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Mechanisms of Ageing and Development
122 (2001) 463–475

mechanisms of ageing and development

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Long-live euthymic BALB/c-nu mice. I. Survival study suggests body weight as a life span predictor

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Received 15 September 2000; received in revised form 4 December 2000; accepted 8 December 2000

Abstract

This paper is the first of a series aimed to show the main physiological and pathological characteristics of male euthymic BALB/c-nu mice, a long-live strain of BALB/c mice bred in our own Institute. In particular, the first two paired papers are respectively devoted to general survival information and disease characteristics, also taking into account very old animals that are of high interest for studies on successful aging. In this paper we report the analysis of survival kinetics, the time course of body weight and the correlation between body weight and time-at-death. The longitudinal study has been performed on 88 male mice, checking individually their body weight and date of death and analyzing survival data by a model built by our own. Survival analysis shows quite higher longevity (median age: about 29 months) in this population when compared with other BALB/c strains. The most relevant finding on body weight is its correlation with longevity until the age of 22 months: thinner subjects live longer and lose weight at a lower rate than their heavier mates. Results have formed the basis on which to plan the cross-sectional experiment to study pathologies and biological parameters at different ages, including a group of mice at very advanced ages (34 months).

Keywords: Survivorship; Long-live animals; Euthymic BALB/c-nu mice; Body weight; Longevity prediction; Mathematical models

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0047-6374/01/$ - see front matter
PII: S0047-6374(00)00256-6

1. Introduction

Interest of our group in what happens at very advanced ages started when we became engaged with the problem of explaining why in most of the populations studied, at least in mammals, mortality kinetics shows a biphasic pattern, increasing roughly exponentially throughout the major part of the curve, then slowing down at the extreme ages (Economos, 1982). In fact, at these ages the population undergoes a strong selection due to mortality, becoming progressively enriched in subjects with higher physiological capacities (Piantanelli, 1986). Another stimulus came from biochemical data on few animals surviving at advanced ages and showing values very close to those from young mature ones: in the very old population there are subjects who age successfully, that is, maintain the efficiency of the main physiological functions relevant to both survival and fitness (Tobin, 1981; Rowe and Kahn, 1987). Further stimuli stemmed from observations reported in human findings on centenarians, where different research groups found age-related parameters which seem unaffected by age (Sansoni et al., 1993).

Most of our experiments on aging studies have been performed on euthymic BALB/c-nu mice. Basically, BALB/c strain shows relatively low incidence of pathologies, and tumor pathology in particular (Staats, 1980; Bronson and Lipman, 1993), thus representing a very interesting characteristic when physiological aging is under scrutiny. Furthermore, our sub-strain of mice is a long-live one; as far as we know, there is no information about maximal life span of euthymic BALB/c-nu mice from other laboratories. This feature is extremely positive as it tells us that mice suffer only little from confounding events such as premature death due to specific pathologies; this is further supported by the good general conditions shown by the animals at gross inspection. Despite these characteristics, we do not know whether some particular pathologies could develop at very advanced ages; the answer to this question becomes a must when studying long-live subjects. The above reported considerations prompted us to undertake a comprehensive cross-sectional study on our strain of mice by sampling at different ages including very old animals (Anisimov et al., 2001).

In the present paper, we report the results of a survival experiment which has been used propaedeutically to properly design the cross-sectional study (Anisimov et al., 2001). For instance, these results on survival data have been used to choose the age groups and their dimension, based on a compromise between the need of achieving our scientific aims and the necessity to use our own animal house facilities with limited capacities. Survival data have been fitted using a mathematical model of survival kinetics (Piantanelli et al., 1992a) built in our lab to overcome pitfalls present in other models, such as Gompertz law (Gompertz, 1825), due to the lack of consideration of interindividual heterogeneity (Economos, 1982; Vaupel et al., 1998). In particular, our model is capable of fitting mortality data also at very advanced ages, which are of obvious interest for planning and studying the group of very old mice. The resulting fitted curve

will also serve as a common reference for the kinetics of biological parameters tested in the cross-sectional study.

Beside these propaedeutical uses, aim of the present paper was to describe the time-course of individual mouse body weights, their rate of changes with age and their eventual correlation with longevity.

2. Methods

2.1. Animals

Experiments have been performed on male euthymic BALB/c-nu mice from our own colony, the same strain we previously used in our studies on aging long ago (Piantanelli et al., 1978; Rossolini et al., 1991; Zaia and Piantanelli, 1996; Basso et al., 1998). The term *nu* refers to the recessive nude mutation introduced into inbred BALB/c mice by crossing them with congenitally athymic nude mutants (nu/nu). Our colony, derived from an original stock of euthymic BALB/c-nu from Copenhagen, had been of great help in early experiments allowing us to compare "haired euthymic" BALB/c (nu/+) and "athymic nude" BALB/c (nu/nu). The aim of these experiments was to study the mechanisms of thymic influence on non-immunological functions, which often we found to be altered in both young athymic nude and old normal mice. In these early experiments, no differences had been observed in the parameters tested between homozygous BALB/c (+/+) and heterozygous BALB/c (nu/+). With the term BALB/c-nu we address a population made up of both heterozygous and homozygous euthymic mice.

Animals were bred as a close colony and maintained under conventional conditions at 23 ± 2°C ambient temperature and 60 ± 15% relative humidity, with minimum ventilation rate of 10 times/h and 14–10/10–14 h light/dark cycles simulating seasonal variations. They were housed 8 per cage (polycarbonate, open on the top and covered with steel wire lid, $26.7 \times 20.7 \times 14.0$ cm^3 deep) and fed with conventional chow (Global diet 2018, Harlan, Italy) and tap water ad libitum. Microbiological monitoring and characterization of animals and environment are routinely performed every 3 months.

2.2. Survival experiment

A made-on-purpose breeding allowed us to include 88 male mice in the survival study. Each subject was marked by ear puncture to follow mice individually all life long. Mice have been checked for dead animals daily and individually weighted every fourth week from about 3 months of age on. Survival data have been fitted using our mathematical model of survival kinetics, which contains two parameters, S_0 and ω, respectively describing stochastic and deterministic components of mortality mechanisms.

2.3. Outline of the mathematical model of survivorship

The model, aimed to fit empirical data from young animals after sexual maturation up to death, uses the concept of vitality with the same meaning used by other authors, an index of efficiency of integrated biological functions which allow the organism to survive (Economos, 1982; Zuev et al., 2000). The maximum achievable vitality value is unity and death is assumed to occur when vitality drops to zero. The assumptions for vitality kinetics are made on the mean $\mu(t)$: it is assumed that $\mu(t)$ decreases continuously with age; in particular, the lower the vitality value the higher the rate of its decrease, according to the equation:

$$\mu(t) = 1 - S_0 + S_0^{10} - S_0^{10} \exp\left(\frac{\omega t}{S_0}\right).$$

The stochastic aspects of mortality kinetics are reflected in the function $S(t)$, the standard deviation of the individual vitality values. A link between $\mu(t)$ and $S(t)$ is given by the following equation:

$$\mu(t) + S(t) = 1.$$

Consequently, the lower the value of mean vitality, the higher the interindividual variability index $S(t)$, accounting for the increasing variability observed on physiological functions with advancing age.

The link between the decrease of biological functions represented by $\mu(t)$ and the kinetics of survivorship function is gained using the probability density function $Z(v,t)$, which allows us to calculate the probability $Z(v,t)\mathrm{d}v$ that an individual of age t has vitality in the range $v–v+\mathrm{d}v$. Thus, the probability that an individual survives up to age t is given by

$$l(t) = \int_0^1 Z(v, t)\mathrm{d}v.$$

$Z(v,t)$ has to present some characteristics: $Z(v,t) = 0$ for $v > 1$ owing to the upper bound of unity for v; the value of the area under the curve $Z(v,t)$ at $t = 0$, representing the number of individuals of the cohort alive at $t = 0$, has to be maintained constant for any time $t > 0$ when summing up individuals dead and alive. For the choice of convenient functions consistent with the aforementioned requirements see the original paper (Piantanelli et al., 1992a).

The model takes into account both deterministic and stochastic components of mortality mechanisms, reflected by the two parameters entering the model, namely ω and S_0. The stochastic component S_0, representative of the fluctuating interactions of the living organism with its environment, accounts for the selection occurring in the heterogeneous population with advancing age, where the progressive death of weaker subjects leads to a population more and more enriched in individuals with higher physiological capacities. The value of the parameter ω gives an estimate of the maximal survival potentiality of the population under study in the given environment. The parameter ω, representative of the deterministic component describing the environmental and genetic influence on physiological functions,

has also been shown to give a good estimate of the rate of aging of the population studied (Piantanelli et al., 1994a). It has also been shown that the life span value times the estimated ω roughly equals an adimensional constant value, about 4. This is the case for survival curves of animals whose life spans range from some days to several years (Piantanelli et al., 1992a): thus, an estimate for maximal life span can be obtained dividing this "constant for longevity" by the estimated ω value. The term "constant for longevity" was coined by Sacher while working with Gompertz parameter α (Sacher and Trucco, 1962); however, α-derived "constant" shows a much higher degree of variability than the ω-derived one (Piantanelli et al., 1992a).

2.4. Statistical analysis

Fitting of empirical survival data with the model has been performed by Newton–Gauss non-linear regression analysis by using "MatLab" Package, MatWorks. Routine survival parameters, such as mean, median and maximal life span have been calculated using SPSS package. The same package has been used to check the correlation and regression parameters between life span and body weight.

Body weight time course has been fitted by non-linear regression analysis using "Fig. P" package, Biosoft.

3. Results

3.1. Survival kinetics

Empirical data of BALB/c-nu mouse survival kinetics, together with the fitted curve obtained by using the model described in the Section 2 are presented in Fig. 1. Some parameters can be easily calculated: mean life span $\pm$ SEM, 29.39 ± 0.69 months; median life span, 29.63 months; median age of the tenth decile of survivorship, 38.50 months; mean age of the tenth decile $\pm$ SEM, 38.99 ± 0.56 months; maximal registered life span, 41.07 months. The values $\pm$ SE of the model parameters estimated from the fitting are: $\omega = 0.1016 \pm 0.0006$ month^{-1}; $S_0 = 0.491 \pm 0.004$. Survivorship curve fitted with these data is used as a reference for further studies.

Fig. 2 shows the age groups for the cross-sectional study, whose first results are reported in the paired paper (Anisimov et al., 2001). The choice has been performed on the basis of information about the percentage of survivors at a given age. We will have two groups of quite different ages showing little difference in survival (4 and 15 months), other two groups in an age range of sharp mortality (22 and 28 months) and, finally, a group of long-live animals (34 months). As an example, when comparing young animals (4 months old) with mice of 22 months of age, we are dealing with populations where mortality-induced selection has had little influence: little less than 90% of young animals reach the age of 22 months. On the contrary, the comparison of the same group of 22 months with the last one (34 months) shows that a strong selection occurs as only about 25% of the animals alive at 22 months will reach the age of 34 months.

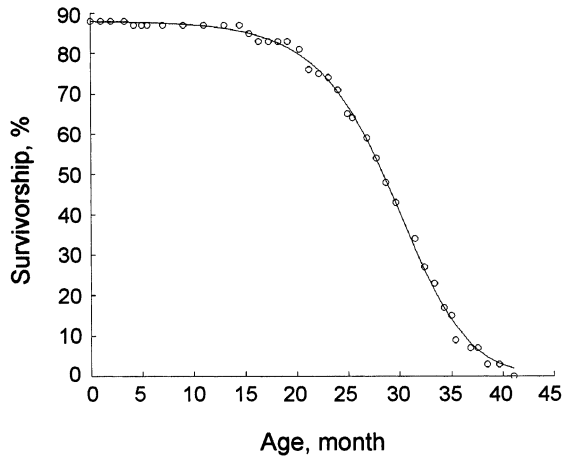

Fig. 1. Experimental absolute survival data (○) and the fitted curve (—) obtained using the mathematical model outlined in Section 2. Values of estimated survival parameters from both empirical and fitted curves are reported in Section 3.

3.2. Body weight

Time course of mean body weight of the cohort under study is reported in Fig. 3. It is worth noting the diphasic trend with a progressive increase in the first part of the curve followed by a slow decrease. Data, which also show the typical saw-tooth-like pattern, could be well fitted with both second and third degree polynomial functions; the figure reports the last one as it is the best in reproducing the empirical pattern.

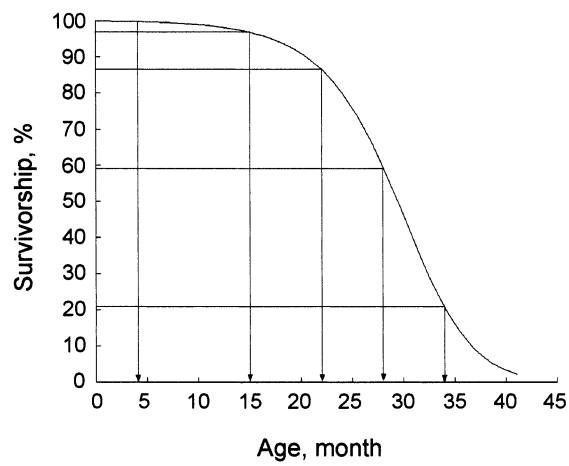

Fig. 2. Fitted survival kinetics as used to plan the age groups for the subsequent cross-sectional experiment. Horizontal and vertical straight lines help to check the percent survivorship at any age. Arrows point to the age chosen for the five groups in the cross-sectional experiment.

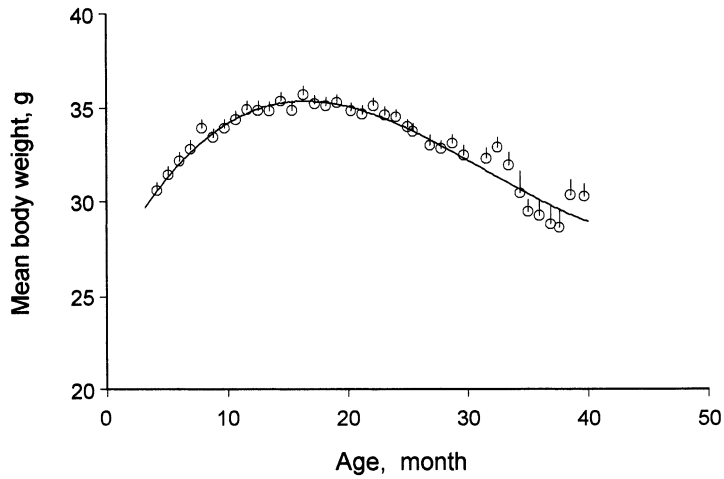

Fig. 3. Body weight pattern throughout the life span: Circles and bars are representative of mean body weight and SEM, respectively. Data are fitted using both quadratic and cubic polynomials. While both give good results, the cubic function is the best and is reported in the figure (—). Estimated values of the parameters entering the function $y = ((ax + b)x + c)x + d$: a, 0.0006 ± 0.0001; b, -0.052 ± 0.006; c, 1.3 ± 0.1; d, 26.1 ± 0.6. Estimated parameter values of the data presented here have also been reported for the quadratic function $y = (ax + b)x + c$: a, -0.016 ± 0.001; b, 0.62 ± 0.05; c, 29.1 ± 0.5. All the estimated values from both quadratic and cubic functions are statistically significantly different from zero ($P < 0.05$).

The correlation between individual body weight and the age-at-death has been analyzed by linear regression at the same five ages chosen for the cross-sectional experiment. Fig. 4 only reports results of the first four linear regression analyses as too few mice remain alive at the fifth age; estimated parameters are reported in the legend. Age-at-death shows a statistically significant inverse correlation with weight measured at 4, 15 and 22 months.

The inverse correlation between body weight and age-at-death suggests that progressive mortality-induced selection of the population can contribute to the body weight decrease observed after adulthood (Fig. 3), as animals with higher weight have a higher probability of dying before. This hypothesis can be verified by comparing body weight mean loss, calculated as the difference of the means from all mice alive at the ages of 22 and 28 months, with the mean loss between the same ages but calculated on the sole animals who survived at the age of 28 or 34 (Table 1). Considering the animals survived at the age of 28, mortality-induced selection accounts for about 40% of the mean body weight loss observed in the ALL group. The existence of a selection effect is further supported by absolute values of body weight as mean body weight of 22 months old mice still surviving at 28 is lower than that calculated on all animals alive at 22. As expected, when considering a highly selected group of animals such as those alive at 34 months of age, both the absolute mean body weight measured at 22 months and the decrease of mean body weight between 22 and 28 months are even lower. From these data it can be

Table 1
Estimate of the influence of mortality-induced selection on the age-related body weight decrease observed after adulthood[a]

Mouse group	Age (month)	Number of mice	Body weight (Mean (g))	Body weight difference (Mean (g))
ALL	22	76	35.07 ± 0.44	2.27 ± 0.84
	28	54	32.80 ± 0.40	
S-28	22	54	34.15 ± 0.42	1.35 ± 0.27
	28	54	32.80 ± 0.40	
S-34	22	17	33.45 ± 0.73	0.76 ± 0.32
	28	17	32.69 ± 0.60	

[a] ALL: mean body weights are calculated over all mice present at 22 and 28 months; S-28: mean body weights are calculated only over animals who survived at 28 months of age; S-34: mean body weights are calculated only over animals who where alive at 34 months. Mean body weight values of each mouse group at 22 months are statistically significantly different ($P < 0.001$). Body weights are expressed as mean $\pm$ SEM. SEM of the first difference in body weight is higher than the others since the two groups are made of different subjects, thus SEM of individual differences cannot be calculated directly; it has been estimated using error propagation. Mean body weight loss in S-28 group accounts for about 60% of the total body weight decrease in the ALL group; thus, in the same interval, weight loss due to mortality-induced selection can be estimated around 40%.

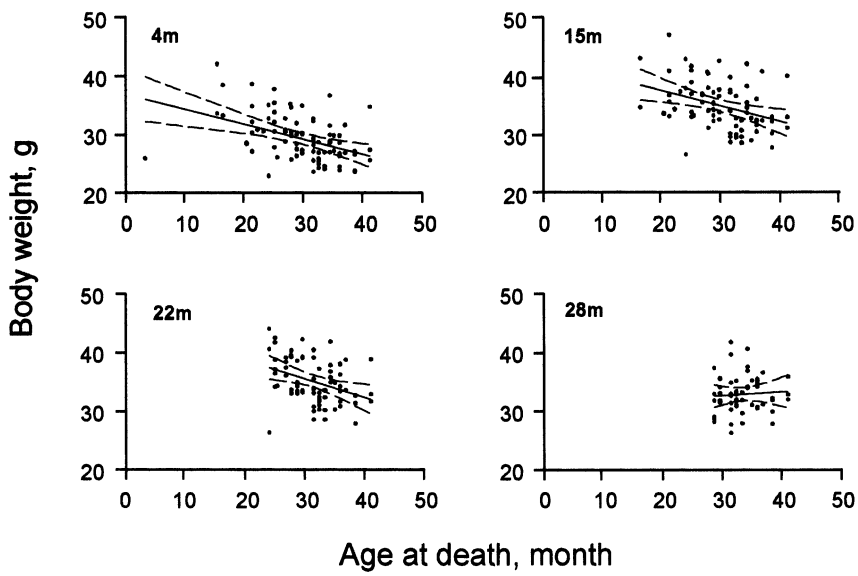

Fig. 4. Linear regression of body weight vs. age-at-death of the mice studied. The analysis has been performed at the five ages chosen for the cross-sectional study in order to facilitate the use of present results in the subsequent cross-sectional experiment. Figure only reports the results of regression analysis (fitted curve: (—); confidence intervals, 95% (- - -)) on the first four ages: there is a statistically significant negative correlation between mouse body weight at 4, 15 and 22 months of age and the age-at-death. Estimated coefficient values entering the function $y = ax + b$ are reported in the following. Four months: a, -0.26 ± 0.06; b, 36.9 ± 1.8; 15 months: a, -0.27 ± 0.07; b, 42 ± 2; 22 months: a, -0.32 ± 0.09; b, 45 ± 3. All parameter values are statistically significantly different from zero ($P < 0.05$).

Table 2
Mean body weight of mice grouped according to their age-at-death intervals, D, at any age chosen for the cross-sectional study[a]

Age (month)	Body weight, g (Number of mice: absolute, %)					
	ALL	$D<15$	D (15–21)	D (22–27)	D (28–34)	$D>34$
4	30.51 ± 0.44 (88, 100)	35.03 ± 5.31 (3, 3.4)	32.87 ± 1.23 (9, 10.3)	32.31 ± 0.85 (22, 25.0)	29.20 ± 0.57 (37, 42.0)	28.98 ± 0.73 (17, 19.3)
15	34.84 ± 0.44 (85, 100)		36.18 ± 1.18 (9, 10.6)	37.14 ± 0.94 (22, 25.9)	33.98 ± 0.59 (37, 43.5)	33.01 ± 0.84 (17, 20.0)
22	35.07 ± 0.44 (76, 100)			37.44 ± 0.85 (22, 28.9)	34.48 ± 0.58 (37, 48.7)	33.45 ± 0.73 (17, 22.4)
28	32.80 ± 0.40 (54, 100)				32.84 ± 0.58 (37, 68.5)	32.69 ± 0.60 (17, 31.5)
34	30.41 ± 0.64 (17, 100)					30.41 ± 0.64 (17, 100)

[a] ALL: mean body weight is calculated over all mice present at the given age; D: age-at-death intervals; $D<15$: mean body weight is calculated only over animals who died before the age of 15 months; D (15–21), D (22–27) and D (28–34): died in the 15–21, 22–27 and 28–34 intervals, respectively; $D>34$: mean body weight is calculated only over animals who were alive at the age of 34 months. It is worth noting that at 28 months, mean body weight of mice surviving up to D (28–34) or $D>34$ are very close in agreement with the lack of correlation between body weight and longevity at this age (Fig. 4). Mean body weights are reported $\pm$SEM.

concluded that mice weighing less live longer and loose their body weight with advancing age in a minor extent.

In an attempt to transfer most of useful information got from survival study to the cross-sectional one, data on the relationship between body weight and longevity has been further analyzed as reported in Table 2. Mice from longitudinal experiment have been grouped according to their age-at-death intervals, D, at the ages of 4, 15, 22, 28 and 34 months. For each age-at-death interval, mean body weight $\pm$ SEM and the relative number of subjects are given. Knowing the total number of the animals alive at any age considered, it is easy to calculate the percent number of mice surviving at any age-at-death interval; thus, surviving mice can be related, more or less tightly, to animal body weight.

These results can be used in interpreting data from cross-sectional studies. As an example, a prediction of longevity for 4, 15 and 22 months old animals can be attempted dividing them, respectively, into clusters on the bases of their body weight. Then, the variability of the values of biochemical and physiological parameters at these ages can be related to the belonging to a specific body weight cluster, characterized by a certain probability to survive up to a given age interval.

4. Discussion

Longitudinal studies on animals undergoing interventions able to modify life span and/or reverse age-related alterations could represent the best way to gain insights into aging processes and to give help in the search for biomarkers of aging (Piantanelli et al., 1992b); even more interesting are the experiments where a sufficient number of very old animals can be studied. However, there are some practical limits, among which the animal group dimension, due to the need of sacrificing part of them in order to assay biologically relevant parameters. The need of an adequate dimension is further requested in studies of long-live animals as increased variability is often observed at very advanced ages; thus, higher number of animals is also required to characterize them. In our approach, we used a preliminary longitudinal survival experiment followed by a cross-sectional one. This procedure allowed us not to challenge the capacity of our animal house raising lots of mice at the same time.

Analysis of survival data reported in the present paper has benefited from the use of our mathematical model of survivorship (Piantanelli et al., 1992a), which can extract some pieces of information useful in interpreting results from both physiological and pathological point of view. The value of the parameter ω gives an estimate of the maximal survival potentiality of the population under study and is representative of the deterministic component of mortality kinetics. The interindividual variability of the population, on the other hand, is estimated by the stochastic parameter S_0, which, therefore, accounts for the selection occurring in the population at advanced ages. It is worth noting that analysis of other survival

experiments performed in the context of our studies shows estimated values of the parameters ω and S_0 very close to those obtained here (Piantanelli et al., 1994b).

The comparison of our data with those present in the literature shows that our strain of BALB/c-nu mice is a long living one (mean life span $\approx$ 29 months); as an example, mean life span of BALB/cj mice is about 16–18 months (Roderick and Storer, 1961), thus about 40% lower than that recorded in our strain bred in our conditions. It is worth noting that data on mean life span in mice and rats of inbred as well as outbred strains kept in different vivaria usually show a quite high degree of variability (Storer, 1966). These variations could depend on different housing conditions, diet, and unidentified causes (Masoro, 1991).When animals are bred under non-SPF conditions, as it is the case of our mice, a non-negligible influence of hormesis can be hypothesized (Masoro, 2000; Rattan, 2000).

Maximal life span also results higher, though this value has to be taken with caution, due to its high degree of variability depending on the number of animals surviving at extreme ages. In our mathematical model of survivorship, maximal registered life span is tightly related to the ω value as their product roughly gives an adimensional constant value around 4 (Piantanelli et al., 1992a). Thus, when necessary, an estimate for maximal life span could be obtained dividing this "constant for longevity" by the value of the ω estimate, particularly useful when only few survival data are available at the tail of the curve. This estimate of maximal life span results in rough but reliable data as it comes from all the data of the survival curve.

It is worth noting that we used the term "maximal" life span, instead of maximum life span, to stress the fact that it is an estimate related to the sample studied, not to the population. Furthermore, in our opinion, we cannot put an upper bound for life span, that is, cannot determine the population maximum life span value. Using an analogy with the radioactive decay, we can imagine that after a certain age the probability to find a subject alive is very low and shows an asymptotic trend towards zero; thus, there is a probability, though very low, to find an age-at-death a little bit higher than that registered till the moment; of course, this probability does depend on the number of subjects in the sample under study.

Data on body weight give rise to some interesting considerations, both when taken alone and in relation to survival kinetics. The inspection of the details of the curve reporting mean body weight vs. age shows a saw-tooth-like pattern, more evident in the middle and final parts. This behavior, also observed in other experiments (Piantanelli et al., 1994b), is most probably due to relevant loss of weight of dying mice, which leads to a decrease of mean body weight followed by a sudden increase after death of these animals. This pattern is particularly evident at the tail of the curve where only very few surviving animals remain.

The biphasic pattern of body weight vs. age, as shown in Fig. 3, deserves some attention about the causes of the decrease observed after adulthood. Firstly, a contribution comes from the selection acting in the population as animals start to die as reported in Table 1. In fact, since the higher the body weight, the higher the probability to die earlier, the population becomes progressively enriched with lower-weight individuals. A second reason is due to the weight decrease measurable

in every subject. At this regard, literature is rich of studies reporting data on body weight loss and longevity. However, most of studies deal with intentional body weight loss in obese humans and do not lead to clear-cut conclusions (Gaesser, 1999; Williamson et al., 1999). Our data show that the lower the body weight the slower its decrease, indirectly suggesting that a slow decrease is associated with higher longevity. In previous studies on humans, a faster body weight decrease in the elderly was found associated with higher risk of mortality (Ho et al., 1994).

The correlation between age-at-death and body weight has been found statistically significant at 4, 15 and 22 months. At these ages, as shown in Table 2, we took advantage of such a relationship to sub-divide animals according to their age-at-death intervals; then, for each sub-group mean body weight has been calculated. This procedure, performed in the opposite direction, permits to roughly estimate potential longevity of animals in cross-sectional experiments on the basis of their body weight, as shown in the paired paper (Anisimov et al., 2001). Literature data on the correlation between longevity and body weight is quite controversial, though findings on humans, when most confounding factors are reduced or eliminated, support the hypothesis that large body mass index is an unfavorable factor for longevity and chronic diseases (Samaras and Elrick, 1999). However, at the time the present paper was being revised, a strong support to our results came from the study by Miller et al. (2000). The authors report, among other findings, data showing that female mouse body weight at 3, 6 and 12 months and peak body weight are all predictors of longevity.

Acknowledgements

The authors thank A. Antognini and F. Marchegiani for their skilful technical assistance.

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