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BIOMARKERS OF AGING: TISSUE MARKERS. FUTURE RESEARCH NEEDS, STRATEGIES, DIRECTIONS AND PRIORITIES

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Abstract — Objective tests that allow early detection of deleterious changes with age are necessary to develop treatments enhancing the health span — the length of healthy life. Here we report tests of eight biological systems that can be performed in mice with no harm to the subjects. Male and female B6, CBA and F1 mice were used. While most test results correlated with chronological age in most genotypes, none predicted subsequent longevities in more than two genotypes. Surprisingly, the open field activity test that most consistently predicted longevities, did not correlate with chronological age. Six tests predicted beneficial effects of food restriction in F1 males, but only one correctly predicted the deleterious effects of the same food restriction regimen in B6 males. These results suggest that different biological systems age at different rates, that rates are affected by genotype and that an antiaging treatment beneficial in one genotype may be harmful in another.

Key Words: aging, physiological change, evaluate antiaging treatments, strain effects

INTRODUCTION

THE ULTIMATE objective of biomedical research on aging is to retard or reverse age-related dysfunctions in all biological systems that damage health and quality of life. This would increase the health span, the length of healthy and enjoyable life. Biomarkers should measure the malfunctions and the damage and provide quantitative, objective assays to diagnose when treatments are necessary to retard or reverse malfunctions. Biomarkers should also evaluate effectiveness of the treatments. Useful biomarkers may measure either the damage to health or the underlying malfunctions that may cause the damage.

Biomarkers contribute to basic research when used to ask how many independent clocks time development of malfunctions with age, how many underlying mechanisms cause these malfunctions and what these causes are. Simultaneously, biomarkers can contribute to practical health-related concerns. They can directly measure how well various treatments retard or reverse declines in health by curing the malfunctions that cause such declines. Biomarkers for a wide range of physiological systems can determine whether a particular treatment benefits different systems proportionally, suggesting that declines with age in those systems have the same underlying cause.

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Biomarker testing must not be harmful, whether dealing with patients or animal models, so that rates of change with age in different biological systems in the same individual can be compared and related to subsequent longevity and moribund pathology. These studies are required to estimate numbers of independent clocks timing aging processes, and to identify underlying causes affecting several systems. Finally, it is vital to determine how much variability in aging patterns results from genetic variability, as this can influence relationships between aging in different physiological systems and responses to treatments. An antiaging treatment that benefits some genotypes may fail to benefit or even harm others (Harrison and Archer, 1987), so it is important to have biomarker tests that identify individuals in whom a particular treatment is not beneficial.

Since biomarkers are measures of age-related changes that reflect underlying malfunctions or damage to organs and tissue systems, it is useful to roughly categorize them on the basis of theoretical interest (measuring changes that may underlie malfunctions), practical interest (measuring systems affecting health) or both. We will illustrate these points with some biomarkers from our past work. Basic measures of growth, such as weight and tail length, provide a useful background; tight wire clinging time and open field activity measure functional levels of a variety of different systems, both those involved with underlying malfunctions, and those directly affecting health; tail collagen denaturation rate reflects the aging of extra cellular macromolecules perhaps due to glycosylation (Kohn *et al.*, 1984), nonenzymatic chemical reactions with glucose that may lead to dysfunctions; urine concentrating ability measures an organ function necessary to health; hair growth after shaving measures how rapidly waves of new hair regrowth pass along the animal, reflecting underlying changes causing hair growth cycling to slow down with age; wound healing and hemoglobin concentrations measure biological functions directly related to health.

MATERIALS AND METHODS

All mice were raised and tested at The Jackson Laboratory, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care. C57BL/6J (B6) females and CBA/CaJ or CBA/CaHT6J (CBA) males were bred in our breeding colony to produce B6CBAF1 hybrids. B6 and CBA males and females were produced in our colony or The Jackson Laboratory Animal Resources colonies, and moved to our experimental animal colony. At four weeks of age, mice were housed four per side, and those in food restriction experiments were divided into two groups: (i) fed mice allowed unlimited access to food (*ad libitum* feeding) and (ii) restricted mice given their rations each day in a single feeding between 12:00 and 2:00 PM, 6 days a week, with double rations on the sixth day, and none on the seventh. These feeding regimens were continued throughout life. Restricted mice were given two-thirds of the amount of food consumed by ad lib fed mice, with one or more food pellets each when fed. There were no unusual losses from fighting among food restricted mice, and the variations in body weights of cage mates were similar in fed and restricted groups, suggesting that the dominant mouse did not consume an unusually large share of food in the restricted cages.

All mice were barrier maintained in an isolated, environmentally controlled room with HEPA filtered air under positive pressure, with temperatures at 22 ± 2°C, and

lighting from 7:00 AM to 7:00 PM. Mice were fed a pelleted, pasteurized diet (96WA, Emory Morse, Guilford, CT), containing 22% protein, 7% fat, 50% nitrogen-free extract (mostly carbohydrates), and 357 kcal/100 g. Details about the diet and animal husbandry have been published (Heiniger and Dorey, 1980). Mice were free from known pathogens, and the colony was clean when tested for 10 standard mouse viruses by Microbiological Associates. A description of the health monitoring procedures used in this colony has been published (Harrison and Archer, 1983). Only mice appearing healthy to an experienced observer were used for tests of physiological systems. Health assessment was based on normal activity and responses to handling, normal external appearance including eyes and pelt, absence of palpable lumps and absence of recent weight loss. Even more important, the tests did not harm the subjects, so subsequent longevities were determined after testing.

Measurements of biological systems

Food (given in food restriction experiments) is the amount eaten recorded as g per day per mouse and measured for 1 week during the period when the physiological systems were being tested.

Weight is body weight in g measured when the other tests were performed.

Tail length is measured in cm from the root of the tail where it joins the rectum to the tip of the tail.

Tight wire is the time in seconds that a mouse can hold itself on a wire suspended above foam padding. Timing is begun after the mouse grips the wire with both front paws and one hind paw. There is no exit from the wire, but mice are removed and testing ends if they cling for 240 s in a single trial. Otherwise, the maximum score of five trials is recorded (Campbell, 1982). Correlations between tight wire clinging times and body weights among mice of the same age and genotype showed that only a small portion of the variance could be explained by increased weight causing decreased clinging times. For example, in B6 males at 500–614 days of age, correlation coefficients (r) between clinging times and body weights for *ad libitum* fed and food restricted +/+ mice and for food restricted *ob/ob* mice were −0.266, −0.159 and −0.409, explaining 7%, 3% and 17%, respectively, of the variances in clinging times (Harrison and Archer, 1987).

Open field is a measure (Sprott and Eleftherious, 1974) of the activity displayed during 5 min in a 80 cm square, well-lighted, open-topped box, measured as the number of 15 cm sided squares that the mouse crosses.

Tail collagen is the number of minutes required for a single tendon fiber from the middle of the tail to be denatured in 7 M urea at 45 °C, so that it is broken by a 2-g weight (Harrison *et al.*, 1978).

Urine concentration is the osmolality in mOs/kg of urine from a mouse deprived of water for 48 h. Urine samples are held in capillary tubes to prevent evaporation, food remains present, and mice are injected with 1.0 ml saline intraperitoneally at time zero (Harrison and Archer, 1983).

Hair growth is the fraction of a 2 cm square shaved area centered on the back near the tail into which hair has begun to regrow after 25 days. The subjective element in estimating the area is minimized by defining regrowth as the first appearance of hair, and dividing the shaved area to be scored into eight equal portions with a transparent screen. Excellent repeatability was found when 22 indistinguishable mice were randomly

TABLE 1. CORRELATIONS BETWEEN TEST RESULTS AND SUBSEQUENT LONGEVITIES*

	R (n) P for the listed genotypes					
Test†	*B6, Male*	*B6, Female*	*CBA, Male*	*CBA, Female*	*F1, Male*	*F1, Female*
Weight	N.S.	N.S.	N.S.	N.S.	0.46(22)0.03	N.S.
Tight wire	N.S.	N.S.	N.S.	N.S.	N.S.	0.34(35)0.05
Open field	0.59(25)0.002	0.51(28)0.005	0.35(30)0.06‡	N.S.	N.S.	N.S.
Tail collagen	N.S.	N.S.	N.S.	N.S.	N.S.	0.30(43)0.05§
Hair growth	N.S.	N.S.	N.S.	0.35(36)0.04	N.S.	0.32(45)0.03
Wound healing	N.S.	N.S.	−0.68(12)0.02	N.S.	−0.57(12)0.05	N.S.
Hemoglobin	0.34(29)0.07‡	N.S.	−0.43(29)0.02§	N.S.	N.S	N.S.
Longevities	905 ± 19	844 ± 26	827 ± 26	927 ± 22	933 ± 33	971 ± 25

*All tests were done at 22 months of age, and mice were followed until they died or became moribund. In *R (n) P*, *R* = correlation coefficient; *n* = number of individuals tested; and *P* = probability of observing the correlation by chance. Longevities are summarized for the tested mice as mean ± SE; numbers ranged from 29–34.
†Only tests showing at least one significant correlation ($P < 0.05$) are listed. NS means $P = 0.05$, except as noted below. Tail length, urine concentration and Avertin anesthesia time showed no significant correlations so were not listed.
‡When $P < 0.10$, but > 0.05 values are listed if correlation for other genotypes were significant.
§Correlations opposite of expected direction (mice with younger values or slower aging rate lived less long).

recaged and retested an hour after the initial trial; 20 were scored exactly the same way both times, and scores of the other 2 varied by one one of the 8 screen divisions (Harrison and Archer, 1987).

Wound healing is the number of days required until the wound in the tail made in removing a portion of one of the dorsal tail tendon bundles for the tail collagen test feels smooth to the touch when running the index finger along the tail. Tails were checked 7 days after the wounds were made, and every 2–3 days thereafter. Since this test is subjective, all animals in an experiment were scored by the same technician. In a blind test of repeatability, the correlation coefficient between successive runs was 0.88 with 16 mice. In the same experiment, one that was designed to evaluate the technician, wounds were made at different times over three weeks, but presented as if all had been made simultaneously. Expected healing times were 27.2, 34.5, 41.2, and 34.8 days for four groups of mice; reported times were 24.5, 36.5, 40.0 and 35.6, respectively, demonstrating good objectivity (Harrison and Archer, 1987).

Hemoglobin is the concentration of hemoglobin in blood, measured by taking blood from the retroorbital sinus in a 70 μl heparinized microhematocrit capillary tube (Fisher), mixing 10 μl blood with 2.50 ml Drabkins' reagent (1.0 g sodium bicarbonate, 0.05 g potassium cyanide, 0.20 g potassium ferricyanide) dissolved in 1000 ml water and measuring absorption 5 to 60 min later using a spectrophotometer (Helena Digispec) at a wavelength of 540 nm.

Statistical significances of differences between groups were tested using the Student–Newman–Keuls multiple range test, while correlation coefficients (r) were calculated for linear correlations.

RESULTS AND DISCUSSION

Biomarkers and subsequent longevity

In basic research, it is vital to determine what causes deleterious changes with age.

TABLE 2. CORRELATIONS BETWEEN TEST RESULTS AND CHRONOLOGICAL AGE

Test	CC (n) for the listed genotypes*					
	B6, Male	B6, Female	CBA, Male	CBA, Female	F1, Male	F1, Female
Weight	0.35(80)†	0.46(73)	0.29(78)†	0.46(77)	0.51(76)	0.60(76)
Tight wire	−0.38(178)	−0.44(81)	−0.52(85)	−0.43(83)	−0.41(115)	−0.38(95)
Open field	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Tail collagen	0.79(119)	0.83(108)	0.71(108)	0.48(120)	0.78(115)	0.68(117)
Hair growth	−0.40(188)	N.S.	0.23(88)†‡	0.32(91)‡	−0.35(147)	0.34(104)‡
Wound healing	0.43(97)	0.28(51)†	0.76(36)	0.43(36)	0.44(55)	0.49(49)
Hemoglobin	−0.52(208)	−0.33(99)	N.S.	N.S.	−0.29(141)	N.S.
Tail length	0.64(161)	0.71(45)	0.65(66)	0.74(62)	0.61(93)	0.58(80)
Urine conc.	−0.38(199)	−0.29(99)	N.S.	N.S.	−0.36(110)	−0.43(135)
Avertin anesth.	0.35(172)	N.S.	N.S.	N.S.	N.S.	N.S.

*In CC (n) the CC = the correlation coefficient and n = number of individuals tested. The probability of observing the correlation by chance was given from Table A13, in Snedecor, GW. Statistical Methods. Iowa State College Press, Ames, 1946. Results include both longitudinal and cross-sectional studies in several independent experiments.
†$P < 0.05$ but > 0.01. Otherwise $P < 0.01$ when numbers given. N.S. means $P > 0.05$ that the correlation occurred by chance.

This question is impossible to address directly, because there are so many changes that it is impossible to separate causes from effects. Biomarkers offer the possibility of detecting malfunctions in a single system before they have become so severe that interacting systems are affected resulting in a confusing pattern of ill health. Table 1 illustrates an attempt to do this by testing mice at 22 months of age, well before their mean life spans, and seeking correlations between test results and subsequent longevities (Harrison *et al.*, submitted). Statistically significant correlations were obtained but differed widely with gender and among genotype. No single test correlated with longevity in more than three of the six genotypes (Table 1). Results were similar for correlations between the different tests (data not shown). The absence of consistent relationships within these six genotypes suggest that individuals with different genetic makeups show different aging patterns. It demonstrates that conclusions based on data from a single inbred strain should not be generalized to the entire species; it is vital to compare a variety of different genotypes before drawing general conclusions.

The correlations with subsequent longevity given in Table 1 illustrate how nongenetic determinants of longevity are associated with scores on the biological systems tested, since only individuals of the same genotype were compared. Genetic differences doubtlessly would cause additional association between longevities and test scores. These might be difficult to evaluate, because there may be many differences between long-lived and short-lived genotypes that are not important in determining longevities.

If a small number of underlying mechanisms cause many changes with age, the results in Table 1 suggest that the factors causing aging have different patterns in animals of different genotypes. Further studies, using a wider variety of biomarkers and genotypes are necessary to establish whether the variability of aging patterns continues to increase as rapidly as additional genetic differences are studied, or whether the variability begins to level off, indicating that there may be a relatively small number of general aging patterns into which most genotypes and biomarkers fall. In such studies it

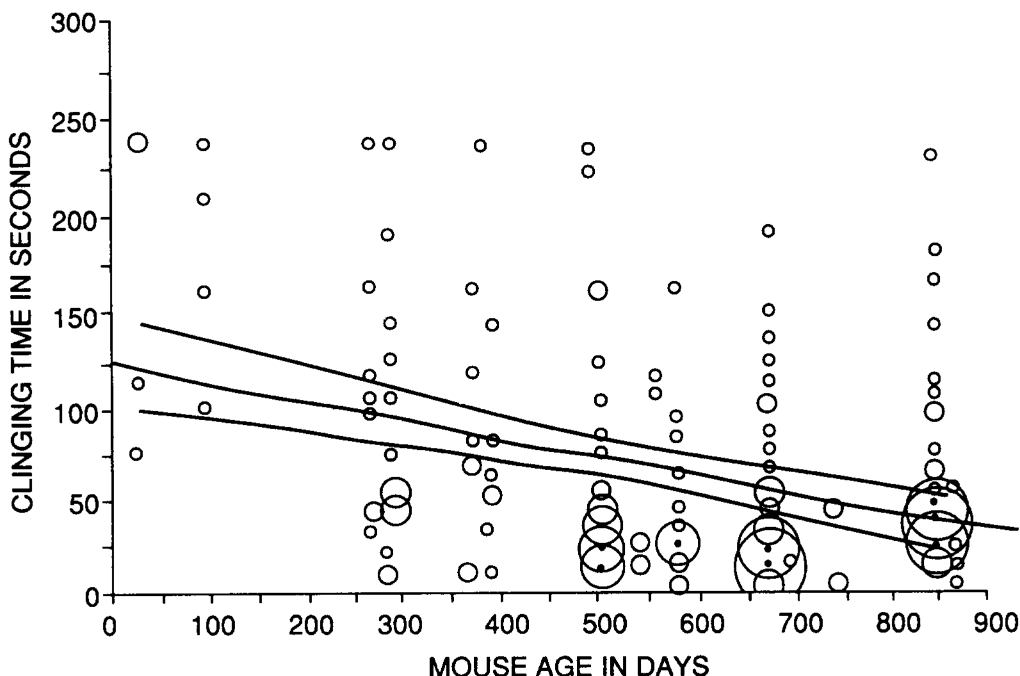

FIG. 1. The change with age in tight wire clinging time in seconds for the 178 B6 males in Table 2 is given as a scatter plot showing all points. In cases of overlaps, points are geometrically enlarged with the areas proportional to the number of overlapping points. The outer lines show 95% confidence bands for the true mean of y, and the central linear regression line is fitted to the data with R squared = 0.14.

is essential to avoid testing diseased individuals, since both their poor performance on biomarkers and their reduced longevities would usually be secondary to the disease.

Biomarkers and chronological age

Table 2 summarizes changes with chronological age in the biomarkers discussed. Changes with age were much more consistent in the six genotypes than relationships to longevity. Tight wire, tail collagen and wound healing changed with age significantly in all genotypes tested, while urine concentration, hair growth and hemoglobin levels changed in several or most genotypes. Figures 1–4 illustrate 95% confidence limits for B6 male changes with age in tight wire clinging time, tail collagen breaking time, urine concentrating ability and hair regrowth rate. In all cases, data were pooled from several independent experiments over several years. Thus, the linear correlation coefficients in Table 2 may underestimate relationships with age found in a single experiment.

Notably, the open field measures that predicted subsequent longevity both at the highest level of significance and in the most genotypes (Table 1), failed to change with chronological age in any of the six genotypes tested (Table 2). This illustrates that measures not changing with chronological age may predict subsequent longevities. Furthermore, biomarkers showing the most consistent changes with chronological age, such as tail collagen denaturation, may fail to predict subsequent longevities. Prediction

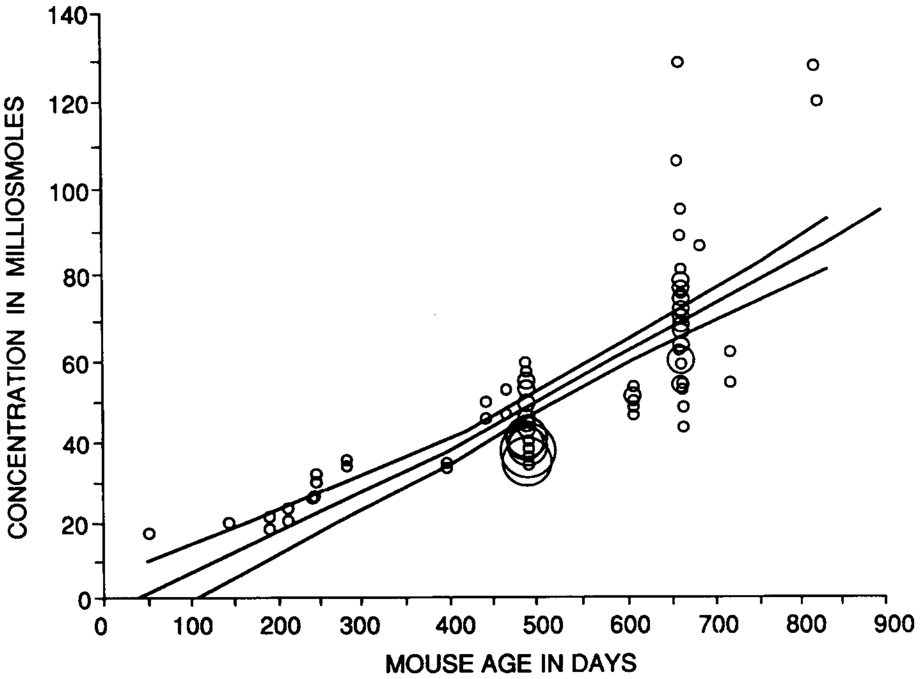

FIG. 2. The change with age in tail tendon collagen denaturation times in minutes for the 119 B6 males in Table 2 is shown following the conventions in Fig. 1; R squared = 0.63.

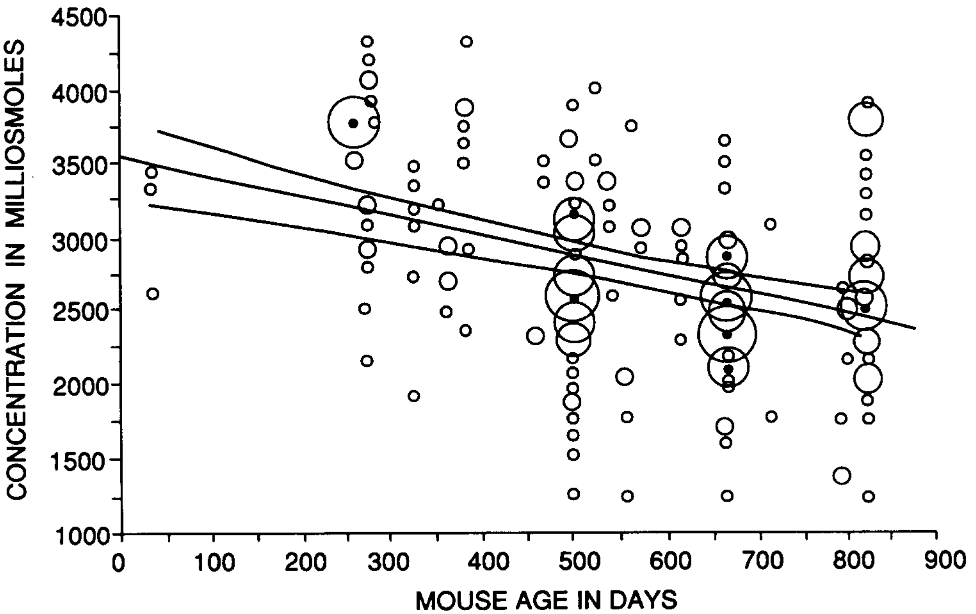

FIG. 3. The change with age in urine concentrating ability in milliosmoles/kg is shown for the 199 B6 males in Table 2 following the conventions in Fig. 1; R squared = 0.14

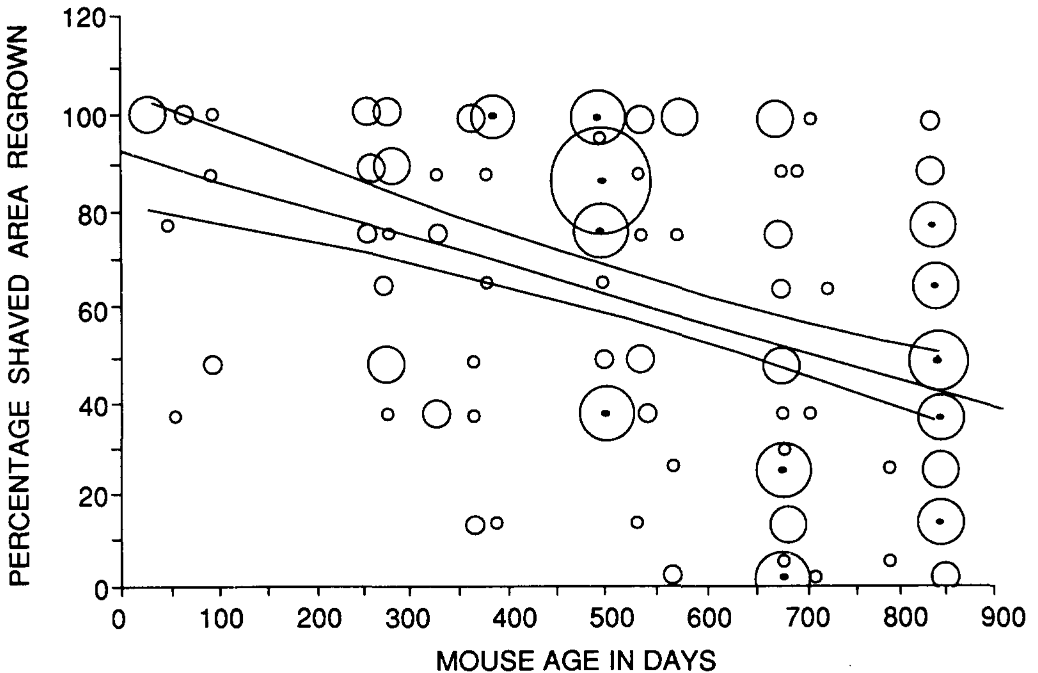

FIG. 4. The change with age in hair regrowth rate, given as the percentage of the shaved area regrown after 25 days, is shown for the 188 B6 males in Table 2 following the conventions in Fig. 1; R squared = 0.16.

of longevity can be a valuable way to validate a biomarker; however, it must be proven by measuring correlation with subsequent longevity; it cannot be assumed as a result of correlations with chronological age.

Validation of biomarkers

Ingram (1983) reviewed criteria to determine which biomarker tests are best related to biological age. He suggested that the best criteria are prediction of subsequent longevity and improvement in test score after a treatment known to increase longevity. Our results suggest that other criteria are also useful to achieve the long-term objective of increasing the health span — the duration of healthy and high quality life. Many tests measure deleterious changes in health directly. In our battery these include tight wire, open field, wound healing, hemoglobin, urine concentration and Avertin anesthesia. Functional losses in the vital biological systems scored by these tests diminish components of health. Therefore, it is important to identify treatments that retard or reverse such losses. Such treatments enhance health, even if they do not extend longevity in certain mouse strains.

We have previously defined four criteria for evaluating a biomarker assay: (1) the results must change significantly with age; (2) changes should be repeatable in the same individual; (3) assays of independent physiological parameters may give similar estimates of age for the same individuals and (4) the degree of aging assayed may correlate

with subsequent longevity. While these criteria remain useful, none of them appear to be absolute. The open field test that filed to meet the first criterion (Table 2), was the best in meeting the fourth (Table 1). Apparently a biomarker need not change with age to be useful, although most do meet this criterion. The second criterion is not met by tests such as hair regrowth rate, whose scores vary widely in each individual. However, hair regrowth proved valuable in group comparisons, correctly predicting that the same food restriction regimen would extend longevities in B6CBAF1 males and reduce them in F6 males (Harrison and Archer, 1987). The third criterion determines whether the same clock times changes with age among apparently independent biomarkers, while the fourth criterion identifies tests related to the earliest occurring dysfunction that causes death. As already discussed, valuable biomarkers may fail to meet the third and fourth criteria; nevertheless, it is of great interest when they are met.

Additional biomarkers

A much greater variety of nonlethal biomarkers than are currently available should be developed and compared in a variety of genotypes to test other biological systems in mice. Immune responses should be measured *in vivo*, as should oxygen consumption and free radical production. Measures of liver function would also be valuable. We measured sleep time after Avertin anesthesia but unfortunately this test correlated with chronological age in only one of the 6 genotypes tested, and test scores at 22 months of age did not correlate with subsequent longevities. Correlations between scores at 28 months of age and longevity in B6 males probably reflected ill health. Thus, better measures of liver function should be developed. Lung function, measured as vital capacity, is one of the best biomarkers in human beings, but is probably impractical for noninvasive measures in rodent, especially when the tests must be done repeatedly on the same individual. Measures of intelligence or learning, reaction time and various sensory functions also should be added to a comprehensive test battery in rodents. Nevertheless, the seven biomarkers plus two parameters of growth that we use here to illustrate possible types of results reflect a wide variety of independently aging physiological systems.

Biomarkers and treatment evaluation

Regardless of how many underlying causes there are for the development of malfunctions with age, biomarkers should be used to evaluate treatments designed to retard or reverse declines with age. Such treatments cannot now be chosen rationally, since patients do not know whether they need the treatment, and have no objective way of evaluating whether it is helping them. Properly designed biomarker studies can directly define how well a treatment retards or reverses declines in health and cures the dysfunctions that cause such declines. Ideally, individual human beings should each be followed as a separate experiment, with frequent testing so that valid and accurate measurements are available for functional levels in important systems while the individual is in good health. With frequent testing, a dysfunction in a biological system would be recognized before it progressed to the point of causing illness. Once treatments began, frequent testing would determine whether the treatments successfully retarded or reversed the dysfunction.

In animal models, each individual genotype should be followed as a separate experiment; individuals should also be followed longitudinally to determine the relationships

TABLE 3. EFFECTS OF FOOD RESTRICTION ON B6 CBAF1 MALES*†‡

	Food restricted		*Ad libitum fed*	
Measure§	*Adult*	*Aged*	*Adult*	*Aged*
Food	2.0	2.1	3.3	3.1
Weight	24.9 ± 0.3*	24.8 ± 0.5*	39.1 ± 0.7	40.0 ± 0.9
Tail length	10.4 ± 0.04*	10.9 ± 0.03	10.8 ± 0.04	11.0 ± 0.04
Tight wire	233 ± 4*	199 ± 10*	178 ± 11	77 ± 10
Open field	105 ± 7*	87 ± 4*	49 ± 4	58 ± 4
Tail collagen	33.5 ± 1.4*	95.9 ± 7.4*	40.6 ± 1.2	205 ± 7
Urine concentration	3929 ± 40*	3453 ± 98*	3156 ± 68	2831 ± 84
Hair growth	69.4 ± 6.3	67.2 ± 6.8*	53.3 ± 5.2	36.3 ± 6.9
Wound healing	19.4 ± 0.7	32.6 ± 3.6	16.2 ± 0.5*	20.0 ± 1.3*

*Values are mean ± SEM, with 30–36 mice per group; measures are defined in the text.
†Adults were 294–389 days old, and aged mice 775–804 days old when tested, with ages matched in the two dietary groups.
‡An asterisk (*) denotes values significantly different from others of the same age on the same line with $P < 0.05$ by the Student–Newman–Keuls multiple range test.
§Units are: food, g/day; weight, g; tail length, cm; tight wire — clinging time in seconds; open field, number of squares crossed; tail collagen, denaturation time in minutes; urine concentrating, mOsm; hair growth, percent of shaved area regrowth in 25 days; wound healing number of days until healed. These data were presented in Harrison and Archer, 1987.

between effects of a treatment on a variety of different biomarkers and on subsequent longevity and pathology for each individual. Such data could detect that aging in two apparently independent systems was timed by the same clock if changes in these systems correlated well in the same individuals.

Treatments that benefit some genotypes may actually harm others. This possibility is illustrated by our studies of food restriction in male B6CBAF1 hybrid and B6 mice (Harrison and Archer, 1987). As expected, food restriction of B6CBAF1 mice improved a variety of biological systems, with older mice giving values like those previously found in young individuals in: tight wire clinging, open field activity, tail tendon denaturation, urine concentrating ability and hair regrowth rate. Wound healing rates, however, were more like values found in old mice as a result of food restriction (Table 3). Both mean and maximum life spans were extended more than 200 days in these restricted mice that were already a long-lived genotype. The maximum longevity of 1742 days in this group may have set a new record for the species Mus (Table 4). The same

TABLE 4. LONGEVITIES OF MALE MICE IN FOOD RESTRICTION STUDIES

Genotype	*Treatment*	*Median*	*Mean*	*SE*	*N*	*Maximum 10%*
B6CBAF1	Restricted	1191	1185	36	34	1432–1742*
B6CBAF1	Fed	985	951	35	35	1188–1296
B6	Restricted	591	593	40	48	967–1145
B6	Fed	878	858	19	45	994–1172

*The longest lived individual was fed ad libitum from 1541–1742 days of age. These data were presented in Harrison and Archer, 1987.

TABLE 5. EFFECTS OF FOOD RESTRICTION ON B6 MALES*†‡

	Food restricted		*Ad libitum fed*	
Measured§	*Adult*	*Aged*	*Adult*	*Aged*
Food	1.8	2.1	3.0	2.8
Weight	22.5 ± 0.8*	24.0 ± 0.9*	33.3 ± 0.4	35.6 ± 0.6
Tail length	9.7 ± 0.06*	10.1 ± 0.04	10.0 ± 0.04	10.1 ± 0.06
Tight wire	149 ± 11.8*	60 ± 12.9*	77 ± 8.4	35 ± 6.2
Open field	116 ± 6.2	111 ± 10.3*	109 ± 5.2	83 ± 6.1
Tail collagen	25.6 ± 1.3	46.8 ± 4.3*	27.8 ± 1.3	60.3 ± 2.5
Hair growth	26.4 ± 3.8	18.3 ± 5.8	68.9 ± 3.7*	40.0 ± 6.6*
Wound healing	31.6 ± 2.8	42.7 ± 3.8*	20.1 ± 0.7*	20.9 ± 0.9

*–§Same as Table 3, except 44–53 mice per adult group and 17–24 per aged group. Adult mice were 260–373 days old and aged mice were 570–679 days old when tested, with ages matched in the two dietary groups. Urine concentration data were not affected by food restriction in B6 males, so were not included.

food restriction treatment, however, had entirely different effects on longevities of male B6 mice, reducing mean and maximum longevities 265 and 27 days, respectively (Table 4). This was surprising, especially since tests of tight wire clinging, open field activity and tail tendon denaturation all were improved by food restriction, despite the fact that longevities were reduced. Only hair regrowth rates correctly predicted the effects of food restriction on longevity in these two strains (Tables 3 and 5).

Why the same life-long food restriction treatment that significantly extended longevities of the B6CBAF1 male reduced longevities in the B6 male is not easy to explain. While an obvious possibility is that an essential nutrient was reduced below the level required by the B6 strain, such a deficiency would be an effect extremely specific to genotype and gender. B6CBAT6F1 hybrids are not sensitive to the deficiency and the effect is eliminated by the obese mutation (Harrison and Archer, 1987). Furthermore, the effect depends on gender since the same food restriction regimen study increased longevity in B6 females (Harrison *et al.*, 1984). This illustrates a potential problem in food restriction studies. It is impossible to avoid malnutrition in food restricted animals without either an excess of essential nutrients in ad lib fed controls, or alterations of nutrient proportions. These proportions were maintained in our study. However, future caloric restriction studies are needed in B6 males, in which all essential nutrients are fed at a constant level to determine whether this benefits longevities.

The data in Tables 3 through 5 suggest that genetic effects may be important in responses to antiaging treatments, even the well defined treatment of food restriction. This illustrates that a wider variety of genotypes should be studied in the future to determine whether different genotypes often show widely disparate responses to antiaging treatments.

Biomarkers in preventative medicine

As test batteries of biomarkers are developed and evaluated, they may be of great value in preventative medicine; i.e., avoiding disease by maintaining optimal health. Biomarker testing can be used to define and monitor optimal health by frequently

TABLE 6. FINDINGS FROM BIOMEASURES OF AGING IN MICE

1. Different biological systems age at different rates.
2. Relationships of aging rates in various systems change with the mouse strain.
3. Differing beneficial treatments affect unique sets of biological systems.
4. The same treatment affects some systems positively and others negatively.
5. Effects of a treatment differ with the mouse strain.
6. These findings imply that aging is not simple, but beneficial treatments may be developed using objective measures of deleterious changes with age in the biological systems to be treated.

measuring the functional abilities of a wide variety of different biological systems. With advancing age, the likelihood of dysfunction in biological systems and consequent ill health increases exponentially. Therefore, treatments to correct malfunctions of age should be developed by using biomarkers.

Treatments to retard or reverse declines with age are the weak link in preventative medicine, as there are no proven effective treatments for human aging. Animal models, using a wide variety of biomarkers, will be vital in suggesting treatments for future work in human beings. Table 6 summarizes our conclusions thus far from studies in mice. There is also a need for standardized tests of biomarkers in man so that results in large numbers of people can be directly compared in a central data base. Many treatments in human beings, such as exercise or nutrition regimens, are being tried voluntarily by large numbers of people; unfortunately the results of these treatments are almost never evaluated objectively, and even when biomarker studies are used, each study is designed with a unique set of biomarkers, and this prevents comparisons. If a standardized test battery were widely used, it would aid treatment development by determining the treatments that had been most effective in people physiologically and genetically similar to the patient being studied. Table 7 summarizes our suggestions for using biomarkers in human beings.

Advances in understanding causes of aging should also suggest improved treatments

TABLE 7. SUGGESTIONS FOR BIOMEASURES OF AGING IN HUMAN BEINGS

1. Each individual is a separate experiment.
2. Measures must be repeated often for accuracy, so must be quick and convenient.
3. Test functioning of biological systems necessary to health and enjoyment of life.
4. Determine normal, healthy values for each individual.
5. Excessive changes with age suggest need for treatments.
6. Use measures to evaluate treatments, expecting individual differences.
7. Learn best treatments for individual of specific types defined by patterns of functional values and rates of change with age, as well as by conventional characteristics such as age, gender, race, socioeconomic status, lifestyle and so on, and with genetic patterns defined by modern molecular genetics.

to enhance the health span, but at the same time knowledge of treatments that retard or reverse aging rates should advance understanding the causes of malfunctions of age. Once objective measures for dysfunctions and health damage that occur with age are available, aging processes may be treated and studied in a productive fashion. This should be the goal of future work with biomarkers.

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