BIOLOGY OF REPRODUCTION 27, 327–339 (1982)

A Longitudinal Study of Estrous Cyclicity in Aging C57BL/6J Mice: I. Cycle Frequency, Length and Vaginal Cytology[1]

JAMES F. NELSON,[2] LÊDA S. FELICIO,[2] PATRICK K. RANDALL, CLIFFORD SIMS and CALEB E. FINCH

Andrus Gerontology Center and Department of Biological Sciences
University of Southern California
Los Angeles, California 90007

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ABSTRACT

Cycle frequency, length, and vaginal cytology were measured longitudinally in three cohorts of singly housed virgin mice staggered across a 3-year interval. The age profiles of these parameters were qualitatively similar, but quantitatively different, among cohorts.

Cycle frequency was initially low (Phase I), due to prolonged cycles and late-starting cyclers, and did not peak (Phase II) until mice were 3–5 months old. Phase II lasted for 7–10 months, depending on the cohort. Thereafter cycle frequency declined steadily (Phase III). The average age of cessation of cyclicity varied among cohorts, occurring between 13 and 16 months of age.

Age changes in cycle length paralleled those of cycle frequency. During Phase II, median cycle length was <5 days and variance was lowest. During Phases I and III, variance was about twofold greater and median cycle length was >5 days. Although median cycle length remained stable for several months during Phase II, the peak period of 4-day cycles was much shorter. In all cohorts, 4-day cycles did not peak until 7–8 months of age and began to decline by 9 months. The decrease in 4-day cycles was associated with a progressive lengthening of cycles—first from 4 to 5 days, then to longer cycles.

The fraction of cycles with extended cornification (>2 days) increased with advancing age from <0.35 during the initial period of cycle lengthening to a maximum of 0.60. The observation that the initial phase of cycle prolongation was not usually associated with extended cornification is consistent with earlier evidence that this period is characterized by a delayed, rather than prolonged, preovulatory rise of estradiol. However, the increased fraction of prolonged cycles with extended cornification at later ages suggests that the preovulatory elevation of estradiol may ultimately be prolonged.

INTRODUCTION

The loss of reproductive function in females is a relatively early event of aging, occurring during midlife in both short- and long-lived mammals (Talbert, 1977). However, the endocrine mechanisms underlying reproductive failure are not well understood. One of the most revealing and readily measurable markers of reproductive decline is the vaginal estrous cycle. Its frequency, length and cytology reflect the hormonal milieu that maintains ovulatory function, and changes in these parameters can thereby give insight into age-related changes in the hormonal control of reproduction. Furthermore, because the measurement of vaginal cyclicity is nonlethal, it can be monitored longitudinally. Thus, previous cycling history can be prospectively correlated with subsequent age-related changes in cyclicity. Such analyses may be useful in identifying etiological factors underlying the loss of reproductive function.

Despite its accessibility and information value, vaginal cyclicity has not been extensively exploited as a research tool in the study of reproductive aging. Although the decline of estrous cyclicity has been qualitatively documented in many strains of rat (e.g., Everett, 1939; Aschheim, 1976; Lu et al., 1979) and mouse (Dickie et al., 1957; Thung et al., 1956), with the exception of a monumental study of humans (Treloar et al., 1967), no detailed quantitative analyses based on longi-

Accepted March 13, 1982.
Received December 31, 1981.

[1] Supported by NIH grants AG 00117, AG 00446 and AG 007670 to C.E.F. and an Eli Lilly Fellowship to J.F.N. Portions of this study were presented at the 30th Annual Meeting of the Gerontological Society, 1977, San Francisco, and at the 3rd Arnold Beckman Conference on the Clinical Chemistry of Aging, Colorado Springs, CO 1979.

[2] Present address: Division of Reproductive Biology, McGill University, Women's Pavilion, Royal Victoria Hospital, 687 Pine Avenue West, Montréal, Québec H3A 1A1.

tudinal data are available. For these reasons, as well as for our need to establish baseline data for experimental studies, we undertook a longitudinal study of estrous cyclicity in the C57BL/6J mouse, a widely used model in aging research.

This report, the first in a series, documents the profile of vaginal cyclicity from its onset to cessation, emphasizing the changes in cycle length and vaginal cytology that mark the declining period of cyclicity.

MATERIALS AND METHODS

Animal Husbandry

C57BL/6J mice were obtained as 8-week-old virgins from the Jackson Laboratory, Bar Harbor, ME. Upon arrival, mice were quarantined and observed for health and deaths. Serum was obtained from a representative sample (2–5% of each shipment) and was checked for a panel of murine viruses (ectromelia, K-virus, lymphocytic choriomeningitis, minute virus of mice, mouse adenovirus, mouse hepatitis virus, pneumonia virus of mice, polyoma, reovirus Type 3, Sendai and Theiler's mouse encephalomyelitis) by Microbiological Associates, Bethesda, MD. No significant titers were present in the several shipments of mice used in this study, and gross inspection revealed no evidence of disease. After 2 weeks in quarantine, the mice were transferred to a limited-access, 450 square-foot room containing an aging colony of female C57BL/6J mice and male C3HeB/FeJ breeders. The number of males in the colony (50–60) was held constant, but the number of females ranged between 300 and 1400 during the study. Mice were housed in 12 × 13 × 30 cm opaque plastic cages with Absorb-dri bedding (Garfield, NJ), and had free access to tap water and food (Purina Rodent Laboratory Chow #5001). Cages and water bottles were changed and autoclaved weekly. The colony was maintained at 23–26°C on a 12L:12D schedule (lights on at 0730 h). Animals that were wounded, appeared weak, showed abdominal swelling, severe weight loss or other sign of disease were promptly culled from the colony to minimize the possibility of contagious disease, a particularly serious jeopardy in longitudinal studies (Kay, 1978).

Vaginal Smears

Smears were obtained daily between 0730 and 1300 h. Each mouse was picked up by the base of its tail and transferred head first into the empty food hopper of an adjacent cage. The fire-polished, shortened tip of a Pasteur pipet was placed at the vaginal orifice. Care was taken not to insert it more than 1 mm to minimize the possibility of inducing pseudopregnancy by cervical stimulation (Sinha et al., 1978). One drop of tap water was gently expelled into the vagina and aspirated back into the tip twice, and then transferred to a MeOH-washed microscope slide. The pipet was rinsed in a tap water, 70% EtOH, tap water sequence between each sampling. Dry smears were fixed in absolute MeOH for 30 sec, drained and stained for a minimum of 30 min in 2% Giemsa blood stain (Medical Chemical Corp., Los Angeles), freshly prepared in distilled water. Staining greatly facilitated identification of cycle stage: Cytoplasm and nuclei stained blue and red, respectively.

Classification of Smears and Definition of a Valid Estrous Cycle

Smears were examined microscopically at 63×, and were classified as to the stage of the cycle according to criteria modified from those of Allen (1922) and Thung et al., (1956) (Table 1). Seven stages were distinguished: Diestrus/ proestrus (DP), proestrus (P), proestrus/estrus (PE), estrus (E), metestrus 1 (M1), mestestrus 2 (M2), and diestrus (D).

An estrus cycle was defined as the period between successive proestrus smears (proestrus=Day 1). A valid cycle met the following criteria, derived from the classical studies of rats (Long and Evans, 1922) and mice (Allen, 1922).

1. The sequence begins with a proestrus smear (DP, P or PE).
2. If there are a series of contiguous proestrus smears at the beginning of the potential cycle, then Day 1 is the highest ranking proestrus smear in that series (i.e., 1st rank, P; 2nd, DP; 3rd PE). If the highest ranking smear occurs more than once in succession, Day 1 is the last highest-ranking smear.
3. A leukocytic smear (see below) must precede Day 1. If Day 1 is the last in a series of proestrus smears, then a leukocytic smear must precede the first smear in that series or that series must begin with DP.
4. Day 1 must be followed by a cornified period at least 1 day long; cornified smears are PE, E or M1; the sequence of cornified smears during this period may follow any order.
5. The cornified period must be followed by a leukocytic (M2, D or DP) period of at least 1 day. Although the usual order of smear types during the leukocytic period is M2-to-D-to-DP, because of occasional reversals, any order is permitted.
6. The leukocytic period must not only be followed by or coincide with a valid proestrus, but that proestrous must be followed by a cornified period of at least 1 day.

Analysis of Cycles

Smears were read by one individual (JFN) to minimize variance. A program written in Basic for an Apple II 48K microcomputer (Apple, Inc., Cupertino, CA) enabled data to be stored according to individual, age and cohort. For each individual, the program identified valid cycles according to the foregoing definition and calculated the number of valid cycles and their respective lengths at 30-day intervals. These data were then analyzed by statistical procedures (described below) using SAS, a statistical computer package (Helwig and Council, 1979).

Experimental Design

Daily vaginal smears were obtained longitudinally from 3 cohorts of virgin mice, each comprising 10–14

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animals. The mice that died during the study, (1 at 11 months and 2 at 16 months) were not replaced. The 3 cohorts were staggered across a 3-year interval to ascertain the replicability of the results. The ages examined and dates of smearing were: Cohort A, 3–18 months, 2/77–5/78; Cohort B, 3–4 months and 7–18 months, 6/77–9/78; Cohort C, 3–15 months, 2/79–2/80. Over 14,000 smears were examined.

The effect of housing density on cycling parameters was measured in the preliminary study. Six-month-old mice were housed singly or in groups of 4, and vaginal smears were collected daily for 30 days. Cycles of singly housed mice were more uniform and about 1 day shorter than those of multiply-housed mice (Table 2). Only 25% of the cycles of singly-housed mice were longer than 5 days, one-third the incidence found in multiply-housed mice ($P<0.001$, chi-square test). Moreover, the variance of cycle length in singly-housed mice was only one-half that of multiply-housed mice ($P<0.01$, F test). Mice used in the longitudinal study were therefore housed singly to minimize the variance of baseline data and thereby optimize conditions for detecting the emergence of age-related irregularities.

RESULTS

Cycle Frequency

Cycle frequency (cycles/month) varied significantly with age (Fig. 1, $P<0.001$, analysis of variance, ANOVA). The age profile of cycle frequency fell into 3 sequential stages: *I*) an initial phase of relatively infrequent and irregular cycles, during which cycle frequency increased linearly with age; *II*) a period of maximal cycling frequency and regularity; and *III*) a period of steadily declining cycle frequency. The apparent absence of Phase I in Cohort C presumably reflects the termination of this phase before sampling had begun; irregular cyclicity of singly housed postpubertal mice has been well documented (Vandenbergh et al., 1972; vom Saal and Bronson, 1980).

Although the age profiles of cycle frequency were qualitatively similar among cohorts, there were marked quantitative differences in the timing of the three phases ($P<0.001$, ANOVA). These differences were assessed quantitatively by demarcating the three phases according to two criteria (Table 3): *1*) the period of peak cyclicity was defined as the interval in which monthly cycling frequency did not fall significantly below the value for the month of maximal cycling frequency ($P>0.05$, Duncan's Multiple Range Test, DMRT), and *2*) cessation

TABLE 1. Classification of stages of the estrous cycle by cell morphology in vaginal smears.

Stage of cycle	Cell type[a]: Leukocytes	Cell type[a]: Nucleated epithelia	Cell type[a]: Cornified epithelia	Smear density
Diestrus/proestrus (DP)	+ to ++[a] (Predominant)	+ Well-formed	0 to +	Thin
Proestrus (P)	0 to + Often degenerating	+ to +++ Well-formed (predominant)	0 to +	Medium
Proestrus/estrus (PE)	0	+ to ++	++ to +++ (Predominant)	Medium
Estrus (E)	0	0	++ to +++ Relatively small cells (predominant)	Medium to heavy
Metestrus 1 (M1)	0 to ++	0	++ to +++ Larger, more flat and clumped than in estrus (predominant)	Medium to heavy
Metestrus 2 (M2)	++ to +++ (Predominant)	+ to ++ Often irregularly shaped and vacuolated	+ to ++	Medium to heavy
Diestrus (D)	+ to +++ (Predominant)	+ Often irregularly shaped and vacuolated	0	Thin

[a]Cell density: 0=none, +=few, ++=moderate, +++=heavy.

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TABLE 2. The effect of housing density on the frequency, length and variance of the estrous cycle.[a]

	1/cage	4/cage
% Cycling[b]	100	100
(N)[c]	(10)	(8)
Cycles/30 days		
(x ± SEM)	4.2 ± 0.4[d]	3.2 ± 0.3
Cycle length		
(x ± SEM)	5.1 ± 0.2	6.4 ± 0.3
(n)[e]	(40)	(25)
Variance (s)	0.6[f]	1.8
Frequency		
4 days	12.5%	0.0%
5 days	62.5	24.0
6 days	20.0	52.0
≥7 days	5.0	24.0
	p<0.001[g]	

[a]Six-month-old virgin mice.

[b]Showing one or more cycles/30 days.

[c]Number of mice.

[d]Significantly greater than the age-matched 4/cage group (p<0.05, *t* test).

[e]Number of cycles.

[f]Significantly less than age-matched 4/cage group (p<0.05, F test).

[g]Significance level of the effect of housing density on the frequency distribution of cycle length (chi-square test).

of cyclicity was determined as the earliest age at which cycle frequency was indistinguishable from zero (P>0.05, DMRT). The magnitude of cycle frequency during Phase II was similar in all cohorts, but its duration ranged from 7 months in Cohort A to 10 months in Cohort B (Fig. 1). This was a result of the variation among cohorts in the ages of onset and cessation of Phase II, which ranged in onset from <3 to 5 months and in cessation from 11 to 14 months. The aforementioned criterion for determining the age of cessation of peak cycle frequency was relatively lenient, and it was clear from examining the data (Fig. 1, Table 3) that cycle frequency began to decline before this age in 2 of the 3 cohorts. For example, if the criterion was made more stringent by adding the requirement that the period of peak cyclicity only included months whose values are significantly different from those of lower frequency periods, (i.e., months showing only *U* classes in Table 3), the age of onset of declining cycle frequency was advanced several months in Cohorts A and B.

Cycle Length

Cycle frequency provides a global index of the cycling status of a population, encompassing changes due to individuals ceasing to cycle, and

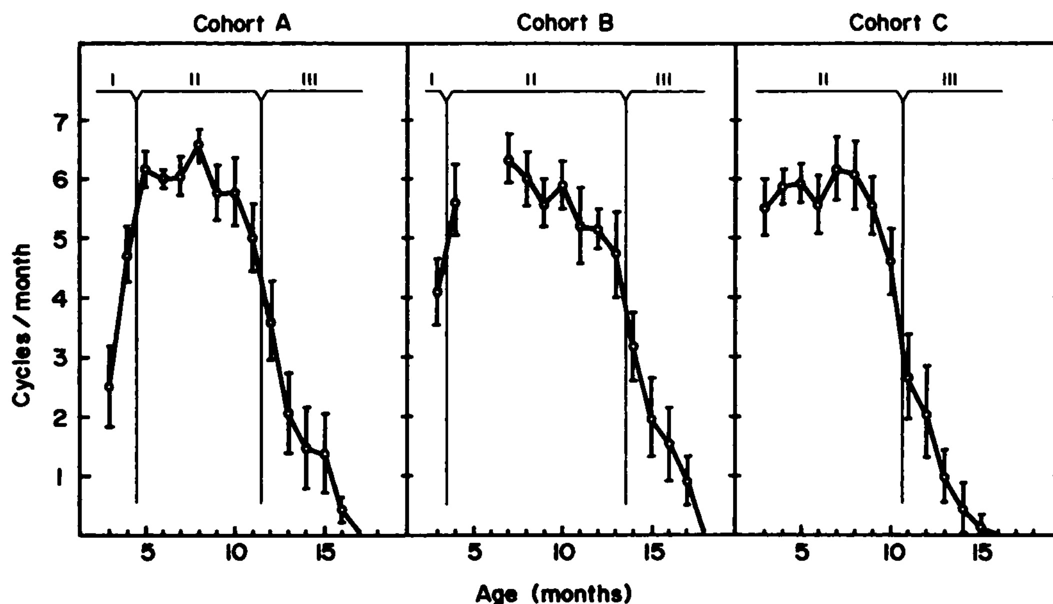

FIG. 1. Average monthly cycle frequency in three cohorts of aging virgin mice (Cohort A, n=10; Cohort B, n=14; and Cohort C, n=10). The effects of both age and cohort were significant (P<0.001, ANOVA). Phase designations refer to the ascending (I), peak (II) and declining (III) periods of cycle frequency, as described in the text.

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TABLE 3. Determination of the duration of the three phases of cycle frequency.

Cohort:	Age (months)																	
	I[a]					II				III		**						
			/							/		/						
A:	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
			(U[b]	U	U	U	U	U	U)[c]									
		V	V	V	V		V	V	V									
		W							W	W								
	X									X								
	Y										Y	Y	Y	Y				
												((Z	Z	Z	Z))[d]			
	I					II						III		**				
		/										/		/				
B:	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
		(U	–	–	U	U	U	U	U	U	U)							
	V	V	–	–			V	V	V	V	V							
	W		–	–					W		W	W						
			–	–								X	X	X				
			–	–									Y	Y	Y			
			–	–										((Z	Z	Z))		
				II					III		**							
									/		/							
C:	03	04	05	06	07	09	09	10	11	12	13	14	15	161	17	18	19	20
	U	U	U	U	U	U	U	U)										
									V	V								
										W	W							
											((X	X	X))					

[a]The phases of cyclicity are demarcated by slashes and represented as follows: Phase I (ascending), Phase II (peak), Phase III (declining), age of cessation of cyclicity=**.

[b]Months with the same alphabetic character have cycle frequencies indistinguishable from each other (p> 0.05, Duncan's multiple range test, DMRT).

[c]Ages enclosed by single parentheses comprise the period of peak cycling frequency, determined as the interval including all months whose cycling frequencies are indistinguishable (p>0.05) from the value of the month with maximal cycling frequency, as determined by DMRT.

[d]Ages enclosed by double parentheses represent months whose cycling frequencies are indistinguishable (p>0.05) from zero. The first month meeting this criterion is defined as the age of cessation of cyclicity for the cohort.

acyclic intervals, as well as changes in cycle length. Thus, cycle length is not necessarily the inverse of cycle frequency. However, in these mice, age-related changes in cycle length closely paralleled and clearly contributed to the age changes in cycle frequency (cf. Figs. 1 and 2). Overlaying the three phases of cycle frequency on the age profile of cycle length subdivided the latter into three phases distinguishable on the basis of differences in variance and median cycle length: *I)* an initial phase with a median length >5 days and 90 percentile values ranging between 6.5 and 8.5 days; *II)* a phase of short cycles (median length <5 days) and minimal variance in cycle length; and *III)* a phase of lengthened cycles (median length >5 days) with markedly greater variance.

Although median cycle length during Phase II remained relatively stable at a minimum value for several months in all cohorts (Fig. 2), the frequencies of 4- and 5-day cycles changed markedly during the same interval (Fig. 3). In all cohorts, 4-day cycles peaked between 7 and 8 months, and declined rapidly thereafter. The initial decline in 4-day cycles was due to an increase in 5-day cycles, which reached a peak

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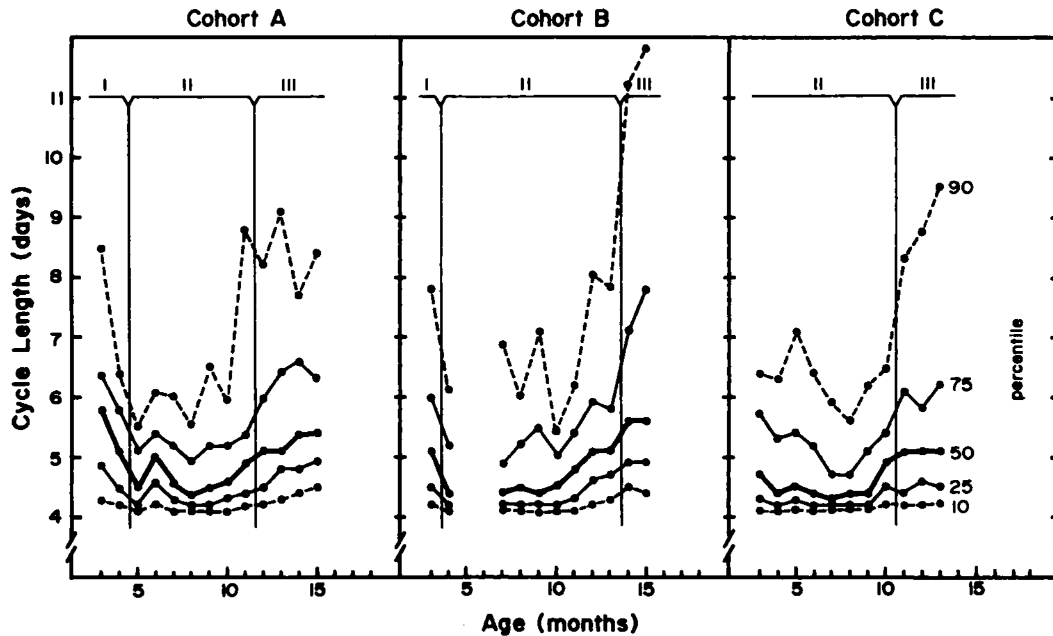

FIG. 2. Median and selected percentile values of monthly cycle lengths in three cohorts of aging virgin mice. To avoid undue weighting of deviant cycle lengths, statistics were based on all cycles from the entire cohort rather than the average monthly cycle length of each individual. Phase designations are described in Fig. 1.

frequency of 47–53% between 3 and 5 months after the peak of 4-day cycles. Cycles >5 days showed no substantial increase until 2–3 months after the onset of declining 4-day cycles, reaching a peak frequency of 36–52% between 1 and 2 months after that of 5-day cycles. These data indicate that cycles lengthened progressively with increasing age.

Cycle Regularity

The frequencies of contiguous pairs of 4-day and 5-day cycles, which may be considered as indices of cycle regularity, never exceeded 60% and 40%, respectively (Fig. 4). Moreover, these peak values were sustained for only 2 to 3 months of Phase II. The age-related profiles of contiguous pairs of 4-day and 5-day cycles closely paralleled those of 4-day and 5-day cycle frequencies (cf. Figs. 3 and 4). There was no age change in the incidence of 4- to 5- and 5- to 4-day cycles, which accounted for about 15% of the total cycle length pairs.

Although the overall frequency of contiguous pairs of 4-day and 5-day cycles was relatively low, when a cycle of either of these lengths occurred, the probability that a cycle of the same length would follow was high (Table 4).

TABLE 4. Conditional probabilities of 4-day cycles following 4-day cycles and 5-day cycles following 5-day cycles.

Age (months)	Conditional probability					
	4-day to 4-day			5-day to 5-day		
	Cohort					
	A	B	C	A	B	D
3	.200	.333[a]	.619	.000	.538	.222
4	.308	.889	.737	.600	.333	.333
5	.733	...	.767	.600	...	.333
6	.455	...	.757	.676	...	.250
7	.913	.800	.824	.667	.333	.100
8	.867	.640	.833	.421	.300	.250
9	.793	.760	.639	.500	.143	.308
10	.720	.739	.286	.619	.600	.640
11	.444	.615	.143	.696	.600	.556
12	.600	.429	.400	.273	.647	.667
13	.000	.000	.500	.167	.600	.333
14	.000	.000	.000	.167	.400	.000
15	.000	.000	.000	.647	.125	.000
16	.000	.000	.000	.000	.000	.000

[a]Phases of cyclicity, as determined in Table 3, separated by horizontal lines: Phase I separated from II by *solid line*; II from III by *dashed line*.

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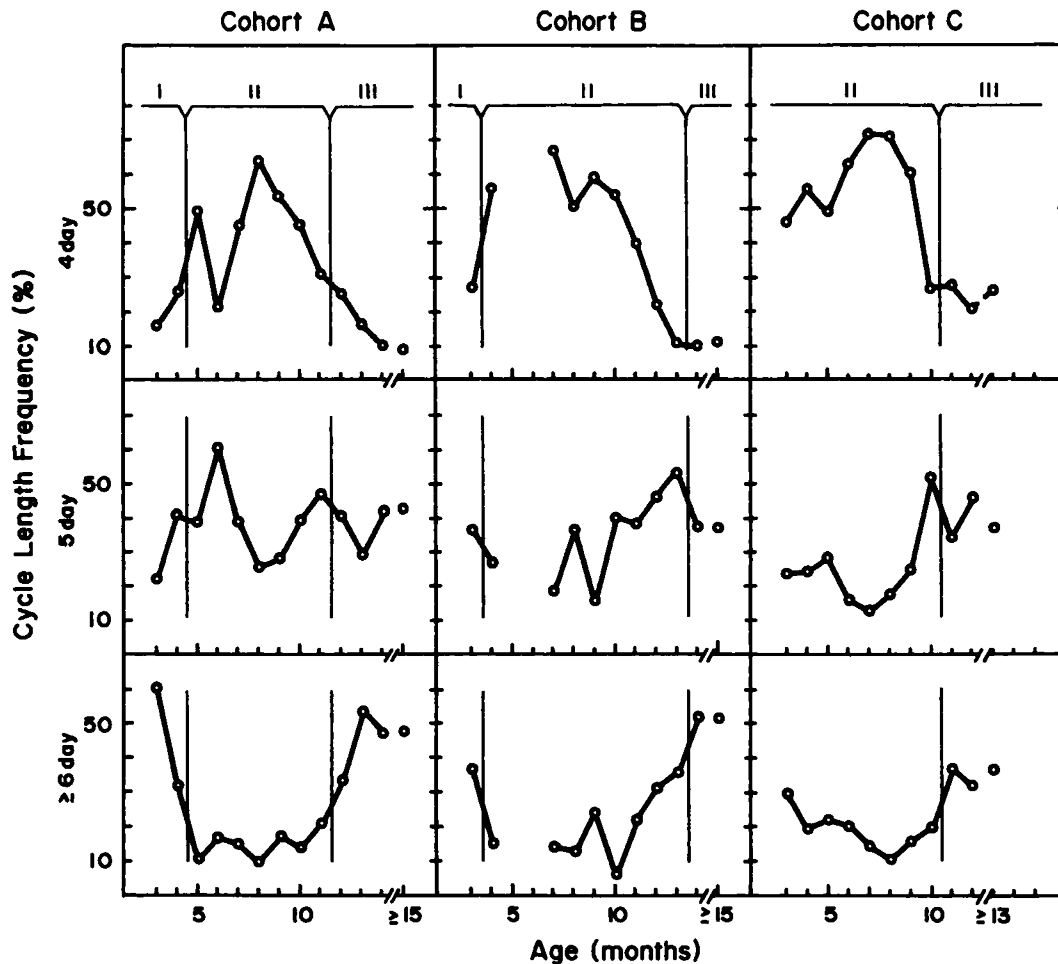

FIG. 3. Frequency profiles of cycle lengths in three cohorts of aging virgin mice. Phase designations are described in Fig. 1.

This is particularly evident for 4-day cycles: during Phase II, the median probability of a 4-day cycle following a 4-day cycle was 0.737 (all cohorts pooled). The corresponding probability value for successive 5-day cycles was lower: 0.421.

Vaginal Cytology

In addition to changes in cycle frequency, length and regularity, there were significant age changes in the frequency of vaginal smear types on Days 2, 4 and 5 of the cycle (Table 5 and Figs. 5 and 6). As with cycle length and frequency, these changes were qualitatively similar among cohorts, although their timing and magnitudes varied.

On Day 1, P was the predominant smear type in all cohorts, accounting for 60–90% of the smears (Table 5). DP was the second most frequent smear type, followed by PE. No age changes in the frequencies of proestrus smear types were present in any cohort on Day 1.

On Day 2 all smears were cornified (E, PE or M1) (Table 5 and Fig. 5). The incidence of E/PE smears varied reciprocally with M1 smears. E/PE smears predominated (70–90%) and remained at a plateau level until midway through Phase II. They began a steady decline approximately concomitant with the decrease in 4-day cycles (cf., Figs. 3 and 5). The distinguishing feature that resulted in the age-related increase of M1 classifications was

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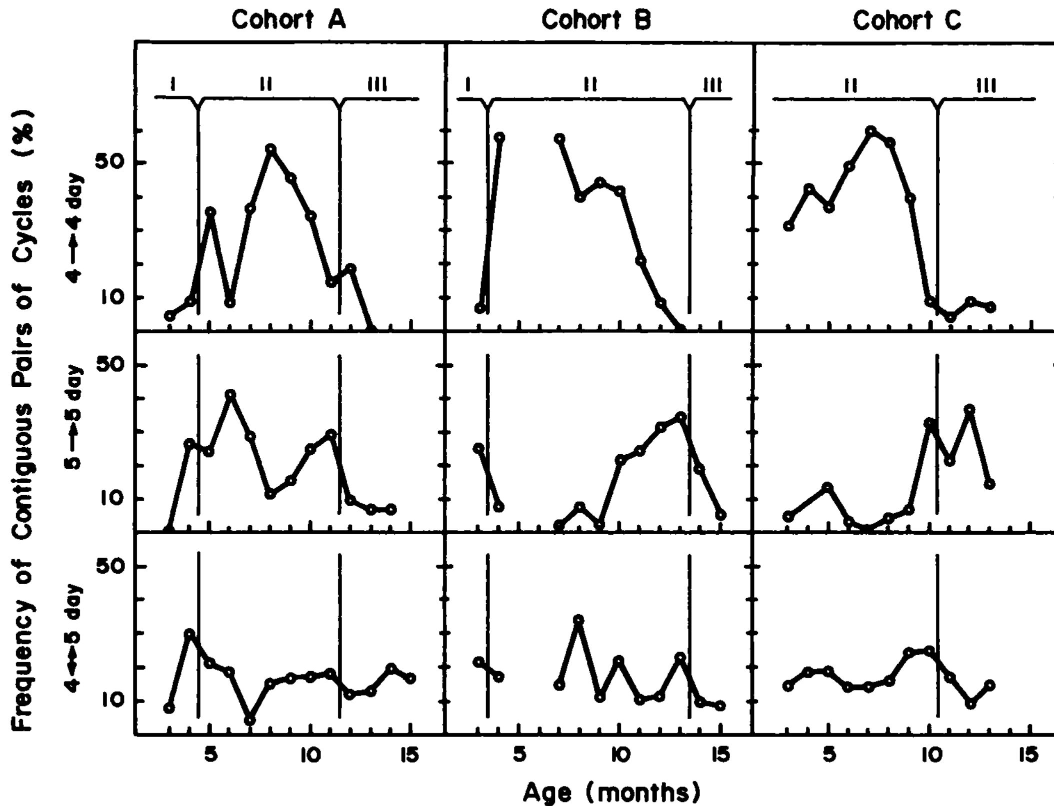

FIG. 4. Frequency profiles of contiguous pairs of 4–4, 5–5 and 4–5 + 5–4 daylength cycles in three cohorts of aging virgin mice. Phase designations are described in Fig. 1.

the enlarged flattened appearance of the cornified epithelial cells rather than the appearance of leukocytes.

On Day 3, over 90% of the smears were cornified (Table 5). M1 smears comprised the majority, followed by E smears. No consistent age changes were observed.

On Day 4, there were marked age-changes in vaginal cytology (Fig. 6). The predominant smear types on this day, M1 and M2, varied reciprocally. During Phase I, M2 was less common, comprising 25–35% of the smears. During Phase II, M2 reached a peak of 60–80% around 8 months of age, concomitantly with the peak of 4-day cycles. Thereafter, the incidence of M2 declined as that of M1 increased steadily to values of 40–60%.

On Day 5, leukocytic smears predominated (Table 5). M2 was the most common smear type, although it declined significantly during Phase III. This decline was accounted for by an increase in M1 as well as D and DP smears.

Analyses of Days >5 were pooled, because the sample sizes were too small for individual days to be evaluated. Leukocytic smears predominated on these days (Table 5), accounting for 50–90% of all smears; M1 smears accounted for the remainder. No consistent age changes in the frequencies of these smear types were apparent in any cohort.

DISCUSSION

General Pattern

The age-related changes in the cyclicity of C57BL/6J mice are qualitatively similar to those previously described for other strains of mouse and rat (Table 6). Estrous cyclicity usually begins shortly after vaginal opening, and rises to a peak level which is usually maintained into midlife. During the subsequent declining phase, cycles are often lengthened and an increasing proportion of the population becomes acyclic.

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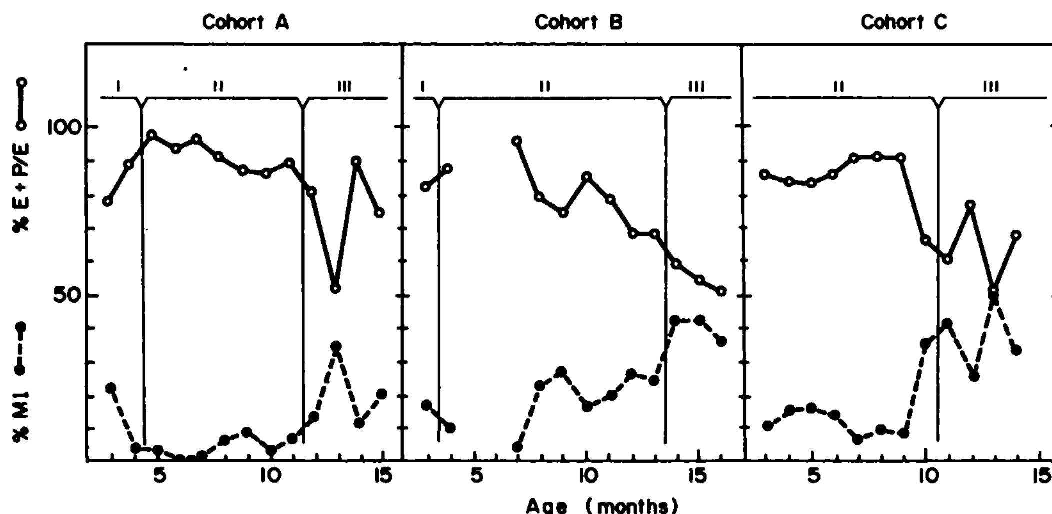

FIG. 5. Profiles of vaginal cytology on Day 2 of the estrous cycle in three cohorts of aging virgin mice.

Cohort Variability

Although the general profiles of cycle frequency, length and vaginal cytology were comparable among cohorts, considerable inter-cohort variation was present in the timing of the transitions between phases. Thus, considerable care must be taken in selecting the age of young control animals for aging studies of cycle-related phenomena. For example, if the cycle frequency of 3- to 4-month-old mice is compared against that of 11- to 12-month-old mice, no age change may be found since young mice do not always attain peak cyclicity until 5 months of age.

The factor(s) responsible for inter-cohort variation in cyclicity have not been elucidated. Genetic variability is an unlikely candidate, since the C57BL/6J strain is inbred. Both male

TABLE 5. Effect of phase of cyclicity on the frequency distribution of vaginal cytological states on successive days of the cycle in aging mice.

Median monthly frequency (%)[a]											
	Vaginal	Phase[b]					Vaginal	Phase			
Day	state	I	II	III	p-value[c]	Day	state	I	II	III	p-value
1	P	74	77	75	N.S.	4	M1	63	27	42	<0.01
	DP	22	17	20	N.S.		M2	30	65	42	<0.01
	PE	5	5	3	N.S.		D + DP	0	6	10	N.S.
							E + PE	2	0	0	N.S.
2	E + PE	81	86	58	<0.01	5	M2	61	61	41	0.01
	M1	17	10	35	<0.01		M1	12	12	22	<0.05
							D + DP	9	18	29	N.S.
3	M1	52	82	72	N.S.	6–14	D + DP	48	37	39	N.S.
	E + PE	41	14	12	N.S.		M2	36	31	25	N.S.
	D + DP	0	0	0	N.S.		M1	12	23	36	N.S.

[a]Values pooled across all three cohorts.

[b]Phases of cyclicity determined according to methods in Table 3.

[c]Significance of differences among phases, determined by Kruskall-Wallis Test. Borderline significance (<0.15) shown numerically.

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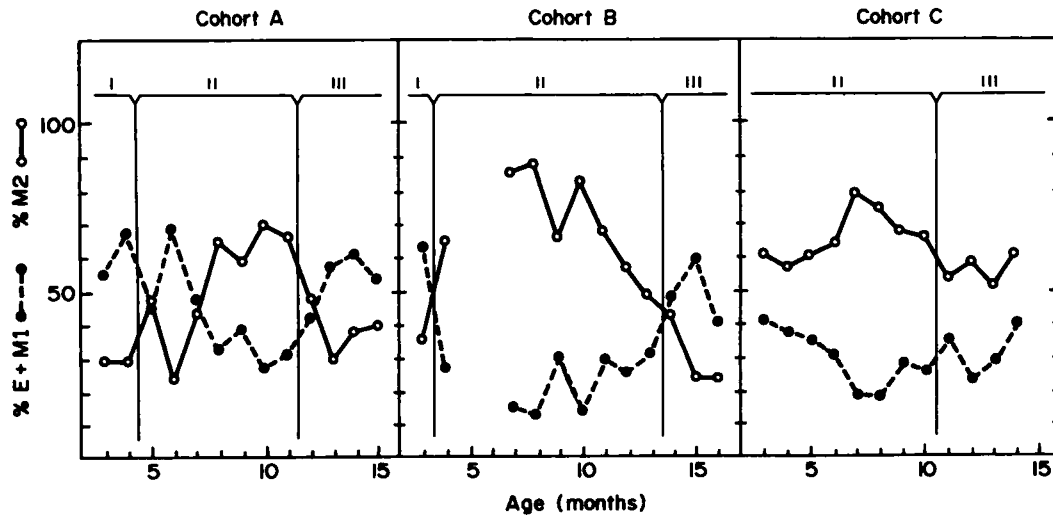

FIG. 6. Profiles of vaginal cytology on Day 4 of the estrous cycle in three cohorts of aging virgin mice.

and female pheromones modulate estrous cyclicity in mice (Whitten and Champlin, 1973), and thus may have played a role. Although the density of males was held constant, that of the females varied nearly 5-fold during the course of the study. Seasonal influences on reproductive function (although not specifically on cyclicity) in temperature- and illumination-controlled settings are well documented in rodents (Pennycuik, 1972; Piacsek et

TABLE 6. Incidence and vaginal cytological status of prolonged cycles in different strains of mouse and rat ranked by age.

Species	Strain	Onset of acyclicity[a] (month)	Prolonged cycles?	Vaginal cytology of extended period[a]		Source
				Leukocytic	Cornified	
Rat	DA	6–7	Yes		X	Everett, 1939
Rat	Sprague-Dawley	6–8	Yes			Everett, 1980
Rat	RxUF1	8–10	No			Van der Schoot, 1976
Rat	Long Evans	10–11	No			Cooper et al., 1980
Rat	Sprague-Dawley	12	Yes		X	Gosden, R., p. comm.
Rat	Sprague-Dawley		Yes			Gray and Wexler, 1980
Rat	Holtzman S/D		Yes		X	Butcher and Page, 1981
Rat	Wistar	12–15	Yes			Aschheim, 1976
Mouse	C57BL/6J	13–16	Yes	X	X	(present data)
Rat	Long Evans	14	Yes		X	Wilkes et al., 1979
Rat	Long Evans	14–15	Yes			Lu et al., 1979
Mouse	(DBAxCE)F1	12–19	Yes		X	Dickie et al., 1957
Mouse	DBAf	15	Yes	X		Thung et al., 1956
Mouse	C57BL/6	16–20	Yes	X		Parkening et al., 1980
Mouse	C3HfC57b/Se	>17	Yes	X		Caschera, 1959
Rat	Wistar	18	Yes	X		Clemens, J., p. comm.
Mouse	RIII/Dm/Se	>20	Yes	X		Caschera, 1959
Mouse	C57BL	20	Yes	X		Thung et al., 1956
Mouse	020	20	Yes	X		Thung et al., 1956
Mouse	(020xDBAf)F1	24	Yes	X		Thung et al., 1956

[a]Blank where not applicable or data is unavailable

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al., 1974; Cohen and Mann, 1979). Preliminary data suggests that both colony density and seasonal cues may have contributed to the inter-cohort variation observed in this study. Cycle frequency was suppressed during the winter and at high colony density, particularly during Phases I and III (Felicio, Nelson and Finch, unpublished).

Onset of Peak Cyclicity

The onset of peak cyclicity occurred several months after puberty. Vom Saal and Bronson (1980) similarly reported that singly housed CF-1 mice in a male-free environment did not exhibit regular cyclicity until they were over 3 months of age. In contrast to mice, limited evidence suggests that the onset of regular cyclicity occurs almost immediately after vaginal opening in rats, although the first cycles are prolonged (Long and Evans, 1922; Gentry and Wade, 1980). This apparent species difference may reflect a greater sensitivity of the mouse's estrous cycle to pheromonal and tactile influences (Whitten and Champlin, 1973). For example, the onset of first estrus in mice can be delayed up to 20 days after vaginal opening if male pheromones are absent (Vandenbergh et al., 1972). Moreover, multiple housing disturbs regular cyclicity in mice (Champlin, 1971; Table 2), but not in rats (Aschheim, 1976; Nelson, Felicio and Finch, unpublished data). In addition, anosmia interferes with estrous cycles in mice (Whitten, 1956; Lamond, 1958), but not in rats (Rosen et al., 1940).

Cycle Regularity

Unlike rats, which characteristically cycle in long series of either 4- or 5-day intervals regardless of environmental conditions (Cooper et al., 1980; Nelson, Felicio and Finch, unpublished), mice, even when housed singly, often cycle less regularly. In this study, cycle regularity, defined as the frequency of contiguous pairs of either 4-day or 5-day cycles, was low during much of the cycling lifespan. However, these cycle pairs together comprised between 65 and 80% of the total pairs during most of Phase II. The frequency and contiguity of 4-day cycles were among the most sensitive and invariant measures of reproductive aging in this study. In all cohorts, both of these parameters peaked at about 60% by 7–8 months of age, and began to decline by 9 months. Thus, in singly housed virgin mice, full maturation of the system governing estrous cyclicity may not occur until shortly before the initiation of its decline. The relatively late onset of peak cyclicity was surprising in view of the evidence that peak fertility in mice usually coincides with the second litter and begins to decline shortly thereafter (Jones and Krohn, 1961; Talbert, 1977). However, given the sensitivity of the mouse's estrous cycle to environmental modulation, it is conceivable that factors such as parity or pheromonal influences may profoundly influence the timing of age-related changes. For example, the onset of Phase III in our colony occurs about 2 months earlier in retired breeders than in virgin mice (Nelson, 1981).

The contiguity of 4-day cycles was relatively low for the population as a whole. However, when a 4-day cycle did occur, the median probability of an ensuing 4-day cycle was >0.7 during much of Phase II, indicating that a subpopulation cycled quite regularly. A corollary to this inference is that there was considerable inter-animal variation in cycle regularity. Although the basis for subpopulational differences in cycle regularity is not clear, one possibility is the recent report that intrauterine proximity of female to male fetuses influences cycle length in adulthood (vom Saal and Bronson, 1980). The inter-animal variation in cycle regularity that is present in virgin C57BL/6J mice provides an opportunity to examine the role of regularity in the timing of cycle cessation and in the duration and qualitative nature of acyclic phenomena (e.g., persistent vaginal cornification or leukocytosis). These questions will be addressed in a subsequent report.

Prolonged Cycles During the Transition to Acyclicity

Progressively lengthening cycles characterize the declining phase of cyclicity in this strain. During the initial phase of decline (10–12 months of age), prolonged cycles are associated with and may be caused by a delay in the preovulatory rise of estradiol (E_2) (Nelson et al., 1981). If this delay is responsible for the initial prolongation of cycles, their progressive lengthening with advancing age may reflect an increasingly impaired preovulatory rise of E_2, which may ultimately become inadequate to trigger an ovulatory surge of gonadotropin.

Although prolonged cycles mark the transi-

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tion to acyclicity in the majority of mouse and rat strains, the vaginal cytology of the extended period of the cycle differs among strains and according to the age of cycle cessation (Table 6). In general, prolonged cornification appears to be more common in rats, which comprise the majority of early terminating strains, whereas extended leukocytic periods are more characteristic of mice or late terminators. The present study demonstrated that the vaginal cytology of the extended period can also vary with age within a strain. During the initial period of declining cyclicity, Days 4 and 5 of the cycle were predominantly leukocytic. However, with advancing age an increasing proportion of the smears on these 2 days were cornified.

Because cornification and leukocytosis reflect differing hormonal milieus, vaginal cytology provides clues to the hormonal changes underlying the transition to acyclicity. The prolonged diestrus associated with the initial phase of cycle lengthening in C57BL/6J mice could indicate luteolytic failure (as in pseudopregnancy). However, this possibility seems unlikely since the midcycle rise of progesterone in 10- to 12-month-old mice is not prolonged (Nelson et al., 1981). Alternatively, extended leukocytosis might reflect insufficient E_2 secretion due to reduced numbers of maturing follicles or a delay in their development. This explanation is consistent with the delayed preovulatory rise of E_2 observed during the initial prolongation of cycles in aging mice (Nelson et al., 1981). The more frequent appearance of extended cornification at later ages suggests an age-related increase in the incidence of cycles with prolonged periods of estrogen secretion, presumably as a result of incomplete or delayed ovulation with concomitantly reduced or delayed luteinization. Prolonged estrogen secretion could be the consequence of a refractory hypothalamic-pituitary complex, unable to release an ovulatory surge of gonadotropin in response to an unimpaired E_2 signal. Alternatively, E_2 secretion might be adequate to maintain vaginal cornification, but insufficient to elicit an ovulatory surge of gonadotropin. Recent evidence suggests that the extended cornification during the prolonged cycles of Sprague-Dawley rats is associated with a prolonged and unimpaired preovulatory elevation of E_2 (Butcher and Page, 1981), indicating that the hypothalamic-pituitary complex is refractory to positive-feedback stimulation of gonadotropin secretion by E_2 in at least one strain of rat. Thus, although prolonged cycles commonly herald the loss of cyclicity, the different vaginal cytologies associated with the extended period of the cycle suggest that the hormonal correlates and, hence, the underlying mechanism may vary according to age or species. This interpretation is consistent with a multifactorial etiology of acyclicity in which ovulatory failure may result from both intrinsic ovarian changes as well as failure of the hypothalamic-pituitary complex to produce an ovulatory surge of gonadotropin.

ACKNOWLEDGMENTS

We thank H. Torres, D. Jacobson and C. Nunez for assistance in data analysis, and G. Kendrick for excellent care of the mouse colony.

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