Delayed Aging in Ames Dwarf Mice. Relationships to Endocrine Function and Body Size

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1 Introduction

There is considerable evidence for genetic control of aging and longevity. For example, there are major differences in life span between different breeds of domestic dogs, between different inbred lines of laboratory mice, and between lines of mice developed by selection for various phenotypic traits. Studies in yeast, in a round worm, *Caenorhabditis elegans*, and in the fruit fly, *Drosophila melanogaster*, provided a wealth of information on the genetic control of aging, including evidence that mutations at specific loci can produce major alterations in the life span. A new term, longevity assurance genes (LAGs), was coined to describe genes involved in the control of the life span. Evidence for the existence of LAGs in yeast, *C. elegans*, and *Drosophila* and the effects of mutations at these loci are described in other chapters in this Volume.

Although existence of LAGs in higher organisms can be suspected from these observ., there are surprisingly few examples of mutations at specific loci affecting aging in mammals or in the human. In the laboratory mouse, mutations at many loci and targeted disruption (knockout) of numerous genes are associated with significant reduction of life expectancy. However, premature death of the affected animals appears to be due to developmental abnormalities or identifiable functional deficits rather than to accelerated aging. There is recent evidence for genetic basis of at least one of several progeria (premature aging) syndromes in the human (Ye et al. 1998) and a mutation causing a syndrome resembling aging was recently described in the mouse (Yamashita et al. 1998). However, it remains to be determined whether these syndromes can be viewed as premature onset and rapid progression of otherwise normal aging or should be regarded as degenerative diseases with symptoms overlapping those that accompany aging. It is against this background that the question of existence of genes that can extend the life span of

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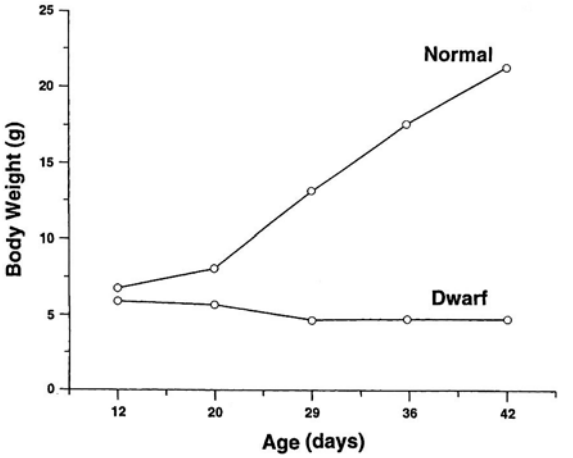
a mammal assumes special significance. One example of delayed aging in mammals caused by mutation at a single locus was provided by our report of markedly increased life span in Ames dwarf mice (Brown-Borg et al. 1996). Comparable extension of life span was demonstrated also in Snell dwarf mice, which are homozygous for a different mutation but phenotypically are very similar, if not identical, to the Ames dwarfs (K. Flurkey and D. Harrison, per. comm., R. Miller, per. comm.). Thus, it would appear that two loci, Prop-1 for the Ames dwarfs and Pit-1 for the Snell dwarfs, may be regarded as the first identified LAGs in a mammal. In this chapter, we will describe the phenotypic characteristics of Ames and Snell dwarf mice, review information on the life span and age-associated changes in these animals, and propose mechanisms that may be responsible for their prolonged survival.

2
Ames Dwarf Mice

Ames dwarf mice are homozygous for a recessive mutation which was discovered in the breeding colony of laboratory mice at Iowa State University in Ames, Iowa. This mutation was originally described by Schaible and Gowen (1961) and named Ames dwarf (genetic symbol, df). The DF locus was mapped to chromosome 7 (currently designated chromosome 11) by analyzing recombination frequency with Rex and waved-2, which were known to be located on this chromosome (Bartke 1965a). More recent studies based on positional cloning resulted in precise mapping of this gene on chromosome 11 (Buckwalter et al. 1991; Sornson et al. 1996). Mice homozygous for Ames dwarfism (df/df) are phenotypically normal at birth, but their growth and development are retarded. Starting at the age of approximately 14 days, Ames dwarf mice are significantly smaller than their normal siblings (df/+ and +/+, which apparently do not differ from each other). Their postweaning growth rate is markedly reduced and adult Ames dwarf mice are diminutive, weighing approximately 1/3 of the weight of their normal siblings (Fig. 1). In addition to reduced body size, Ames dwarfs are distinguished from normal mice by somewhat infantile body proportions, with a short snout being perhaps the most striking feature. Although less active than normal mice, the Ames dwarfs appear healthy and, under conditions of our animal colony, outlive their normal siblings by an average of at least 1 year.

The mechanisms responsible for retarded development, diminutive body size, and infertility of Ames dwarf mice are well understood. In most elegant studies, Sornson and his colleagues (Sornson et al. 1996) demonstrated that this mutation prevents differentiation of specific cell lineages in the anterior pituitary on embryonic day 12.5–13 (Fig. 2). Since the affected cell types would normally express the homeotic factor Pit-1, these investigators named



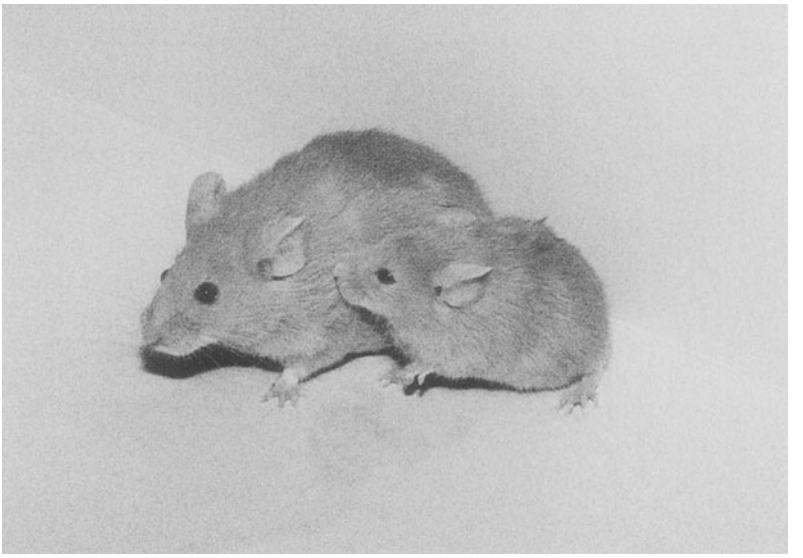


Fig. 1. *Top.* Body weight of Ames dwarf (df/df) and normal (+/?) mice. Each *point* represents the mean value of five to seven animals. (After Romero and Phelps 1993). *Bottom.* Adult Ames dwarf mouse (*right*) and its normal sibling (*left*). (Photo courtesy Dr. W. Hunter)

the Ames dwarf locus Prophet of Pit-1, abbreviated Prop-1. As the result of developmental arrest in the pituitary of the Ames dwarf mouse, the following three cell types fail to differentiate: (1) the somatotrophs which normally produce growth hormone (GH), (2) the lactotrophs, which normally produce prolactin (PRL), and (3) the thyrotrophs which normally produce thyroid-stimulating hormone (TSH). Consequently, Ames dwarf mice are GH-, PRL-, and TSH-deficient. This has been amply documented by histological and

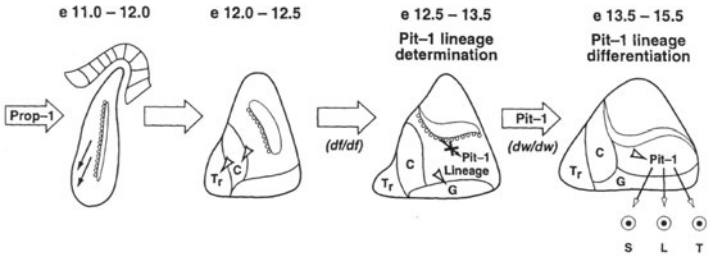


Fig. 2. Model of determination and differentiation events in pituitary ontogeny. The determination of corticotrope (*C*), rostral tip thyrotrope (*Tr*), and gonadotrope (*G*) cell phenotype between embryonic days (*e*) 8.5 and 11.5, and their subsequent differentiation occur before determination of the Pit-1 lineages on ~e12.5–13.0, with Pit-1 initially detected on e13.5. Determination/migration is denoted by *small arrows*, differentiation by *open arrowheads*. The Prop-1 mutation in df/df results in a failure of determination of the Pit-1 lineages (e12.5-e13.0). *Cross* determination block in df/df mouse; *Pit-1* gene activation and initial differentiation is followed by the appearance of three distinct cell types: the somatrotrope (*S*), lactotrope (*L*), and thyrotrope (*T*) cell types. (After Sornson et al.)

immunocytochemical studies of the pituitary (Bartke 1964; Andersen et al. 1995), radioimmunoassay measurements of hormone levels in peripheral circulation (Barkley et al. 1982), and results of hormone replacement studies (Bartke 1965b; Doherty et al. 1980; Soares et al. 1984; Chandrashekar and Bartke 1993; Andersen et al. 1995). Most of the phenotypic characteristics of Ames dwarf mice represent the expected consequences of complete, life-long deficiency of GH, PRL, and TSH. Thus, GH deficiency leads to dramatically reduced levels of insulin-like growth factor I (IGF-I, the key mediator of GH action) in peripheral plasma (Chandrashekar and Bartke 1993), reduced growth, and diminutive body size. Deficiency of PRL accounts for luteal failure and consequent sterility of Ames dwarf females (Bartke 1965c, 1979a). In the mouse, PRL is an essential component of the hormonal complex controlling production of progesterone by the corpora lutea of the ovary and maintenance of early pregnancy (Bartke 1965c, 1973). Deficiency of TSH leads to hypothyroidism (Bartke 1964; K. E. Borg and A. Bartke, unpubl. observ.) and persistence of infantile body proportions in the adult. Ames dwarf mice have been used extensively as an animal model for defining the physiological role of adenohypophyseal hormones. Results of these studies included the demonstration that development and functional activity of tuberoinfundibular dopaminergic neurons in the hypothalamus are PRL-dependent (Romero and Phelps 1993), and that both PRL and GH can stimulate testicular function (Bartke 1966a, 1979a; Chandrashekar and Bartke 1993).

Phenotypic characteristics of Ames dwarf mice which are of particular interest in the context of their prolonged longevity are those that resemble the effects of caloric restriction. Caloric restriction can delay aging and prolong life span of mice (Duffy et al. 1990; Masoro 1995; Weindruch and Sohal 1997). These characteristics include reduced body size and delayed

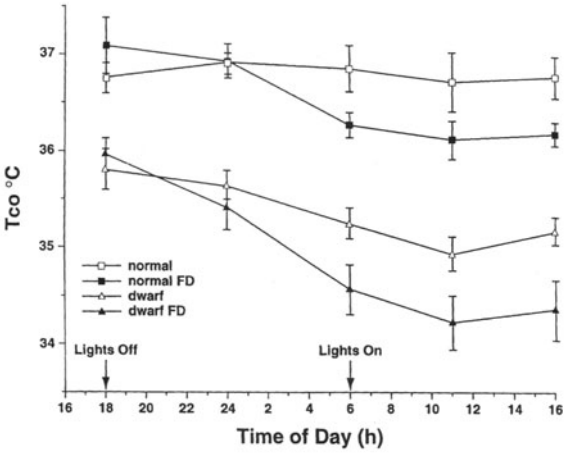


Fig. 3. Body core temperature (*Tco*) of Ames dwarf mice and their normal siblings during two consecutive 24-h periods. Each *point* represents mean data from six mice averaged over the preceding time periods (2, 6, or 5 h) ± SEM. *Open squares* (normals) and *open triangles* (dwarfs) indicate data collected during 24-h baseline period and *solid squares* (normals) and *solid triangles* (dwarfs) represent data collected during the succeeding 24-h food deprivation period. Please note similar Tco responses to food deprivation in dwarf and normal mice. (After Hunter et al. 1999)

puberty, reduction in core body temperature (Bartke et al. 1998; Hunter et al. 1999; Fig. 3), reduction in plasma IGF-I and glucose, reduction in insulin levels in females, and increase in corticosterone levels in males (Borg et al. 1995). However, under standard housing conditions, i.e., constant access to food, dwarf mice consume more food per gram body weight than their normal siblings (Mattison and Bartke, unpubl.), and frequently become obese. Thus, longevity of dwarf mice does not appear to be due to "voluntary" caloric restriction. These findings will be discussed in some detail later in the chapter. Counter-intuitive in the context of delayed aging are several indices of reduced immune function in Ames dwarf mice (Duquesnoy 1972; Esquifino et al. 1991).

3
Snell Dwarf Mice

In 1929, Snell (1929) reported the existence of a recessive mutation which caused dwarfism in mice, and named it dwarf (genetic symbol dw). Deficiency of pituitary function in dw/dw dwarf mice was demonstrated in a series of developmental and hormone replacement studies (review in Grüneberg 1952). Demonstration that this mutation causes primary defect in adenohypophyseal rather than hypothalamic function was obtained

in studies involving reciprocal pituitary transplantation between dwarf and normal mice (Carsner and Rennels 1960). More recent studies demonstrated various secondary alterations in the hypothalamus, some of which could be corrected by appropriate replacement with anterior pituitary hormones (Morgan et al. 1981; Webb et al. 1985; Phelps 1994). The DW locus is located on the chromosome 16 and thus is unrelated to DF. In 1990 (Li et al. 1990), it was shown that dwarf mice, which in the meantime were renamed Snell dwarf mice, are homozygous for a mutation at the Pit-1 locus. As was mentioned earlier in this chapter, Pit-1 is involved in the differentiation of somatotrophs, lactotrophs, and thyrotrophs on embryonic days 13.5–15.5 (Sornson et al. 1996). It binds to the promoters of the GH, PRL, and TSHβ genes and stimulates their expression (Schaufele 1994; Holloway et al. 1995). Thus, Pit-1 mutants, similarly to Prop-1 mutants, lack somatotrophs, lactotrophs, and thyrotrophs and are GH-, PRL-, and TSH-deficient. In other words, phenotypes of Snell dwarf mice are very similar, if not identical, to the phenotypes of Ames dwarfs even though the corresponding mutations are genetically unrelated and located on different chromosomes. Quantitative differences in various traits of Snell and Ames dwarf mice as well as quantitative differences between findings of different investigators studying the same mutant are almost certainly due to differences in genetic background. For example, gonadal development of Snell dwarf mice is more advanced in animals on a heterogeneous genetic background than in those derived from an inbred strain (Bartke and Lloyd 1970). Although both Snell and Ames dwarf mice reach approximately 30–35% of the body weight of their normal siblings, the absolute body weight of dwarf animals can differ substantially, in proportion to differences in body weight between normal animals from the corresponding lines (Bartke and Lloyd 1970; A. Bartke, unpubl. observ.).

Snell dwarf mice were used extensively as a model of congenital GH deficiency and hypothyroidism (Grüneberg 1952). Evidence that they are also PRL-deficient became available only 35 years after their initial description (Bartke 1965b; Bartke 1966a,b). They continue to be a popular animal model for the study of the GH-IGF-I axis, immune function, etc.; their phenotype has been characterized in considerable detail. The characteristics of Snell dwarf mice which could have some relevance to their delayed aging include small body size, low metabolic rate and body temperature (Boettiger et al. 1940), reduced number of mitotic divisions during development leading to reduced cell number in the various organs (Viola-Magni 1965; Winick and Grant 1968), hypogonadism (Grüneberg 1952; Bartke and Lloyd 1970; Bartke 1979b), hypothyroidism (Grüneberg 1952; Lewinski et al. 1984), and extremely low levels of IGF-I (Holder et al. 1980; van Buul Offers et al. 1986). Similarly to Ames dwarf mice, immune function in the Snell dwarfs appears to be compromised (Duquesnoy et al. 1970; Fabris et al. 1972; Pelletier et al. 1976).

4
Development and Longevity of Dwarf Mice

As was mentioned earlier in this chapter, the Ames dwarf mice, in comparison to their normal siblings, are much smaller, have infantile body proportions, are less active, and retarded in their sexual development. In our colony with heterogeneous genetic background derived from crosses of several lines, male dwarf mice undergo pubertal development with obvious testicular growth and scrotal descent and, similarly to normal males, exhibit aggressive behavior toward nonfamiliar male dwarfs. They have complete spermatogenesis and attain testicular size which is smaller than normal in terms of absolute weight but larger than normal when expressed per gram body weight (Bartke 1979a; Chandrashekar and Bartke 1993). However, most males are infertile and only an occasional animal will sire a few litters if housed with very young (i.e., small) normal females. Sexual maturation of females is retarded, as judged by the age of establishment of vaginal opening and many females never reach puberty. Sexual maturation can be induced by treatment with thyroxine (T_4), GH, or a combination of T_4 and GH (Bartke 1979b; Soares et al. 1984). Transplantation of pituitary glands from normal females under the renal capsule of the dwarfs can also induce sexual maturation (Bartke 1979b). Most of the Ames dwarf females that achieved sexual maturation spontaneously or as a result of hormonal treatment will mate but never become pregnant. Typically, the matings will occur at regular 4–6-day intervals corresponding to the length of the estrous cycle in normal female mice. This indicates that the animals have ovulatory cycles of approximately normal length, but they fail to respond to stimuli associated with mating by activation of the corpora lutea. In normal female mice, mating induces twice daily surges of PRL (Barkley et al. 1978) which lead to persistence of corpora lutea and maintain pregnancy (Bartke 1973). Sterile matings in normal animals are similarly followed by induction of PRL surges and thus lead to luteal activation, the so-called pseudopregnancy. Pseudopregnancy delays the next ovulation and mating for 10–11 days (Bartke et al. 1972). In Ames dwarf females, pregnancy can be maintained by treatment with injections of PRL or with ectopic transplants of normal pituitaries which are known to secrete PRL (Bartke 1979a). Dwarf females with a normal pituitary transplanted under the renal capsule can produce a series of litters and raise their pups to weaning (Bartke 1965c, 1979a). Collectively, these data indicate that luteal failure and infertility of Ames dwarf females are due to PRL deficiency.

Our study of longevity of Ames dwarf mice was prompted by chance observ. that these animals appear to remain in excellent general condition longer than their normal siblings. They tend to become obese (which is also common in the normal animals from this line) and some experience hair loss

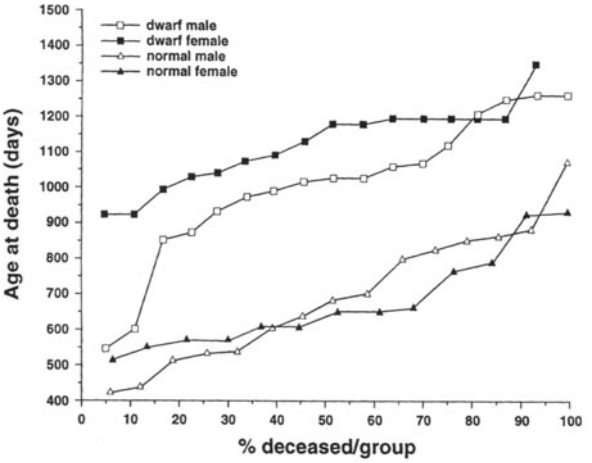


Fig. 4. Longevity in male and female normal and Ames dwarf mice. Each *point* on the graph represents an individual animal surviving to the specific age indicated versus the percentage of animals deceased per group. *Open symbols* males; *closed symbols* females; *triangles* normals; *squares* dwarfs. Dwarfs live longer than normal mice regardless of gender ($P < 0.0001$). (Brown-Borg et al. 1996)

but otherwise appear very healthy. When a group of 34 dwarf and 28 normal mice were allowed to live their natural life span, the dwarfs outlived the normal mice by more than a year (Fig. 4). The difference in the average life span was 353 days for males and 488 days for females (Brown-Borg et al. 1996). This corresponds to extension of the life span by 49% in males and by the remarkable 64% in females. Two of the female dwarfs lived 4 years and 2 months. Most of the dwarfs remained in very good body condition well past the average life span of their normal siblings and exhibited age-related decline (weight loss, sluggishness, occasional cataracts) only toward the end of their life. A study of locomotor activity revealed that 3-year-old Ames dwarf mice did not exhibit any evidence of age-related reduction and, in fact, were more active than young dwarfs in terms of both ambulatory and stereotyped behaviors (Meliska and Bartke, unpubl. observ.). Normal animals examined in the same study were more active than dwarfs. However, at the age of approximately 20 months, their ambulatory and stereotyped movements tended to decline rather than increase, in comparison to the values measured in young normal mice (Meliska and Bartke, unpubl. observ.).

We are currently studying histopathological changes in aging Ames dwarf and control (normal) mice. Results available to date indicate that incidence of lung tumors and liver tumors as well as incidence of all types of tumors in Ames dwarf mice dying of natural causes are comparable to the incidence of these lesions in normal animals dying from natural causes. Since dwarf mice live longer, there is reason to suspect that they develop tumors at a more advanced chronological age, and additional studies to test the validity of this interpretation are indicated. Data on tumor incidence in aging Ames dwarfs imply that age-related pathological changes in these animals are not prevented or altered although they are likely to be delayed. This suggests yet another similarity between the effects of hereditary dwarfism and effects of

caloric restriction and argues in favor of considering the Ames dwarf mice as a novel model system to study delayed but otherwise normal aging.

The observed incidence of tumors in aging Ames dwarf mice was not anticipated from the reports of "tumor resistance" in Snell dwarf mice. Snell dwarf mice were reported to develop fewer spontaneous or chemically induced tumors than the normal animals (Bielschowsky and Bielschowsky 1961; Chen et al. 1972) and to exhibit reduced growth of transplanted tumors (Rennels et al. 1965). The natural history of tumors in dwarf mice is of particular interest in the context of the recent evidence that IGF-I is an important cancer risk factor and that it can promote growth of different types of tumors (Werner and LeRoith 1996).

5
Longevity of Snell Dwarf Mice and the Issues of Husbandry

An early report (Fabris et al. 1972) claimed that Snell dwarf mice are extremely short-living with an average life span of 4–5 months. This was ascribed to reduced immune competence of these animals and the consequent susceptibility to infectious disease (Fabris et al. 1972). These findings must have been unique to the particular population of mice used for these studies or, more likely, to the husbandry conditions, because soon after their publication, three different laboratories responded by reporting that Snell dwarf mice in their colonies live at least as long as normal animals (Shire 1973; Schneider 1976; Bartke 1977). However, these investigators did not report how long their animals live, and this problem was apparently not systematically investigated until the mid 1990s. Since that time, it was determined in two laboratories that Snell dwarf mice live significantly longer than normal animals from the same strain (R. Flurkey, D. Harrison, and R. Miller, pers. comm.). We believe that the discrepancies between data on longevity of Snell dwarf mice in different laboratories are almost certainly due to differences in husbandry and to the general improvement in hygiene and animal health standards in the animal colonies during the past 50 years. Although Snell dwarf mice are immunodeficient (Duquesnoy et al. 1970; Fabris et al. 1972), this may be of little consequence for survival of the animals in modern facilities which are free of most, if not all, of the common murine pathogens.

Since both Snell and Ames dwarf mice have reduced body temperature (Schonholz and Osborn 1949; Hunter et al. 1999) and limited ability for thermoregulation at low ambient temperatures (Grüneberg 1952), their survival can probably be influenced by temperature and housing conditions, e.g., single vs. group housing. Interactions among the group-housed animals can also be important. For example, normal adult males persist in trying to mate

with dwarfs of both sexes and apparently can also be aggressive toward these animals. In contrast, housing dwarfs with normal females or with several other dwarfs appears to promote their well being, presumably by helping them to keep warm by huddling with their cage mates. Survival of very young dwarfs presents additional problems since at the usual age of weaning they are tiny and appear to have difficulties in eating food pellets placed in the food hopper or cage lid. Our standard practice is to wean the dwarfs at the age of 6–8 weeks, i.e., 1 month after weaning their normal siblings or at the time of weaning normal animals from the next litter, whichever comes first. After weaning, the dwarfs are housed with other dwarfs or with normal females, in groups of four to five animals in cages equipped with filter (micro-isolator) tops. After weaning, regrouping of the animals is kept to absolute minimum. To avoid fighting, male dwarf mice that were housed since weaning in different cages are never combined. The temperature in the room is maintained at 22 ± 2°C, the light cycle is 12:12, and food and water are available ad libitum.

Although no systematic studies of the effects of housing conditions on the survival of Snell or Ames dwarf mice appeared to have been conducted and there is little published information on their husbandry, it can be assumed that these mutants will not thrive when single-housed or exposed to low ambient temperatures, particularly in wire-bottom cages. Weaning the dwarfs at the age of 3 weeks is likely to interfere with their health and survival unless special arrangements are made to improve food accessibility and palatability and to prevent excessive heat loss. After the age of 1 1/2–2 months, dwarf mice thrive under standard conditions when housed with normal females or with other dwarfs.

6
Possible Mechanisms of Delayed Aging in Dwarf Mice

The remarkable longevity advantage of Ames dwarf mice raises an obvious question, namely: which characteristics of these animals are responsible for prolongation of their life span? Information available to date and comparison with data obtained in other models of delayed aging suggest a number of possibilities which will be briefly discussed below.

6.1
Reduced Blood Glucose and Increased Sensitivity to Insulin

As was mentioned earlier in this chapter, plasma glucose levels in Ames dwarf mice are lower than in the corresponding normal controls, while plasma insulin is normal in female dwarfs and reduced in male dwarfs (Borg et al.

1995). Thus, these animals appear to have enhanced sensitivity to the actions of insulin. Concomitant reduction in glucose and insulin levels is consistently observed in genetically normal animals subjected to caloric restriction (Duffy et al. 1990; Masoro 1995; Weindruch and Sohal 1997). Mice with targeted disruption knockout of the GH receptor gene have extremely low plasma insulin levels and reduced levels of blood glucose (N. Danilovich, J. Mattison, and A. Bartke, unpubl. observ.), and preliminary data suggest that they may live longer than normal animals. Nonenzymatic glycation of proteins and other components of living cells is believed to represent an important mechanism of aging and links elevated blood glucose levels with reduced life expectancy (Reiser 1998). In the human, diabetes and insulin resistance are important risk factors for a number of diseases and for reduced life span. Transgenic mice overexpressing GH are insulin-resistant (Balbis et al. 1996; Dominici et al. 1998), exhibit various symptoms of accelerated aging (Pendergast et al. 1993; Steger et al. 1993; Miller et al. 1995), and have a drastically reduced life span (Wolf et al. 1993; Cecim et al. 1994; Rollo et al. 1996). The possibility of functional and evolutionarily conserved role of energy metabolism and insulin signaling in the control of aging is suggested by the demonstration that a "longevity gene" in a worm, *Caenorhabditis elegans*, is related to the insulin receptor gene in higher organisms (Kimura et al. 1997).

6.2 Hypothyroidism

Both Ames and Snell dwarf mice are severely hypothyroid due to primary TSH deficiency (Grüneberg 1952; Bartke 1964; Li et al. 1990; Sornson et al. 1996). In normal animals, caloric restriction reduces thyroid hormone levels (Meites 1993; Weindruch and Sohal 1997). In rats, experimentally induced hypothyroidism is associated with increased longevity (Denckla 1974; Ooka et al. 1983), while hyperthyroidism is associated with shortened life span (Ooka and Shinkai 1986). Beneficial effects of hypothyroidism on the life span could be related to the role of thyroid hormones in the control of body temperature, metabolic rate, and thus generation of oxygen radicals.

6.3 Reduced Body Temperature and Metabolic Rate

Core body temperature in Ames dwarf mice is reduced by approximately 1.5°C (Hunter et al. 1999). This suggests that metabolic rate is also reduced. Reduced metabolic rate in Snell dwarf mice was described in several studies dating back to the 1930s (Benedict and Lee 1936; review in Grüneberg 1952). In normal animals, caloric restriction reduces body temperature (Lane et al. 1996). Metabolic rate is initially reduced in CR animals but thereafter sta-

bilizes at a level appropriate to the reduction in lean body mass (Duffy et al. 1989, 1990). It is widely accepted that generation of free oxygen radicals (often referred to as reactive oxygen species, ROS) in the course of mitochondrial energy metabolism represents an important, if not the key mechanism of aging. Therefore, it can be assumed that reductions in metabolic rate and in body temperature can prolong life by reducing exposure of the cells to ROS.

6.4 Improved Capacity to Remove Reactive Oxygen Species

Oxidative damage, believed to represent a major mechanism of aging, is related not only to the rate of generation of ROS, but also to the rate of their removal (Cutler 1985). Removal of ROS depends on the actions of several enzymes including catalase and glutathione peroxidase, and we have recently shown that the activity of catalase is significantly greater in the liver and the kidney of Ames dwarf mice than in the corresponding organs of normal animals (Bartke et al. 1998). In the liver, the activity of glutathione peroxidase was also greater in dwarf than in normal mice (H. M. Brown-Borg et al. unpubl. data).

These findings suggest that the tissues of dwarf mice may be less vulnerable to oxidative damage by virtue of increased ability to detoxify free oxygen radicals. Consistent with this interpretation, the levels of inorganic peroxides in the liver were found to be lower in dwarf than in normal mice (Bartke et al. 1998). The possible link between the activity of enzymes involved in scavenging ROS and longevity is supported by evidence for reduced activity of these enzymes and for the increase in oxidative processes in GH transgenic mice which have greatly reduced life span (Rollo et al. 1996; H. M. Brown-Borg, pers. comm.).

6.5 Hypogonadism

In dwarf mice, puberty is delayed and gonadal function is suppressed (reviews in Bartke 1979b, 1982). Production of testosterone by the testes is significantly lower than in normal mice (Bartke et al. 1977; Chandrashekar and Bartke 1993). In Snell dwarf mice from an inbred strain, Dwarf/J, the reproductive system is infantile and spermatogenesis is suppressed (Bartke and Lloyd 1970). Most of the Ames dwarf and Snell dwarf females fail to undergo sexual maturation and thus can be assumed to experience estrogen deficiency throughout their life span. Although effects of gonadal function (more specifically, gonadal steroids) on aging and longevity are not well understood, there is evidence for increased life span of castrated, as compared to intact animals (Drori and Folman 1976). Sex steroids have anabolic

effects and contribute to pubertal growth and thus could influence longevity by increasing metabolic rate and body size. Relationships of growth and body size to longevity are discussed in another section of this chapter (6.7).

In support of the possible role of hypogonadism in delayed aging of dwarf mice, the longevity advantage (defined as extension of the life span in relation to the normal animals) is greater in females than in male dwarfs (Brown-Borg et al. 1996). Female dwarfs are profoundly hypogonadal while gonadal function in males is much less affected.

6.6 Deficiency of GH and IGF-I

In dwarf mice, GH is not produced and the levels of IGF-I in peripheral circulation are profoundly suppressed and remain near or below the levels of detectability by radio immunoassay (Pell and Bates 1992; Chandrashekar and Bartke 1993). There is considerable evidence for a reciprocal relationship between the function of the GH-IGF-I axis and longevity. In normal rats, caloric restriction leads to significant suppression of IGF-I levels and this effect has been proposed as a likely mechanism for delayed onset of age-related diseases (especially tumors) and for increased longevity (Breese et al. 1991). In these animals, plasma GH levels also decline (Meites 1993), although in older CR rats, GH levels were found to be higher than in ad libitum-fed controls because CR prevents age related decline in GH pulsatility (Sonntag et al. 1995). Mechanisms responsible for suppression of IGF-I levels in older CR animals in which pulsatile GH secretion is preserved remain to be clarified. Preliminary data suggest that GH receptor knockout mice which are GH-resistant and consequently IGF-I-deficient live longer than normal mice (Kopchick and Coschigano, pers. comm.). Studies in different breeds of domestic dogs suggested a reciprocal relationship between plasma IGF-I levels and life span (Patronek et al. 1997). In transgenic mice overexpressing GH genes, plasma GH and IGF-I levels are greatly increased (Naar et al. 1991) and life span is reduced (Wolf et al. 1993; Cecim et al. 1994; Bartke et al. 1998). In the human, the syndrome of acromegaly involves elevation of plasma GH and IGF-I levels due to hypersecretion of GH by anterior pituitary tumors and is associated with reduced life expectancy (Bengtsson et al. 1988; Orme et al. 1998).

The possible mechanisms linking activity of the GH-IGF-I axis and longevity include anabolic and thermogenic actions of GH (Strobl and Thomas 1994) and the dominant role of this axis in determining the rate of growth and the adult body size. There is considerable evidence that, within the species, body size is negatively correlated with longevity (details and references in the next section of this chapter).

Mitogenic actions of IGF-I may be specifically related to the reciprocal relationship between IGF-I levels and longevity. In Snell dwarf mice, mitotic

divisions appear to cease early in life and the number of cells in the various organs is reduced (review in Bartke 1979b), implying reduced number of cell divisions. The number of cell divisions in cultured cells determines their remaining "replicative potential" and life span (Hayflick 1998), and cells removed from an older individual's bone reduced potential to undergo divisions in culture (Hayflick 1998). Interestingly, reduced replicative potential was detected in GH transgenic mice in which GH levels were elevated and life expectancy reduced (Pendergast et al. 1993).

However, it may be premature to consider delayed aging of Ames and Snell dwarf mice as the expected outcome of deficiency of GH and IGF-I. Although the evidence for reciprocal relationship between the activity of the GH-IGF-I axis and longevity summarized above appears to be compelling, important issues concerning this relationship remain to be clarified. In the human, both congenital and adult onset GH deficiency are associated with serious functional deficits and increased risk for premature death from cardiovascular disease (Sacca et al. 1994; DeBoer et al. 1995; Bates et al. 1996). Although no data on longevity of humans chronically treated with GH are available, GH-deficient patients clearly benefit from GH replacement therapy (Sacca et al. 1994; Pfeifer et al. 1999). Moreover, numerous symptoms of aging resemble symptoms of GH deficiency, presumably represent consequences of age-related decline in GH levels, and can be corrected by GH replacement therapy (Rudman et al. 1990; Corpas et al. 1993). The possible reasons for discrepancy between these findings and the prolonged survival of GH-deficient animals will be discussed below.

6.7
GH-IGF-I Axis, Body Size, and Aging

Although the present understanding of the mechanisms of normal, delayed, or accelerated aging is limited, causal relationships can be tentatively identified from associations of various phenotypic characteristics with alterations of life span in different models. For example, demonstration of reduced core body temperature, blood glucose, and IGF-I levels in both hereditary dwarf mice and CR animals strengthens the argument that these characteristics may be important in increasing longevity. Analysis of findings in these and other models of delayed aging points to the body size as a key correlate and perhaps a key determinant of longevity. Thus, life span in mice can be extended by hereditary dwarfism (Brown-Borg et al. 1996; Flurkey and Harrison, pers. comm.; Miller, pers. comm.), selection for reduced body size (Roberts 1961), or CR which also reduces growth and body size (Duffy et al. 1990). In rats and mice, CR initiated at the time of weaning has greater impact on body size and causes greater extension of life span than is observed in animals subjected to CR after reaching sexual maturation, or at middle age (Weindruch and Sohal 1997). Remarkably, longevity of rats can be extended by hypophysectomy

providing that the animals receive thyroid and glucocorticoid replacement (Everitt et al. 1980). Hypophysectomy produces numerous functional deficits which include arrest of growth, and thus leads to reduced body size in comparison to intact controls. Negative correlation of body size and life span within a species applies not only to mice but also to invertebrates (Austadt 1997), domestic dogs (Patronek et al. 1997), and probably also to humans (Samaras and Storms 1992; Micozzi 1993). At the present time, it is unclear whether small body size per se imparts a longevity advantage or provides a marker for some physiological characteristic or characteristics that affect life span. Small body size could favor longevity by increasing efficiency of the cardiovascular system within the confines of a genetically determined, species-specific body plan (Promislow 1993), or by some other mechanisms specifically related to body dimensions, mass/surface ratio, or weight. Relationship of body size to longevity could also reflect correlation of body size with the amount of metabolic energy processed per unit of time, with the amount of ROS-related damage incurred in the process of metabolism and growth, with regulation of glucose metabolism and thus glycation-related damage of proteins, or with other physiological characteristics related to aging. The impressive longevity advantage of GH-deficient mutants and of CR animals in which the GH-IGF-I axis appears to be suppressed can perhaps be reconciled with the detrimental effects of GH deficiency in humans by assuming that the benefits of reduced body size in genetically dwarf or CR mice outweigh the "risks" of GH deficiency on cardiovascular system or immune function. Naturally, these comparisons are complicated by differences between the species and by differences between the environmental conditions of laboratory rodents and human beings. Limited opportunity or need for physical activity, constant access to high energy food, constant ambient temperature, and effective protection from pathogens are characteristic of "standard" conditions in colonies of laboratory rodents and clearly differ from the conditions and challenges of human existence.

It is also unclear whether evidence that GH can prevent or reverse some of the physiological changes associated with aging in the human (Rudman et al. 1990; Pfeifer et al. 1999) or in experimental animals (Sonntag et al. 1985, 1997) can be taken as indication that GH normally acts to prevent rather than to accelerate aging. There are no data on the effects of GH treatment on longevity in these studies; in fact, the initial report of highly beneficial outcome of GH replacement in the elderly (Rudman et al. 1990) was followed by reports of various detrimental side effects of treatment with GH or IGF-I (Borst et al. 1994; Thompson et al. 1995; Papadakis et al. 1996).

The issue of the dose-response relationship of the actions of GH (and possibly also IGF-I) introduces yet another complication to interpretation of the data concerning GH levels and life span. GH actions can be biphasic with regard to the dose (Ultsch and deVos 1993) and, therefore, it is quite likely that the relative GH deficiency occurring naturally in old age may be detri-

mental and correctable by treatment with low (Toogood and Shalet 1999) or moderate doses of GH, while GH excess is clearly detrimental to survival (Bengtsson et al. 1988; Orme et al. 1998).

7
General Conclusions and Future Directions

The remarkable extension of life span in dwarf mice clearly establishes these animals as a unique and potentially valuable model for the study of delayed aging in a mammal. Availability of a new model system for this type of investigation is very important because, up to now, CR represented the only well-established experimental model for mechanistic studies of aging in mammals. The effects of CR on the life span and on numerous physiological parameters are well documented and CR animals are generally considered as the system of choice and a "gold standard" in experimental gerontology. However, there are inherent limitations in any attempt to identify causal relationships from the study in a single experimental system, and there are obvious limits to useful generalizations from data generated in any particular model. For example, it can be argued that dramatic extension of life span in CR animals is due in large measure to preventing overeating and obesity, which are characteristic of ad libitum-fed laboratory rodents housed in standard (i.e., relatively small) cages with no access to running wheels, opportunities to exercise, or need to expend energy to find food.

In comparison to the vast literature concerning effects of CR in rats, mice, and, more recently, monkeys (Duffy et al. 1990; Lane et al. 1996; Weindruch and Sohal 1997), the amount of information relevant to prolonged survival of Ames and Snell dwarf mice is limited. However, information available to date and comparisons with findings in CR animals already allow some novel and useful, even if tentative, conclusions. For example, we can assume that while the activity of the GH-IGF-I axis, body size, body temperature, and plasma glucose levels may be critical for prolonged survival, leanness, suppression of insulin levels, and elevation of corticosterone levels are probably much less important or not absolutely required.

The fact that the effects of both Prop-1 Pit-1 genes, as well as primary defects in development and endocrine function of Ames and Snell dwarf mice are well understood, should allow linking specific developmental events and specific aspects of neuroendocrine function to the control of aging. Thus, both of these genes are primary candidates for study as longevity assurance genes (LAGs) in mammals.

The major impact of hereditary dwarfism on aging emphasizes the potential importance of other animal models for this field of investigation. For example,

preliminary data suggesting increased longevity of GH receptor knockout mice (Coschigano and Kopchick, pers. comm.) raise a very exciting possibility that a single genetic defect in GH signaling may be sufficient to affect life span. If confirmed, these findings would focus attention on the role of the GH-IGF-I axis and body size in aging. However, caution must be exercised in interpreting these preliminary findings because, as of now, there is no evidence for prolonged survival of little (lit/lit) mice with isolated GH deficiency or in transgenic mice with GH resistance due to overexpression of an antagonistic GH analogue and, indeed, there is evidence to the contrary (D. Harrison, pers. comm.; Bartke and Kopchick, unpubl. data). However, there is little doubt that mutants, knockouts, and transgenic animals will soon assume a major role in research directed at identifying factors that control mammalian aging.

The relationship of findings in Ames and Snell dwarf mice and other genetic models to the human is of obvious importance. Direct application of findings in Ames or Snell dwarf mice, and, parenthetically, also in the CR animals, to the human is exceedingly unlikely. Hypopituitarism, hypothyroidism, stunted growth, and starvation certainly do not represent acceptable clinical interventions or public health measures. Mutations homologous to Prop-1 and Pit-1 have been identified in the human (Tatsumi et al. 1992; Pfäffle et al. 1996; Flück et al. 1998), and the affected children obviously require GH and thyroid hormone replacement. No one would seriously question the benefits of these treatments in these or other hypopituitary patients. However, it is most intriguing that patients with multiple pituitary hormone deficiency due to mutation at the PROP-1 locus were recently reported to reach very old ages, exceeding the average life expectancy in the general population (Krzisnik et al. 1999).

Comparative studies in Ames and Snell dwarfs, other mutants, GH-R-KO mice, and CR animals, as well as in various KO and transgenic animals yet to be produced, will undoubtedly lead to identification of the physiological characteristics linked to the rate of aging and the mechanisms involved. Information on the importance of some of these factors, e.g., insulin sensitivity, IGF-I levels, or tall stature may help identify individuals at risk for premature aging or, more importantly, for premature onset of age-related diseases and suggest appropriate testing and perhaps also interventions.

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