compared with controls. Thus when two experimental disruptions of the cycle, similar to (but perhaps not identical with) those seen in old age were induced for 4 months in adults, they were not followed by a persisting "aging" change of hypothalamic regulation of ovarian function. Sixteen-month-old rats in PE, induced to cycle by hCG, were "biologically old" when tested at the fourth or fifth estrus following the treatment. Reinstatement of cyclicity does not signify rejuvenation of the control mechanism. Multiparous cyclic rats aged 13 months, with a mean of eight previous pregnancies, the last one occurring at 11 months, provided a significantly older test response than nulliparous controls.

4.2.2. Changes in Feedback Regulation and Pituitary Response to LHRH

These changes are generally similar to those described in old acyclic rats (see Section 3.2.2). The post-ovariectomy rise of circulating LH was attenuated at about 12 months of age in regular or irregular cyclers and in recently initiated PE rats (Steger *et al.*, 1980; Gray *et al.*, 1980; Gray and Wexler, 1980; see also our unpublished results in Table 2, seventh line). The negative feedback action of estradiol on LH was maintained or reduced and the positive feedback of estrogen and/or progesterone on LH release was absent or reduced (Lu *et al.*, 1977; Steger *et al.*, 1980; Gray *et al.*, 1980; Gray and Wexler, 1980). As for the rise of FSH after ovariectomy or the positive steroidal feedback on FSH release, an age effect has not yet been detected in these animals (Steger *et al.*, 1980). The pituitary response to LHRH was decreased in ovariectomized, formerly irregularly cycling rats, but was partially restored after steroidal priming (Steger and Peluso, 1979). In intact middle-aged rats which had just become acyclic, the pituitary response to LHRH was not altered in PE, but was decreased in RPP rats (Wise and Ratner, 1980). Like the

biological parameters shown in Table 2, some of the hormonal changes can already be detected at the age of 8–9 months. During aging of cyclic rats, decreasing hypothalamic sensitivity to estrogen feedback on LH release is not inconsistent with increasing hypothalamic sensitivity to estrogen feedback on PRL release, since they could result in a decreased output of LHRH or PRL-inhibiting factor, respectively.

5. Experimental Manipulations of Hypothalamic-Hypophysial-Ovarian Aging

In the past, various experimental approaches contributed widely to a better understanding of the aging problem, e.g., use of heterochronic grafts of ovaries and pituitary, reinstatement of cyclicity in PE rats, etc. As will be shown in this and the next section, such manipulations continue to provide interesting models for further analysis.

5.1. Suspended Aging

At 375 days after hypophysectomy of 40-day-old rats, resumption of ovarian cycles occurred after a successful graft of a young pituitary (Smith, 1963; Table 3). When ovariectomy at 6 or 12 months was followed at 24–27 months of age by graft of young or old ovaries, cycling was restored (Aschheim, 1964/5; Table 3). During the postovariectomy interval, aging of the neuroendocrine regulating system was suspended by lack of steroidal action. Hormonal characterization and testing of the biological age of the restored cycles were not yet feasible in those years. As emphasized in Section 3.1.3, early ovariectomy prevented the development of gliosis in the arcuate nucleus area normally found in old PE rats (Brawer *et al.,* 1980; Schipper *et al.,* 1981).

Very interesting results have been reported recently in an abstract by Elias *et al.* (1979). In old rats, ovariectomized at 2 months of age and studied 22 months later, circulating LH was not maintained at castration levels reached 2 months after ovariectomy, and was no longer secreted in a pulsatile fashion (Table 3). As pituitary stores of LH were not reported, it is possible that there was an age-related dissociation between LH synthesis and release. But it is more likely that the decrease in circulating LH was due to a deficiency of LHRH activity, for stimulation by exogenous LHRH resulted in a normal pituitary response as in young ovariectomized controls. The positive steroidal feedback on LH was also similar in the two groups. The putative deficiency of LHRH activity is

Table 3. Long-Term Effects of Early Hypophysectomy (Hx) or Ovariectomy (Ovx) in Aging Rats

Source	Age at operation	Time elapsed	Results	Interpretation
[a]	26 days + Hx	7–24 months	Proliferation of ovarian epithelial cords; thickening of basement membranes (epithelial cords, testis-like tubes, small follicles)	Intrinsic aging of these ovarian structures
[b]	26 days + Hx	> 11 months	Intramitochondrial inclusions in ovarian insterstitial cells after hCG stimulation	Intrinsic aging seen only after stimulation
[c]	40 days + Hx	12–13 months	+ young pituitary graft: resumption of cycles	Suspended aging during Hx
[d]	6 or 12 months + Ovx	21 or 12 months	+ old or young ovarian graft: resumption of cycles	Suspended aging during Ovx
[e]	60 days + Ovx	22 months	Castration levels and pulsatile fluctuations of LH: not maintained; LH responses to E,[f] E + P[g] and LHRH: maintained	See text, no aging of pituitary sensitivity
[h]	45 days + Ovx	→ 12 months	Decrease of prolactin secretion *in vitro;* stimulation by E, inhibition by dopamine added *in vitro:* maintained	Intrinsic pituitary aging? no aging of pituitary sensitivity

[a] Crumeyrolle-Arias and Aschheim, 1981.
[b] Crumeyrolle-Arias, 1979.
[c] Smith, 1963.
[d] Aschheim, 1964/5.
[e] Elias *et al.*, 1979.
[f] E = estradiol.
[g] P = progesterone.
[h] Rasolonjanahary, 1979.

not necessarily age-dependent in a linear way, since data are available only for 4- and 24-month-old rats, and LH levels may decrease abruptly in middle age and remain constant thereafter.

Prolactin secretion *in vitro* by pituitaries originating from rats ovariectomized at 45 days of age and sacrificed at regular intervals for 11 months demonstrated a significant decrease with age and/or a post-ovariectomy interval (Rasolonjanahary, 1979; Table 3). Since PRL production *in vitro* is considered to be spontaneous, and since the number of secretory cells does not increase after early ovariectomy, the decreased secretion may denote intrinsic cellular aging. But when estradiol or DA was added to the incubation medium, pituitary sensitivity to stimulation or inhibition, respectively, was maintained in all groups. As a rule, endocrinological aging results on the contrary, in maintained basal levels of hormones and deficiencies are observed after adaptive tests.

5.2. Retarded Aging

By addition of L-tyrosine to the diet, starting at 7.5 months of age, Cooper and Walker (1979) reported a significant extension of the period of ovarian cyclicity in rats, which possibly resulted from achieving an appropriate catecholamine to serotonin balance within the central nervous system.

It is well-known that caloric restriction in rodents extends lifespan and retards growth and sexual maturity. Growth and reproduction are resumed after return to *ad libitum* feeding. Merry and Holehan (1979) reported that a particular pattern of chronic dietary resriction (continuous adjustment so that body weight was 50% of that of controls) resulted in enhanced longevity, retarded onset of puberty, and a longer duration of fertility during treatment. Their data demonstrated the persistence of cyclicity for more than 18 months, as compared to less than 12 months in controls. Unless the additional cycles are hormonally abnormal, these two examples do not favor the hypothesis of a strictly programmed cumulative effect of estrogen on the hypothalamus which self-limits cyclicity.

The third example even suggests that the hypothalamus can be desensitized to estrogen. Ethinyl-estradiol, mestranol (5–7 μg/day), or a progestogen (700 μg/day) estrogen mixture was administered orally to rats for 4 or 8 months, starting at the age of 4–5 months (Aschheim, 1975). After cessation of treatment, cyclicity resumed. As compared to controls, there was a high percentage of cyclic rats and a significant decrease of the biological age of these cycles. The delay of aging was maintained for at least 4 months. At autopsy at 16–18 months, experi-

mental rats demonstrated significantly fewer tumorous or hypertrophied pituitaries than controls. When steroid treatments were begun at 10 months of age in cyclic rats, they lost most of their effectiveness. Progestogen given alone did not produce the age-retarding effects. Delayed aging may be explained by a central "desensitization" to estrogen during treatment, counteracting the normally occurring age-related increase in sensitivity to estrogen and subsequent secretion of PRL.

5.3. Advanced Aging

Early hemiovariectomy advances the biological age of the hypothalamic-hypophysial-ovarian axis (Aschheim, 1979). It does so in a very stereotyped way. For several months after hemiovariectomy at 30 days of age, the usual compensatory hypertrophy of the remaining ovary manifests itself through maintained cyclicity, a true ovulatory and luteal compensation (10–12 eggs and fresh corpora lutea), and a resulting increase in ovarian weight. But progressively functional compensation ceases in an increasing number of rats (Table 2, fourth line) and the nature of the persistent gain in ovarian weight changes. Cycles are maintained, but the ovary ovulates no more than 5–8 eggs and shows a similar number of fresh corpora lutea. The maintained ovarian weight increase is now due to an increased persistence of old corpora lutea. A very close correlation exists between results of the aging test and ovulatory compensation. Rats with a "young" test ovulate 10–12 eggs, whereas those ovulating only 5–8 eggs give an "old" test result. At 11 months of age, the experimental group is significantly "older," according to the aging test, than the control group of intact rats. At 12 months of age, an important shift from ovarian cycles to PE occurs in the hemiovariectomized rats which is advanced by 4 months as compared to intact rats. The mechanism of this interesting model for advancing reproductive aging is presently unknown.

6. Extrinsic and Intrinsic Aging

Hypophysectomy (Hx) in early life isolates the ovary from the hormonal imbalance normally induced by aging of the neuroendocrine regulatory centers. Hx may thus produce experimental conditions allowing primary aging of ovarian structures to emerge. Jones and Krohn (1961), for example, reported that Hx in mice clearly retarded but did not abolish oocyte loss with time. Crumeyrolle-Arias and Aschheim (1981)

reported on post-Hx ovarian senescence compared to the structural ovarian changes observed in intact aged rats (see Section 3.1.3). Most of the ovarian changes in intact old rats were due to extraovarian aging. For example, in old rats Hx when young, proliferation of the surface epithelium or phagocytes filled with lipofuscin granules was lacking in the ovaries. Intrinsic aging was restricted (Table 3) to thickening of basement membranes and proliferation of epithelial cords (whereas their induction was of extraovarian origin). Ovarian interstitial cells remained in a deficient condition after Hx, without alterations due to aging, and they could be restored in old age by hCG treatment (Crumeyrolle-Arias, 1979). However, the morphological reactivation was submaximal compared to results obtained earlier in life, and at 12 months of age and later, hCG induced large unusual mitochondrial inclusions. The physiological significance of intrinsic aging changes in both Hx and intact animals, such as proliferation of epithelial cords observed at 12 and 22 months of age, respectively, is probably unimportant since these old ovaries can function in a cyclic manner when grafted into young ovariectomized rats.

The decreased secretion *in vitro* of PRL cells originating from old rats, ovariectomized when young, has already been discussed in Section 5.1. Provided that it expresses intrinsic hypophysial aging, it is completely masked in SDC of intact rats by proliferation of PRL cells and decreased hypothalamic PRL release-inhibiting factor (PIF) activity, resulting in increased PRL secretion.

Recently observed hypothalamic alterations (Section 3.1.3) are by no means necessarily intrinsic. In Chapter 5, Peng demonstrated that aged males do not display the neuronal loss observed in several hypothalamic nuclei of females. It would be interesting to investigate the neuronal loss in the hypothalamus of old females that are ovariectomized when young. On the other hand, the neurotoxic effect of estrogen on the hypothalamus, leading to a chemical deafferentation in PE, is lacking in old ovariectomized rats.

It is paradoxical that intrinsic aging changes are exhibited by proliferation of ovarian cellular cords which do not play any discernible role in aging of ovarian function or of its central regulation, whereas aging changes in steroid-sensitive hypothalamic neurons do not presently provide evidence for their intrinsic nature. Moreover, it is clear that such a system assuming a regulatory function, can express aging only when it is responsive to stimuli and thus is necessarily exposed to extrinsic influences. One may speculate whether investigation of intrinsic aging changes in such neurons is not meaningless despite their postmitotic condition, and whether intrinsic aging *in vivo* is not limited to cells (or subcellular compartments) with terminal activity, without any feedback.

7. Conclusions

Considering what is known of the relation of the neuroendocrine system to the reproductive decline in female rats today, I would propose the following conclusions:

(1) Aging of the hypothalamic-hypophysial-gonadal axis differs in female and male rats. Neuronal loss and functional impairment of residual neurons are obvious in some steroid-sensitive areas of the hypothalamus in females; no neuronal loss is observed in old males (see Chapter 5, by Peng). There is an age-related decrease of hypothalamic sensitivity to negative estrogen feedback in females, but an increase of sensitivity to testosterone in males (see Chapter 8 by Steger and Huang).

(2) The reproductive aging pattern of female rats is common to all investigated strains, but differs from those observed in other rodents, including mice (see Chapter 9 by Finch), particularly the CBA strain.

(3) Reproductive aging is characterized by a persistent, but modified ovarian function displaying senile deviations of the estrous cycle. A common factor of SDC is their age-related increase of circulating PRL. This implies alterations in hypothalamic sensitivity to control input (hormonal or environmental) and changes in neurotransmitter activity. There is no doubt that the predominant factor responsible for the reproductive decline of female rats is aging of the neuroendocrine regulatory mechanisms. Some of these hypothalamic aging changes have recently been described. Hormonal input, especially estrogen, modulates these changes, but this does not mean that the starting-point of aging is in the ovary. In this respect, the results of heterochronic ovarian transplantation experiments remain conclusive. The influence of estrogen on hypothalamic aging is varied, depending probably on the pattern of exposure to the hormone. Estrogen removal by ovariectomy suspends aging, whereas administration of ethinyl-estradiol can be desensitizing and is then followed by a delay of aging. However, estrogen can also become neurotoxic, hastening PE by selective hypothalamic deafferentation.

(4) The deficiency of hypothalamic control mechanisms awaits further analysis at a cellular and subcellular level. It accounts for the plasticity of reproductive aging in rats, the facility of its experimental manipulation, the reversibility of SDC, and the early changes already occurring during ovulatory cycles which can be detected by a biological aging test. There are critical periods for aging in adulthood, just as there are critical periods for development at about the time of birth or puberty. Neuroendocrine aging is a "historical" process, recording and/or integrating past events.

Therefore, longitudinal investigations are meaningful, and hormonal snapshots of old age are not.

(5) Oocyte depletion in rats displays a very unusual "aging" pattern. Instead of exhibiting an increasing level of vulnerability with age, or at least a constant one as in mice, the proportion of oocytes lost per unit time decreases with age! Verification of this puzzle is needed.

ACKNOWLEDGMENTS. I am grateful to N. Breugnot for help in typing this manuscript and to J. Meites for correction of deficient English. Experimental work in this laboratory was supported by grants from INSERM and Faculté de Medecine, Paris Ouest, Université René Descartes.

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