Chapter 19

AGING IN THE HYPOTHALAMIC-HYPOPHYSEAL OVARIAN AXIS IN THE RAT

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SUMMARY

Normal functioning of the hypothalamic-hypophyseal ovarian axis which is seen in the succession of estrous cycles does not cover even the first half of the total life duration in a population of rats. Senile deviations of the cycle, in the form of permanent estrus and the repetitive pseudopregnancies, occupy the major part of the life time. There is no cessation of either ovarian function or its hypothalamo-hypophyseal control, but there is modification of the mode of central regulation. The ovary is not primarily responsible for this senile change; we have no arguments in favor of a primary role for the hypophysis, although we cannot formally dismiss it. On the other hand convergent facts point to a primary responsibility of the hypothalamic areas which control the gonadotropic function of the hypophysis. The senile change manifests itself first in an overall hypersensitivity of these hypothalamic areas to estrogen. It becomes evident, beginning with adulthood in cyclic rats, by a growing aptitude to react by pseudopregnancy to a decreasing external estrogenic stimulation. This fact is used as the basis of a test for aging. Later on, this hypersensitivity leads to a hypofunctioning of the hypothalamic centers which regulate tonic LH secretion. This is followed by the senile deviations of the cycle caused by inadaptation to the environmental modifications previously compensated for and to their neuro-endocrine expression. The senile deviations of the cycle are for the most part reversible. The aging of the hypothalamic-hypophyseal ovarian axis can be advanced by early hemicastration. It is suspended during castration or hypophysectomy, followed by the replacement of the removed organ, that is to say, during the cessation of the steroid information of ovarian origin. It is delayed after chronic treatment with estrogen or estrogen-progestin.

INTRODUCTION

Various dialectic approaches to the study of the senile ovary have been followed during the past hundred years (Waldeyer, 1870). One concerns

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the double ovarian function, the external or reproductive, and the internal or hormonal function. How do they age? Simultaneously or differentially? Independently or not? Another dilemma is presented by comparative physiology: the menopause, the cessation of the double ovarian function, and the structural regressions that occur is a phenomenon which has been progressively recognized as being limited to humans. What is it that replaces the menopause in animals? Is there nevertheless a common process underlying the ovarian aging in humans and animals and, if so, what are its limits? One other problem is that of comparative sexual senescence in the male and the female.

Depending on the period, emphasis has been placed on one or the other of these different aspects.

The first phenomena investigated were the *morphological modifications of the human ovary* after the menopause, as well as the *diminution with age in the reproductive performance* of different species of animals.

At the time of the "explosive" period of sexual endocrinology (since 1920) and up to 1945, the *hormonal aspect* (hypophyseal and gonadal) has been considered most important. The understanding of the human menopause has undeniably benefitted from this, but the therapeutic deductions drawn, with a view to a general "rejuvenation" on a gonadal basis, have partly discredited endocrinology. Its true place was thus restricted at the very moment when the basis of experimental gerontology was being laid (see respectively Korenchevsky, 1961 and Chap. 9 of Verzár, 1963). From this time, date the first morphological studies (Romeis, 1931; Wolfe, 1938, 1943) and experimental data (Zondek and Aschheim, 1927; Hoffman, 1931) on hypophyseal-ovarian relationships in senile rodents.

The diminution in the number of oocytes with age has then been the dominant preoccupation for about 15 years. This oocyte "depletion," an incontestable fact in all mammals studied, has become for many authors the central event in the aging of the ovary (Thung, 1961). The excesses of the "hormonal" period have been succeeded by the dogma of the primacy of the egg. This position clashes with the following facts: "The decline in oocyte numbers is best described by an equation which implies that a constant proportion (and not a constant number) of the population is lost per unit time. This finding implies further, that oocytes maintain a constant level of vulnerability. They do not behave, therefore, like a classical aging system which should become increasingly vulnerable with advancing age" (Krohn, 1967a). The greatest loss of oocytes comes about in the stage prior to puberty. The regression line representing the decrease of oocytes in relation to age never reaches zero till after death in most of the species studied (except for some strains of mice). Furthermore, it is known that after irradiation of the ovaries with x-rays or grafting of

ovaries preserved by refrigeration, the endocrine function persists for a certain time, even if it is modified, in the presence of a small number of follicles or even in their absence. The intrasplenic ovaries after tumorous transformation, which produce steroids, are equally devoid of oocytes.

For the last 15 years the new impetus to the study of the senile ovary has come from *neuroendocrinology*. This is why this chapter is entitled "The Aging of the Hypothalamic-Hypophyseal Ovarian Axis in the Rat," and is found in a work devoted to the relation of the hypothalamus and pituitary to senescence. We will in fact study *the aging of a system of regulation,* the one that controls the gonadotropic activity of the hypophysis in the female. In all the classical treatises on sexual endocrinology (Young, 1961; Chester Jones and Ball, 1962; Rowlands and Parkes, 1966) the need for such a study is stressed when the function of senile gonads is discussed. From the experiments of Krohn (since 1957), of Lipschütz, *et al.* (1963, 1965) with the mouse, from observations of Bloch and Flury (1959, 1961) of Mandl and her group (from 1958 to 1961) on the rat, there emerges the notion of an extraovarian, hypophyseal factor "responsible" for ovarian aging.

Since 1961 my own work has implicated the hypothalamus in this process, and thus completes the regulatory circuit by introducing a "postmitotic" element (the hypothalamic neurons do not divide). New perspectives result therefrom:

1. the hypothalamus is at the center of the regulatory system under study,
2. the aging of its nonrenewable neurosecretory cells is certain,
3. it manifests itself by a modification of the sensitivity of the specialized neuronal areas to the circulating steroids,
4. the hypothalamus occupies a central position in other regulatory functions (hormonal or other),
5. their aging, which is characterized above all by an increasing inadaptability, is said to be differential. If that is the case, how does it work inside the same regulatory organ, the hypothalamus? Are there correlations in the senescence of its different functions of control?

The information furnished by the vaginal smear will act as a leading thread for our study. The *estrous cycle and its senile deviations* reflect, through the ovary, the activity of the follicle-stimulating hormone (FSH), the luteinizing hormone (LH) and the luteotropic hormone (prolactin) of the hypophysis and the hypothalamic control over this activity. The division of our aging animals into cyclic rats, those in permanent estrus (PE) and those in repetitive pseudopregnancy (RPP), with each category corresponding to a different and well defined neuroendocrinological situa-

tion, has created a rational basis for experimental analysis compared with most of the earlier work. We will analyze and summarize these data in the first part of this chapter. The second part will specify the localization, the characteristics and the nature of the senile disturbances of the estrous cycle. The third part will deal with the temporal shifts of the onset of the deviations and the biological measure of the aging of the function under study.

We will not discuss the subject of senile sterility raised in the next chapter. It has been considered in a number of recent publications (Aging and Reproduction, *Journal of Reproduction and Fertility,* Suppl 12, 1970).

THE ESTROUS CYCLE AND ITS VARIATIONS IN AGING RATS

Numerical Data

Fifty percent of our Wistar rats alive at 1 month reach the age of 24 months. The extreme longevity of some animals is 36 to 40 months. These figures are comparable with those of Verzár (1963).

With increasing age the estrous cycles of 4 or 5 days have an increasing tendency to become irregular and are replaced from the age of 12 to 15 months on by two senile deviations of the estrous cycle: the senile permanent estrus (SPE) and the senile repetitive pseudopregnancy (SRPP). Table 19-I gives the percentage distribution of these modalities as a function of age. It combines two different longitudinal studies (nulliparous from 6 to 12 months and multiparous from 15 to 27 months). In cross-sectional studies over a period of 10 years we have repeatedly found a similar distribution.

Table 19-I shows that SPE predominate in the second year and SRPP during the third year. The cyclic females represent between 5 and 10 percent after the age of 18 months. The passage from one state to another cannot be deduced from the Table because it does not take into account mortality, which is noticeable after 12 months. In most cases the cyclic rats undergo only one transformation to a terminal state of either SPE or SRPP. The passage from cycles to SPE and then to SRPP is rare, but unquestionable and interesting for it implies the apparently spontaneous restoration of the ovulatory mechanism suspended furing PE. The "cycles" which oc-

TABLE 19-I

PERCENTAGE DISTRIBUTION OF ESTROUS CYCLES, PERSISTENT ESTRUS, AND REPETITIVE PSEUDO-PREGNANCIES IN RELATION TO AGE

Age in months	6	10	12	15	20	23	27
Estrous cycles	89	72	60	40	8	8	8
Persistent estrus	7	9	20	40	64	52	20
Repetitive pseudo-pregnancies	4	19	20	20	28	40	72

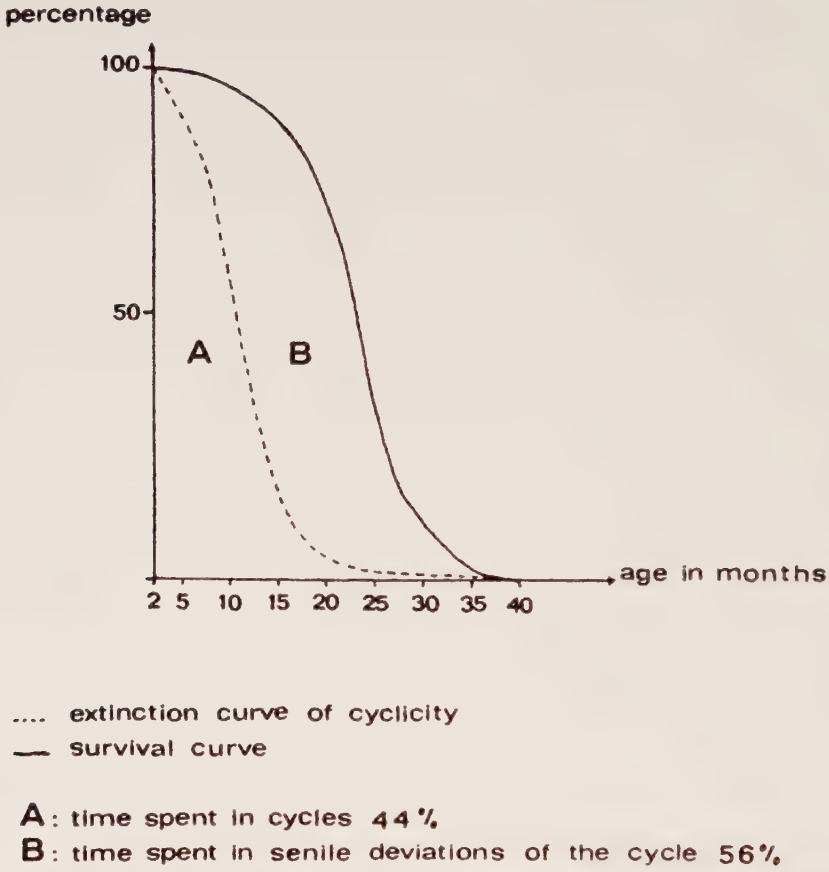

Figure 19-1. Time spent in estrous cycles (A) or in senile deviations of the cycle (B) by a rat population. Curve on left: percentage of cyclic rats. Curve on right: percentage of surviving rats.

casionally follow a PE are mostly anovulatory and aluteal; but again there are a few rare ovulatory cycles. These are more frequent in other strains of Wistar rats, which have a higher rate of PE at 10 to 14 months, but which only stabilize 4 to 6 months later. Everett (1970) had available a strain of rats (DA) almost all of which had PE at 6 to 7 months of age. Our proportions of SPE are comparable to those given by S. Bloch (1959). Mandl (1961) reports a greater number of cyclic rats in the senile multiparous than in the nulliparous group.

Figure 19-1 is a diagram applicable to our nulliparous rats. The coordinate and two curves, on the right the percentage of rats surviving, on the left the percentage of cyclic rats, adjusted for the mortality, define two areas. One can see that after the age of puberty (about 2 months) and up to the disappearance of the population, the total time spent by rats in

estrous cycles (surface A) is less than that spent in senile deviations of the cycle (surface B), 44 percent as opposed to 56 percent. This is a graphic justification of the study of the senile period.

Description

In this histophysiological description of the estrous cycle and its senile deviations, we will mention the *experimental* facts only in as much as they serve to identify the different categories.

Senile cyclic rats. The estrous cycles of aging rats are often more irregular than those of young adults. Their duration can range from 4 or 5 days to 6, 7 or 8 days, either from a lengthening of the periods of estrus or from a lengthening of the diestrus. The ovary has a mean weight of 30 to 40 mg. The number of eggs ovulated and corpora lutea formed is normal (Aschheim, not published), in spite of the diminution of oocytes which has been thoroughly studied by Mandl and Shelton (1959). Follicles and cyclic corpora lutea are comparable to those of adults. As regards the interstitial tissue which shows morphological and functional signs of senescence, we will study it later for all of the senile categories. The appearance of the genital tract is normal and the mammary glands are neither proliferated nor secreting.

Rats in SPE or in SRPP experimentally reset in estrous cycles show the same histophysiological picture as the spontaneously cyclic senile rats.

Senile permanent estrus (SPE). This has been known for a long time (see the bibliography in Bloch and Flury, 1959). It is characterized by a persistent cornified smear. One can assume that there is permanent estrus when one recognizes this cytological aspect in 80 to 90 percent of the smears taken on successive days. The polynuclears which appear from time to time among the cornified cells can be due to a uterine inflammation, but sometimes there are also "pseudocyclic sequences" (Everett, 1939) without ovulation.

Permanent estrus is an anovulatory state. The ovaries are quite small (mean weight of 20 to 30 mg per ovary), devoid of corpora lutea but with healthy and atretic follicles of every size. There are always a few ripe follicles, sometimes cystic ones. The interstitial cells are qualitatively similar to those of other senile categories but occupy a relatively more important place, due to the absence of corpora lutea. The biochemical study of the steroids produced has been effected only *in vitro*. According to Weisz and Lloyd (1965) there is in these PE ovaries an increase in the conversion of labeled progesterone into estrogen and an accumulation of testosterone and androstenedione. The genital tract may be the seat of inflammatory phenomena. The uterine horns are stimulated by estrogen, but are not turgescent. The vagina is cornified. The mammary glands are hypertro-

phied and secreting (Aschheim, 1962); the hypophysis shows a hyperactivity and a proliferation of the prolactin cells (Aschheim and Pasteels, 1963), characteristics which SPE share with all the other categories of senile rats, except the cyclic.

Senile repetitive pseudopregnancy (Aschheim, 1961). This is characterized on the vaginal smear by an estrus which intervenes every 12 to 14 days; sometimes the diestrous interval continues for 30 days or more. Estrus can last 1 or 2 days, be transient or be replaced by a proestrus-postestrus sequence. Ovulation is qualitatively and quantitatively normal at the time of estrus; it can be seen in more than half the cases when a postestrus follows a proestrus (Aschheim, not published). The use of a marker, such as Evans blue, indicates that every pseudopregnancy results from the formation of a new generation of functional corpora lutea. Sometimes an estrous cycle of 4 or 5 days occurs between two pseudopregnancies. The SRPP have a tendency to lengthen progressively.

The ovary of such rats weighs between 40 and 50 mg. As the RPP is a later senile deviation than PE, there is here an exception to the rule of ponderal involution of organs with age. The functional corpora lutea measure between 1.3 and 1.4 mm (pseudogestational size). The ovary of the SRPP contains at least 3 generations of corpora lutea at a time, while that of adults in experimental RPP (by renal graft of a supplementary pituitary) has only one generation (Quilligan and Rothchild, 1960). Follicles of all sizes are present. In diestrus the uterine horns are thin and the vagina shows a mucification of the lactational type (a layer of basal cells surmounted by a single layer of mucous cells), indicating a very low or nonexistent estrogen production, plus progesterone. The mammary glands and the pituitary indicate as before a hypersecretion of prolactin.

The pseudopregnant character of prolonged diestrus (or the secretion of progesterone by the corpora lutea) is demonstrated in 2 ways: 1) by the ready development of traumatic deciduoma after the passage of threads in the uterine horns on the 4th or 5th day after estrus (Aschheim, 1961); 2) at any moment of PP, by the fact that the injection of 1 μg of estradiol benzoate daily for 3 days does not result in a vaginal keratinization, as with castrates of the same category, but in a mucification of the gravid type, indicative of a progesterone estrogen synergism (or a progesterone antagonism to keratinization). In this way we have been able to ascertain the functional character of these pseudogestational corpora lutea up to more than 30 days after their formation (Aschheim, not published).

Senile rats "androgenized" at birth. We have followed the senescence of rats, which, after injection with 1.25 mg of testosterone propionate at the age of 5 days, enter into permanent estrus at the time of their puberty. They are sterile (androgen-sterilized = AS) because they are anovulatory.

At the time of their senescence the vaginal estrus persists, however in a less regular manner. The ovaries, still deprived of corpora lutea, contain fewer follicles than before, but up to the age of 23 months there is no depletion of the oocyte stock. The mammary glands hypertrophy and become secretory (Aschheim, not published).

Senile rats in anestrus. An occasional senile rat does not fit into the preceding categories. The vaginal smear shows a prolonged diestrus of several weeks, interrupted irregularly by an estrus which can last one or several days. The ovary is often very small (10-15 mg), and devoid of corpora lutea, but primary follicles and occasional maturing or mature follicles are always present. The uterus is atrophic, the vagina is unstimulated, without mucification or keratinization. The mammary glands are developed as before.

Anestrus coupled with an atrophic ovary is, according to Bloch and Flury (1959), a terminal state rarely attained by senile rats. One may question however, whether one still deals with a normal senescence, for this atrophy is usually seen only in our apparently sick animals as it is in adults under severe chronic stress (Selye, 1939). In no case is there oocyte exhaustion before death in the rat (Bloch and Flury, 1959; Mandl and Shelton, 1959) as there is with the CBA mouse from the age of 14 months on (Jones and Krohn, 1961a) or in the human female after menopause. Even the Wistar rats of the W_{12} variety of the CNRZ (kindly placed at my disposal by Mr. Mauleon), which undergo a very rapid oocyte loss in the first weeks of life (Mauleon and Rao, 1963), retain a few ovarian follicles between 21 and 24 months, although the ovarian weights indicate a strong atrophy (Aschheim, not published).

The ovaries of animals in anestrus respond to gonadotropic hormones both exogenous (Bloch and Flury, 1959) and endogenous (intrasplenic ovary, Aschheim, 1968b). The formation of corpora lutea, the repair and then the hypertrophy of the interstitial cells in the intrasplenic ovarian graft indicate: 1) that the ovary *in situ* exerted a restraining action through negative feedback on the hypophysis, and, 2) that the latter is capable, once unrestrained, of functioning as it does in the case of castration. Therefore senile anestrus in the rat is not a spontaneous condition similar to that of the human menopause.

Cytohormonal Data

The ovarian interstitial tissue, its deficiency cells, and the circulating LH. A certain number of morphological signs of senescence are common to the ovaries of all types of old rats. These changes develop progressively after 12 months. There are cells (probably macrophages) filled with senile pigment (lipofuscin). There is always an important proliferation

of the germinal epithelium. Above all, formations appear which resemble "testis-like tubules" (Romeis, 1931) as well as cords of cells of epithelial shape. These elements have been described in detail in the mouse by Thung (1958). It has been shown recently that testis-like tubules derive from atretic follicles as a special consequence of the decrease in circulating gonadotropins (Arias and Aschheim, 1974) and that the cellular cords can be "luteinized" by HCG (Arias-Crumeyrolle, *et al.*, to be published).

Another senile feature is the existence of deficiency cells (DC) or "wheel-cells" in the ovarian interstitial tissue. They have a specific morphological aspect. Their functional meaning is known: the absence or insufficiency of the circulating LH. The DC have been described by Wolfe (1940) and Mandl (1959) in old rats, by Green (1957) and Jones and Krohn (1961a) in old mice. They are repaired for the most part by the daily implantation of a male rat's pituitary (Burack and Wolfe, 1959); 20 IU of PMS are effective in mice (Green, 1957). We have seen the DC in *all* senile categories after 13 months, including those that ovulate periodically, releasing a physiological amount of ovulatory LH. We have shown that the DC have not become insensitive to the gonadotropic hormone, since they resume a normal appearance 1) in a young adult gonadotropic environment (by heterochronic graft of the ovaries) 2) in the senile rat itself, in an intrasplenic graft (they hypertrophy because of the increase in LH due to castration) 3) after injection of HCG (Aschheim, 1968b). The existence of the DC clearly indicates a reduction in the level of basal circulating LH compared with that of adult rats.

There is no vascular sclerosis in the ovary of the aged rats.

The senile pituitary and its content of prolactin, LH and FSH. The "chromophobic adenoma" of the hypophysis is frequent in the old rat, especially in the female (Wolfe, *et al.*, 1938). It has been established (Aschheim and Pasteels, 1963) that the prolactin cells are hyperactive in all our senile *noncyclic* Wistar rats including castrates. This explains the mammary secretion, already indicated. On the other hand, these same elements proliferate, ranging from small clusters of giant prolactin cells, to limited adenomas whose cells retain their typical erythrosinophilic granulations, and to the previously mentioned "chromophobic" tumors.

The hyperactivity of the prolactin cells in the senile pituitaries is reflected both by an increase in the circulating hormone (Aschheim, SRPP 1961, SPE 1962, SAS 1965) and by an elevated prolactin content of the pituitary (Clemens and Meites for the SPE, 1971).

The total gonadotropin content is elevated according to Lauson, *et al.* (1939) in 3 rats aged 2½ years, only one of which had corpora lutea.

Matsuyama, *et al.* (1966) confirmed this observation in a pool of 4 hypophyses from SPE aged 12 months.

The same authors report in the same case of SPE a low content of LH measured by Parlow's ovarian ascorbic acid test. Kabak and Sokolova (1963), with a nonquantitative method, find a concentration of LH greater than that of the cyclic rat at the time of estrus, which is minimal.

Table 19-II shows our results (Aschheim, 1968a) of the hypophyseal LH content by the Parlow test in the different categories of senile rats. After ovulation, the cyclic rats have a comparable content at 4 and at 19 months. Similarly in rats androgenized at birth (AS), the LH content is the same at 5 and 19 months. The amount in the SPE is low, being 3 to 4 times lower than that of the SRPP, at the end of pseudopregnancy. Ovulation in the latter is accompanied by a significant reduction in the hypophyseal LH. SPE and SRPP have respectively the amounts of LH corresponding to

TABLE 19-II[a]

THE AMOUNT OF HYPOPHYSEAL LH IN DIFFERENT TYPES OF SENILE FEMALE RATS

Age in Months	*Number of Pituitaries Per Group*	*Content in μg of LH[b] Per Gland*	*95% Confidence Limits*	*Functional Type*	*Time of Autopsy*
12-24	5	35.0	11.1-111.0	SRPP[c]	Proestrus
23-25	4	36.5	10.9-122.5	SRPP	Diestrus of 9-11 days
24-25	5	24.6	11.7- 52.0	SRPP	Diestrus of 7-10 days
33	4	17.9	8.2- 39.4	SRPP	Diestrus of 4- 8 days
30	1	26.4	13.5- 51.8	SRPP hemicastrated	Diestrus of 14 days
23	1	22.3	10.3- 48.6	SRPP hemicastrated	Diestrus of 12 days
30	1	22.0	9.9- 49.0	SRPP hemicastrated	Diestrus of 10 days
20	1	16.3	8.4- 31.3	SRPP hemicastrated	Diestrus of 5 days
22	1	8.5	3.5- 20.9	SRPP hemicastrated	Post estrus
20-27	6	5.5	2.3- 13.2	SPE[d]	
21-24	6	8.1	5.6- 11.7	SPE	
24-26	6	6.8	3.1- 14.7	SPE	
22	4	11.9	5.6- 23.5	SPE	
20	3	10.9	4.5- 26.9	SPE	
22	4	13.7	7.0- 26.9	SPE hemicastrated	
19	6	22.8	12.1- 43.0	AS[e] senile	
5	8	19.5	8.3- 45.4	As adult	
19	6	8.0	3.5- 18.3	Cyclic senile	Post estrus
4	5	8.4	5.1- 14.0	Cyclic adult	Post estrus

[a] From Aschheim (1968a), simplified.
[b] LH-NIH-S_3, gift of Endocrinology Study Section, NIH, Bethesda, Md.
[c] SRPP—senile repetitive pseudopregnancy.
[d] SPE—senile permanent estrus.
[e] AS—androgen-sterilized.

those of adults affected experimentally with the same anomalies of cycle (PE under permanent light or after anterior hypothalamic lesion; PP by cervical stimulation). In summary at the same age, the hypophyseal LH content of senile rats varies with the nature of their estrous and ovarian rhythm. In the same neuroendocrinological situation adult and senile rats have a comparable content of LH.

Labhsetwar (1969) found a raised LH content (Parlow test) in irregularly cyclic rats of age 9 months, estimated from their weight, killed in diestrus and with corpora lutea in their ovaries. It is the same increased amount of LH that we have shown in our pseudopregnant rats and may reflect the same endocrinological condition. The pituitary of these animals is 2 to 3 times richer in FSH (Steelman and Pohley test) than that of younger rats.

As to FSH, Takasugi (1963) indicates that adult mice placed in parabiosis with intact senile ones show PE just as they do with adult castrates, whereas when united with intact adults, they remain cyclic, as do their parabiont partners. This activity of the senile gonadotropic hormones cannot be seen in the ovaries of the senile mice, which do however remain responsive, as shown by the parabiosis with adult castrated mice! These results seem to me difficult to interpret (for example by hypersecretion of FSH), in the absence of data from adult hypophysectomized mice parabiosed with senile mice, with or without ovaries.

The hypothalamus of rats in SPE contains as much prolactin-inhibiting factor (PIF), less luteinizing-hormone-releasing-factor (LRF), but more FSH-releasing-factor (FRF) than those of adults (Clemens and Meites, 1971). We lack data concerning the modifications with age in the production of hypothalamic monoamines and in the vascularization of the region.

There is little certainty as to the diminution in the number of hypothalamic neurons with age, a phenomenon quite evident in other sectors of the central nervous system, such as the cortex, the cerebellum, the thalamus. On the contrary, Andrew (1956) insists on 2 points: 1) there is no cellular destruction in the supraoptic and paraventricular nuclei of the human senile hypothalamus, nor any proof of real degenerative changes, 2) Buttlar-Brentano (1954) describes the following modifications in these nuclei: an increase in the cellular volume, leading to giant neurons, with a surface 8 to 10 times the adult size. These cells are bi- or trinuclear, with an increase in the nuclear "basophilia," and 2 to 6 nucleoli. Andrew interprets this as a reactive or defensive phenomenon. In any case, we know nothing about similar age changes in the rat's neurons responsible for gonadotropic regulation: those of the preoptic area and the arcuate and ventromedial nuclei. In a recent paper, Babichev (1973) states that in old cyclic female

rats, these same neurons become less sensitive to estradiol, although those of the arcuate region remain more sensitive than those of the preoptic region.

Summary and Neuroendocrinological Significance

Table 19-III attempts to summarize the hormonal status in the different senile conditions, either by means of biological or biochemical measurements or by the histophysiological study of the appropriate target organs.

TABLE 19-III

HORMONAL STATUS IN THE SENILE FEMALE RAT

	Cyclic adults	Cyclic seniles	SPE	SRPP	SAS	Means of detection
Estrogen (ovarian in vitro)	N		↗			biochemistry
Estrogen (circulating)	N	N	→[a]	↘	→[a]	histological aspect of target organ
Testosterone (ovarian in vitro)	N		↗			biochemistry
Progesterone (circulating)				↗		histological aspect of target organ
Total gonadotropins (hypophyseal)	N		↗			bioassay
FSH (hypophyseal)	N		↗	↗[b]		bioassay
LH (hypophyseal)	N	N	↘[c]	↗[c]	N[d]	bioassay
LH (circulating)	N	↘	↘	↘	↘	histological aspect of target organ
Prolactin (hypophyseal)	N		↗			bioassay
Prolactin (circulating)	N	N	↗	↗	↗	histological aspect of target organ
FRF (hypothalamic)	N		↗			bioassay
LRF (hypothalamic)	N		↘			bioassay
PIF (hypothalamic)	N		N or ↘			bioassay

SPE senile permanent estrus

SRPP senile repetitive pseudopregnancy

SAS senile androgen-sterilized rats

N normal value or situation for adults within the limits of variation during the cycle.

↗ increase ↘ decrease in senile rats compared with cyclic adults

a persistent secretion of estrogen; amount ignored.

b if the information from Labhetswar (1969) concerns the SRPP

c but N in comparison with adults, in persistent estrus by continuous illumination or hypothalamic lesion, or pseudopregnant after cervical stimulation.

d N by comparison with androgen-sterilized adults.

What seems to emerge from the table is not so much the need to fill the empty spaces, but the overall significance that leads to an experimental approach. Two facts are dominant:

ALL THE SENILE GROUPS HAVE A DIMINISHED BASAL CIRCULATING LH while the hypophyseal content of the hormone varies significantly from one group to another. Synthesis and release are diminished or slowed down in the SPE. In the SRPP, the lack of release during pseudopregnancy is accompanied by a progressive accumulation of LH in the pituitary which does not indicate the intensity or speed of synthesis. After ovulation, the reduction in the hypophyseal LH content is greater in absolute value than in the cyclic rat.

ALL THE SENILE NONCYCLIC GROUPS SHOW A HYPERSECRETION OF PROLACTIN. The hypothalamic control of this secretion being inhibitory, this means that there is a hypothalamo-hypophyseal "disconnection" for the function studied. It develops *in situ*. It concerns only prolactin, since the ovulatory LH "passes" at the beginning of each pseudopregnancy and since the production of estrogen in the SPE necessitates a basal secretion of FSH and LH. It will be seen later that it is for a great part reversible. At any rate it serves to draw attention to the senile hypothalamus.

EXPERIMENTAL STUDY OF THE SENILE MODULATIONS OF ESTROUS REGULATION

This section deals with the localization of the senile disorder in the regulation of the estrous cycle, its characteristics and its nature. This *experimental* study and the methods used are relevant both to gerontology and to neuroendocrinology.

Primary Responsibility of the Hypothalamus

The hypothalamus of the noncyclic senile rat thus functions in a different manner from that of the cyclic adult female. Does the primary cause of this difference reside in the hypothalamus itself? Or do the hypothalamic regulating centers merely reflect a disturbance of peripheral origin? The part played in this respect by the ovary and the hypophysis can be specified by their "heterochronic graft."

HETEROCHRONIC GRAFT OF THE OVARY. The experiment (Aschheim, 1964-65) consists of grafting, to a female Wistar rat deprived of its own ovaries, the ovaries from a rat of a different age. These homografts are inserted under the renal capsule or in the anterior chamber of the eye.

Whether the replacement of the ovary is immediate or is done one month after castration, the results are identical and can be systematized in this way:

Pre-puberal ovaries (28 to 32 days) grafted into senile female rats pre-

viously in permanent estrus, cause renewal of the permanent estrus. Likewise, pre-puberal ovaries implanted into senile females previously in repetitive pseudopregnancy restore this type of function.

Conversely, senile ovaries, originating from rats in permanent estrus or in repetitive pseudopregnancies assume normal cycles when they are grafted into a young adult rat. We have already noted that under these conditions, the deficiency cells of the senile ovary resume a normal appearance.

It is not the age of the ovary that is responsible for the manner of its endocrine functioning, but the age of the hypothalamo-hypophyseal control system to which the ovary is subjected.

Moreover, if a cyclic rat is castrated at between 8 and 12 months and receives at 24 to 27 months an either immature or senile ovarian graft, it resumes estrous cycles, a very exceptional occurrence in intact controls of the same age. After this long period of castration during which the higher centers are deprived of all steroid information of ovarian origin, they show the same sensitivity as before.

Zeilmaker (1969) has confirmed my results by exchanging ovaries between adults and SPE. A more complete confirmation comes from Peng and Huang (1972) who extend the experiment to senile anestrus rats.

In the mouse, the previous experiments of Krohn seem more difficult to interpret. Jones and Krohn (1961b) transplant ovaries from CBA mice hypophysectomized when young and allowed to become old into some adult CBA's and into a senile CBA. In the adult, the ovary grafted into an orthotopic position assumes cycling and gravidity. However in the senile mouse, both before and after the graft, the estrogen stimulation is weak and irregular. The ovary develops some graafian follicles but no corpora lutea. The interstitial tissue is deficient. We shall return later to the role of hypophysectomy in conserving the stock of oocytes. This experiment clearly indicates that the functioning of this ovary which is chronologically old, but has been for a long time inactive, depends on the gonadotropic environment, which is normal in the adult recipient but is deficient in the senile recipient.

An orthotopic heterochronic graft of an ordinary senile CBA ovary into the adult assumes only a very irregular estrogen stimulation (Krohn, 1962). This is due to the fact that such ovaries no longer possess follicles and have become refractory to the adult gonadotropins. Senile CBA × A ovaries, still possessing oocytes, function in a cyclic manner in the adult. It is the inverse experiment that is disturbing. In the senile CBA mice that have been grafted with young ovaries, there is a resumption of estrous cycles where previously they had stopped or a normalization of the rhythm in the mice previously in prolonged estrus or with irregular cycles. For Krohn (1967b), these transplanted ovaries have a normal appearance. The

author concludes (Krohn, 1962) that the gonadotropic function is normal, whereas he speaks of an LH deficiency in the very same senile CBA recipient mouse mentioned in the previous paragraph (see also the discussion of Ber (1968), p. 63).

In my opinion, the following hypothesis remains: if certain CBA mice stop secreting ovarian steroids at about 10 to 12 months and others do not, then the first group could be, until the grafting of young ovaries, in the situation of my rats, castrated young and grafted when senile, or in the situation of the rats of Smith (1963), that were hypophysectomized young and grafted with hypophyses 12 months later (see further on). In this first group the hypothalamic sensitivity to the ovarian steroids would be maintained unchanged in their absence and allows the resumption of cycles after the ovarian graft. The mouse of Jones and Krohn (1961b) would be, on the other hand, an example of the second group.

In the case of the rat there is no primary ovarian deficiency with age. However, this does not signify that ovarian aging is nonexistent, but means that it is not responsible for the reorganization of the hypothalamo-hypophyseal ovarian regulation appearing with age.

Heterochronic graft of the pituitary. Smith (1963) hypophysectomizes Long-Evans female rats 40 days old and grafts into their sella turcica 60, 200 or 375 days after hypophysectomy, the hypophysis of a pre-puberal male. The experiments are well controlled and great care is taken to ensure vascular reconnection. Two thirds of the females start their normal vaginal cycles again, the ovaries resume a normal weight and appearance, the interstitial cells become normal, even those in the group of rats grafted at the age of 415 days and autopsied at 16 months. The only anomaly is an absence or a deficiency in the lactation of the females after parturition. In the case of female rats that remain acyclic, there is a gain of weight, stimulation of bone growth and of the thyroid as in the preceding groups, but the adrenal cortex and the ovary remain atrophic as with the hypophysectomized rat.

It is important to note that in the oldest group, the graft still allows at the age of 16 months a normal functioning of the estrous cycle with a normal ovarian interstitial tissue, when at this age intact rats usually have developed deficiency cells. During 375 days, the absence of any ovarian secretion preserves the hypothalamic sensitivity to steroids.

Pecile, *et al.* (1966) hypophysectomize females of 40 days. One month later they transplant into the sella turcica 3 mg of hypophyseal tissue from rats aged respectively 30 days, 8 months and 2 years. The grafts of all ages exhibit good functional capacity with regard to growth hormone but not for the corticotropic and gonadotropic hormones. The gonadotropic function remains nil, except with the pituitaries of 30 days. The transfer of

3 mg of hypophyseal tissue leads one to think that only glandular fragments from the groups of 8 and 24 months have been grafted. Under these conditions, it is as easy for a hypothalamic-hypophyseal reconnection to succeed as with an entire gland? It is difficult to understand that the pituitaries of 8- and 24-month-old rats are equally incapable of being stimulated by a young hypothalamus.

Peng and Huang (1972) replace pituitaries of young females by those from old ones, grafted under the median eminence. In 10 out of 30 cases, there is a resumption of vaginal cycles, 3 of them being also luteal, and one case of fertility. Thus, old pituitaries can function normally in young females, but they seem to do so much less frequently than young pituitaries grafted under the median eminence of young recipients. The graft of young pituitaries into senile recipients has not yet been done.

Some Characteristics of Senile Deviations of the Estrous Cycle

THESE DEVIATIONS ARE NOT FIXED. Bloch and Flury (1959) have described ovulatory cycles which spontaneously follow PE. We have observed (Aschheim, not published) that this is so in certain Wistar strains, although not in the Wistar strain that we have mostly studied. But in the latter the SPE can "spontaneously" pass to SRPP. We cannot indicate the percentage of SPE which change the rhythm in this way, but we do know that about 20 percent of the SRPP come from SPE (Aschheim, 1961).

SRPP exposed to permanent light enter into PE and when put back into alternating light they return to SRPP (Aschheim, 1961).

So these animals, while remaining under a senile neurohumoral command, are led to produce the one ovarian hormone, progesterone or estrogen, which was previously lacking.

THESE DEVIATIONS ARE REVERSIBLE. Unlike the preceding situation here an experimental procedure restores ovulatory cycles which appear self-sustained after the initial, inducing modification.

In 1927, Zondek and Aschheim described the morphological and functional reactivation of the ovary of the mouse in anestrus for 5 months, by the implantation of the cow's pituitary. Hoffman (1931) in the senile mouse, and Romeis (1931) in the senile rat, likewise both obtained the resumption of estrous cycles.

We have reactivated the SPE rats by intravenous injection of LH (1 to 3 μg of LH-NIH), by subcutaneous injection of 10 IU of HCG or by subcutaneous implantation of the pituitary of a rat in SRPP (Aschheim, 1965).

In almost 70 percent of the cases the gonadotropic hormone induces corpora lutea which become functional through the prolactin secreted by the SPE. Pseudopregnancy is followed by ovulatory cycles, due this time

to the ovulatory release of *endogenous* LH. At the same time the secretion of prolactin is inhibited, the mammary glands which previously were secreting, regress, the new corpora lutea are small. It would be interesting to study the hypophyseal prolactin cells previously hyperactive and often tumoral. On the average, these rats have 11 autonomous cycles (5 to 24) of which 75 percent have a duration of 4 or 5 days. The final evolution is towards repetitive pseudopregnancy; only exceptionally is there a return to PE.

The injection, 2 days after the gonadotropic hormone, of ergocornine, a drug which interrupts the secretion of prolactin, and hence the secretion of progesterone by the new formed corpora lutea, leads to a resumption of PE. When exposed to continuous light, the reactivated rats enter permanent estrus, but when returned to alternating light they resume cycles, again preceded in most cases by a pseudopregnancy. Senile androgen-sterilized rats react to the injection of LH by a pseudopregnancy, but this is not followed by a release of endogenous ovulatory LH and the rats return to PE.

The reactivation of the SPE is also possible by provoking a release of endogenous LH, which is achieved by placing in darkness. Persistent vaginal estrus is now replaced by vaginal cycles and in half of the cases this is accompanied by the formation of cyclic corpora lutea (Aschheim, 1965; see also Everett, 1943 and 1970). The daily injection of 0.25 mg of epinephrine for 10 days leads in half the cases to the restoration of numerous normal cycles (Clemens, *et al.*, 1969). The aptitude for the ovulatory release of LH in SPE rats with their small amount of hypophyseal LH is demonstrated by electrical stimulation of the preoptic area of the hypothalamus (Clemens, *et al.*, 1969; Everett, *et al.*, 1970) and through the administration of the LH releasing factor (Aschheim, 1963, not published).

The SRPP rats can also be rendered cyclic again, but the efficiency of the treatment is clearly less. The housing of adult mice in groups leads to pseudopregnancy (Van der Lee and Boot, 1955). It does not in adult rats (Heinecke, *et al.*, 1960). But in senile rats, the opposite operation, the isolation of grouped SRPP rats, renders them cyclic in 5 times out of 7 at 16 to 19 months of age and once in 9 rats aged 20 to 26 months (Aschheim, 1966).

To conclude, the "reactivation" of senile rhythms is more or less easy to realize and generally of limited duration. Its mechanism raises several neuroendocrinological problems, in particular the cessation of the secretion of prolactin. But the correction of senile deviations of the estrous cycle is a fact which implies 3 consequences:

1. Reactivation produces a large number of senile cyclic rats and so per-

mits the comparative study with the few senile rats which remain spontaneously cyclic and with adult cyclic animals.

2. Among the efficient means of reactivation the external environmental factors are interesting. Isolation from light for the SPE, social (olfactory) isolation for the SRPP could create for the animal the "basic" conditions facilitatory for the self maintenance of the cycles, thus masking the increasing inability of the aging organism to adapt to changing conditions of the environment.

3. The reversibility of the senile deviations helps us to understand the cause of aging in the hypothalamo-hypophyseal-ovarian axis. A reversible state seems to be incompatible with the idea of *primary* faults in hormonal production or release of whatever origin (ovarian, hypophyseal, hypothalamic). It points more to a *primary* trouble in the reception or transmission of information, to changes in the sensitivity of the regulatory structures which have already been localized in the hypothalamus. Anyhow, it is this hypothesis of a modification with age of the hypothalamic sensitivity to ovarian steroids and especially to estrogen which we have tried to verify.

The Nature of Aging in the Central Regulatory Mechanism of the Gonadotropic Function

A recent report (Aschheim, 1970) deals with the feed-back of ovarian steroids on the regulation of the LH function in senile rats. The effects of castration and of injection of estrogen in the castrate on the hypophyseal and on the circulating LH and the effect of hemicastration or the administration of steroids on other parameters in the noncastrated female are studied.

Feed-back due to castration or administration of estrogen in castrates. Senile rats are divided according to their estrous rhythm, whether cyclic or in senile deviation. The duration of castration is one month. The treated female castrates receive during this month 5 μg of estradiol benzoate every 3 days. Other groups consist of rats castrated when young and allowed to become old, some remain without treatment and others receive 5 μg of estradiol benzoate every 3 days during the last month of their life; groups at autopsy are spread out up to 28 months of age.

The hypophyseal LH is determined according to the Parlow method, circulating LH is evaluated by the histophysiological appearance of the ovary grafted into the spleen, the formation of corpora lutea and the aspect of the ovarian interstitial tissue.

Results

a. Castration results in the increase of circulating LH in all senile categories.

b. Castration results in the increase of hypophyseal LH in all senile categories (Table 19-IV).
c. The magnitude of feed-back differs according to the categories studied (Table 19-V).
d. Prolactin restrains the increase of hypophyseal LH after castration.
e. The restraint put on the LH of castration by estrogen is more efficient in the senile previously cyclic rats than in the adult previously cyclic rats (Table 19-VI).
f. The animals which are castrated young retain their hypophyseal sensitivity to the withdrawal and administration of estrogen throughout their senescence.

Comments

a. This proposition is derived from the study of more than 80 ovarian intrasplenic grafts. The categories (SPE, SAS) whose ovaries *in situ* are devoid of corpora lutea form these in the intrasplenic grafts one month after the operation. The interstitial tissue, which is deficient in the ovary *in situ* in all categories, is restored and then undergoes hypertrophy in the intrasplenic position. The administration of estrogen during castration causes the deficiency cells to reappear in the splenic ovary.

b. In all the castrated groups, the increase of hypophyseal LH is significant by comparison with the corresponding intact females. Table 19-IV shows that the hypophyseal content is about 80 μg of LH per gland in the castrated adult or senile rats which were previously cyclic (the same in the adult castrates previously androgen-sterilized and not shown in the Table),

TABLE 19-IV[a]

HYPOPHYSEAL CONTENT OF LH IN μg OF LH-NIH[b] PER GLAND MEASURED BY THE OVARIAN ASCORBIC ACID DEPLETION TEST

	Intact Female	*Female Castrated for 1 Month*	*Female Castrated and Estrogenized for 1 Month*[c]
Cyclic rats, 3-5 months old	10.9 ±[d] 2.5 (2)[e]	74.5 ±6.6 (8)	37.3 ± 3.5 (3)
Cyclic rats, 12-14 months old	12.4 ± 4.4 (2)	80.0 ±6.9 (6)	20.2 ± 6.8 (3)
Rats in senile permanent estrus	9.4 ± 1.3 (6)	43.9 ±3.6 (5)	20.9 ± 3.5 (2)
Rats in senile repetitive pseudopregnancy	28.5 ± 4.4 (4)	57.8[f] ±3.0 (2)	46.3 ±13.4 (2)
Senile androgen-sterilized rats	28.8[f] (1)	47.3[f] ±6.7 (3)	28.2[f] (1)

[a] From Aschheim (1970), modified.
[b] LH-NIH-S_3 and S_{12}, gift of Endocrinology Study Section, NIH, Bethesda, Md.
[c] 5 μg of estradiol benzoate every third day.
[d] ± standard error.
[e] ( ) number of assays.
[f] Without an aberrant value.

and the LH content is about 50 μg for castrated senile rats previously in RPP, PE or AS.

c. Table 19-V illustrates this dichotomy by the relative potencies of LH. They are respectively of 6-7 for the first 3 groups and of 2-3 for the last 3 groups. The column of castrated adults indicates an identical behaviour between cyclic and AS rats. In the column of the senile castrates, the cyclic rats are different from all the other senile categories which behave identically. The line of cyclic rats does not show a change with age, whereas the AS line shows a difference in relative potency according to age.

d. It is prolactin which is responsible for the smaller increase in LH after castration of senile, noncyclic rats.

The suppression of the secretion of prolactin by "reactivation" of cycles previously to castration increases the hypophyseal content of LH from 50 μg to 80 μg one month after ovariectomy. Thus the magnitude of the increase in LH after castration which is clearly less in the most representative senile groups, SPE and SRPP (and also in the SAS), is not a *direct* effect of aging, but a consequence of the *preliminary* modification of the hypothalamic regulation of the estrous cycle, a direct consequence of hypersecretion of prolactin.

e. On the other hand the cyclic senile rats behave like adults with respect to castration. Thus, although very rare, they are of fundamental importance. The easy experimental transformation of SPE into senile cyclic rats similar to the former allows one to increase at will the number of animals in this group. It is indeed this category which, from a gerontological point of view is directly comparable with adults. The restraint put on the increase of LH after castration by estrogen is clearly more efficient in old cyclic rats than in adults; in the former the hypophyseal LH is no more

TABLE 19-V[a]

RELATIVE POTENCY OF HYPOPHYSEAL LH IN FEMALE RATS AFTER ONE MONTH OF CASTRATION[b]

	Adult		*Senile*
Cyclic	6.0	6.2 (3.7-10.5)[c] 5.8 (3.5-10.0)	6.3	8.0 (4.0-16.0) 4.6 (2.0-10.8)
Senile repetitive pseudopregnancy			3.1	3.4 (1.2- 9.6) 2.8 (0.8- 9.7)
Senile permanent estrus			2.7	3.4 (1.7- 6.8) 1.9 (0.8- 4.5)
Androgen-sterilized	7.0	(3.2-15.6)	2.0	2.6 (1.5- 4.8) 1.3 (0.6- 3.2)

[a] From Aschheim (1970).
[b] Compared to intact female rats of same age and category.
[c] ( ) 95% confidence limits.

TABLE 19-VI[a]

RELATIVE POTENCY OF HYPOPHYSEAL LH IN FEMALE RATS CASTRATED AND ESTROGENIZED[b] FOR ONE MONTH[c]

	3-5 Months of Age	*12-14 Months of Age*
Cyclic before castration	4.9 (2.8-8.5)[d]	1.2 { 1.7 (0.4-6.7) / 0.7 (0.3-2.1)

[a] From Aschheim (1970), modified.
[b] 5 μg of estradiol benzoate every third day.
[c] Compared to intact female rats of same age.
[d] ( ) 95% confidence limits.

significantly different from that of intact controls; whereas in the latter, it is about 5 times more potent (Table 19-VI). Thus, contrary to what appears in 90 percent of senile rats (those in senile deviations of the cycle), there is an *increase* with age in hypothalamic sensitivity to estrogen when comparison is made between cyclic rats differing only by age.

f. The results of prolonged maximal working of the hypothalamo-hypophyseal complex are studied in female rats castrated early in life and left to age. Castration is performed between 3 and 6 months of age. The hypophyseal LH content estimated at varying times between 2 and 25 months after the operation is approximately 80 μg per gland, which is similar to that obtained one month after castration. The injection of 5 μg of estradiol benzoate every 3 days for a month reduces this amount to about 20 μg per gland. The persistence of the sensitivity of the system to the withdrawal or administration of estrogen should be compared to another fact already mentioned: the reestablishment of estrous cycles in rats, castrated or hypophysectomized when young, to which the missing organ is grafted at an old age. A prolonged period of ovarian inactivity preserves the central regulatory potentialities.

Griesbach and Purves (1960) have shown in their rats of both sexes, castrated when young and allowed to grow old, a high proportion of hypophyseal tumors of gonadotropic cells. Castration is believed to be solely responsible for this change. The adrenal has been excluded. Houssay, *et al.* (1951) have described in the rat castrated for a long time, adrenal tumors which do not seem to secrete sex hormones active on the genital tract (Houssay, *et al.*, 1952). Estrogen stimulation in these castrated rats which is frequently mentioned in the literature is attributed by these authors (1952) to an incomplete castration.

But what happens when the hypothalamo-hypophyseal system is already aged at the time of castration? We have seen that one month after castration, the hypophyseal LH content is significantly increased in all types of

senile rats by comparison with corresponding intact rats. However it is not known if this value will be maintained for the rest of life. Does prolactin play again a discriminatory role between the different groups? The mediocre development (at the end of 6 months) of intrasplenic ovaries of SRPP grafted at more than 24 months of age, coupled with a low LH content of their pituitaries will lead us to the study of continuous maximal functioning imposed on a system already aged and therefore perhaps no longer homogeneous.

Bøe, *et al.* (1954) found that the splenic autograft of the ovary in rats aged from 18 to 24 months was only weakly developed at the end of 4 to 5 months. In the mouse the splenic homograft leads to tumors both of the young ovary grafted to a senile mouse and of the senile ovary grafted to a young animal (Klein, 1953). According to Ber (1968) the ovary possesses an intrinsic property which controls its capacities for growth, diminishes with age and is independent of the hypophyseal environment.

The feedback of steroids administered to intact senile rats. The existence of deficiency cells in the ovarian interstitial tissue of all groups of senile rats indicates a hyposecretion of LH. Their presence in the anovulatory females (SPE, SAS) shows that this hypofunctioning affects the basal or tonic secretion of LH, regulated by the arcuate and ventro-medial nuclei of the hypothalamus. One of the causes of the hyposecretion of basal LH could well be the increase with age in the sensitivity of these nuclei to estrogen which we have just seen in castrated rats which were previously cyclic. In the intact animal, the situation is the following:

The aptitude for pseudopregnancy triggered by the injection, at estrus, of minute quantities of estrogen increases with age.

Alloiteau (1957) has shown that, in the adult rat, the single injection of 50 or 100 μg of "nonesterified" estradiol at estrus, induces pseudopregnancy in 50 percent of the cases. Gilmore and McDonald (1969) successfully use 5 μg of estradiol benzoate. Acker and Chabardès (1970) with 5 μg obtain 70 to 90 percent of pseudopregnancies by injecting at proestrus, 55 percent at estrus. All rats were "adult" without indication of age.

We have studied this aptitude for pseudopregnancy in relation to age (Aschheim, 1972). Table 19-VII shows in Wistar rats, that for each dose used the percentage of pseudopregnancies triggered by estradiol benzoate increases with age.

Two remarks: (1) In the oldest rats, and only in these, another type of response to the injection of estrogen is added: not pseudopregnancy, but persistent estrus with disappearance of corpora lutea, lasting mostly 10 to 30 days. (2) The pre-puberal rat, in which corpora lutea are induced by human chorionic gonadotropin, requires considerable doses of estradiol benzoate to make the corpora lutea functional.

TABLE 19-VII

THE INCREASING APTITUDE WITH AGE TO REACT BY PSEUDOPREGNANCY TO ESTRADIOL BENZOATE INJECTED AT ESTRUS IN WISTAR RATS

Estradiol Benzoate in μg	*30 Days*[a]	*2 Months*	*3-6 Months*	*7-8 Months*	*9 Months*	*11-15 Months*
50	10/16[b] 62.5%		12/15 80.0%	13/15 86.7%		
10	5/15 33.3%	15/24 62.5%	9/15 60.0%		9/11 81.8%	
5		6/17 35.5%				11/12 91.7%
3		4/15 26.7%	12/34 35.3%	45/80 56.3%	11/15 73.3%	18/23 78.3%
1		0/15	5/35 14.3%	10/38 26.3%	17/42 40.5%	18/29 62.0%
0.5				0/6		7/18 38.9%

[a] Corpora lutea induced by HCG.
[b] Numerator: number of pseudopregnancies; denominator: number of injected rats.

We will see later on that, derived from data like those in Table 19-VII, a biological aging test for the central regulation of the estrous cycle can be set up.

Steroids injected into intact rats with a senile deviation of the cycle (SPE and SRPP) have the same effects as in adults in spontaneous persistent estrus or in experimental pseudopregnancy.

Thus progesterone (4 mg daily for 3 days) induces ovulation in 60 percent of the SPE (Clemens, *et al.*, 1969) as it does in adult or senile PE of the DA strain (Everett, 1940). Likewise estrogen (10 μg of estradiol benzoate during 1, 2 or 3 days) injected in the SRPP, produces ovulation in 50 percent (Aschheim, 1970), a result which agrees with that obtained by Everett (1947) in pseudopregnant or pregnant adult rats.

One can then admit that the preoptic area, responsible for the ovulatory release of LH can be reactivated in those senile rats in which it is spontaneously out of use or functions only at long intervals. However there is no quantitative data on its sensitivity to steroids.

The consequences of hemicastration in various types of senile rats. Ingram (1959) indicates that the hemicastration of 7 senile rats at the age of 20 to 23 months leads 6 weeks later to a 1.5 fold increase in the weight of the remaining ovary. Labhsetwar (1967) reports no compensatory increase in the weight of the ovary (10 days after hemicastration) in rats estimated to be 9 months old according to their weight.

We have examined the consequences of hemicastration all together on the maintenance of the pre-existing vaginal rhythm and on ovulatory, luteal and ponderal compensation of the remaining ovary (Aschheim, 1970). The period of observation has been of one month. As usual the cyclic rats and those in permanent estrus and in repetitive pseudopregnancies have been considered separately.

The weight gain of the remaining ovary is statistically nonsignificant in rats in PE or in RPP at 24 months and similarly in cyclic rats of 17 or 20 months. However, there are functional changes.

In the senile cyclic rats, only about 40 percent maintain the cyclic rhythm after hemicastration at 13 to 17 months (20% between 20 and 24 months). Nearly all of these show then an ovulatory, luteal and weight compensation in the remaining ovary one month after operation (for example at 13 months, 11 rats out of 12 show weight and luteal compensation and 9 out of 12 ovulatory compensation). The other senile cyclic rats change rhythm shortly after hemicastration. Thirty percent of the initial animals pass into a persistent estrus which leads to the absence of ovulation and corpora lutea and to a regression of ovarian weight. This explains the absence of weight gain in the remaining ovaries of the whole group of senile cyclic rats. Thirty percent pass into RPP or into alternating cyclic

and pseudopregnant sequences. This leads in the 13-month-old rats to 8 out of 8 weight and luteal compensations and to 4 out of 8 ovulatory compensations. In older animals, the change to pseudopregnancy less often involves compensations.

In rats in SPE, ovulation and luteinization of the ovary remaining after hemicastration are exceptional. The majority of them take up persistent vaginal estrus after a more or less long diestrus. The remaining ovary does not compensate in the number of large follicles nor in weight. The number of eggs ovulated and corpora lutea formed immediately after the injection of LH in these hemicastrated SPE rats is from about 5 to 6 (that is, no compensation). But as this same injection triggers the re-establishment of *autogenous* cycles, in most cases, an ovulatory, luteal and weight compensation is then found (Aschheim, not published).

The rats in SRPP generally continue their rhythm after hemicastration. At 13 months 7 out of 9 compensate for the ovarian weight and the number of corpora lutea and 6 out of 9 for the number of eggs. From 20 to 29 months compensation rarely occurs.

In summary the senile rats in which the inhibitory control of the hypothalamus on the secretion of hypophyseal prolactin is abolished are barely able to compensate functionally for hemicastration. When the inhibitory control is re-established (by the reactivation of the SPE), the compensation reappears. In senile cyclic rats hemicastration forms a test of adaptation, from which a majority, increasing with age, escapes through a change into one of the senile deviations of the estrous rhythm. The operation selects, therefore, among a population of given age those individuals which are still adaptable and those that are no longer. Our aging test, performed before hemicastration, allows one to forecast this potential difference in an apparently homogenous group of senescent cyclic rats (Aschheim, 1971).

Conclusions on the nature of aging of the regulation of the estrous cycle. The ovary being excluded from the primary responsibility for aging of the hypothalamo-hypophyseal-ovarian axis, a modification of sensitivity to ovarian steroids has been proposed as the expression of aging of the central regulation, and its direction and its magnitude have been investigated. In really comparable situations (cyclic rats differing only by age) there appears to be an overall hypothalamic hypersensitivity to estrogen in old age.

However, this notion must be understood "per animal," "per hypothalamus," not "per neuron." It is the result of the action of at least 2 unknown contributing factors which may counteract each other, the number of active neurons and their individual sensitivity. Does the senile decline in neurons of 30 to 40 percent, known for other sectors of the central

nervous system, also apply to the hypothalamus? If it does, the increased inhibition of LRF and PIF manifested by the hyposecretion of LH and the hypersecretion of prolactin, is compatible with all the hypotheses on individual neuronal sensitivity to estrogen, increased like the overall response, maintained or diminished. Even in this last case, an unchanged quantity of estrogen facing a reduced number of only slightly sensitive neurons could bring about a response of increased inhibition. It is known that the hypothalamic contribution to the onset of puberty consists of an abrupt decrease in hypothalamic sensitivity to estrogen. There is no need then to postulate an inverse process for old age in each of the still existing neurons.

Other questions remain equally open. Firstly that of the feedback of other ovarian steroids. Above all that of the hypothalamic areas affected by the overall increase of sensitivity to estrogen. The region of the arcuate and ventro-medial nuclei, regulating the tonic LH, is certainly involved. The tuberal region whose stimulation induces pseudopregnancy (Everett and Quinn, 1966) could also be involved considering what we have said of the increasing aptitude with age to react by pseudopregnancy to estrogen. Now this region includes the dorso and ventromedial nuclei. Nothing is known about the possible implication of the preoptic area which controls the release of ovulatory LH. However, as already mentioned, the administration of estrogen as well as its deprivation (by hemicastration) involves, as age increases, besides the pseudopregnant response, another response of the persistent estrus type. This evokes the results of Pasteels and Ectors (1968) and Ectors and Pasteels (1969). These authors obtain pseudopregnancy or persistent estrus by the implantation in the anterior hypothalamus of progesterone, medroxyprogesterone or estradiol benzoate. Thus none of these areas can be at present excluded from possible participation in the process of aging under study. This is an important fact because it is consistent with the hypothesis of a similar or even simultaneous aging of all neurons sensitive to estrogen.

These changes in the sensitivity of the central regulation system are also responsible for the transition to senile deviations of the estrous cycle. This is obvious for the injection of minute quantities of estrogen. The deprivation of estrogen by hemicastration which leads to similar results could act in the same way. In fact the change towards permanent estrus or pseudopregnancy rarely occurs suddenly after the operation, more often after one or sometimes two vaginal cycles. One could imagine that by an increased feedback on the hypothalamus, hemicastration could result in the supplementary secretion of estrogen before the estrus of such an intermediary cycle and that it is this supplement which starts the senile deviation of the cycle.

When the senile deviation of the cycle is established, then the central sensitivity to castration or to estrogen appears reduced. We have seen that this is related to the hypersecretion of prolactin. These senile animals behave like the castrated rats of Halasz and Gorski (1967) which have undergone an interruption of neural afferents to the medial basal hypothalamus, leaving inside the feedback loop only the arcuate and ventro-medial nuclei.

TEMPORAL ASPECTS AND MEASUREMENT OF AGING OF THE HYPOTHALAMO-HYPOPHYSEAL OVARIAN AXIS

The orientation of this section is gerontological. Because the known facts are few it is in part prospective.

Without a test of aging, it is difficult to assess any procedure intended to retard or advance the onset of senescence, to slow down or accelerate its progression. In the case of thermoregulation for example, the decline in adaptation with age can be measured, because the framework to which the biological test refers remains unaltered, a difference of temperatures or a time needed for restoration of temperature after cooling. The regulation of the estrous cycle is, however, more complex. During cyclicity, it is possible to measure a change with age in a process of adaptation (see page 406). But at a certain point this quantitative change produces qualitative changes, the senile deviations. And then, the system of reference is no longer the same. It is then impossible to measure any further progression in an aging process which is now masked.

Masked Aging

The inverse situation shall also be considered. The senile deviations are reversible. For a variable time and with a variable efficiency, rats in SRPP and SPE are transformed into cyclic rats similar to the spontaneously cyclic ones of the same age. Does that mean "rejuvenation" at least for the central regulation? These animals retain a deficient ovarian interstitial tissue, evidence for their hyposecretion of tonic LH which differentiates them from young cyclic rats. It is conceivable that these rats, when cyclic again, utilize more steroid sensitive neurons, due to restoration of activity in the preoptic area, than at the time of their permanent estrus. This could also explain the restoration of the inhibitory control on the secretion of prolactin. Thus it would be interesting to apply the aging test to "reactivated" rats, especially at an age when no more cyclic controls exist. Meanwhile, the situation is that of a masked aging.

Suspended Aging

This is what is obtained by castration or hypophysectomy carried out in the young adult. The prolonged absence of steroid information apparent-

ly conserves the hypothalamic sensitivity at a level permitting the resumption of cycles when the missing organ is substituted for. For castration (Aschheim, 1964-65) this delay has been such that there no longer exists an intact cyclic rat of the same age. During 25 months the amount of hypophyseal LH in the castrated animal remains constant, just as the LH content remains constant (4 times less) in the castrated animal, estrogenized during the last month before autopsy. It is quite clear that here is simply a suspension of the regulatory *function* under study, the senescence of this function evidently cannot be expressed until after its resumption.

It seems likely that this is what happens in the spectacular experiments which prolong life by caloric restriction as recorded by McCay (McCay, *et al.*, 1935) and confirmed by later workers (see Everitt and Porter, Chap. 30 of this book). Whatever the mode of action of the food restriction, whether or not it acts on the hypothalamus to achieve what is sometimes called a pseudo-hypophysectomy, there is a suspension of the estrous cycle. The latter can be reactivated simply by the reestablishment of the caloric allowance (Asdell and Crowell, 1935).

A report by Zeilmaker (1969) has to be mentioned even if it is not evident that it deals with "suspended aging." Rats receive Lyndiol (mixture of 30:1 of lynestrenol and mestranol), orally at 9 weeks, and for varying lengths of time. The ovaries atrophy while conserving their potentiality. The cessation of the treatment after 6 months leads in 7 out of 9 cases to a resumption of the estrous cycles which continue for 14 ± 1 months; 2 rats enter PE. The cessation of treatment after 14 months leads in 7 out of 9 cases to a resumption of ovarian activity as persistent estrus; 2 out of 9 cases resume cycles for 3 to 4 months. The pituitaries of rats treated for 14 months and then fed normally are, at 20 to 22 months of age, smaller than those of the controls and do not show hemorrhagic foci. The author concludes that the treatment may slightly retard the process of hypothalamic aging.

If there is a suspension of ovarian activity during the treatment, the latter on the other hand continuously supplies steroid information to the hypothalamus. The effect on its biological age of the antifertility steroids may differ according to their hormonal composition and should benefit from the set up of the test of aging. This experiment has now been done. Cycles resume after 4 or 8 months of steroid treatment and their biological age is then considerably younger than in control rats of the same age. This applies to estrogen as well as to estrogen-progestin treatment, but not to progestin alone (Aschheim, 1974). Does long term oral administration of estrogen (about 5 μg per day) act as a hypothalamic-hypophyseal desensitizer counteracting the natural increase with age in the central sensitivity to the hormone?

Retarded Aging

I am not aware of any situation in which there is a true retardation of the aging of the estrous regulation once the system is functioning in a cyclic manner. Besides the aging test with estrogen, the study of deficiency cells in the ovarian interstitial tissue (present in almost all cases after 13 months of age) could be useful here. An important delay in the appearance of these cells would have gerontological significance.

Advanced Aging

Early hemicastration produces such a situation (Aschheim, 1970; Aschheim, 1971).

The long term effects of early hemicastration have been studied on reproductive performance; among others by Biggers, *et al.* (1962a, b) and Finn (1963) in the mouse. The result is a reduction to half of this performance, affecting the total number of offspring as well as the length of the period of reproduction. In other words during the first half of the period of reproduction of intact animals, the hemicastrated mice show compensation; but during the second half they are sterile. This is due probably to the premature aging of the overburdened functional uterine horn. Adams (1970) reports results in the rat and the rabbit. In the rat the total number of young produced after early hemicastration is 50 percent less than that of intact rats, but this time the reduction results from both a reduction in the number of young born per litter (especially after the sixth) and the reduction in the number of litters, the period of reproduction lasting as long as in the controls.

To my knowledge, only 2 authors discuss the long term physiological compensation of the single ovary from the endocrine point of view. King (1911), cited by Biggers (1962a) states that the ovary of hemicastrated rats shows only little or no compensatory hypertrophy "several months after the operation." Thung (1961) notes that in mice hemicastrated at 13 months, there is, 8 months later, in comparison with intact mice, twice as many (12% compared with 6%) animals whose vaginal smears show an irregular estrogenic activity, including a more or less continuous estrogenic stimulation.

According to the criteria already mentioned (type of the vaginal smears, ovulatory, luteal and weight compensation of the remaining ovary) we have compared (Aschheim, 1970), in the course of their aging, rats hemicastrated at 21 days and their intact controls. The number of animals with disturbed cycles remains equally small in the 2 groups up to the age of 10 months. At 11 months the situation changes abruptly: 9 out of 45 intact rats are in permanent estrus at 11 and at 12 months; in the hemicastrated

rats this number increases to 19 out of 46 at 11 months, 21 out of 45 at 12 months (46.7%). This percentage is not achieved by the intact rats at 17 months of age. Hemicastration has then notably advanced the onset of one of the senile deviations of the estrous cycle. The single ovaries of these PE rats do not compensate.

Moreover, the 24 other hemicastrated females which did not enter PE do not show further functional compensation. Six have vaginal cycles, but are in fact anovulatory rats, with a small ovary devoid of corpora lutea, comparable to the SPE ones. Five others are pseudopregnant rats, either already repetitive or still alternating with cycles; the remaining ovaries containing numerous corpora lutea are very large: 75.8 mg (as against 40.9 mg for one ovary of the intact SRPP rats of the same age). A single female ovulates 11 eggs; the others a mean of 6.3. Thirteen hemicastrated rats remain cyclic with an average weight of 50.8 mg per remaining ovary and 5.9 eggs (as against 31.1 mg for one ovary in 17 intact cyclic rats of the same age and 5.3 eggs for the corresponding oviduct). Histological examination of the serially sectioned ovaries reveals that the number of fresh corpora lutea is only slightly in excess of the number of ovulated eggs. There is no increase in the number of corpora lutea with retained ova. The weight increase of the ovary is due to a deficient luteolysis, maintaining numerous old corpora lutea.

Two recent papers confirm that ovulatory compensation is not maintained for a long time after early hemicastration. It is stopped 6 months after the operation in the 8-month-old rats of Peppler (1971) and after ten cycles in the rats of Chatterjee and Greenwald (1972) operated at about 2 months of age.

In a second experiment (Aschheim, to be published), we have followed the rats hemicastrated at 23 days during the entire period that they maintain estrous cycles. Ovulatory compensation (which means 10-12 eggs versus 5-6 eggs in case of noncompensation) is continuously diminishing. It is maintained in 11 out of 14 rats at 3 months of age, in 17 out of 30 at 7 months, in 3 out of 8 at 10 months, in 0 out of 13 at 12 months in the group described above. Ovarian "hypertrophy" persists in all animals, but its significance changes. Whereas in rats with ovulatory compensation, the hypertrophy due to the increase in fresh corpora lutea is truly compensatory in the others, the weight gain results from maintained old corpora lutea.

In a third experiment (Aschheim, 1971), rats hemicastrated at 23 days, undergo a test of aging (3 μg of estradiol benzoate) at 7 months, which triggers 57 percent of pseudopregnancies, 43 percent of the animals remaining cyclic. After that, 2 cycles are allowed to pass, then the eggs in the remaining oviduct are counted. All the rats which gave a response of the

"young" type to the test (that is, no pseudopregnancy) show ovulatory compensation (10 to 12 eggs); all those that do not compensate (5 to 8 eggs) have given an "old" type response to the test (induced pseudopregnancy).

Early hemicastration then advances aging of the estrous regulation.

Other Situations

Are there changes in the rapidity of aging of the estrous function (acceleration, slowing)? If so are they correlated or not with the onset of senescence, normal, advanced, retarded? Nothing is known about this.

Biological Measure of Aging of the Hypothalamic Regulation of the Estrous Cycle

Cyclic rats show an increasing aptitude with age to react by pseudopregnancy to estradiol benzoate injected at estrus (Table 19-VII) (Aschheim, 1970, 1972). That means that there is an increasing facility with age to release prolactin in response to an estrogenic stimulation at estrus, which in turn results probably from the increasing facility for estrogen to inhibit the hypothalamic prolactin-inhibiting-factor (PIF). This applies to a twentyfold range of doses of estrogen. For a given dose of the hormone, the curve showing the percentage of induced pseudopregnancies in relation to age is S-shaped. The curve representing the quantities of estrogen efficient at the 50 percent level in different age groups is hyperbolic.

At least two different biological criteria must be used to assess the diagnosis of pseudopregnancy, persistent estrus or a maintained cycle, the 3 types of response which can occur. The "old" response is pseudopregnancy or occasionally persistent estrus, the "young" response is a maintained or slightly lengthened cycle.

As for specificity, the frequency of pseudopregnant responses, not to estrogen, but to the stress of injection at the day of estrus is 7 percent, but is not age-dependent. The nonreproducibility of the aging test is also about 7 percent. It does not change with age. Repeated trials, until 4 tests at 3 to 4 week intervals, can be done without altering the response.

The aging test is then a functional test for adaptability, dealing only with physiological stimulation and responses and leaving the rat intact. It can be carried out during the whole period of cyclicity, but must be used with caution at the end of this period, when cycles are "spontaneously" replaced by their senile deviations (12-14 months). It reveals the latent heterogeneity of an apparently homogenous, cyclic population. This heterogeneity is age-dependent.

We do not yet know if this aging test expresses *the* aging of the central

regulation of the cycle in female rats or only one of its aspects. But it is a striking fact that the mechanism underlying the spontaneous senile deviations and the senile responses to the test in cyclic rats is the same.

It may be possible that the rats which remain cyclic when aging represent a population selected for the stability of the central regulation and that this would account for the flattening of the last part of the S-shaped curve. The problem of selection exists in all aging phenomena, but in this particular case, it could be studied experimentally. Indeed, the possibility of reactivating, of "recycling" rats in senile persistent estrus, should permit us to test these animals, which have not been selected for stability (or negatively) and to compare them with spontaneously cyclic rats of the same age.

FINAL CONSIDERATIONS AND PROSPECTS

The Senile Ovary

In the rat it is clear that the ovary is not responsible for the senile sterility which takes place before the oocyte stock is exhausted (Jones, 1970). The ovary is not primarily responsible for senile deviations of the estrous cycle (Aschheim, 1964-65). We are now far from the earlier view that oocyte depletion is the central event of ovarian aging which moreover would lead to a loss of sensitivity to gonadotropic hormones. The question of the possible relation between ovarian senescence, the number of oocytes, the production of ovarian steroids, and their hypothalamo-hypophyseal feed-back in my opinion is as follows.

Can we reveal or create in the rodent a situation like that found in women after the menopause, that is, a "biological" castration with disappearance of follicles and gonadotropic hyperfunction of the pituitary? One thinks of CBA mice whose ovaries are deprived of oocytes in the second half of their life. The gonadotropic activity of their pituitary has not yet been studied; but the presence of deficiency cells in the interstitial tissue of their ovaries makes a gonadotropic hypersecretion unlikely. After the disappearance of the oocytes, ovarian tumors form (Thung, 1961), which do not appear to secrete biologically active steroids. The C57-BL/6-W^v-W^v mice whose ovaries, through mutation, are practically deprived of oocytes from the very beginning, rapidly develop ovarian tumors which are hormonally inactive: tubular adenomas derived from the germinal epithelium (Russell and Fekete, 1958). The experimental destruction of oocytes and follicles after local irradiation by X-rays (Mandl and Zuckerman, 1956) also leads to the formation of tumors in the ovary of the rat. But here estrogen is secreted for several months in the absence of oocytes and follicles. In the terminal stage of hormonal inactivity of the ovary, the

pituitary reacts as in castration by an increase of its FSH content (Mandl and Zuckerman, 1956) and of its total gonadotropin secretion (Westman, 1958).

Do such sequences result from ovarian aging or simply from destruction of oocytes? The aging test could determine this in certain circumstances.

The Senile Pituitary

Throughout this study the gonadotropic function of the pituitary during senescence has appeared as a passive vehicle of a hypothalamic command altered in its sensitivity to estrogen. Now, this hormone can exert a direct influence on the pituitary, inhibiting the gonadotropic cells (Bogdanove, 1963; Ramirez, *et al.*, 1964), stimulating the prolactin cells (Nicoll and Meites, 1962). (However, we do not know if this action plays a physiological role in the regulation of the estrous cycle.) Moreover the pituitary undergoes structural alterations with age.

Autonomous aging of the gonadotropic function of the pituitary. Only the result of a heterochronic graft of the pituitary in contact with the median eminence can attribute the respective responsibilities to the hypothalamus and to the pituitary in the aging of the estrous function. In the experiment of Peng and Huang (1972), already mentioned, this responsibility seems to be shared. In any case the reversibility of the senile deviations of the cycle leads us to impute the failure of the organ to a primary defect of information, of sensitivity, not to a primary disturbance of hormonal production or excretion. And in fact the senile pituitary retains all its capacities for the elaboration of the gonadotropic hormones. At the same age, its LH content differs significantly according to the category of estrous regulation; in the same category, the content does not differ with age. The decrease in hypophyseal LH after ovulation of the SRPP is clear and significant. Castration leads, after one month, to a similar increase of LH in the senile and adult cyclic rats. The pituitaries freed of inhibition by castration at a young age maintain their maximal amount of LH throughout their senescence. For Labhsetwar (1970) castration, like hemicastration, of an "aged" rat leads to the same important amount of hypophyseal FSH, similar to that of a castrated adult. There is only the long term restraint from inhibition, caused by castration of the already aged rat, which might perhaps demonstrate an ultimate fading of the feedback (but at what level?).

Like the ovary, the pituitary ages, but there is no evidence for its primary responsibility for the senile changes in gonadotropin regulation. While the ovary can be formally eliminated owing to the results of its heterochronic graft, a kind of hypophyseal collusion remains possible.

Hypophysectomy and aging. Jones and Krohn (1961b) studied the evo-

lution of the ovary of mice hypophysectomized at the age of 40 and 50 days. The operation clearly retards but does not abolish the oocyte loss with time. The intact CBA mice lose 28 percent of their oocytes in periods of 20 days, the hypophysectomized ones 11 percent. The ovaries of mice hypophysectomized 300 days previously, ovulate after orthotopic grafting into a young recipient, and lead to pregnancies as successfully as young normal ovaries grafted in the same way. Senile ovaries of mice hypophysectomized when young have less lipofuscin pigment and anovulary follicles than those of intact mice of the same age.

But, as mentioned before, oocyte loss throughout life is not an "aging" process which should be accentuated with age and give an S curve like for example the disappearance of estrous cycles (Fig. 19-1) or the aptitude for pseudopregnancy.

The aging of tail tendon collagen continues, but in a clearly retarded manner, in the hypophysectomized rat, both male (Olsen and Everitt, 1965) and female (Verzár and Spichtin, 1966) whilst longevity, another criterion of aging, diminishes (Everitt and Cavanagh, 1965; Verzár and Spichtin, 1966).

The effects of caloric restriction leading to a "pseudohypophysectomy" are manifest by a retardation in the aging of collagen (Chvapil and Hrüza, 1959) and an increase in longevity (see Chap. 30). From the sexual point of view, there is a cessation or nonappearance of the estrous cycles and an ovarian atrophy with preservation of numerous young follicles (Huseby, *et al.*, 1945; mice), but without corpora lutea. These ovaries, evidence of the lack of circulating gonadotropic hormones, remain sensitive to the injection of these hormones (Mulinos and Pomerantz, 1941). It is interesting to note that the pituitary accumulates, at least for a short term, the hormones which it is unable to release (Rinaldini, 1949; Meites and Reed, 1949; Maddock and Heller, 1947; Srebnik, *et al.*, 1961). After a longer time the gland is deficient in gonadotropic substances (Werner, 1939).

If one adds that early castration does not influence longevity (Asdell, *et al.*, 1967), nor the biological age of collagen (Árvay, *et al.*, 1963; Árvay and Takács, 1966) in comparison with the nulliparous intact rat, one can set up Table 19-VIII. It can be seen that the 3 conditions which "suspend" hypothalamo-hypophyseal-ovarian aging have divergent effects on longevity and on the biological age of collagen. Thus it is not through the gonadotropic function of the pituitary that these parameters are acted upon.

Hypophysectomy and aging of the ovarian target cells. The gonadotropic hormones induce in the ovary of the rat temporary morphogenetic and steroidogenetic phenomena. Hypophysectomy and replacement therapy allow the study of ovarian target cells in different, stable and continuous conditions of time. Thus, artificial types of cellular aging may be realized.

TABLE 19-VIII

RELATIONSHIP BETWEEN GONADOTROPIN LEVELS AND TWO CRITERIA OF SENESCENCE

	Gonadotropins		Aging of collagen	Survival
	pituitary	circulating		
Castrated rat	↗	↗	N	N
Hypophysectomized rat	O	O	↘	↘
Caloric restricted rat	at first ↗ later ↘	↘	↘	↗

Value in relation to intact nulliparous rat

N normal

↗ increace

↘ decrease

Hypophysectomy changes the interstitial cells of the ovary into deficiency cells or inactive "wheel"-cells (Selye, *et al.*, 1933). Moreover, the operation stops the formation of new interstitial cells which originate from the thecal cells of atretic follicles. The deficiency cells persist in the hypophysectomized rat. Their morphology and function can be repaired by gonadotropic hormones with "ICSH" activity (LH, HCG, PMS) up to 30 to 60 days after the operation (Rennels, 1951; Marti and Green, 1965). Four months after hypophysectomy of an immature rat, 50 IU of HCG, previously sufficient for repair, have lost much of their efficiency (Arias and Aschheim, to be published). Does this indicate an "aging" of these dormant cells, or does it result from the general metabolic consequences of hypophysectomy at that time?

The corpora lutea present in adult rats (or in pre-puberal rats artificially luteinized) at hypophysectomy are maintained in an inactive state for a very long time. When stimulated by HCG or LH, these luteal cells are able to produce estrogen, up to 9 months after hypophysectomy (Parlow, 1961). But for Chamorro (1945) corpora lutea of rats hypophysectomized

at 18 months are unable to do so despite a strong, prolonged treatment with HCG, started 3 weeks after the operation.

The luteal cell in the hypophysectomized rat whose gland has been regrafted in an ectopic position where it becomes a source of prolactin, is active and maintains its production of progesterone for at least 104 days (Everett, 1956).

Thus, hypophysectomy leads to simplified experimental situations which deserve time-related investigations by modern methods of subcellular exploration.

The Senile Hypothalamus

THE STEROID-SENSITIVE HYPOTHALAMUS. With age the hypothalamic target structures for steroids modify their sensitivity to estrogen in the normal cyclic rat. The other sex steroids have not yet been examined. This modification appears as an overall increase in sensitivity. That of the individual neurons concerned remains unknown in the absence of qualitative and quantitative morphological data. What are their cyto-physiological and ultrastructural aspects? What is their number? It is only on these bases that future results of dynamic studies on the uptake of labelled steroids, the hormone-dependent electrophysiological activity or the neuropharmacological reactions could be interpreted.

CORRELATIONS BETWEEN VARIOUS REGULATORY FUNCTIONS OF THE HYPOTHALAMUS. Kennedy and Mitra (1963) have considered body weight and food intake as initiating factors for puberty in the rat. In their opinion the decrease in sensitivity to estrogen which characterizes puberty at the hypothalamic level, may originate from a decrease in the metabolic rate. The latter is a consequence of the decrease in energy balance which is required for the adaptation of the growing organism to its environment, especially as regards thermoregulation.

Nutrition, light, and temperature are well known as initiating factors of sexual activity in seasonal animals.

What can be said about their correlations in the senile hypothalamus? Rothchild (1967), in a brilliant speculation has formed a scheme for it. He suggests that the "syndrome of old age" could be due to the decrease of inhibitory activity of the ventro-medial nucleus on the far-lateral area. Activation of this area increases the appetite (Kennedy, 1966). According to a second hypothesis activation of the far-lateral area leads also to a series of inhibitions, resulting in the decrease of caloric loss (and hence the increase in temperature), of sexual receptivity, locomotor activity, maximal secretion of LH and in the increase of the secretion of prolactin (by inhibition of PIF). One can discuss the second hypothesis, the need to consider the far-lateral area as the starting point of the inhibitory influence

acting upon the functions enumerated which effectively diminish with age. But the initiating role attributed to food intake (Aschheim, 1964-65; Rothchild, 1967), as in puberty, requires consideration.

The decreased activity (or hypofunction) of the ventro-medial and of the arcuate nuclei in the gonadotropic LH regulation of the senile rat is an established fact (Aschheim, 1970). Furthermore, this hypofunctioning is secondary; it results from an altered perception of the steroid environment. Is the inhibitory control upon the far-lateral area and the stimulatory control upon the gonadotropic activity of the pituitary exerted by the same neurons of the ventro-medial nucleus? If so, is the senescence of the two functions necessarily simultaneous? Does a caloric restriction applied to rats in senile deviations of the cycle restore estrous cycles? Thus, once more, but now via the hypothalamus, we face the relationship between food intake, pituitary, ovary and aging.

There is less data (see Chap. 5) available on the correlations of the different hypophysiotropic functions of the senile hypothalamus than there is on the relations between gonadotropic regulation in the senile female and appetite (Kennedy, 1953) or locomotor activity (Farris, 1945). Even more astonishing, scarcely anything is known about aging of the hypothalamo-hypophyseal-testicular hormonal axis (see Bishop, 1970). In a single assay of hypophyseal LH in 4 groups of intact males and castrates aged 6 and 21 months, we found a significant increase in LH after 1 month of castration (at 6 months 3.6 times; at 21 months 2.7 times). The amount of LH in intact males like that of castrates did not change with age (Aschheim, not published). Some interesting studies (Larsson and Essberg, 1962; Jakubczak, 1967) have been made of the sexual behavior of the male rat. There is not an overall decrease in sexual activity with age but a different temporal distribution. These neurological changes occur before any detectable testicular hormonal deficiency develops.

CONCLUSION

We have studied the aging of a specific regulatory system. The primary failure resides in its central link, the senile hypothalamus. But, at present, the latter appears as a "black box." The different tests of adaptation which demonstrate the modification of its sensitivity to estrogen are triggered upside and are recorded downside. This applies also to the described aging-test of the estrous cycle. However, with its use, one can hope for new information on the control and understanding of experimental situations susceptible to modify the temporal course of aging. But the direct study of the neuroendocrine regulatory cell in relation to its age remains to be done.

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