COMPARATIVE VS ONTOGENETIC PARADIGMS FOR TESTS OF THE INTRINSIC MUTAGENESIS HYPOTHESIS OF AGING

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COMPARATIVE VS ONTOGENETIC PARADIGMS FOR TESTS OF THE INTRINSIC MUTAGENESIS HYPOTHESIS OF AGING*

George A. Sacher

This paper examines certain aspects of the biology of longevity and aging that bear on the role of DNA in the ontogenetic aging process, and on the genetic basis for the differences of longevity among mammalian species.

Let us begin with a phenomenological analysis of a most important manifestation of the aging process, i.e., the increase of death rate with age. Figure 1 shows the survivorship curves of two mammalian species, the house mouse, Mus musculus, and a North American field mouse, Peromyscus leucopus. Both show sigmoid survival curves, indicative of an increase of mortality rate with age, but Peromyscus lives about 2.5 times longer than does Mus. P. leucopus has an average lifespan of 48 months and a maximum span of 100 months, while wild-type Mus musculus has an average span of 20 months and a maximum of about 45 months (Sacher and Hart, 1978).

Figure 2 is a plot of the adult age-specific mortality rate, q_x, of Mus and Peromyscus, on a logarithmic scale against age. This relation is linear throughout adult life, so that we can readily estimate the two parameters of the actuarial aging process in these two species. The slope, in units of reciprocal days, specifies the constant rate of increase of logarithm of mortality rate with age. The trend is exponential, like the increase of capital at compound interest. The slope coefficient, α (alpha), can be identified as the actuarial aging rate of the population. A more easily grasped number is the doubling time of mortality rate, $T_d = 0.693/\alpha$. We have found that the doubling time for P. leucopus is 422 days, as against 180 days for Mus (Sacher and Hart, 1978). These times are in a ratio of 2.3:1, almost the same as the 2.4:1 ratio for the mean longevities (Table 1.)

Table 1;

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The straight line relation of log q_x to age has an intercept at zero age, log q_0, that is an estimate of the initial, intrinsic vulnerability to the age-related diseases of adult life, and hence says something about the inherent stability of the genome, its genetically determined capacity to resist disease.

We have determined the life tables for a number of rodent species, and gathered published and unpublished data for a number of others. All species have approximately linear q_x plots, as in Figure 2. Table 1 gives the q_0 and T_d values for five species (Sacher, 1978a), and it can be seen that T_d increases by a factor of 17 from mouse to man, while life expectation increases 46-fold. In other words, the increase of life expectation from mouse to man is greater than can be accounted for by the increase of T_d alone.

Table 1 also shows that initial vulnerability decreased by a factor of about 200 from mouse to man while life expectation increased by a factor of 46. This 200-fold factor of decrease of initial vulnerability is responsible for about half of the increase of lifespan from mouse to man.

Two conclusions can be drawn: first, that the decrease of the initial vulnerability, q_0, has been equal in importance to the decrease of the actuarial aging rate, alpha, in contributing to the evolutionary increase of mammalian longevity; and second, that as longevity increases in the evolution of mammals, the rectangularity or determinacy (Sacher, 1966a), of the survival curve increases also. This increase of determinacy contributes to the reproductive fitness of long-lived species (Sacher, 1978a).

The most noteworthy feature of the actuarial aging process is the exponential increase of death rate with age. However, the decrement with age of the physicochemical integrity of the organism, and the decrements in the vital functions that depend on the physicochemical makeup most directly, typically are nearly linear, and of comparatively small magnitude, in the range of tens of percent over the lifespan (Shock, 1977).

It has been shown (Sacher, 1956; Sacher and Trucco, 1966) that a reconciliation of these contrasting trends of physicochemical aging and actuarial aging can be accomplished by a mathematical model which postulates that the living organism is a dynamically metastable system, i.e., is self-restoring within a closed region, but has a finite probability of escaping from the stable region as a result of fluctuations of state arising from large perturbations of either internal or external origin. An escape is equivalent to a transition from living to dead, or from healthy to diseased. Figure 3 schematizes the potential well for a one-dimensional mortality process arising from the fluctuations of a single physiological variable. The cubic curve is the potential function, while the parabolic curve, the first derivative of the potential, is the strength of the restoring force. At the rim or "divide" of the potential function the restoring force falls to zero, and at larger displacements of the physiological variable the force is positive, implying that once the divide is crossed the system moves away from its stable region. This irreversible transition is a "death," and the mathematical problem is to determine the escape rate as a function of the system parameters and external parameters.

The depth of the well in the model can be equated to the vulnerability of a living population, and it can be shown (Sacher, 1956; Sacher and Trucco, 1966) that a linear decrement of the system parameters with age will lead to a linear decrease of the depth of the well, and thereby bring about an exponential increase of the escape rate with age. In application to the theory of aging, this model tells us that the rapid increase of mortality in a population near the end of the life span does not require the postulate that biochemical or genetic senescence processes accelerate with comparable rapidity at advanced age. Therefore: a molecular aging process that proceeds at a constant rate throughout the course of the individual's adult life is both mathematically and biologically consistent with the observed exponential increase of morbidity and mortality rates with age.

This is a key statement for this symposium on the molecular genetics of aging, because it widens the range of possible models for the time course of molecular aging processes that can be consistent with the data of senescent morbidity and mortality.

It has been argued that the aging process and life table characteristics of mammals cannot be the result of natural selection because animals in their natural habitats do not live long enough to experience senescence. This is a fallacious inference, which is contradicted by several lines of evidence. In particular, the increased rectangularity of the survivorship curve suggests that the life curve is the outcome of natural selection, for just such an increase of rectangularity is necessary to maintain reproductive fitness in the large, long-lived mammals. Their reproductive rates are lower, and maturation times longer, than those for small, short-lived mammals, and the replacement time for long-lived mammals is a disproportionately greater fraction of their lifespans than for short-lived mammals (e.g., 33% for Homo sapiens, as against 10% for Mus musculus).

The requirement for the risk of reproductive failure to be low over the entire duration of the replacement time is sufficient to assure that species with longer replacement times will be selected to have more rectangular survival curves. In other words, the rectangular form of the survivorship curve is the result of selection acting not on the survival curve itself, but rather on the reproductive risk function, which is linked with the actuarial risk function by the senescence process that they have in common.

We must examine one further aspect of the phylogeny of longevity, namely the degree to which species longevity is correlated with certain constitutional variables. In a study in which the dependence of maximum species lifespan on four constitutional variables--brain weight, body weight, specific metabolic rate, and deep body temperature--was determined by multiple regression (Sacher, 1976), the outcome was that about 85% of the longevity variance in this set of mammalian

species is accounted for by these four variables, even though they are measured on different samples of animals, and the data are drawn from heterogeneous published data. This result makes it possible to draw the further inference that mammalian longevity did not evolve independently but rather as part of a covarying set of anatomical and physiological characteristics that have complex relationships among themselves and with the reproductive process, and that contribute to species fitness.

The high correlation of species lifespan with brain weight and other constitutional variables makes it difficult to give credence to the hypothesis that the limits on longevity are imposed by senescence genes (Sacher, 1978a, b). Thus, another important implication of comparative longevity research for this symposium is that structural DNA pathology in somatic cells does not arise from inherited structural pathology in the germ line, such as senescence genes or the Szilardian "faults" (Szilard, 1959).

The ontogenetic aspect of DNA structural pathology, i.e., the observed pattern of its accumulation with age, is another important consideration in the choice of an optimal research paradigm. In brief, my argument on this point is based on the assumption that structural DNA pathology, and in particular chromosome deletions and rearrangements, accumulates more slowly than in proportion to age. This assumption is supported by the data of Curtis (1963) for the age-dependence of chromosomal abnormalities in mice and dogs. If mutations occur at a constant rate at all ages, then the accumulated number should increase in linear relation to age unless the mutations impose a selective disadvantage on the cells bearing them, in which case the frequency of mutations will grow more slowly than in proportion to age, and in the extreme case of rapid selective elimination can approach a steady state level that is independent of age.

Insofar as the evidence points to no more than a linear accumulation of structural DNA pathology with age, we are justified in adhering to the working

assumption that the rate of mutation is constant, and not necessarily a rapidly increasing function of age, as is assumed by the error catastrophe theory of aging (Orgel, 1963; Harrison and Holliday, 1967). This has the paradoxical implication that the factors governing the generation of fundamental aging pathology in DNA are themselves age-independent. If this is so, then the factors that govern the rate of production of structural pathology in somatic cell DNA need not be studied in old animals. The use of old animals for this purpose confers no advantage and entails considerably greater cost. Also, insofar as studies of old animals lead to a preoccupation with the secondary pathology of senescence, they can be counterproductive to the goal of establishing the basic molecular mechanisms of aging, for the accumulated manifest pathological changes are effects of senescence, and however fascinating and important they are in their own right, they cannot be traced back to their causes.

On the basis of the above considerations, it is possible to outline a paradigm for research on the causal factors governing the rate of occurrence of structural pathology in DNA.

Assumptions:

1. The species survival characteristic, the mathematical function which expresses the average longevity and the shape of the survivorship curve, is a fitness variable, on which natural selection operates in close interaction with several other constitutional and reproductive variables.

2. The importance of the survival characteristic, and the need for it to vary in close conjunction with other variables, led to the evolution of a set of positive longevity-assurance systems at all levels of organization, enzymatic, physiological, and behavioral.

3. The evolution of vertebrate longevity--usually in the direction of increased mean longevity and decreased relative dispersion of survival--is achieved in considerable degree by means of quantitative modification of the expression of this set of structural longevity-assurance genes, which are assumed to all mammalian species have in common.

4. The assumption of senescence genes that have their expression at the end of the lifespan can therefore be replaced by the more parsimonious assumption of genes for positive life-stabilizing functions that have constant effect throughout the lifespan (this in regard to senescence only; developmental sequence genes in the adult phase, such as those controlling menopause, are not covered by this assumption).

5. In the absence of selection for a particular time-dependent developmental program, the two parameters accessible to selection for maintenance of reproductive efficiency and survival are the slope and intercept of the log rate-of-mortality (Gompertz) function.

Given these assumptions, the longevity-assurance research paradigm proceeds by looking for correlations of constitutive biochemical, physiological, and behavioral systems with longevity. Such relations, when found, are not considered to be causal unless further evidence supports that assumption. The comparisons can be within species, e.g., among laboratory mouse strains or hybrids, or among Drosophila strains, but there must be clear evidence about whether the genotypes in question differ only in their vulnerability parameters, as is usually the case (Sacher, 1966b; Storer, 1978) or whether they differ in the aging rate parameter as well. The comparisons between populations would be made at comparable degrees of progression toward maturity or senescence, e.g., on animals at 10% of their maximum lifespans, and would preferably, though not necessarily, be made on young animals (Sacher and Hart, 1978). The basic assumption is that the factors responsible for differences in survival characteristic between populations are usually constitutive, and are operative from an early age. The species compared should, moreover, be well defined in regard to genetic makeup, diet, disease susceptibility, etc., similar in function and structure, and closely related taxonomically, although comparisons across orders (rodents versus primates, for example) would be appropriate at a later stage of research.

A concrete instance of the utility of the comparative paradigm is the comparison of Mus musculus and Peromyscus leucopus in regard to the capacity of cultured primary explant fibroblasts from young donors of these two species to carry out excision repair of 260 nm ultraviolet ray damage to their DNA. The proliferative capacity of cultured Mus cells falls off about twice as rapidly with passage number as does the proliferative capacity of Peromyscus, and the rate of DNA repair of early-passage cells, as measured either by unscheduled DNA synthesis or by BUdR photolysis, proceeds at a 2.5-fold greater rate in Peromyscus cells than in Mus cells (Sacher and Hart, 1978; Hart et al., 1979).

We saw above (Table 1) that Mus and Peromyscus differ by a factor of 2.4 in life expectation and that this is attributable almost entirely to a 2.3-fold difference in aging rate, alpha, with no significant difference in the initial vulnerability parameter, q_0. Does this signify that DNA repair systems in mammals operate primarily to govern the aging rate parameter, and not the vulnerability parameter? The tenfold variation of repair rate across species (Hart and Setlow, 1974; Hart, these proceedings) is about the same as the range of variation of alpha, and so is consistent with the hypothesis that DNA repair governs the species aging rate. In particular, it can be hypothesized that laboratory mouse strains and hybrids, which usually differ only in q_0, and not in alpha (Sacher, 1966b; Storer, 1978), will show little variation in DNA repair rate among strains, and a low correlation of repair rate with strain longevity. The appropriate experiments are eagerly awaited.

REFERENCES

Andersen, A. C. 1970. General pathology. In: The Beagle as an Experimental Dog, A. C. Andersen, ed. Iowa State University Press, Ames, pp. 520-546.

Andersen, A. C., and L. S. Rosenblatt. 1974. Survival of beagles under natural and laboratory conditions. In: Dogs and Other Large Mammals in Aging Research: I, A. C. Andersen, E. A. Boyden, and J. H. Dougherty, eds. MSS Information Corp., New York, pp. 18-23.

Comfort, A. 1958a. The longevity and mortality of thoroughbred mares. J. Gerontol. 13: 342-350.

Comfort, A. 1958b. Coat color and longevity in thoroughbred mares. Nature (London) 182: 1531-1532.

Curtis, H. J. 1963. Biological mechanisms underlying the aging process. Science 141: 686-694.

Harrison, B. J., and R. Holliday. 1967. Senescence and the fidelity of protein synthesis in Drosophila. Nature 213: 990-992.

Hart, R. W., and R. B. Setlow. 1974. Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species. Proc. Nat. Acad. Sci. USA 71: 2169-2173.

Hart, R. W., G. A. Sacher, and T. L. Hoskins. 1979. DNA repair in a short- and a long-lived myomorph rodent species. J. Gerontol. (in press).

Norris, W. P., S. A. Tyler, and G. A. Sacher. 1976. An interspecies comparison of responses of mice and dogs to continuous ^{60}Co gamma irradiation. In: Biological and Environmental Effects of Low-Level Radiation. International Atomic Energy Agency, Vienna, Vol. I, pp. 147-155.

Orgel, L. E. 1963. The maintenance of the accuracy of protein synthesis and its relevance to aging. Proc. Nat. Acad. Sci. USA 49: 517-521.

Sacher, G. A. 1956. On the statistical nature of mortality, with especial reference to chronic radiation mortality. Radiology 67: 250-257.

Sacher, G. A. 1966a. Abnutzungstheorie. In: Perspectives in Experimental Gerontology, N. W. Shock, ed. Charles C. Thomas, Springfield, pp. 326-335.

Sacher, G. A. 1966b. The Gompertz transformation in the study of injury-mortality relationship. Application to late radiation effects and ageing. In: Radiation and Ageing, P. J. Lindop and G. A. Sacher, eds. Taylor and Francis, London, pp. 411-441.

Sacher, G. A. 1976. Evaluation of the entropy and information terms governing mammalian longevity. In: Interdisciplinary Topics in Gerontology, R. G. Cutler, ed. Karger, Basel, Vol. 9, pp. 69-82.

Sacher, G. A. 1977. Life table modification and life prolongation. In: Handbook of the Biology of Aging, J. Birren, C. Finch, and L. Hayflick, eds. Van Nostrand Reinhold, New York, Ch. 24, pp. 582-638.

Sacher, G. A. 1978a. Evolution of longevity and survival characteristics in mammals. In: The Genetics of Aging, E. L. Schneider, ed. Plenum Press, New York, pp. 151-168.

Sacher, G. A. 1978b. Longevity and aging in vertebrate evolution. BioScience 28: 497-501.

Sacher, G. A., and R. W. Hart. 1978. Longevity, aging, and comparative cellular and molecular biology of the house mouse, Mus musculus, and the white-footed mouse, Peromyscus leucopus. In: Genetic Effects on Aging, D. Bergsma and D. E. Harrison, eds. Birth Defects:Original Article Series, The National Foundation-March of Dimes, Alan R. Liss, New York, pp. 71-96.

Sacher, G. A., and E. Trucco. 1966. Theory of radiation injury and recovery in self-renewing cell populations. Radiat. Res. 29: 236-256.

Shock, N. W. 1977. Systems integration. In: Handbook of the Biology of Aging, J. Birren, C. Finch, and L. Hayflick, eds. Van Nostrand Reinhold, New York, pp. 639-665.

Storer, J. B. 1978. Effect of aging and radiation in mice of different genotypes. In: Genetic Effects on Aging, D. Bergsma and D. E. Harrison, eds. Birth Defects:Original Article Series, The National Foundation-March of Dimes, Alan R. Liss, New York, pp. 55-70.

Szilard, L. 1959. On the nature of the aging process. Proc. Nat. Acad. Sci. USA 45: 30-42.

Table 1. Life Expectation E_0, Initial Vulnerability q_0, and Doubling Time of Mortality Rate T_d for Five Mammalian Species.[a]

Species	E_0 (days)	$q_0(day)^{-1}$	T_d (days)
Mus musculus (house mouse)	602[b]	3.0×10^{-4}	180
Peromyscus leucopus (white-footed mouse)	1,420	1.2×10^{-4}	422
Canis familiaris (beagle dog)	3,617	2.7×10^{-5}	812
Equus caballus (thoroughbred mare)	6,329	6.0×10^{-6}	1332
Homo sapiens (U.S. white female, 1969)	27,700	1.5×10^{-7}	3100

[a]Life expectations are from birth; in the cases of the rodents they do not include the deaths from birth to weaning. Data for the rodent species from Sacher and Staffeldt (unpublished); for beagle dog, from Andersen (1970), Andersen and Rosenblatt (1974), and Norris et al. (1976); for thoroughbred mare, from Comfort (1958a,b). Reproduced, with modifications, from G. A. Sacher (1977).

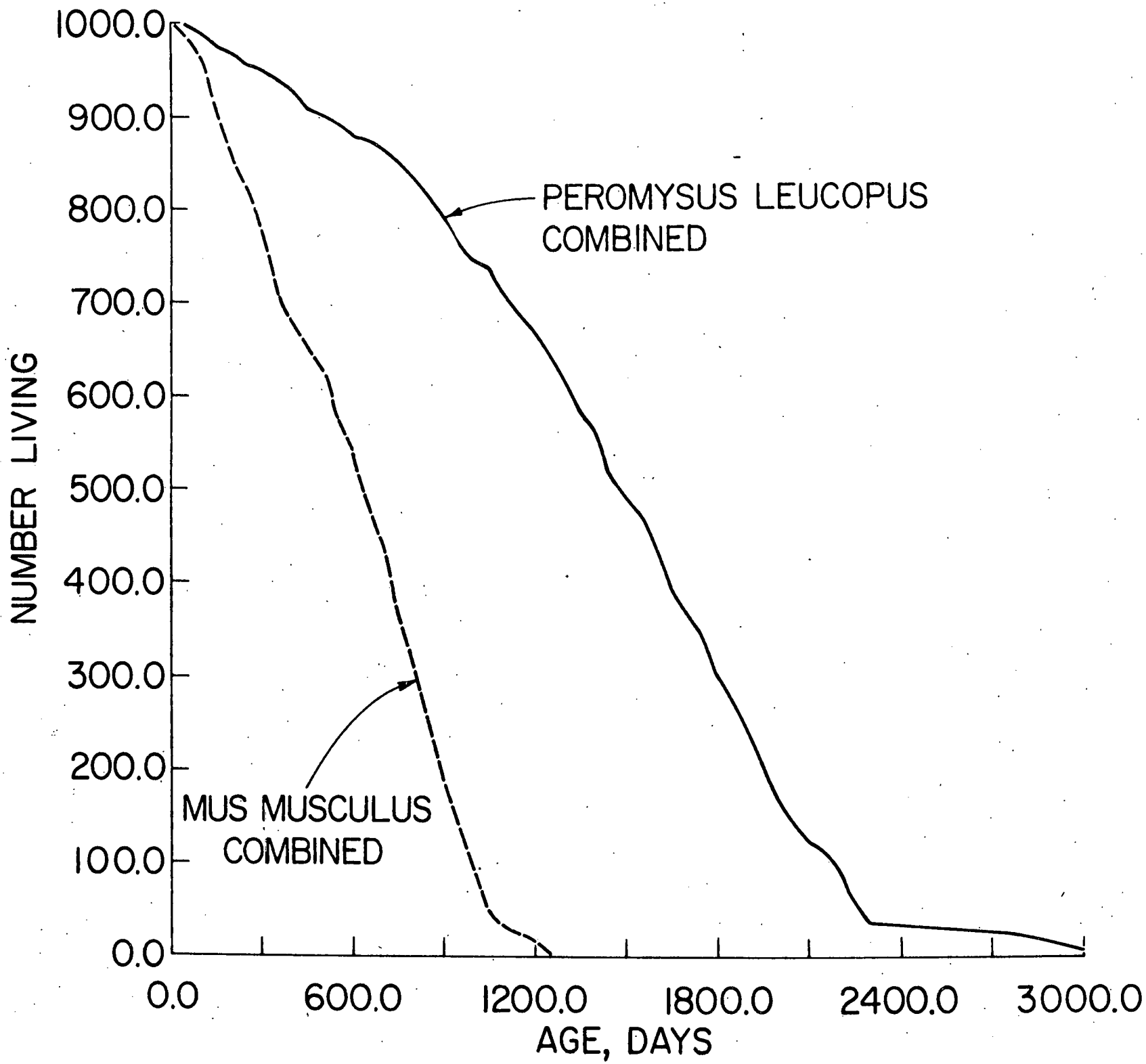
[b]Median survival time.

FIGURE LEGENDS

Fig. 1. Survivorship curves for combined sexes of two muroid rodent species, Mus musculus and Peromyscus leucopus, bred and maintained in captivity. Peromyscus, though similar in size and ecological adaptation to Mus, lives about 2.5 times as long. Reproduced from Sacher and Hart (1978).

Fig. 2. Plots of logarithm of age-specific mortality rate (Gompertz function), on a logarithmic scale, vs. age for combined sexes for Mus musculus (o) and Peromyscus leucopus (●). Both species have approximately linear Gompertz equation. They have comparable q_0 values, but Peromyscus has a slope (actuarial aging rate) about half that for Mus (see Table 1). Reproduced from Sacher (1978a).

Fig. 3. The potential function for a hypothetical physiological variable, V(y), and the restoring force function, K(y). The stable equilibrium of the system is at y = 0, and the point of instability is at y = Λ.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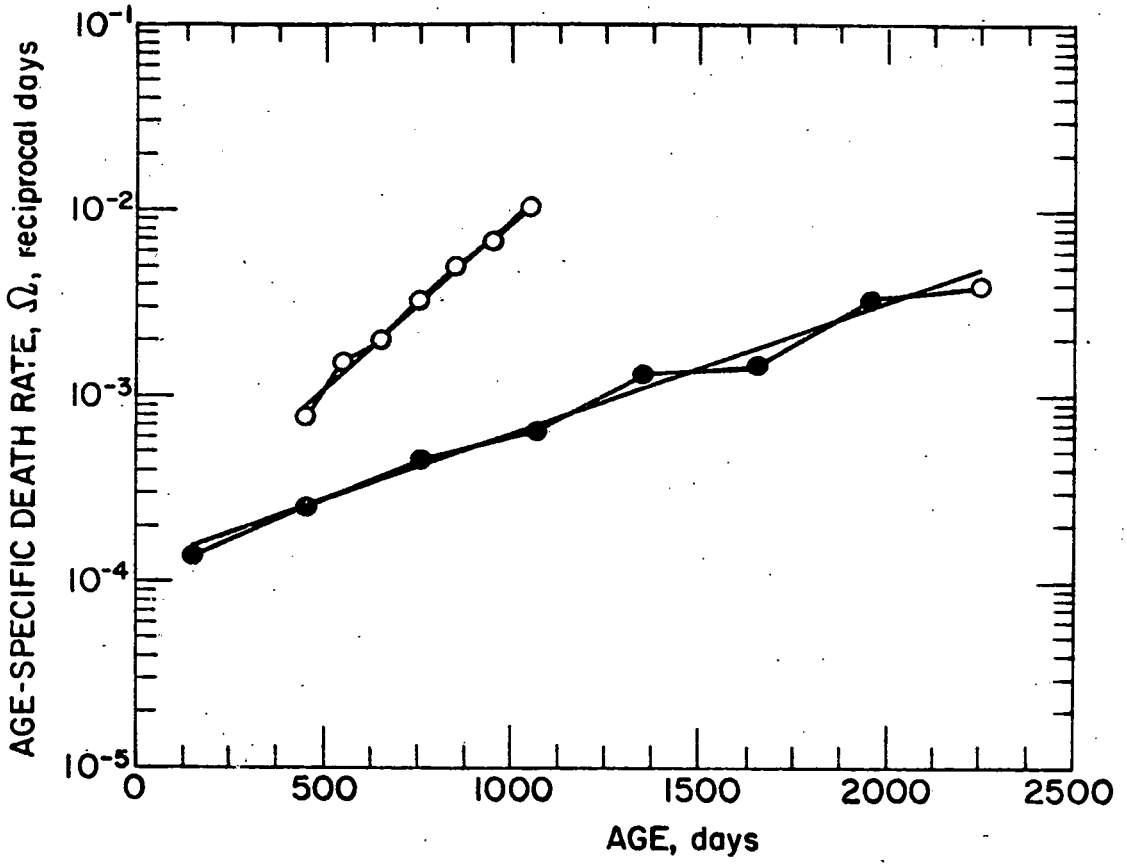
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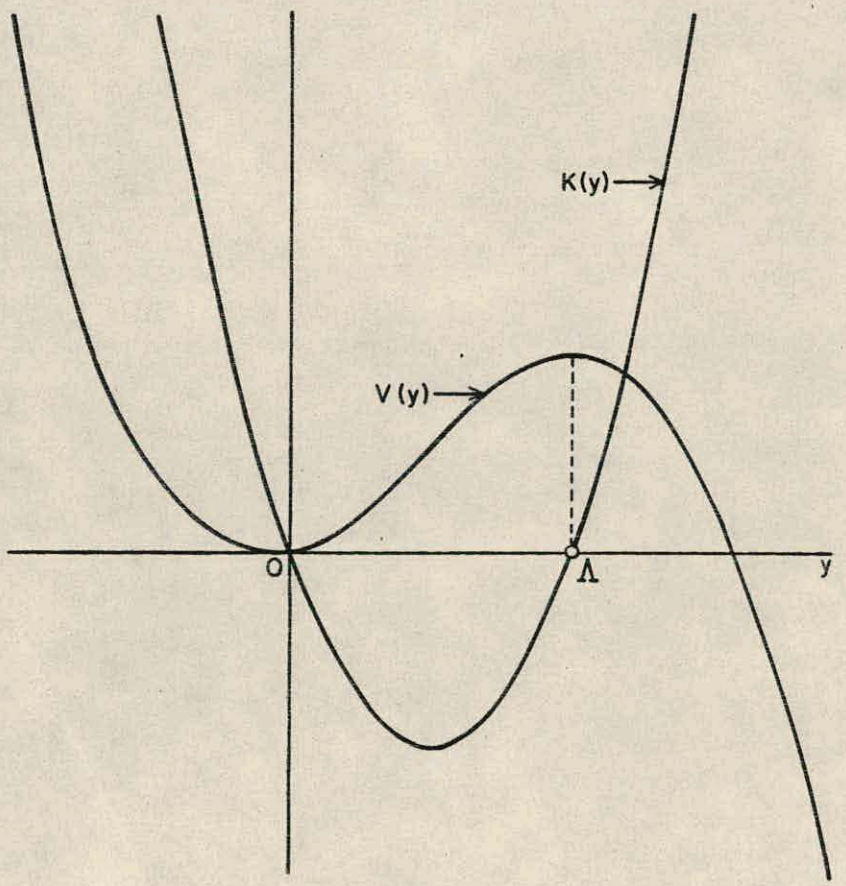
AGE, days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