Routledge
Taylor & Francis Group

Experimental Aging Research

ISSN: 0361-073X (Print) 1096-4657 (Online) Journal homepage: http://www.tandfonline.com/loi/uear20

Physiological assays for biological age in mice: Relationship of collagen, renal function, and longevity

David E. Harrison & Jonathan R. Archer

To cite this article: David E. Harrison & Jonathan R. Archer (1983) Physiological assays for biological age in mice: Relationship of collagen, renal function, and longevity, Experimental Aging Research, 9:4, 245-251, DOI: 10.1080/03610738308258459

To link to this article: http://dx.doi.org/10.1080/03610738308258459

Experimental Aging Research, Volume 9, Number 4, 1983, ISSN 0361-073x

Physiological Assays for Biological Age in Mice: Relationship of Collagen, Renal Function, and Longevity[1]

DAVID E. HARRISON[2] AND JONATHAN R. ARCHER[2]

Tests of physiological changes with age are illustrated by collagen denaturation times of tail tendon fibers, and urine concentrating abilities; the tests are evaluated using the following four criteria: change with age, repeatability, relationship to other assays, and relationship to longevity. These tests usually showed highly significant changes with age when mice of different ages were compared for nine mouse genotypes, however neither appeared to be related to subsequent longevities of individual mice. When average values for eleven mouse genotypes were compared, the mean longevities of the genotypes were not significantly correlated with their mean collagen denaturation times or mean renal concentrating abilities, tested at two different ages. The relationships between all three factors—collagen denaturation times, urine concentrating abilities, and longevities—were tested in the same individuals for mice of six different genotypes at 600–700 days of age. Only one marginally significant correlation appeared out of 21 tested; this probably occurred by chance. We conclude that tail tendon collagen denaturation times and urine concentrating abilities change with age independently of each other; furthermore, these changes are unrelated to subsequent longevities, at least when linear relationships are tested. These data suggest that aging is timed by more than one mechanism and demonstrate that strong correlations with chronological age do not necessarily indicate that independent tests will be correlated with longevity or with each other.

We have defined four criteria that are useful for evaluating physiological age assays [9]:

1. *Change with age.* The assay must show significant changes with age.
2. *Repeatability.* Changes should be repeatable in the same individual over a short time, showing that the assay measures stable changes.
3. *Relationships to other assays.* Independent assays may give similar results in the same individuals. This criterion may not be met if changes with age in the assays compared are timed by different mechanisms.
4. *Relationships to longevity.* The results of the assay predict subsequent longevities. This criterion may not be met if the assayed changes with age are independent of those causing morbidity.

In order to meet the criteria 2–4, it is necessary that performing the assay not cause any long-term deleterious effect on the individual tested. Criteria 3 and 4 need not be met by physiological age assays, but are important to test in order to understand how the aging process is timed. If aging is timed by a single factor that causes the measured physiological changes with age and determines subsequent longevity, then criteria 3 and 4 would be met. If aging is multifactorial, and aging in each system is timed independently, criterion 3 would not be met because the different physiological assays would not be related. Furthermore, longevities would be determined by the first physiological system that declined so greatly with age that life could no longer be sustained. If this was not the one that was tested, the physiological assay would not correlate with subsequent longevity.

A major advantage of studying aging in the mouse model system is the availability of genetically and environmentally defined animals [17]. Mice of several different genotypes (inbred strains and their F_1 hybrids) should be used, however, to avoid confusing specific characteristics of a single genotype with general characteristics for a species [17]. Environmental definition includes temperature and humidity control, precise definition of diets, control of microorganisms and elimination of pathogens. The latter is particularly important because older animals are likely to be more vulnerable to pathogens than younger animals, causing changes with age to be confounded with increasing vulnerability to pathogenic organisms. Thus the rigidly controlled artificial environments that should be used with mouse model systems minimize variability from causes other then the aging process.

We report here studies of collagen and renal aging, using physiological tests that do not injure the mouse being tested. According to a previous report, the test of collagen denaturation times had already met the first two criteria, changing significantly with age and showing repeatable changes when the same individuals were retested 22 or 31 days later [11]. Our results confirm this and show that urine concentrating abilities also decline with age. However, neither test correlates with subsequent life expectancies of individuals or genotypes; they also fail to correlate with each other.

Methods

Mice

All mice were genetically defined members of inbred strains or F_1 hybrids of two inbred strains. Mice of each strain have been brother/sister mated for at least 60 generations and are therefore completely inbred (homologous for alleles at all genetic loci) except for mutations [2]. Genotypes of mice

[1]This work was partially supported by NIH grants AG 01755 and AG 00594 from the National Insitute on Aging. We thank E. Farrin and B. Stork for dependable technical assistance.

[2]From The Jackson Laboratory, Bar Harbor, Maine 04609, U.S.A.

used included the following: C57BL/6J (B6), CBA/HT6J (CBAT6), CBA/CaJ (CBA), and their F_1 hybrids (B6CBAT6F_1, B6CBAF_1). Both sexes were studied in most cases, and each sex is considered a separate genotype. Aging animals were housed in isolated, environmentally controlled rooms under positive pressure with filtered air, and with temperatures, humidity and light cycles controlled. Mice were housed four per side in double-sided plastic cages (12 by 6 in. per side) covered with filter hoods. Every six months, at least 20 old mice were submitted to The Jackson Laboratory's diagnostic laboratory for routine animal health status assessment. In addition, any mice that appeared ill without an explainable cause, such as extreme age or experimental treatment, were submitted as soon as they were found. Mice from this colony were free of extoparasites, nematodes, cestodes, and trematodes. Two species of intestinal protozoa, *Tritrichomonad spp* (occasional) and *Spironucleus (Hexamita) muris* (rare) were observed. *Pasteurella pneumotropica* was commonly isolated from the upper respriatory tract. Routine cultures of intestines for *Salmonella spp, Pseudomonas spp,* and *Citrobacter freundi 4280* were negative. The predominant intestinal flora included *Proteus spp* and other coliforms. Routine cultures of tracheas or middle ears were negative for mycoplasma and other recognized pathogenic bacteria such as *Streptococcus pneumonia, Bordetella bronchiseptica,* or Corynebacterium kutscheri. Lesions suggestive of Tyzzer's disease were not observed. Serological tests conducted at the Murine Virus Diagnostic Laboratory, Microbiological Associates, Bethesda, MD, indicated that the mice were free of the following viruses: Ectromelia, Sendai, K, Mouseadeno, Polyoma, Lymphocytic choriomeningitis, Reovirus III, and GD VII viruses. Titers exceeding 1:20 were not observed in hemaglutination inhibition tests for antibody to pneumonia virus of mice. From July 1979 to June 1980 but not thereafter significant compliment fixing antibody titers to mouse hepatitis virus (MHV) were observed. (The foregoing report was prepared by Terrie Cunliffe Beamer, DVM, MS, of The Jackson Laboratory.)

Mice were fed a pasteurized diet (The Emory Morse Co., 96WA containing 357 C/100 g, 22% protein, 7% fat and 50% nitrogen free extract (NFE) which is mostly carbohydrates); they had *ad libitum* food, and chlorinated water acidified to prevent growth of *Pseudomonas*. The room temperature was $22 \pm 2°C$, and it was lighted from 6 A.M. to 6 P.M. All mice were bred and maintained at The Jackson Laboratory, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

Tail tendon denaturation times were assayed as previously reported [10; 11]. Urine concentrating abilities were assayed according to the following schedule: water bottles were removed, and mice were intraperitoneally injected with 1.0 ml 0.9% saline at approximately noon. Food remained available throughout the experiment. Urine was sampled 48 hours later, and the urine osmolality was measured on a WESCOR vapor pressure osmometer using 5 μl samples, diluted as necessary for accurate measurements. Correlation coefficients (r) and t values were calculated by standard methods (R. Sokal and J.F. Rholf, *Biometry,* W.H. Freeman and Co., San Francisco, 1969). The number of degrees of freedom (group $n = 2$) and t values were used with a standard t-test table to determine significance.

Results

Data on longevity in our aging colonies are summarized in Table 1. Medians were higher than means in all inbred strains because there were more early than late deaths, however this was not true in three of the four F_1 hybrid mouse genotypes.

Table 1

Longevities of Mice of Several Inbred Strains and Their F_1-Hybrids

Genotype	Sex	Lifespan (days) Median	Lifespan (days) Mean ± SE(n)	Longest-Lived 10% (Range)
C57BL/6J	M	851	815 ± 16 (169)	1040–1180
C57BL/6J	F	809	780 ± 13 (154)	950–1170
CBA/HT6J	M	862	830 ± 14 (132)	1000–1050
CBA/HT6J	F	874	856 ± 10 (223)	1020–1150
B6CBAT6F_1	M	888	893 ± 15 (115)	1060–1250
B6CBAT6F_1	F	928	889 ± 17 (145)	1180–1210
CBA/CaJ	M	804	770 ± 11 (181)	920–1000
CBA/CaJ	F	932	898 ± 12 (190)	1060–1280
B6CBAF_1	M	918	925 ± 18 (70)	1120–1230
B6CBAF_1	F	944	970 ± 32 (37)	1200–1320

Lifespans are given from weaning at 28 ± 3 days of age for mice born in 1972 to 1976. Environmental controls were improved during this period by using pasteurized food and maintaining a more narrow range of temperatures. No pathogens known to affect mouse longevity were found in either colony; however, some old mice had MHV (mouse hepatitis virus) and pinworms (both eliminated eventually). Some healthy 24- to 31-month-old animals were removed for experiments, and are not included in these groups. The number removed was less than 10% of any group. Longevities of B6D2F_1 mice from a separate colony with better environmental controls and no mice removed for experiments were: male 975 ± 18 (113) and females 879 ± 18 (120, giving data as mean ± SE (n).

Comparisons of the CBA/HT6J and CBA/CaJ genotypes suggest that there is no important deleterious effect on longevity from homozygous *T(14;15)6Ca* (T6) chromosome translocations. B6CBAT6F_1 mice are heterozygous for the T6 chromosome translocation and have slightly shorter lifespans than B6CBAF_1 mice. Figure 1 illustrates longevities for B6, CBAT6, and their F_1 hybrid virgin male mice. Although mean longevities of CBAT6 males are slightly longer than those of B6 males, the longest lived B6 individuals substantially outlived the longest lived CBAT6 mice (Figure 1). This is because the CBAT6 mice showed a remarkedly consistent time of death.

The relationships of tail tendon collagen denaturation times with chronological age are shown in columns 3–5 and with longevities in columns 6–8 of Table 2. Correlations with chronological age are remarkably good, as we have previously reported [11]. However, correlations with longevity do not appear to be significant, with the one case of statistical significance almost surely occurring by chance. The relationship between collagen denaturation times and chronological age for B6 male mice is illustrated in Figure 2. Similar relationships for many mouse genotypes have been previously reported [11].

The same test was used to compare mean values for mice of 11 different genotypes; those in Table 2 plus male B6D2F_1 mice. The average collagen denaturation times and mean longevities for the 11 genotypes were correlated. This was done for denaturation times at two ages, 400–550 days and 600–750 days. The correlation coefficients are −0.41 in the former and +0.41 in the latter case; these are not statistically significant and are in opposite directions. Thus the genotypes having more rapidly aging collagen do not tend to have shorter lifespans.

The relationships of urine concentrating abilities and chronological age are shown in columns 3–5 and with longevities

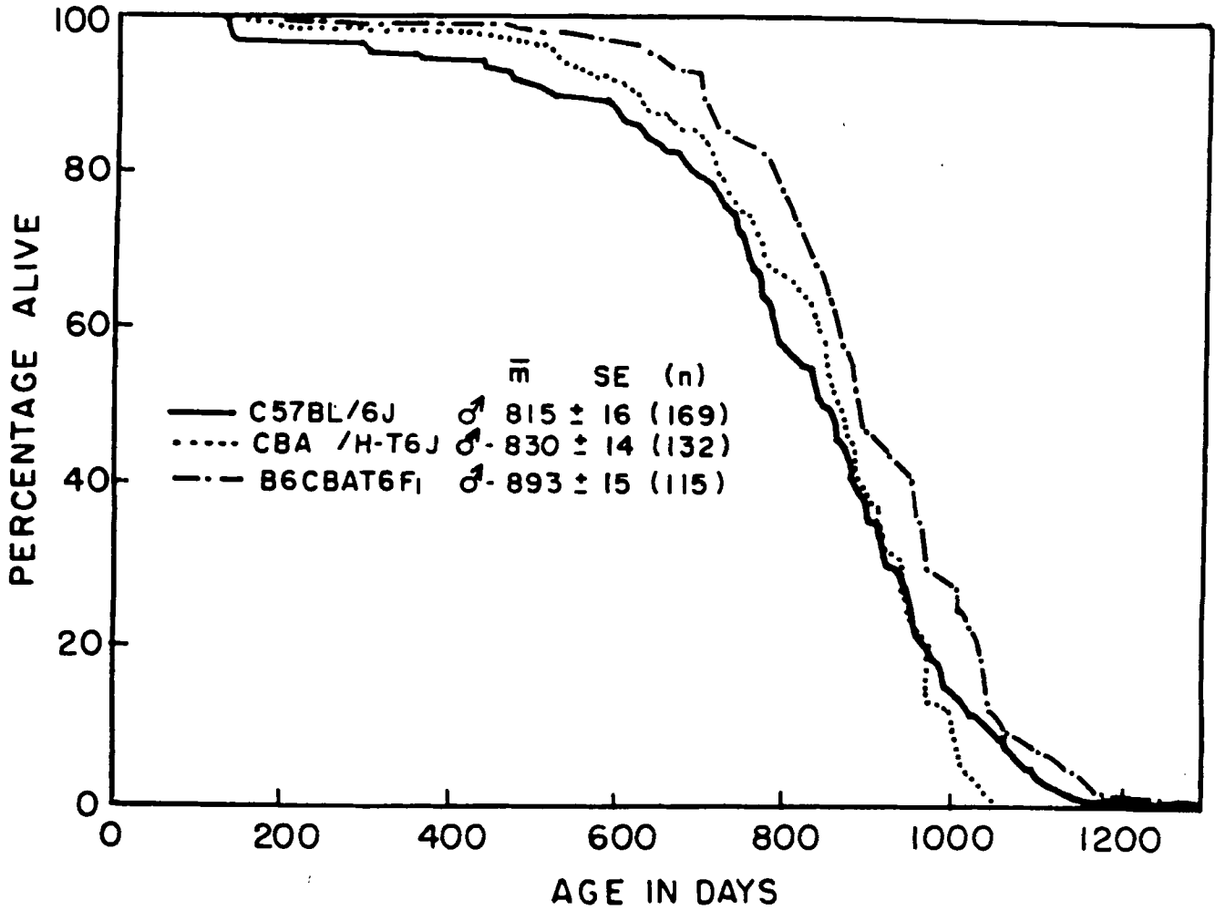

Figure 1. Longevity curves for B6, CBAT6, and B6CBAT6F$_1$ male mice.

Table 2

Collagen Denaturation Time: Correlations with Chronological Age and Longevity[a]

		With Chronological Age			With Longevity[b]		
Genotype	Sex	*n*	CC	Significance	*n*	CC	Significance
All	Both	908	0.54	<0.01	130	0.02	NS
C57BL/6J	M	52	0.92	<0.01	11	−0.32	NS
C57BL/6J	F	56	0.88	<0.01	8	−0.32	NS
CBA/HT6J	M	37	0.90	<0.01	14	−0.58	<0.05
CBA/HT6J	F	43	0.56	<0.01	11	0.21	NS
B6CBAT6F$_1$	M	37	0.77	<0.01	7	0.45	NS
CBA/CaJ	M	33	0.81	<0.01	8	−0.16	NS
CBA/CaJ	F	60	0.68	<0.01	15	−0.19	NS
B6CBAF$_1$	M	23	0.64	<0.01	10	0.52	NS
B6CBAF$_1$	F	21	0.81	<0.01	11	0.12	NS

[a]For each correlation tested, *n* is the number of mice tested and CC is the correlation coefficient. Significance is the probability of this correlation occurring by chance according to Student's *t*-test. NS means not significant because this probability is greater than 0.05 ($p<0.05$).

[b]Mice tested at 650–700 days of age; then longevities were determined.

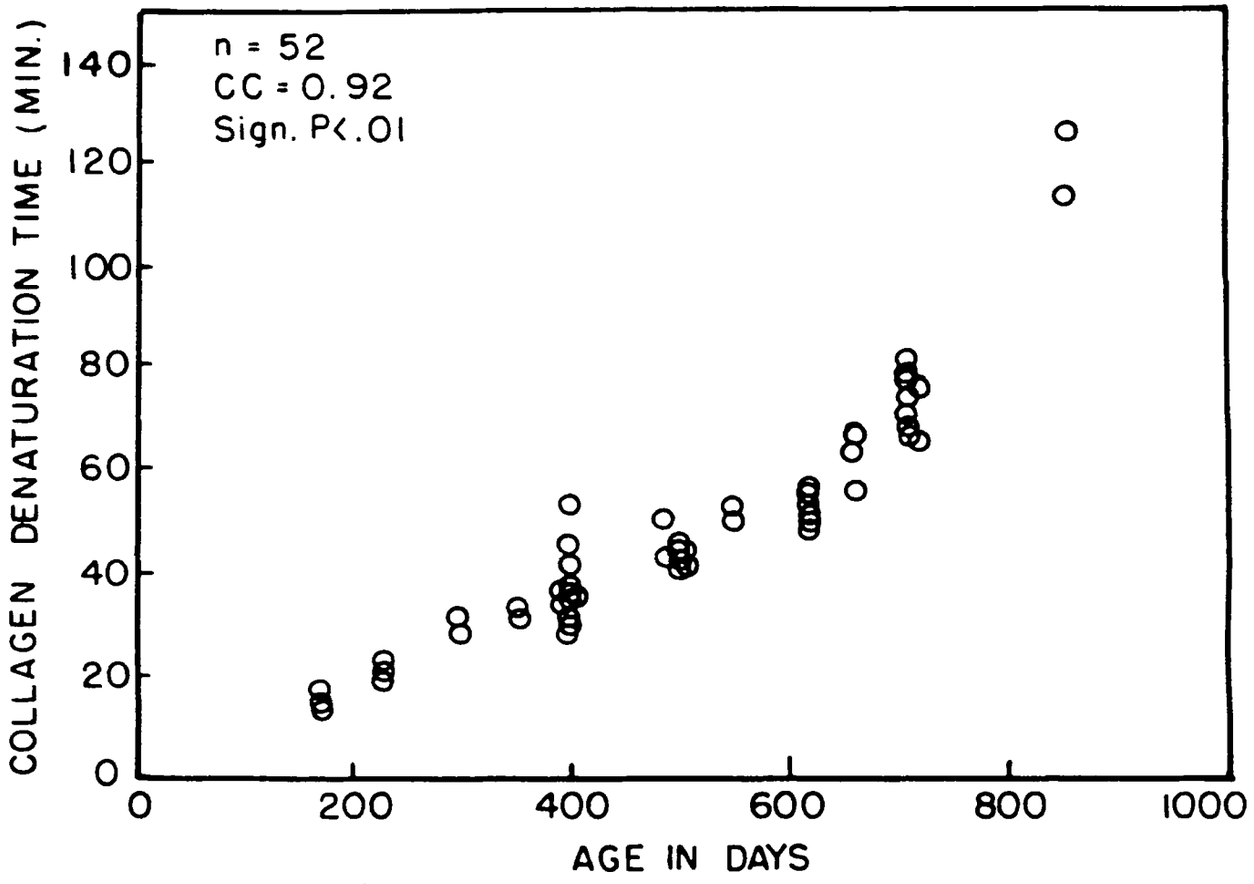

Figure 2. Relationships between tail tendon fiber denaturation and age of B6 male mice.

Table 3

Urine Concentrating Ability: Correlations with Chronological Age and Longevity[a]

		With Chronological Age			With Longevity[b]		
Genotype	Sex	*n*	CC	Significance	*n*	CC	Significance
All	Both	597	−0.56	−0.01	62	−0.07	NS
C57BL/6J	M	55	−0.67	<0.01	9	−0.38	NS
C56BL/6J	F	74	−0.66	<0.01	2	–	NS
B6CBAT6F$_1$	M	25	−0.49	<0.02[c]	9	−0.25	NS
CBA/CaJ	F	25	−0.49	<0.05[c]	3	0.74	NS

[a]For each correlation tested, *n* is the number of mice tested and CC is the correlation coefficient. Significance is the probability of this correlation occurring by chance according to Student's *t*-test. NS means not significant because this probability is greater than 0.05 ($p<0.05$).

[b]Mice tested at 650–700 days of age; then longevities were determined.

[c]Measurements only made over a short age range (16–22 months), causing poor correlations within these genotypes, and in five others not shown. However, young B6 mice have no higher urine concentrating abilities than young CBA and B6CBAF$_1$ mice. Therefore, pooled data show a valid change with chronological age.

in columns 6–8 of Table 3. For a number of genotypes, the measurements were only made on 16- to 22-month-old individuals, and changes with age could not be detected. However, in most cases while correlations are not as precise as with collagen, there is a highly significant decline in urine concentrating ability with age. This is illustrated in Figure 3 for B6 male mice. No significant correlations with longevity were seen.

When the same 11 genotypes discussed above were compared for this test, their average urine concentrating abilities at the same two age ranges and mean longevities were correlated. The correlation coefficients are 0.28 and −0.55 at the two age ranges. These are not statistically significant and are in opposite directions. Thus, the genotypes having more rapidly aging urine concentrating abilities do not tend to have shorter lifespans.

Collagen denaturation times, urine concentrating abilities, and subsequent longevities were determined on each individual from a group of mice representing six genotypes at 600 to 700 days of age. Table 4 shows that there were no statistically significant correlations between collagen denaturation times and longevities (columns 3–5), one significant at $p<0.05$ between urine concentrating abilities and longevities (columns 6–8), and no significant correlations betwen collagen denaturation times and urine concentrating abilities (columns 9–11). For the number of correlations studied in Table 4, it is likely that one would be found by chance at the 0.05 level, so we conclude that no significant correlations existed.

Discussion

The four criteria noted in the *Introduction* can be used to evaluate the physiological age assays for collagen denaturation times and urine concentrating abilities. The assays met criterion 1, as results changed significantly with chronological age (Tables 2 and 3, Figures 2 and 3). Matched groups of tested and untested mice had similar longevities (data not shown), so these assays did not affect the mice tested and can be validly evaluated using criteria 2–4. Collagen tests have been previously shown to meet criterion 2, repeatability [11]; results with urine concentrating abilities may not be reliable, because mice may require a long period to completely recover from the dehydration necessary for the test. Criterion 3 was not met by these assays, as their results showed no significant correlations (Columns 9–11, Table 4). This suggests that the underlying mechanisms timing the declines with age in collagen denaturation rates, and in urine concentrating abilities, are not related. Criterion four was also not met, suggesting that the defects developing with age that cause death in the types of animals used were not related to the changes with age assayed by these physiological tests.

There has been a strong interest in developing a set of tests measuring physiological age in man [4]. By using a number of different tests, coefficients of multiple correlations to the chronological ages of human beings ranged between 0.74 and 0.83 in one study [12], and reached 0.8 to 0.96 in another [6]. However, these were studies of populations and the precision possible for age estimates in individuals was not given. Young and Rickert [19] have pointed out that these are much less precise than estimates for populations. Although aging is commonly thought to begin in human beings at about age 30, physiological tests in cross sectional studies may peak as early as 3 to 20 years of age [1]. Therefore, it is wise to study a variety of ages and determine the shape of physiological changes across the lifespan in human beings, as well as in laboratory animals [11].

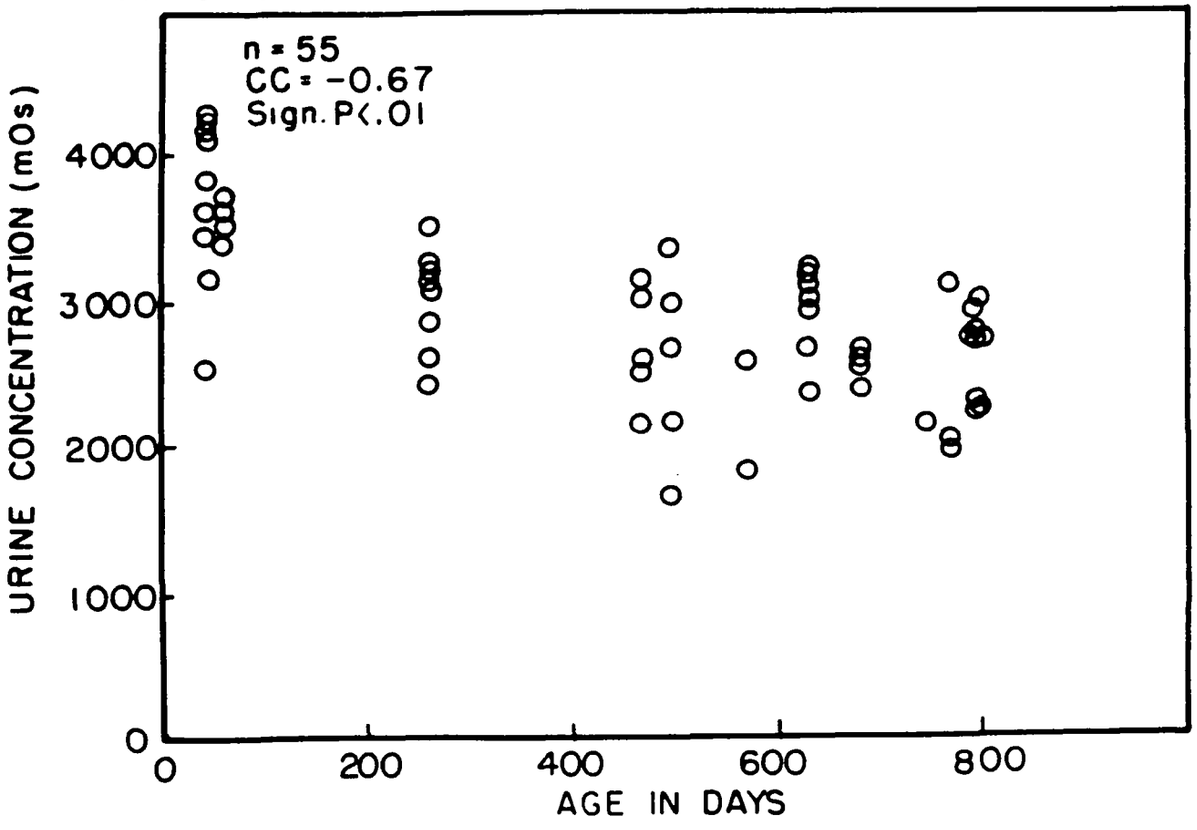

Figure 3. Relationships between urine concentrating abilities and age of B6 male mice.

Table 4

Absence of Correlations[a] Between Collagen Denaturation, Longevity, and Urine Concentration[b]

		Collagen/Longevity			Urine Concentration/Longevity			Collagen/Urine Concentration		
Genotype	Sex	*n*	CC	Significance	*n*	CC	Significance	*n*	CC	Significance
All	Both	91	0.11	NS	73	0.265	<0.05	70	0.07	NS
C56BL/6J	M	16	0.02	NS	12	−0.058	NS	12	−0.356	NS
C56BL/6J	F	16	−0.11	NS	12	0.396	NS	12	−0.509	NS
CBA/HT6J	M	14	−0.47	NS	12	0.100	NS	11	0.127	NS
CBA/HT6J	F	15	−0.14	NS	12	0.560	NS	11	0.477	NS
B6CBAT6F$_1$	M	16	−0.04	NS	13	0.128	NS	13	0.297	NS
B6CBAT6F$_1$	F	14	0.428	NS	12	0.296	NS	11	−0.044	NS

[a]For each correlation tested, *n* is the number of mice tested, and CC is the correlation coefficient. Significance is the probability of this correlation occurring by chance according to Student's *t*-test; NS means not significant because this probability is greater than 0.05 (>0.05).

[b]Both collagen denaturation rates and urine concentrating abilities were tested on individual mice at 600–700 days of age; then longevities were determined.

In animal model systems, the ability of mice to do maximum work declined with age very reliably [13]. Ludwig and Smoke [16] have discussed in detail some examples illustrating the quantitative measurement of biological age in individuals. Our study suggests that the interrelationships of such measurements and their abilities to predict longevity must not be assumed, but must be demonstrated.

The assay to test tail tendon collagen denaturation times was developed by Everitt and his colleagues using rats [3]. Walford studied ratios of soluble to insoluble collagen [18], but relationships with subsequent longevities and other physiological tests were not determined. Although changes with age in tail tendon collagen may not have important physiological significance, we found that they occurred more slowly in a longer-lived mouse species [11] and correctly predicted the effects of food restriction on increasing longevities of genetically obese animals, while measures of immune response failed to predict this increase [9]. Thus tail collagen aging may reflect the aging patterns shown by collagen in systems important to the animal's health.

Tests of renal function did not show as precise correlations with chronological age as did tests of collagen denaturation, however some correlations with chronological age were highly significant (Table 3, Figure 3). The high variability between individuals of the same age suggested that there might be a significant relationship between urine concentrating ability and subsequent longevity. This was not observed (Tables 3 and 4), suggesting that loss with age in this functional ability was not a major cause of morbidity in our animals. In more sophisticated measures of renal aging, Hackbarth and Harrison [7] showed that glomerular filtration rates decreased with age most rapidly in B6 mice, less rapidly in CBAT6 mice, and did not change over the portion of the lifespan measured (to about 700 days of age) in the B6CBAT6F$_1$ hybrid. Tests of renal sodium conservation in humans have shown significant changes with age [5], however correlations with subsequent longevity are not available. Changes with age in the thickness of the renal glomerular capillary basement membrane were extremely regular [15; 16], however relationships of this measure to renal function, subsequent longevity, or other physiological tests are not available.

Ingram and his colleagues [14] found significant relationships between physiological and behavioral measurements on a cohort of 28-month old male B6 mice. Through the use of multiple regression techniques, about one-third of the variance in lifespan could be explained by a combination of physiological variables, including hematocrit and hemoglobin levels and collagen denaturation times, and about two-fifths could be explained by a combination of behavioral variables including exploratory activity and passive-avoidance learning. However, the age of the B6 mice used was past the mean life expectancy for this genotype. Therefore, it is likely that many animals already were showing the effects of pathological lesions that would eventually kill them. The physiological tests may have measured how severely such lesions had already undermined the animals' general health, rather than assessing basic physiological changes with age.

When the purpose of physiological assays is to determine how aging is progressing, while the individual is in otherwise good health, animal experiments offer several important advantages. The animal colony used may be rigorously defined [7-9; 17] and animals such as mice or rats age about 30 times faster than human beings. This allows such tests to be done and their results to be verified within a reasonable length of time for repeated experiments. Furthermore the pathological lesions that the aging animals will show can be accurately predicted, so that physiological testing can be scheduled before the aging process has resulted in severe deterioration of general health.

Our results suggest that aging rates in the physiological systems tested are timed independently. Of course, tests of aging rates in other physiological systems may be related to each other and to longevity. Even the tests that we used may show such relationships if studied at other ages, or if analyzed by methods other than linear correlation. Nevertheless our data show that a strong correlation with chronological age may not predict that correlations exist with longevity or even between independent tests. Thus, there may not be a central mechanism timing the overall aging process.

If aging is centrally timed, the development of accurate assays for the functional age of each physiological system will become extremely important. Such assays would indentify the systems whose aging was the fastest in each individual. Measures to retard or reverse this loss of functon could be instituted before aging of the system progressed to the point of irreversible damage. Used this way, assays of physiological age would lead

to the ultimate in preventative medicine. The accurate control of genotype and environment and the rapid aging rates shown by mouse or rat model systems will make these valuable for developing the assays to measure a system's physiological age and for evaluating treatments designed to retard or reverse the functional changes with age in that system.

References

1. Bafitis, H., & Sargent, F. Human physiological adaptability through the life sequence. *Journal of Gerontology,* 1977, *32,* 402-410.

2. Bailey, D.W. Sources of subline divergence and their relative importance for sublines of six major inbred strains of mice. In H.C. Morse III (Ed.), *Origins of inbred mice.* New York: Academic Press, 1978, pp. 197-215.

3. Boros-Farkas, M., & Everitt, A.V. Comparative studies of age tests on collagen fibers. *Gerontologia,* 1967, *13,* 37-49.

4. Comfort, A. Test-battery to measure aging-rate in man. *Lancet,* Dec. 27, 1969, pp. 1411-1415.

5. Epstein, M., & Hollenberg, N.K. Age as a determinant of renal sodium conservation in normal man. *Journal of Laboratory and Clinical Medicine,* 1976, *87,* 411-417.

6. Furukawa, T., Ionue, M., Kajiya, F., Inada, H., Takasugi, S., Fukui, S., Takeda, H., & Abe, H. Assessment of biological age by multiple regression analysis. *Journal of Gerontology,* 1975, *30,* 422-434.

7. Hackbarth, H., & Harrison, D.E. Changes with age in renal function and morphology in C57BL/6, CBA/HT6 and B6CBAF$_1$ mice. *Journal of Gerontology,* 1982, *37,* 540-547.

8. Harrison, D.E. Genetically defined animals valuable in testing aging of erythroid and lympthoid stem cells and microenvironments. In D. Bergsma (Series Ed.) & D.E. Harrison (Sci. Ed.), *Genetic effects on aging.* National Foundation-March of Dimes Original Articles Series, New York: A.R. Liss, 1978, pp. 187-196.

9. Harrison, D.E. Experience with developing assays of physiological age. In M.E. Reff & E.L. Schneider (Eds.), *Biological markers of aging.* U.S. Department Health and Human Services, 1982, NIH Publ. No. 82-2221, pp. 2-12.

10. Harrison, D.E., & Archer, J.R. Measurement of changes in mouse tail collagen with age: temperature dependence and procedural details. *Experimental Gerontology,* 1978, *13,* 75-82.

11. Harrison, D.E., Archer, J.R., Sacher, G.A., & Boyse, F.M. Tail collagen aging in mice of thirteen different genotypes and two species: relationship to biological age. *Experimental Gerontology,* 1978, *13,* 63-73.

12. Heikkinen, E., Kiiskinen, A., Kayhty, B., Rimpela, M., & Vuori, I. Assessment of biological age. *Gerontologia,* 1974, *20,* 33-43.

13. Hensley, J., McWilliams, P.C., & Oakley, G.E. Physiological capacitance: a study in physiological age determination. *Journal of the Gerontology Society,* 1964, *19,* 317-321.

14. Ingram, D.K., Archer, J.R., Harrison, D.E., & Reynolds, M.A. Physiological and behavioral correlates of lifespan in aged C57BL/6J mice. *Experimental Gerontology,* 1982, *17,* 295-303.

15. Ludwig, F.C., & Silverman, L. Measurement of biological age in samples of kidney and brain obtained at autopsy from rats. *Verhandlungen der Deutschen Gesellschaft fur Pathologie,* 1975, *59,* 242-251.

16. Ludwig, F.C., & Smoke, M.E. The measurement of biological age. *Experimental Aging Research,* 1980, *6,* 497-522.

17. Russell, E.S. Genetic considerations in the selection of rodent species and strains for research in aging. In D.C. Gibson (Ed.), *Development of the rodent as a model system of aging.* NICHD, DHEW Pub. No. (NIH) 72-121, 1972, Special Documents U.S. Gov. Print. Off. Stock No. 1746-0010, pp. 33-53.

18. Walford, R.L., Sjaarda, J.R., & Anderson, R.E. Lifespan and soluble/insoluble collagen (hydroxproline) ratios in normal and irradiated hamsters. *Experimental Gerontology,* 1965, *1,* 117-125.

19. Young, J.C., & Rickert, W.S. Concerning the precision of age estimates based on biological parameters. *Experimental Gerontology,* 1973, *8,* 337-343.