Plasma and pituitary concentrations of LH, FSH and prolactin in aged female C57BL/6 mice

T. A. Parkening, T. J. Collins and *E. R. Smith

*Departments of Anatomy and *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.*

Summary. Plasma and pituitary concentrations of LH, FSH and prolactin were determined by radioimmunoassay in 2-month-old (young) and 16–20-month-old (old) C57BL/6 mice. There were no statistical differences in hormonal levels between aged females in oestrus (those exhibiting a copulatory plug) and those in constant dioestrus. In the old females plasma levels of LH ($P < 0.002$) and FSH ($P < 0.001$) were significantly elevated, while levels of prolactin ($P < 0.001$) were significantly depressed when compared with those from young animals. Pituitary homogenates from old females also contained more gonadotrophins ($P < 0.001$) and less prolactin ($P < 0.001$) than those of the young females. A radioreceptor assay utilizing a plasma membrane of luteinized rat or mouse ovaries indicated that LH from 2-month-old animals bound better to ovarian receptors ($P < 0.05$) than did LH from old mice, although radioimmunoassay of the same samples gave higher ($P < 0.01$) plasma LH levels for the old mice. Since the radioreceptor assay is considered to be a more sensitive test for biologically active LH, the results from these two types of assays suggest that there may be an alteration in the mouse LH molecule with age.

Introduction

After reaching sexual maturity, female rodents exhibit a period of maximum fecundity until their 4th to 6th litters, depending upon the strain and species of animal. Each animal then normally enters a reproductive phase in which the size of the litter becomes progressively smaller as the animal ages, until no young are produced. In C57BL/6 mice a decline in litter size is readily apparent by 12 months of age, and most animals by the age of 16 months are incapable of bearing young (Harman & Talbert, 1970; Talbert, 1971). All organs of the reproductive system have been implicated as the prime organs for initiating a decline in the litter size of laboratory rodents (for recent reviews see Peluso, 1976; Steger, 1976; Finch, 1977, 1978; Talbert, 1977, 1978; Soriero, 1978; Meites, Huang & Simpkins, 1978). The hypothalamic–hypophysial complex is thought by many researchers to be the primary cause of reproductive senescence (Aschheim, 1964/5; Labhsetwar, 1970; Howland & Preiss, 1975; McPherson, Costoff & Mahesh, 1977; Huang, Steger, Bruni & Meites, 1978; Riegler & Miller, 1978), although others consider an ageing ovary which is no longer capable of responding to or secreting hormones largely to blame (Thorneycroft & Soderwall, 1969; Blaha, 1970; Gosden, 1975; Leathem & Murono, 1975; Chan & Leathem, 1977). The advent of techniques for the radioimmunoassay of hormones has led to studies on aged laboratory rats to determine which part of the reproductive system is influenced first or to the greatest degree by the ageing process (Howland & Preiss, 1975; McPherson *et al.*, 1977; Huang *et al.*, 1978; Riegler & Miller, 1978). In contrast, with the exception of a few recent reports, little research of a similar nature has been done on

ageing mice (Gosden, Jones & Jacks, 1978; Holinka, Tseng & Finch, 1978; Parkening, Lau, Saksena & Chang, 1978). The present study was therefore conducted to determine plasma and pituitary concentrations of LH, FSH and prolactin in 2-month-old and 16–20-month-old C57BL/6 mice and to compare these hormonal levels with those previously reported in the senescent rat.

Materials and Methods

The C57BL/6 mice were acquired from the NIH Institute of Aging Colony maintained by Charles River Laboratories. Virgin females were studied at 2, 16, 18 and 20 months of age; hereafter they are referred to as 'young' (2 months) or 'old' (16–20 months). The animals were provided with mouse chow and water *ad libitum* and housed in separate quarters illuminated daily with 14 h artificial light. After allowing 5 days for the mice to adjust to their new environment, vaginal smears were taken daily and those exhibiting a pro-oestrous smear were placed individually with a C57BL/6 male (3–6 months of age) of proven fertility in the evening and checked the following morning for a copulatory plug. Those females which had mated were bled unanaesthetized the same morning (10:00–12:00 h) by cardiac puncture with a heparinized syringe. Females which were not cycling (58% of the aged females) were similarly bled after exhibiting 8–15 persistent dioestrous or oestrous vaginal smears. Only one aged female showed cornified epithelial cells for 8 consecutive days and was not included in this study. All females were killed within 4 weeks of their arrival. After bleeding, the pituitary was immediately removed, weighed and cut in half. One-half was placed in fixative for future immunocytochemical studies while the remaining half was reweighed and homogenized in 0.5 ml 0.01 M-phosphate-buffered saline (PBS) containing 0.05 M-EDTA and 0.1% sodium azide (pH 7.6). The ovaries and adrenals were also removed, weighed and fixed in Bouin's fluid or 10% neutral formalin. Of those females which mated, the oviducts were excised before the removal of the ovary and placed in Hanks' solution containing 0.05% hyaluronidase. The ampulla of each oviduct was opened with dissecting needles to allow the eggs within the cumulus mass to exude into the medium for the subsequent dispersion of the cumulus cells. The ova were then mounted, fixed, stained and examined as previously described (Parkening & Chang, 1976). Blood and pituitary homogenates were centrifuged at 1000 g for 15 min at 4°C and the plasma and pituitary supernatants were stored at –80°C. A sufficient amount of plasma was obtained from each mouse to permit the radioimmunoassay of at least 2 of the 3 hormones being examined. The homogenized pituitaries consisted of enough supernatant when diluted to assay all 3 hormones.

Radioimmunoassay for hormones

Plasma and pituitary LH and FSH concentrations were measured using the NIAMDD rat LH and FSH kits. Mouse LH and FSH have been shown to cross-react with rat antibody (Beamer, Murr & Geschwind, 1972; Kovacic & Parlow, 1972), and in the present study serial dilutions of C57BL/6 mouse plasma and pituitary homogenates were parallel to standard curves formed by using NIAMDD-rat-LH-RP1 and NIAMDD-rat-FSH-RP1 reference preparations. Single 100 µl and 200 µl samples of plasma were measured for LH and FSH levels, while pituitary gonadotrophins were assayed in duplicate 200 µl aliquots of a 1:100 dilution of the supernatant from pituitary homogenates for LH and a 1:10 dilution for FSH. Plasma and pituitary levels were expressed in terms of ng equivalents of NIH-LH-S1 or NIH-FSH-S1/ml plasma or µg pituitary wet weight. Inter- and intra-assay variations were 11.8 and 9.4% respectively for the LH assay and 8.5 and 9.5% respectively for the FSH assay. The sensitivities of the assays were 1 ng LH/tube and 10 ng FSH/tube.

Unlike assays with LH and FSH, there is little immunological cross-activity between mouse

and rat prolactin (Sinha, Selby, Lewis & VanderLaan, 1972). Mouse prolactin for standards, having a potency of 25 i.u./mg, and an antiserum to mouse prolactin were provided by Dr Y. N. Sinha. Duplicate 50 μ l samples of plasma and duplicate 200 μ l aliquots of pituitary supernatant diluted 1:20 with buffer were assayed according to the procedures described by Sinha *et al.* (1972). The prolactin concentration was expressed as ng standard/ml plasma or μ g pituitary wet weight. To eliminate inter-assay variance, all prolactin samples were analysed in a single assay. The intra-assay variation for prolactin was 9.9% and the sensitivity was 0.2 ng/tube.

Radioreceptor assay for LH

A crude plasma membrane fraction was prepared from ovaries of Wistar rats (50–70 g) or pseudopregnant mature random-bred mice according to the procedures of Lee & Ryan (1972). The final plasma membrane pellets containing the ovarian receptors from rat or mouse ovaries were diluted to a concentration of 10 mg equivalents wet ovarian weight/100 μ l, snap frozen in 0.2–0.5 ml aliquots, and stored at -20°C until used (2–3 weeks). The incubation media used for the binding assay contained 100 μ l mouse plasma or 200 μ l of a 1:10 pituitary dilution (in duplicate), iodinated rat LH (Lee & Ryan, 1973) totalling approximately 20 000 c.p.m. and 5 mg equivalents of the receptor fraction. The final volume was adjusted to 1 ml with the addition of 0.1% BSA–Tris buffer. The assay was incubated in a Dubnoff shaker at 4°C for 48 h and the plasma membranes were recovered by centrifugation (5000 g for 15 min). The pellet was carefully washed, recentrifuged, and the same process repeated before it was counted for radioactivity in a Packard Tri-carb Spectrometer (70% efficiency). Total binding was 34% of the radioactivity added to the incubation mixture, with non-specific binding to the membranes constituting less than 1% of the total counts. Serial dilutions of mouse plasma and pituitary supernatants paralleled those in standard curves generated against the NIAMDD LH-I-4 reference preparations in the rat and mouse receptor systems. Concentrations of LH were expressed in terms of ng equivalents of NIAMDD LH-I-4/ml plasma or μ g pituitary wet weight. All samples were processed in the same assay and the intra-assay variation was 9.3%.

Ovarian histology

Ovaries embedded in paraffin wax were serially sectioned (5 μ m) and stained with haematoxylin and eosin or PAS. Sequential sections of ovaries selected from 9 young and 16 aged females were examined and the total numbers of ovarian oocytes (excluding those in primordial follicles) were counted. The oocytes were classified according to Pederson & Peters (1968), except that type 5b follicles (no antrum), which they considered as large follicles, were included in the medium category in this study.

Statistical analyses

Comparisons of mean organ weights and mean concentrations of hormones were made using Student's *t* test. Statistical significance was determined at the 0.05 probability level.

Results

The young females were significantly lighter in body weight than older females (Table 1). When organ weights were adjusted to compensate for body size the ovaries of oestrous (those exhibiting copulatory plug) and constant dioestrous old females were significantly lighter than those in young females. The pituitary weights of constant dioestrous aged females were also significantly less than those of the young mice, but there were no statistical differences in the weights of pituitaries from oestrous old and young females (Table 1).

Table 1. Body and organ weights (mean $\pm$ s.e.m.) from 2-month-old and 16–20-month-old C57BL/6 mice

Age and state	No. of mice	Body wt (g)	Organ wet weights (mg)			Organ wet weights (mg/100 g body wt)		
			Ovaries	Pituitary	Adrenals	Ovaries	Pituitary	Adrenals
2 months oestrous	24	21.0 $\pm$ 0.3	6.9 $\pm$ 0.4	1.7 $\pm$ 0.3	6.8 $\pm$ 0.2	32.7 $\pm$ 1.7	7.9 $\pm$ 0.3	32.3 $\pm$ 1.1
16–20 months oestrous	27	23.7 $\pm$ 0.4‡	5.2 $\pm$ 0.3†	1.8 $\pm$ 0.3	8.0 $\pm$ 0.3†	21.9 $\pm$ 1.4‡	7.6 $\pm$ 0.2	33.8 $\pm$ 1.2
Constant dioestrous	38	24.3 $\pm$ 0.3‡	4.9 $\pm$ 0.2‡	1.7 $\pm$ 0.3	7.9 $\pm$ 0.2†	21.0 $\pm$ 1.0‡	7.0 $\pm$ 0.2*	32.7 $\pm$ 1.1

Significantly different compared with values for 2-month-old females: * $P < 0.01$, † $P < 0.002$, ‡ $P < 0.001$.

All of the young mice exhibited normal 4–5 day oestrous cycles and mated, while only 66% of the old females that were not considered to be in constant dioestrus mated. Not all of the older females that mated showed a normal oestrous cycle and 10% did not mate until the 2nd or 3rd pro-oestrous vaginal smear. Within each group of females which mated some failed to ovulate even though all had a prominent copulatory plug; this was most apparent in the 20-month-old mice for which ova could not be found in the oviducts of 5 of the 8 animals (Table 2). The mean number of ova collected from the females that ovulated was similar except for a marked decline in the number of eggs in 20-month-old mice. However, fewer ova were fertilized from all groups of the aged mice when compared with those in the young animals (Table 2). Of the ova fertilized, 95% from the young females contained both male and female pronuclei, whereas only 63% contained pronuclei from senescent mice. The numbers of fragmented and degenerating ova recovered at ovulation increased with age, representing 3% in 2-month-old animals and 15, 27 and 64% in 16-, 18- and 20-month-old females respectively.

Table 2. The numbers of cyclic females which mated and ovulated and the mean numbers of ova fertilized in C57BL/6 mice of different ages

Age (months)	No. of females			Mean $\pm$ s.e.m. no. of ova*	No. of ova fertilized (%)
	Studied	Mated	Failed to ovulate		
2	24	24	5	8.0 $\pm$ 0.6	125–152 (82)
16	17	10	2	7.6 $\pm$ 0.9	40–61 (66)
18	13	9	3	7.2 $\pm$ 0.5	27–44 (61)
20	11	8	5	4.3 $\pm$ 0.9	0–14 (0)

* Ovulating females only.

To determine to what degree the numbers of ovarian oocytes declined with age, some ovaries were randomly selected from each age group and the total numbers of oocytes (excluding primordial follicles) were counted. There was a progressive decline in the numbers of follicles with the age of the female (Table 3), but the ovaries of 20-month-old mice still contained all sizes of follicles. Interstitial cells in the medullary portion of the ovaries were also examined to determine if the nuclei contained condensed chromatin arranged in a pattern resembling the “wheel cells” (or deficiency cells) first described in the ovaries of hypophysectomized rats (Selye, Collip & Thomson, 1933). The cytoplasm was diminished and the nuclei were more condensed in many of the interstitial cells of older mice compared with similar cells in the young mice. These cells were most notably present in the ovaries of the oldest females.

There were no statistical differences in either plasma or pituitary concentrations of LH, FSH or prolactin when aged oestrous and constant dioestrous females were compared either as a

Table 3. Mean $\pm$ s.e.m. total numbers of oocytes (excluding primordial follicles) present in ovaries of C57BL/6 mice of different ages

Age (months)	No. of ovaries	Follicle size		
		Small	Medium	Large
2	9	269 $\pm$ 38	106 $\pm$ 12	28 $\pm$ 4
16	5	91 $\pm$ 19	24 $\pm$ 7	10 $\pm$ 3
18	5	56 $\pm$ 16	24 $\pm$ 4	5 $\pm$ 2
20	6	24 $\pm$ 9	11 $\pm$ 2	6 $\pm$ 1

group (Table 4) or individually at 16, 18 and 20 months of age. Since differences between oestrous and constant dioestrous females were insignificant, the hormone data were combined for the 3 groups of old mice for comparison with those of young mice (Table 5). Highly significant differences existed between the old and young animals in all comparisons except for plasma LH values of the young compared with the 16-month-old females. Plasma and pituitary prolactin concentrations were significantly lower, while plasma and pituitary LH and FSH levels were statistically higher in aged females (Table 5).

Table 4. Mean $\pm$ s.e.m. plasma and pituitary concentrations of LH, FSH and prolactin in 16–20-month-old oestrous and constant dioestrous C57BL/6 mice

Hormone	Mice (no. in parentheses)		<i>t</i> value
	Oestrous	Constant dioestrous	
Plasma (ng/ml)			
LH	65.8 ± 9.3 (16)	54.6 ± 6.5 (15)	0.979
FSH	961.7 ± 83.6 (14)	981.3 ± 74.2 (29)	0.160
Prolactin	78.1 ± 5.2 (11)	75.5 ± 3.9 (18)	0.405
Pituitary (nf/μg)			
LH	4.6 ± 0.4 (26)	5.1 ± 0.3 (38)	0.888
FSH	3.9 ± 0.3 (26)	4.6 ± 0.3 (38)	1.829
Prolactin	0.6 ± 0.1 (11)	0.6 ± 0.1 (19)	0.188

Table 5. Mean $\pm$ s.e.m. plasma and pituitary concentrations of LH, FSH and prolactin in C57BL/6 mice of different ages (no. in parentheses)

Age (months)	Plasma (ng/ml)			Pituitary (ng/ μ g)		
	LH	FSH	Prolactin	LH	FSH	Prolactin
2	38.7 $\pm$ 2.0 (16)	335.6 $\pm$ 22.4 (18)	111.6 $\pm$ 6.1 (12)	1.9 $\pm$ 0.2 (29)	1.9 $\pm$ 0.1 (29)	1.1 $\pm$ 0.1 (11)
16	47.0 $\pm$ 5.4 (10)	937.2 $\pm$ 82.3 $\ddagger$ (14)	81.8 $\pm$ 6.7* (9)	5.0 $\pm$ 0.5 $\ddagger$ (20)	4.1 $\pm$ 0.3 $\ddagger$ (20)	0.7 $\pm$ 0.1 $\ddagger$ (10)
18	74.7 $\pm$ 8.6 $\ddagger$ (11)	1021.4 $\pm$ 126.3 $\ddagger$ (16)	72.4 $\pm$ 4.7 $\ddagger$ (10)	4.6 $\pm$ 0.4 $\ddagger$ (22)	4.6 $\pm$ 0.4 $\ddagger$ (22)	0.5 $\pm$ 0.1 $\ddagger$ (10)
20	69.2 $\pm$ 9.7 $\ddagger$ (10)	958.0 $\pm$ 63.5 $\ddagger$ (13)	75.8 $\pm$ 4.7 $\ddagger$ (10)	5.3 $\pm$ 0.5 $\ddagger$ (22)	4.2 $\pm$ 0.3 $\ddagger$ (22)	0.6 $\pm$ 0.1 $\ddagger$ (10)
16–20 (combined)	64.0 $\pm$ 5.0 $\ddagger$ (31)	974.9 $\pm$ 56.4 $\ddagger$ (43)	76.5 $\pm$ 3.1 $\ddagger$ (29)	5.0 $\pm$ 0.3 $\ddagger$ (64)	4.3 $\pm$ 0.2 $\ddagger$ (64)	0.6 $\pm$ 0.1 $\ddagger$ (30)

Significantly different compared with values for 2-month-old females: * $P < 0.005$, $\ddagger P < 0.002$, $\ddagger P < 0.001$.

To assess the biological activity of the LH measured by radioimmunoassay, the binding of plasma and pituitary LH to ovarian receptors was examined using the procedure of Lee & Ryan (1972). Sufficient amounts of plasma remained from some of the mice to allow use of the radioreceptor assay on samples from females for which plasma and pituitary values for LH had already been obtained. Plasma LH levels in the old females were significantly lower than those of the young animals (Table 6). Sufficient samples of pituitary supernatants remained from all females to permit analysis of LH levels by the radioreceptor assay. The same females from which the plasma had been examined using the radioreceptor assay plus some additional randomly selected females were chosen for this study. Pituitary LH levels were significantly higher in all aged animals as were receptor values in 16- and 18-month-old females, but not 20-month-old females, compared with those in 2-month-old animals (Table 6). In the plasma and pituitary samples there was a trend in which the radioreceptor to radioimmunoassay ratio decreased with increased age (Table 6).

Table 6. Mean $\pm$ s.e.m. plasma and pituitary concentrations of LH, determined by radioimmunoassay (RIA) and ovarian radioreceptor assay (RRA), in mice of different ages

Tissue	Age (months)	No. females	RIA	RRA	RRA:RIA
Plasma (ng/ml)	2	10	40.2 $\pm$ 2.2	56.0 $\pm$ 2.4	1.39
	16–20	8	57.9 $\pm$ 5.8†	47.2 $\pm$ 3.2*	0.82
Pituitary (ng/μg)	2	10	2.1 $\pm$ 0.2	1.2 $\pm$ 0.1	0.57
	16	8	5.0 $\pm$ 0.3‡	1.8 $\pm$ 0.3*	0.36
	18	7	6.4 $\pm$ 0.6‡	2.0 $\pm$ 0.1‡	0.31
	20	7	5.4 $\pm$ 0.3‡	1.6 $\pm$ 0.1	0.30

Significantly different compared with value for 2-month-old females: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Discussion

In an initial study (Parkening *et al.*, 1978) there were no differences in the plasma levels of circulating LH in 3–5-month-old and 12–15-month-old pregnant C57BL/6 mice. However, in a second study (unpublished data) conducted on this strain of mice to investigate in greater depth gonadotrophin levels in pregnant females of the same age group, there was evidence that statistically significant higher levels of plasma LH and pituitary LH (homogenates) existed during certain stages of pregnancy in older females. The only detectable difference noted in a critical examination of the two studies was the inclusion of a larger number of 14–15-month-old females in the second study. This was the impetus for conducting the present study which indicates that significantly higher levels of plasma and pituitary LH exist in 16–20-month-old C57BL/6 mice.

Circulating levels of gonadotrophins and prolactin in the ageing female C57BL/6 mouse are different from those reported for senescent rats. Ageing irregularly cycling and non-cycling female rats exhibit, in general, a reduced capacity to secrete LH and FSH and an increased capacity to secrete prolactin when compared with younger mature females (Meites *et al.*, 1978; Riegle & Miller, 1978). The higher prolactin levels in some old rats, particularly anoestrous females, apparently result from pituitary tumours (Meites *et al.*, 1978) which are thought to be responsible for altering hypothalamic mechanisms which cause a reduction in gonadotrophin secretion (Huang *et al.*, 1978; Meites *et al.*, 1978). Finch *et al.* (1977) have shown that the

reproductive hormonal levels of ageing male C57BL/6 mice are also considerably different from those of ageing male rats.

In the present study it has been assumed that the metabolic clearance rates of gonadotrophins are similar in 2-month-old and 16–20-month-old mice. Previous studies have shown that the metabolic clearance rates of LH and FSH are similar in pre- and post-menopausal women (Kohler, Ross & Odell, 1968; Coble, Kohler, Cargille & Ross, 1969), indicating that the elevation of gonadotrophins in post-menopausal women results from differences in the secretory rates of the hormones by the pituitary. The gonadotrophin and prolactin levels in plasma of C57BL/6 female mice paralleled those reported for peri- and post-menopausal women. Differences in the secretion of oestrogen and elevated levels of FSH first appear in older mice (Parkening *et al.*, 1978), followed by a gradual elevation of LH in some females still capable of producing young (unpublished). A similar pattern of hormonal secretion has been detected in plasma of women just before the menopause (Sherman, West & Korenman, 1976; Reyes, Winter & Faiman, 1977). In post-menopausal women, circulating gonadotrophins rise, FSH to a greater extent than LH, while prolactin levels become lower and there is a concomitant decrease in oestrogen and progesterone levels (Scaglia *et al.*, 1976; Reyes *et al.*, 1977). Climacteric symptoms in pre-menopausal women 35–39 years of age have been attributed to lower levels of oestrogen and elevated levels of LH (Abe *et al.*, 1977). While oestrogen concentrations were not analysed in the present study, it can be assumed that such levels were low because of the depressed levels of prolactin. Oestrogens are known to be potent stimulators of prolactin secretion in man (Yen, Ehara & Siler, 1974) and rat (Chen & Meites, 1970; Ajika, Krulick, Fawcett & McCann, 1972; De Léan, Garon, Kelly & Labrie, 1977). The low levels of plasma prolactin in senescent mice are probably an indication that circulating progesterone levels are also low, since prolactin has been reported to be essential for maintaining progesterone secretion in the rat (Meites *et al.*, 1972).

The pituitary hormone contents observed may not be a true reflection of their values since the stored hormone may be different from the secreted form (Bogdanove & Nansel, 1978). However, the rise in pituitary gonadotrophins and the fall of pituitary prolactin in older mice do coincide with similar changes occurring in the plasma of the same animals.

The presence of “wheel cells” or “deficiency cells” in ovaries of ageing rodents has been taken *a priori* to mean that these animals have depressed levels of gonadotrophins (Gosden *et al.*, 1978). In the present study the morphological appearances of interstitial cells in the medullary portion of aged ovaries were similar to those described by Green (1957) for the same strain of mouse. It is not known why “deficiency cells” persist in older mice which have significantly higher levels of LH and FSH (measured by radioimmunoassay) than do younger females. However, the decrease in LH measured by the radioreceptor assay suggests that the ovary of the old animal is not exposed to as much biologically active LH as is the young animal. Still, it is doubtful that the decrease in LH potency alone would explain the presence of the “deficiency cells” observed in the ovaries of aged mice. There appeared to be no differences in the morphology of interstitial cells whether from mice which had mated or those exhibiting constant dioestrus. While it is tempting to speculate that the gonadotrophin receptors lose their binding affinity with age, there is evidence this may not be true for the binding of gonadotrophins in aged rat ovaries (Steger, Peluso, Huang, Hafez & Meites, 1976; Erickson, Hsueh & Lu, 1979). It is possible that the receptors lose their sensitivity with increasing levels of gonadotrophins. Green (1957) injected 20 i.u. PMSG into aged mice to restore interstitial cells to an appearance similar to that found in younger females; this dosage is twice that needed for superovulation. Specific binding of hormones in target tissues is known to occur in association with defined physiological states (Cheng, 1976). The relationship of levels of gonadotrophins and ovarian steroids to the numbers of binding sites in the ovary is not known. Meaningful information might be obtained from conducting studies to determine any changes in receptor-binding sites for LH and FSH in mature and aged mouse ovaries. LH acting on granulosa cells

of preovulatory rat follicles could cause a decline in its own receptor by mechanisms other than occupancy (Rao, 1979) and the synergistic relationship of oestradiol, FSH and LH acting at specific times on target tissues could increase or decrease receptors of these hormones (Richards, 1979). In the ageing ovary endogenous gonadotrophins may no longer influence or stimulate receptors to the same degree as at an earlier time in the animal's life; and with quantitative changes in plasma and pituitary gonadotrophins and prolactin, as well as what appear to be qualitative changes in LH, changes must certainly take place in receptors of these hormones. A simpler explanation for the presence of "deficiency cells" in ovaries of mice with elevated gonadotrophin levels may result from atrophy and age-related changes in the blood vessels supplying the ovary. During ovariectomy, very little bleeding occurs after excising ovaries not previously ligated in 18–20-month-old females compared with those from 12–13-month-old mice (unpublished data). Exogenous hormones injected intraperitoneally should, therefore, temporarily restore interstitial cells to the appearance of such cells found in younger animals.

Inhibitory substances found in follicular fluid could explain the considerable elevation of plasma and pituitary concentrations of FSH in aged mice. One such substance, thought to be a polypeptide and termed "follicular fluid inhibin" or "folliculostatin", appears specifically to inhibit the secretion of FSH from the pituitary (de Jong & Sharpe, 1976; Schwartz & Channing, 1977; Lorenzen, Channing & Schwartz, 1978). A reduction of ovarian follicles with the subsequent lowering of fluid inhibin has been postulated as a cause for elevated levels of FSH in post-menopausal women (Sherman & Korenman, 1975; van Look, Lothian, Hunter, Michie & Baird, 1977). With the corresponding reduction of follicles in the ageing mouse ovary, a similar interruption of the feedback mechanism may exist.

While the technique of radioimmunoassay of gonadotrophins is very sensitive and can readily distinguish between LH and FSH, it has been recognized that iodination damage of hormones can provide an over-estimation of the biologically active hormone. The radioreceptor assay is considered to be highly sensitive and able to discriminate between the damaged and undamaged hormone, and is also in close agreement with the ovarian ascorbic acid depletion assay for LH potency (Lee & Ryan, 1972, 1976). The significantly lower binding of LH to corpora lutea using the radioreceptor assay when testing 16–20-month-old females indicates that there is some modification of the LH molecule in aged animals and therefore at least a portion of the higher values of LH detected by radioimmunoassay in the plasma of senescent females may be a reflection of changes in the LH molecule. The change might be due to post-synthetic modifications of the hormone, as postulated for some altered enzymes found in senescent animals (Rothstein, 1979). Another possibility is that a large molecular form of LH (possibly representing biosynthetic precursors of LH) may be released by the ageing pituitary as has been reported for thyrotrophin in older rats (Klug & Adelman, 1977). The lower radioreceptor assay:radioimmunoassay ratio found in pituitary homogenates than in plasma samples in the present study could be explained on the basis of similar changes since the pituitary homogenates should contain LH molecules in various stages of processing and alterations may result from incomplete peptide chains released from ribosomes, "prohormone", or hormone without its complete carbohydrate component. An alteration of the LH molecule may not, however, be totally responsible for the elevation of LH, as detected by radioimmunoassay, in aged C57BL/6 mice. The increased secretion of LH by the older animal probably reflects an attempt to compensate for the decrease in serum oestrogen levels. It is not known if the rise in radioimmunoassayable serum LH from post-menopausal women (Sherman *et al.*, 1976; Scaglia *et al.*, 1976; Reyes *et al.*, 1977) reflects an alteration of the molecule.

While there are considerable species differences between rats and mice and man, it appears that the hormonal changes occurring in the senescent C57BL/6 mouse more closely parallel those in post-menopausal women than do those reported for the ageing rat. This strain of mouse may prove to be a better animal model than the rat for investigating ageing pituitary and hypothalamic changes in man.

We thank Dr Y. N. Sinha, Scripps Clinic and Research Foundation, La Jolla, California, for supplying materials for mouse prolactin RIA and the NIAMDD Pituitary Hormone Distribution Program for supplying rat LH and FSH radioimmunoassay kits. This research was supported in part by NIH Grant No. AG-00757.

References

- Abe, T., Furuhashi, N., Yamaya, Y., Wada, Y., Hoshiai, A. & Suzuki, M. (1977) Correlation between climacteric symptoms and serum levels of estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone. *Am. J. Obstet. Gynecol.* **129**, 65–67.
- Ajika, K., Krulick, L., Fawcett, C.P. & McCann, S.M. (1972) Effects of estrogen on plasma and pituitary gonadotropins and prolactin, and on hypothalamic releasing and inhibiting factors. *Neuroendocrinology* **9**, 304–315.
- Aschheim, P.P. (1964/5) Résultats fournis par la greffe hétérochrone des ovaires dans l'étude de la régulation hypothalamo-hypophyso-ovarienne de la ratte sénile. *Gerontologia* **10**, 65–75.
- Beamer, W.G., Murr, S.M. & Geschwind, I.I. (1972) Radioimmunoassay of mouse luteinizing and follicle stimulating hormones. *Endocrinology* **90**, 823–827.
- Blahe, G.C. (1970) The influence of ovarian grafts from young donors on the development of transferred ova in aged golden hamsters. *Fert. Steril.* **21**, 268–273.
- Bogdanove, E.M. & Nansel, D.D. (1978) Biological and immunological distinctions between pituitary and serum LH in the rat. In *Structure and Function of the Gonadotropins*, pp. 415–430. Ed. K. W. McKerns. Plenum Press, New York.
- Chan, W.C. & Leatham, J.H. (1977) Aging and ovarian steroidogenesis in the rat. *J. Geront.* **32**, 395–401.
- Chen, C.L. & Meites, J. (1970) Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. *Endocrinology* **86**, 503–505.
- Cheng, K.W. (1976) Changes in rat ovaries of specific binding for LH, FSH and prolactin during the oestrous cycle and pregnancy. *J. Reprod. Fert.* **48**, 129–135.
- Coble, Y. D., Kohler, P.O., Cargille, C.M. & Ross, G.T. (1969) Production rates and metabolic clearance rates of human follicle-stimulating hormone in premenopausal and postmenopausal women. *J. clin. Invest.* **48**, 359–363.
- de Jong, F.H. & Sharpe, R.M. (1976) Evidence for inhibin-like activity in bovine follicular fluid. *Nature, Lond.* **263**, 71–72.
- De Léan, A., Garon, M., Kelly, P.A. & Labrie, F. (1977) Modulation of pituitary thyrotropin releasing hormone receptor levels by estrogens and thyroid hormones. *Endocrinology* **100**, 1496–1504.
- Erickson, G.F., Hsueh, A.L.W. & Lu, K.H. (1979) Gonadotropin binding and aromatase activity in granulosa cells of young proestrous and old constant estrous rats. *Biol. Reprod.* **20**, 182–190.
- Finch, C.E. (1977) Neuroendocrine and autonomic aspects of aging. In *Handbook of the Biology of Aging*, pp. 262–280. Eds C. E. Finch & L. Hayflick. Van Nostrand Reinhold Company, New York.
- Finch, C.E. (1978) Reproductive senescence in rodents: factors in the decline of fertility and loss of regular estrous cycles. In *The Aging Reproductive System. Aging*, Vol. 4, pp. 193–212. Ed. E. L. Schneider. Raven Press, New York.
- Finch, C.E., Jones, V., Wisner, J.R., Sinha, Y.N., Devellis, J.S. & Swerdloff, R.S. (1977) Hormone production by pituitary and testes of male C57BL/6J mice during aging. *Endocrinology* **101**, 1310–1317.
- Green, J.A. (1957) Some effects of advancing age on the histology and reactivity of the mouse ovary. *Anat. Rec.* **129**, 333–347.
- Gosden, R.G. (1975) Ovarian support of pregnancy in ageing inbred mice. *J. Reprod. Fert.* **42**, 423–430.
- Gosden, R.G., Jones, E.C. & Jacks, F. (1978) Pituitary-ovarian relationships during the post-reproductive phase of inbred mice. *Expl. Geront.* **13**, 159–166.
- Harman, S.M. & Talbert, G.B. (1970) The effect of maternal age on ovulation, corpora lutea of pregnancy, and implantation failures in mice. *J. Reprod. Fert.* **23**, 33–39.
- Holinka, C.F., Tseng, Y.-C. & Finch, C.E. (1978) Prolonged gestation, elevated preparturitional progesterone, and reproductive aging in C57BL/6J mice. *Biol. Reprod.* **19**, 807–816.
- Howland, B.E. & Preiss, C. (1975) Effects of aging on basal levels of serum gonadotropins, ovarian compensatory hypertrophy, and hypersecretion of gonadotropins after ovariectomy in female rats. *Fert. Steril.* **26**, 271–276.
- Huang, H.H., Steger, R.W., Bruni, J.F. & Meites, J. (1978) Patterns of sex steroid and gonadotropin secretion in aging female rats. *Endocrinology* **103**, 1855–1859.
- Klug, T.L. & Adelman, R.C. (1977) Evidence for a large thyrotropin and its accumulation during aging in rats. *Biochem. Biophys. Res. Commun.* **77**, 1431–1437.
- Kohler, P.O., Ross, G.T. & Odell, W.D. (1968) Metabolic clearance and production rates of human luteinizing hormone in pre- and postmenopausal women. *J. clin. Invest.* **47**, 38–47.
- Kovacic, N. & Parlow, A.F. (1972) Alterations in serum FSH/LH ratios in relation to the estrous cycle, pseudopregnancy, and gonadectomy in the mouse. *Endocrinology* **91**, 910–915.
- Labhsetwar, A.P. (1970) Ageing changes in pituitary-ovarian relationships. *J. Reprod. Fert., Suppl.* **12**, 99–117.
- Leatham, J.H. & Muroso, E.P. (1975) Ovarian Δ^3 - β -hydroxysteroid dehydrogenase in aging rats. *Fert. Steril.* **26**, 996–1000.
- Lee, C.Y. & Ryan, R.J. (1972) Luteinizing hormone receptors: specific binding of human luteinizing hormone to homogenates of luteinized rat ovaries. *Proc. natn. Acad. Sci., U.S.A.* **69**, 3520–3523.
- Lee, C.Y. & Ryan, R.J. (1973) Interaction of ovarian

- receptors with human luteinizing hormone and human chorionic gonadotropin. *Biochemistry, N.Y.* **12**, 4609–4615.
- Lee, C.Y. & Ryan, R.J.** (1976) Specific binding of human luteinizing hormone and human chorionic gonadotropin to ovarian receptors. In *The Endocrine Function of the Human Ovary*, pp. 181–194. Eds V. H.T. James, M. Serio & G. Giusti. Academic Press, New York.
- Lorenzen, J.R., Channing, C.P. & Schwartz, N.B.** (1978) Partial characterization of FSH suppressing activity (folliculostatin) in porcine follicular fluid using the metestrus rat as an in vitro bioassay model. *Biol. Reprod.* **19**, 635–640.
- McPherson, J.C., Costoff, A. & Mahesh, V.B.** (1977) Effects of aging on the hypothalamic-hypophyseal-gonadal axis in female rats. *Fert. Steril.* **28**, 1365–1370.
- Meites, J., Lu, K.H., Wuttke, W., Welsch, C.W., Nagasawa, H. & Quadri, S.K.** (1972) Recent studies on function and control of prolactin secretion in rats. *Recent Prog. Horm. Res.* **28**, 471–526.
- Meites, J., Huang, H.H. & Simpkins, J.W.** (1978) Recent studies on neuroendocrine control of reproductive senescence in rats. In *The Aging Reproductive System. Aging*, Vol. 4, pp. 213–235. Ed. E. L. Schneider. Raven Press, New York.
- Parkening, T.A. & Chang, M.C.** (1976) In-vitro fertilization of ova from senescent mice and hamsters. *J. Reprod. Fert.* **48**, 381–383.
- Parkening, T.A., Lau, I.F., Saksena, S.K. & Chang, M.C.** (1978) Circulating plasma levels of pregnenolone, progesterone, estrogen, luteinizing hormone, and follicle stimulating hormone in young and aged C57BL/6 mice during various stages of pregnancy. *J. Geront.* **33**, 191–196.
- Pederson, T. & Peters, H.** (1968) Proposal for a classification of oocytes and follicles in the mouse ovary. *J. Reprod. Fert.* **17**, 555–557.
- Peluso, J.J.** (1976) Aging of mammalian ova. In *Aging and Reproductive Physiology*, pp. 35–50. Ed. E. S. E. Hafez. Ann Arbor Science, Ann Arbor, Michigan.
- Rao, M.C.** (1979) Hormone receptor regulation by luteinizing hormone: an active, inducible process. In *Ovarian Follicular Development and Function*, pp. 325–331. Eds A. R. Midgley & W. A. Sadler. Raven Press, New York.
- Reyes, F.I., Winter, J.S.D. & Faiman, C.** (1977) Pituitary-ovarian relationships preceding the menopause. I. A cross-sectional study of serum follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol, and progesterone levels. *Am. J. Obstet. Gynec.* **129**, 557–564.
- Richards, J.S.** (1979) Hormone regulation of hormone receptors in ovarian follicular development. In *Ovarian Follicular Development and Function*, pp. 225–242. Eds A. R. Midgley & W. A. Sadler. Raven Press, New York.
- Riegle, G.D. & Miller, A.E.** (1978) Aging effects on the hypothalamic-hypophyseal-gonadal control system in the rat. In *The Aging Reproductive System. Aging*, Vol. 4, pp. 159–192. Ed. E. L. Schneider. Raven Press, New York.
- Rothstein, M.** (1979) The formation of altered enzymes in aging animals. *Mech. Ageing Dev.* **9**, 197–202.
- Scaglia, H., Medina, M., Pinto-Ferreira, A., Vázques, G., Gual, C. & Pérez-Palacios, G.** (1976) Pituitary LH and FSH secretion and responsiveness in women of old age. *Acta endocr., Copenh.* **81**, 673–679.
- Schwartz, N.B. & Channing, C.P.** (1977) Evidence for ovarian “inhibin” suppression of the secondary rise in serum follicle stimulating hormone levels in proestrus rats by injection of porcine follicular fluid. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5721–5724.
- Selye, H., Collip, J.B. & Thomson, D.L.** (1933) On the effect of the anterior pituitary-like hormone on the ovary of the hypophysectomized rat. *Endocrinology* **17**, 494–500.
- Sherman, B.M. & Korenman, S.G.** (1975) Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J. clin. Invest.* **55**, 699–706.
- Sherman, B.M., West, J.H. & Korenman, S.G.** (1976) The menopausal transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. *J. clin. Endocr. Metab.* **42**, 629–636.
- Sinha, Y.N., Selby, F.W., Lewis, U.J. & VanderLaan, W.P.** (1972) Studies of prolactin secretion in mice by a homologous radioimmunoassay. *Endocrinology* **91**, 1045–1053.
- Soriero, A.A.** (1978) The aging uterus and fallopian tube. In *The Aging Reproductive System. Aging*, Vol. 4, pp. 85–126. Ed. E. L. Schneider. Raven Press, New York.
- Steger, R.W.** (1976) Aging and the hypothalamo-hypophyseal-gonadal axis. In *Aging and Reproductive Physiology*, pp. 51–65. Ed. E. S. E. Hafez. Ann Arbor Science, Ann Arbor, Michigan.
- Steger, R.W., Peluso, J.J., Huang, H., Hafez, E.S.E. & Meites, J.** (1976) Gonadotrophin-binding sites in the ovary of aged rats. *J. Reprod. Fert.* **48**, 205–207.
- Talbert, G.B.** (1971) Effect of maternal age on postimplantation reproductive failure in mice. *J. Reprod. Fert.* **24**, 449–452.
- Talbert, G.B.** (1977) Aging of the reproductive system. In *Handbook of the Biology of Aging*, pp. 318–356. Eds C. E. Finch & L. Hayflick. Van Nostrand Reinhold Company, New York.
- Talbert, G.B.** (1978) Effect of aging of the ovaries and female gametes on reproductive capacity. In *The Aging Reproductive System. Aging*, Vol. 4, pp. 59–83. Ed. E. L. Schneider. Raven Press, New York.
- Thornycroft, I.H. & Soderwall, A.L.** (1969) Ovarian morphological and functional changes in reproductively senescent hamsters. *Anat. Rec.* **165**, 349–354.
- van Look, P.A., Lothian, H., Hunter, W.M., Michie, E.A. & Baird, D.T.** (1977) Hypothalamic-pituitary-ovarian function in perimenopausal women. *Clin. Endocr.* **7**, 13–32.
- Yen, S.S.C., Ehara, Y. & Siler, T.M.** (1974) Augmentation of prolactin secretion by estrogen in hypogonadal women. *J. clin. Invest.* **53**, 652–655.

Received 5 June 1979