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53. We thank J. Campisi, H. Warner, and V. Lundblad for critical reading of this manuscript and P. Love for secretarial assistance.

Menopause: The Aging of Multiple Pacemakers

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Menopause signals the permanent end of menstrual cyclicity in a woman's life. Its impact reaches far beyond just the reproductive system. An understanding of the factors that interact and govern the process of aging in the reproductive system will help us to develop strategies for alleviating the negative aspects of menopause and may help us to better comprehend the process of biological aging.

Current interest in the natural decline of fertility in middle-aged women and the onset of menopause is a result of three converging areas of understanding. First, the average life-span of humans has increased during the past 100 years. Thus, an increasing proportion and a larger total number of women will live a larger fraction of their lives in the postmenopausal state. Currently, 35 million American women are postmenopausal and over a million enter menopause each year (*1*). The dramatic and fairly rapid endocrine changes brought about by menopause have biological, societal, and cultural implications that profoundly influence the latter half of a woman's life. Therefore, a better understanding of the consequences of prolonged exposure to low estrogen levels, as occurs in menopause, is increasingly important to our society.

Second, over the past decade the perception that estrogen is merely a female reproductive hormone that influences predominantly classical reproductive tissues, such as the hypothalamus, anterior pituitary, mammary glands, uterus, and vagina, has changed considerably. We now are beginning to appreciate the fact that estrogen also affects a number of other functions, including urinary continence (*2*), nutrient absorption and metabolism (*3*), bone and mineral metabolism (*4*), blood pressure and cardiovascular function (*5*), memory and cognition (*6*), organization and expression of daily rhythms (*7*), and the progression of age-related diseases (*8, 9*). Thus, the cessation of menses and the resulting hypoestrogenicity affect multiple physiological systems and can lead to significant morbidity in later life. Finally, because menopause occurs relatively early during the life-span of many mammalian species, the female reproductive system serves as an excellent model system in which to study the aging process in the absence of confounding pathological changes that complicate many gerontological studies.

By the time a woman is 65 years old, the ovary is virtually devoid of follicles (*10, 11*) and is no longer the primary site of estradiol or progesterone synthesis (*12*). Also, inhibin, a glycoprotein that is synthesized in granulosa and luteal cells of the ovary and that selectively suppresses follicle-stimulating hormone (FSH) secretion, becomes undetectable in the blood (*13*). In response, the anterior pituitary gland secretes copious amounts of both of the gonadotropins FSH and luteinizing hormone (LH). Amounts of FSH increase by the time women are 45 to 50 years old, while they are still menstruating, whereas amounts of LH increase later, when women are postmenopausal. In addition, concentrations of the hypothalamic releasing hormone, gonadotropin-releasing hormone (GnRH), in the mediobasal hypothalamus are low, perhaps because of prolonged high levels of release and decreased synthesis (*14*).

For many years, the prevailing view was that menopause resulted from an exhaustion of ovarian follicles. More recently, the relative contribution of the ovaries and the hypothalamic-pituitary unit in the menopausal transition has been intensively debated. One view maintains that the impending exhaustion of the pool of growing ovarian follicles triggers the menopausal transition: the hypothalamic-pituitary changes that accompany menopause are a consequence of compromised ovarian function. The alternative perspective is that age-related changes in the central

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nervous system are the driving force that initiates the menopausal transition: the exhaustion of ovarian follicles is a consequence of the altered temporal organization of neural signals. In this review, we discuss the cogent arguments on both sides of this controversy [for comprehensive reviews, see (*15–18*)].

To understand the pacemakers that trigger the transition to the menopausal state, investigators have examined the period before permanent acyclicity is established, that is, the fourth decade of life in women or the equivalent stage in experimental animal models. Long before the follicular reserve is exhausted, fertility and fecundity decrease markedly, reproductive cycles become increasingly irregular in length, and patterns of gonadotropin secretion are altered (*18*). We investigated here what critical variables control these changes.

An Abundant But Nonrenewable Reserve of Follicles in Ovaries

Some investigators maintain that there is "little doubt that ovarian exhaustion of follicles is the pacemaker of reproductive senescence in women" (*18*, p. 1241). If this is the case, then the two critical factors are the number of follicles in the initial endowment and the steps that regulate the rate of loss of this stockpile of primordial follicles. Little is known of the factors that determine the size of the initial primordial follicle pool. In mammals, during early fetal development germ cells proliferate outside of the embryo proper and migrate into the indifferent gonad, stop dividing, enter meiosis, then become organized into primordial follicles (germ cells surrounded by pregranulosa cells). At birth, female mammals are endowed with a vast, but finite, nonrenewable, and postmitotic reserve of dormant follicles that will later be recruited to grow, differentiate, and acquire the ability to synthesize estradiol.

Coincident with the initiation of meiosis in these cells, large numbers of germ cells undergo atresia. In fact, most follicles are destined to die early during development and will never differentiate into Graafian follicles nor undergo ovulation [for review, see (*19*)]. Virtually nothing is known about the factors that regulate proliferation, that govern the organization of cells into discrete follicles, or that influence the extent of cell loss during the early embryonic stage.

Many believe that the rate of loss of the follicular pool during postnatal life is determined by the number of dormant follicles that reawaken and are recruited into the growing pool. This number, in turn, is determined by the number of follicles that remain in the ovary (*20*). Some investigators believe that an enormous excess of follicles is required to supply a constant stream of follicles for the developmental pipeline. When the number of follicles falls below a critical number, fine-tuning is lost, the number of follicles that enter the growing pool increases, the number of ova ovulated during each estrous or menstrual cycle and the length of time between cycles becomes more variable, and as a consequence fertility declines (*21, 22*). Interestingly, the logarithmic loss of follicles is not linear throughout the reproductive life-span (*11, 23*). Instead, there is a doubling of the rate of loss of follicles in middle-aged women around the age of 35, when about 25,000 primordial follicles remain and, coincidentally, when perimenopausal symptoms begin to appear. It is possible that approximately 25,000 follicles is the "critical number" below which fine-tuning is lost. In chronologically matched, middle-aged women, those that exhibited irregular menstrual cyclicity had only a tenth of the follicles in their ovaries that women who continued to cycle regularly had (*11*). There is a similar relation between imminent oocyte exhaustion and the transition to acyclicity in mice (*22*).

Changing patterns of FSH secretion have been interpreted to indicate altered follicular growth. FSH is clearly a key regulator of the exponential growth and terminal differentiation of follicles, although many other growth factors interact with FSH to recruit the follicles to undergo ovulation and to ensure their final maturation [for a review, see (*24*)]. It remains controversial whether these same factors regulate the reentry of dormant primordial follicles into the growing pool and to what extent they regulate the early slow-growth phase. It is also unknown whether altering the later stages of rapid growth and final differentiation influences the reawakening of the remaining follicles in the stockpile or the initial stages of the growth of small follicles.

Nevertheless, knowledge about whether and why FSH secretion changes during menopause may help us to understand the sharp decrease in the follicular pool that occurs before menopause. Particularly during the early follicular phase, plasma concentrations of FSH are elevated in middle-aged women who continue to cycle (*25–27*), before any detectable change in LH secretion. Similar elevations in FSH concentrations have been observed in middle-aged rats (*28*). The cause of the rise in FSH concentrations has been studied in both humans and laboratory animals. In many instances, the change in FSH levels is paralleled by diminished levels of plasma estradiol (*25*) and inhibin (*27, 29, 30*). In addition, when given clomiphene and human menopausal gonadotropin in preparation for in vitro fertilization, women over 35 years of age revealed lower inhibin responses, which predicted lower success rates of pregnancy (*31*). These findings have led investigators to conclude that changes in FSH reflect "diminished folliculogenesis as age progresses and the numbers of primordial follicles in the ovary as the menopause approaches" (*27*).

Researchers have tested the hypothesis that a diminished number of primordial follicles in the reserve alters the dynamics of follicular reawakening and growth. If the size of the primordial follicle endowment determines the duration of a woman's fertile period, then a dramatic decrease in the endowment by surgical manipulations—such as unilateral ovariectomy or pharmacological manipulations that decrease the follicular reserve—should hasten the onset of reproductive decline. In support of this concept, unilateral ovariectomy accelerates the onset of irregular cyclicity, increases the loss of primordial follicles, and decreases the total number of possible offspring (*32–35*). Interestingly, unilateral ovariectomy only hastens the loss of primordial follicles in old rats and not in younger animals and is correlated with elevated FSH concentrations (*33*). When rat embryos are exposed to busulfan, a drug that destroys primordial germ cells in the embryos, there is an inverse correlation between the number of primordial follicles in the ovary at birth and the rate at which they begin to grow (*36*). At the highest dose, all of the remaining follicles grow at an earlier time in life than normal, exhausting the follicular reserve during the prepubertal period. Together, these observations suggest that reduction of the number of follicles in the ovarian pool, which occurs normally during middle age, disrupts the dynamic equilibrium between the dormant and growing pool of follicles, causes alterations in the regularity of cycles, and compromises feedback to the neuroendocrine axis, leading ultimately to menopause.

The Hypothalamus, Follicle Development, and the Ovulatory Surge of LH

Some investigators maintain that the hypothalamus is the pacemaker that initiates the cascade of events leading to menopause. They contend that alterations in the pattern of neurochemical signals that govern the secretion of GnRH, LH, and FSH may cause the increased rate of depletion of follicles seen during middle age as well as after unilateral ovariectomy in older rodents. Intriguingly, changes in the hypothalamus—as measured by the incidence of

hot flashes, sleep disturbances, and changes in the pattern of gonadotropin secretion—become apparent in women when they are between 35 and 40 years old, at the same time as the rates of follicular loss increase.

Therefore, the acceleration of follicular loss may not reflect the need to maintain a threshold number of follicles in the reserve, but rather a change in the pattern of neuroendocrine messages that govern the dynamics of follicular reawakening, recruitment, growth, and differentiation. In addition, elevated amounts of FSH are not always temporally correlated with decreased estradiol or inhibin levels in individual women (*25, 37, 38*). Thus, a simple decrease in the levels of ovarian steroids or peptides cannot always explain changes in gonadotropin secretion.

The pattern of pulsatile GnRH secretion from the hypothalamus during all stages of the menstrual and estrous cycle determines the secretory patterns of LH and FSH. Because hypothalamic releasing hormones cannot be detected in peripheral plasma, LH and sometimes FSH have been used as indirect indices of GnRH secretion; the frequency of LH pulses is assumed to be a faithful mirror of GnRH pulse frequency. In middle-aged, regularly menstruating women, the frequency of LH pulses decreases and the width of the peak increases during the follicular phase, before any change in the amount of plasma estradiol (*39*). These changes are accompanied by elevated FSH concentrations during the early follicular phase.

These data are particularly provocative for what they may tell us is happening in the neuroendocrine axis. Changes in the frequency of LH pulses and broadening of the width of the pulses usually reflect imprecision in the hypothalamic GnRH pulse generator as a result of the gradual uncoupling of the generator from the neurochemical factors that coordinate GnRH secretion. One of the first signs of the impending transition to menopause is elevated FSH levels during the follicular phase, as observed here. Such elevated levels have been assumed to indicate changes in ovarian estradiol and inhibin feedback. However, decreased frequency of GnRH pulses (*40, 41*) or lowered concentrations of GnRH (*42*) can lead to a preferential increase in the release of FSH compared to the release of LH. Therefore, increases in FSH levels do not necessarily reflect changes in ovarian function.

Parallel changes have been reported in rodent models. Middle-aged rats with regular estrous cycles exhibit alterations in the preovulatory pattern of LH and FSH secretion (*28, 43–46*). In addition, alterations in the steroid-induced surge (*46*), where estradiol was clamped to equivalent levels in both young and middle-aged rats, suggest that changes in the steroidal milieu do not account for the differences in the patterns of gonadotropin secretion. Changes in the ultradian frequency of pulsatile LH secretion also occur in ovariectomized rats (*47*).

GnRH secretion is regulated by multiple neurotransmitters and neuropeptides [for a review, see (*48*)]. During middle age, the precise, synchronized, and interactive patterns of hypothalamic neurotransmitter and neuropeptide activity, which are critical for the maintenance of a specific pattern of GnRH secretion, become less ordered. First, hot flashes, a hallmark of the deterioration of the hypothalamic thermoregulatory centers, occur in normally cycling women during the fourth decade, when more than 25,000 follicles should be present in the normal ovary (*4, 49*). Second, in rodent models, changes in neurotransmitter activity, neurotransmitter receptor densities, and levels of mRNAs that encode for GnRH-regulating neuropeptides [for a review, see (*17*)] occur during middle age, when animals still exhibit regular estrous cyclicity. Some researchers propose that this deterioration in communication among the neurotransmitters that regulate GnRH secretion causes the initial changes in patterns of gonadotropin secretion and that these changes herald the imminent transition to the perimenopausal state. The recurring and pervasive feature of many of these neurochemical alterations is that they are a result of perturbations in the daily rhythmicity of these parameters (*46, 50–52*).

The finding that the rhythmicity of so many neurotransmitters changes with age has led to the hypothesis that the deterioration of the biological clock underlies these desynchronizations. The suprachiasmatic nuclei (SCN) of the hypothalamus are the master circadian pacemakers, or biological clocks, in mammals. This bilateral dense cluster of neurons exhibits inherent 24-hour rhythmicity. Neurons from the SCN communicate extensively with each other, arborize broadly to many regions of the brain, and drive the timing of multiple outputs so that almost all physiological functions show a pervasive daily rhythm. Female reproductive cyclicity is fundamentally grounded on a circadian foundation. This is most evident in rodents that are maintained in controlled laboratory conditions (*53, 54*).

But even in human populations, where activity, light-dark cycles, and sleep are not rigorously controlled by experimental protocols, reproductive functions exhibit a diurnal rhythmicity (*55–58*). Several neuropeptides that are abundantly expressed in the SCN and whose rhythmicity is critical to coordinated clock function send projections to GnRH neurons (*59, 60*) and may communicate temporal information to the reproductive axis. A fundamental deterioration in this neural pacemaker or the coupling to its outputs may initiate the gradual disintegration of the temporal organization of neurotransmitter rhythms that are critical for stable, precise, and regular cyclic LH secretion. This deterioration may initiate a cascade that leads to the transition to irregular cycles and ultimately contributes to acyclicity (see Fig. 1). Indeed, changes in the daily rhythms of several parameters in the SCN of middle-aged rats occur while they are still cycling, including changes in α_1 adrenergic receptors (*51*), monoamine turnover rates (*52, 61*), local cerebral glucose utilization (*62*), and the ability of light to induce Fos expression (*63*). Furthermore, the expression of the neuropeptides of the SCN that are in neurons projecting to GnRH neurons decreases with age, and manipulations that mimic these effects result in changes in LH secretion that are like those found in aging organisms (*64, 65*).

The possibility that the biological clock itself or its coupling to an array of outputs may deteriorate with age is bolstered by numerous reports that multiple circadian rhythms are compromised in aging organisms: the period of rhythms decreases, the

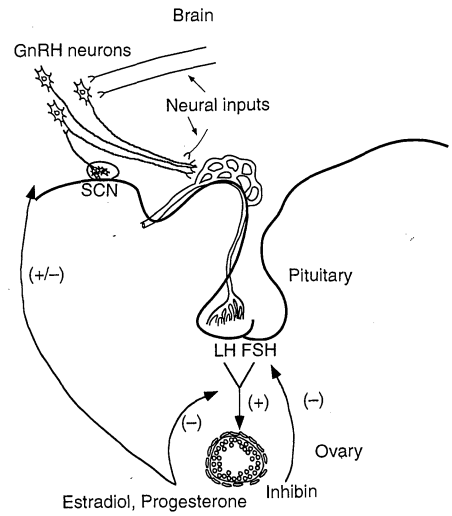

Fig. 1. Schematic diagram of the relations between the brain, pituitary gland, and ovary. GnRH neurons are influenced by neural inputs from several hypothalamic and extra-hypothalamic regions of the brain. Among these, neural communication from the SCN synchronizes GnRH secretion according to daily environmental cues. The pattern of GnRH secretion is also influenced indirectly by ovarian steroidal input. GnRH regulates the pattern of LH and FSH secretion from the anterior pituitary gland, which in turn regulates the terminal differentiation of the ovarian follicles and secretion of estradiol, progesterone, and inhibin. Data suggest that multiple changes occur at the level of the brain and ovary and initiate the menopausal transition (*17*).

phases of many outputs of the clock advance, and the amplitude of multiple rhythms is attenuated [for review, see (66, 67)]. In addition, there is an increased incidence of temporal desynchronization of two or more rhythms, fragmentation of circadian rhythms, and altered responsiveness to stimuli that induce phase shifts. Thus, declining reproductive function may be only one of many physiological endpoints to be affected by the fragmentation of the temporal organization of physiological functions.

The evidence that both the ovary and the brain are key pacemakers in menopause is compelling. We probably should not debate which component of the reproductive axis deteriorates first. It is likely that multiple redundant pacemakers govern the precise orchestration of physiological, cellular, and molecular events that weave together and lead to reproductive cyclicity. Instead, our goal should be to better understand the constellation of factors that interact to maintain regular reproductive cyclicity and how this precise dynamic balance changes with age. In so doing, we will come to understand the complex fabric of the functioning system and the multiple triggers that lead to reproductive decline.

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The Aging Immune System: Primer and Prospectus

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Changes in T lymphocyte populations underlie much of the age-related decline in the protective immune response. Aging leads to the replacement of virgin T cells by memory T cells and to the accumulation of cells with signal transduction defects. Studies of antibody gene assembly, accessory cell function, post-thymic T cell development, skewed selection of T cell receptor repertoire, and the clinical concomitants of immune senescence will shed new light on the causes and consequences of age-dependent immune failure.

Like a citadel astride key trade routes, an immune-system perspective gives a commanding view of both basic and applied gerontology. Immune cells offer powerful models for study of how aging affects gene expression, cell communication, and homeostatic regulation; few other multicellular systems perform so well in vitro after dissection and reassembly. Immune senescence also attracts intervention-minded geriatricians seeking to protect elderly patients from infection and, perhaps, from neoplasia. The past two decades of research have produced consensus in some areas, confusion in others, and provocative findings that are worth further pursuit. Figure 1 depicts some components of the immune system that exhibit age-dependent alterations.

The immune system comprises many cells with distinct functions, and the challenge to the immunogerontologist has been to map age-associated changes in immune responses to underlying cellular alterations. Aged people and rodents show declines in

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