J. Anat. (2000) **197**, pp. 521–528, with 3 figures Printed in the United Kingdom

An integrated theory of ageing in the nematode *Caenorhabditis elegans*

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(*Accepted 10 January 2000*)

ABSTRACT

Numerous theories of ageing have been proposed, and many have been tested experimentally, particularly using nematode models such as *Caenorhabditis elegans*. By combining those theories of ageing that remain plausible with recent findings from studies of *C. elegans* life span mutants, an integrated theory of ageing has been devised. This is formed from 3 interconnected elements: the evolutionary theory of ageing, the oxidative damage theory of ageing, and a nonadaptive programmed ageing theory. This tripartite theory of ageing gives rise to a number of predictions that may be tested experimentally.

Key words: Theories of ageing; oxidative damage; programmed ageing; evolutionary theory of ageing; *Caenorhabditis elegans*; nematode.

INTRODUCTION

The 20th century saw a cornucopia of theories of ageing put forward—over 300 by 1 estimate (Medvedev, 1990). In recent decades many of these have been put to the test experimentally, and falsified. A smaller number of theories have stood up to scrutiny, and appear to have the capacity to explain ageing, at least in some of its aspects, in particular the evolutionary and oxidative damage (or free radical) theories (Medawar, 1952; Harman, 1956; Williams, 1957; Rose, 1991; Sohal & Weindruch, 1996). Yet each of these alone is inadequate to provide a global description of the causes of ageing. For example, the evolutionary theory, while successfully explaining how and why ageing evolves, is uninformative about the specific mechanisms underlying ageing, although attempts have been made to draw inferences (Kirkwood & Rose, 1991; Partridge & Barton, 1993; Kirkwood, 1995). Likewise, while oxidative damage accumulation is a determinant of the rate of ageing, it remains unclear (1) whether it is a primary cause of ageing, or a secondary event, (2) why it occurs at such different rates in different species, and (3) whether such differences reflect programmed or stochastic mechanisms.

In this review a novel, integrated theory of ageing is proposed. The evidence for this theory draws heavily on recent studies of ageing using short-lived nematode models, and its primary purpose is to provide a possible explanatory framework with which to understand ageing in the nematode *Caenorhabditis elegans*, currently the subject of intense investigation (reviewed in Kenyon, 1997; Gems, 2000). The proposed integrated theory assumes that the inferred evolutionary theory and the evidence-based oxidative damage theories are essentially correct. To these a third element is added, a theory of nonadaptive programmed ageing. This proposes that underlying the traversal of adulthood in animals is the expression of a succession of age-specific developmental genetic identities. Such a theory of programmed ageing can provide an explanatory link between the evolutionary theory and the oxidative damage theory. The capacity of such a tripartite model to explain recent findings is explored, and some testable predictions which follow from it are set out.

THEORIES OF AGEING FALSIFIED IN NEMATODES

Recent investigations suggest that the following proposed mechanisms of ageing are incorrect, as least

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as far a nematodes are concerned. Ageing in *C. elegans* is highly unlikely to be due to telomere shortening, since all somatic cells in adults of this species are postmitotic. Nor does it appear to be due to DNA damage: in a comparison of the efficiency of DNA repair mechanisms in *C. elegans* recombinant inbred lines with mean life spans varying from 10–30 d, no difference was detected (Hartman et al. 1988). However, DNA repair synthesis and removal of pyrimidine dimers did decrease with age in the nematode *Turbatrix aceti* (Targovnik et al. 1984). Nematode ageing does not appear to be caused by increased frequency of errors in protein synthesis, leading to an 'error catastrophe' (Orgel, 1963). This theory predicts the appearance of large numbers of proteins with altered primary structure during ageing, which are not seen (Rothstein, 1980; Johnson & McCaffrey, 1985; Vanfleteren & De Vreese, 1994). In contrast to many other species (e.g. the fruitfly *Drosophila melanogaster*) there is no evidence that life span in *C. elegans* is affected by variation in fertility: alteration of fertility does not affect it (Friedman & Johnson, 1988; Kenyon et al. 1993; Gems & Riddle, 1996), nor are genetically specified increases in life span correlated with reductions in fertility (Brooks & Johnson, 1991; Larsen et al. 1995; Gems et al. 1998). Finally, a number of *ced* (*ce*ll *d*eath) genes have been identified which control programmed cell death (apoptosis) in *C. elegans*. However, there is no evidence for common mechanisms controlling apoptosis and ageing (Hengartner, 1997), and mutations in *ced* genes do not affect life span (Kenyon, 1997).

THE OXIDATIVE DAMAGE THEORY OF AGEING

In many species ageing is accompanied by an accumulation of damage to proteins, lipids and DNA caused by free radicals, such as superoxide ($O_2^{\cdot -}$) and the hydroxyl radical (OH^-), produced partly as a byproduct of mitochondrial respiration (Harman, 1956; Sohal & Weindruch, 1996). In wild-type *C. elegans*, hyperoxia reduces life span and increases the rate of ageing, expressed as the Gompertz mortality rate, whereas hypoxia (1% oxygen) increases mean life span by 15%, and reduces the Gompertz mortality rate (Honda et al. 1993). The role of oxidative damage in ageing has also been studied extensively using mutations affecting the genes *mev-1* and *age-1* (Ishii et al. 1990; Honda et al. 1993; Adachi et al. 1998). The *mev-1*(*kn1*) mutation results in increased sensitivity to the oxygen free radical generator *me*thyl *v*iologen (Paraquat) (Ishii et al. 1990). The ageing rate of *mev-1* mutants is hypersensitive to oxygen concentration, and their longevity is reduced by about a third under atmospheric oxygen. That increased oxygen and mutation of *mev-1* shorten life span by accelerating the ageing process rather than some other deleterious effect is supported by the finding that both accelerate the rate of accumulation of the age pigment lipofuscin (Hosokawa et al. 1994), and of protein carbonyl (Adachi et al. 1998), which are markers of ageing in many animal species. Long-lived *age-1* mutants show increased resistance to oxidative damage, e.g. from hyperoxia (Adachi et al. 1998), and other pro-oxidants (Larsen, 1993; Vanfleteren, 1993), and their rate of increase in protein carbonyl content with age is reduced (Adachi et al. 1998).

The work of Ishii and coworkers, in particular, provides strong evidence that damage by oxygen free radicals is, if not the cause of ageing itself, then closely linked to it. What has not yet been demonstrated in nematodes is that overexpression of genes determining resistance to oxidative damage can extend life span, although this has been demonstrated in fruitflies (Orr & Sohal, 1994; Parkes et al. 1998; Sun & Tower, 1999). The oxidative damage theory also predicts that treating nematodes with antioxidant compounds should extend their life span. This is not generally the case, or where life extension occurs, it does not appear to be a consequence of their antioxidant properties (Harrington & Harley, 1988; reviewed in Gems, 2000).

THE EVOLUTIONARY THEORY OF AGEING

The key inference of the evolutionary theory of ageing is that the force of natural selection decreases with age after the onset of reproduction, and has little influence on the effects of genes at ages exceeding the life expectancy of a species in its natural habitat, where death is almost invariably due to extrinsic factors, e.g. predation, starvation or disease (Medawar, 1952; Williams, 1957; Rose, 1991). Consequently, mutations causing deleterious effects at advanced ages are not removed from the population, especially where there are positive pleiotropic effects on fitness early in life, and the resulting accumulation of mutations gives rise to ageing. Thus, ageing is akin to a late acting genetic disease, and the timing of its onset is related to life expectancy in the wild (Edney & Gill, 1968). By this view, ageing is genetically determined but non-adaptive, and the condition of persistence at an age beyond natural life expectancy is comparable to that

of an atrophied vestigial organ, such as the human appendix, or the remnants of eyes in blind cave fish.

NONADAPTIVE PROGRAMMED AGEING

Central to the present discussion is the relation between development and the evolution of ageing. This may be considered by reference to ageing in nematodes. The phylum Nematoda includes species with a broad range of longevities (reviewed in Gems, 2000), from free-living nematodes such as *Mesodiplogaster biformis*, with a mean life span of around 8 d (Sohlenius, 1969) to parasitic nematodes such as *Onchocerca volvulus*, which causes river blindness, and (unfortunately) can live for over 14 y (Plaisier et al. 1991). This enormous difference in life span potential is likely to reflect differences in the levels of extrinsic mortality experienced by these 2 species in the past, such that selection for the effects of genes at much later ages occurs in the parasite. Given that *O. volvulus* adults remain healthy and fecund for over 14 y, it can be inferred that a mutation causing deleterious effects on *O. volvulus* after, say, 12 y will reduce competitive fitness.

How may we envisage the mode of action of a mutation that has no effect for more than 11 y, and then in y 12 reduces fitness, e.g. by reducing fertility or viability? One possibility is that this is a long-term stochastic effect, of the sort that occurs in complex systems such as cars. For example, due to differences in construction and design, 1980 Toyotas were longer-lasting than 1980 Chevrolets (Vaupel, 1997). An alternative possibility is that just as an *O. volvulus* first stage larva is distinct in developmental genetic terms from a third stage larva, an 11-y-old adult is distinct from a 12-y-old adult. By this view, in developmental genetic terms, an 11-y-old worm is distinct from a 12-y-old worm, i.e. each has a distinct, determinative pattern of gene expression. Thus, the marking of biological age, or biological time, during adulthood and ageing is akin to that acting during development: a concerted sequence of changes in gene expression.

In the past, the idea of ageing as an extension of development, or 'programmed ageing', has tended to be lumped with the view that ageing is adaptive (see e.g. Kirkwood, 1995). An early, influential adaptive theory of ageing saw it as beneficial to the species, e.g. as preventing competition for limited resources (Weismann, 1891, cited in Rose, 1991). Such group selectionist arguments are not supported by contemporary evolution theory (for a discussion see e.g. Kirkwood, 1995), although they are still sometimes invoked in discussions of age-related changes in gene expression (see e.g. Roy, 1997). By contrast, in the model suggested here, ageing is viewed as programmed, but nonadaptive.

Developmental genetic theories of ageing are in themselves nothing new. For example, according to the dysdifferentiation hypothesis, ageing results from the nonprogrammed derepression of genes (Ono & Cutler, 1978; Cutler, 1982). One branch of ageing research has focused on the complex changes in gene expression that occur throughout adulthood and ageing in mammals. Expression levels of a number of genes rises and/or falls during ageing in mammals, controlled by changes in transcription factor activities (reviewed in Papaconstantinou et al. 1996; Roy, 1997). However, it remains unclear whether such changes drive the ageing process, or are mere downstream effects, or what are the evolutionary significance of such changes.

A TRIPARTITE MODEL OF AGEING

As proposed, there are 3 facets of ageing: a declining force of natural selection with increasing age after onset of reproduction, the marking of biological time by developmental genetic changes, and the accumulation of oxidative damage. These may be integrated into a single model of ageing as follows. For a species to evolve a longer life span, it must evolve fit, healthy developmental genetic identities for more advanced ages. According to this model, at advanced ages, the developmental programme assuring fitness starts to expire. This results in a gradual loss of homeostasis, and increasing fragility. Given this loss of cellular homeostasis, one of the first aspects of cellular function to break down is mitochondrial function, leading to increased production of free radicals, and increased free radical damage. Thus, free radical damage is widely observed during ageing across species, since it is a consequence of the loss of homeostasis within cells resulting from expiration of the genetic programme specifying successive fit, healthy, age-specific adult developmental identities. The evolutionary theory of ageing potentially explains why this expiration occurs, and its timing. This tripartite theory of ageing is summarised schematically in Figure 1. According to this theory, to understand the genetic determination of longevity, we must understand the mechanisms determining the length of the developmental programme of healthy adulthood, and the rate at which it is expressed. A key prediction of the theory is that experimentally induced dyshomeostasis will result in increased free radical production and molecular damage.

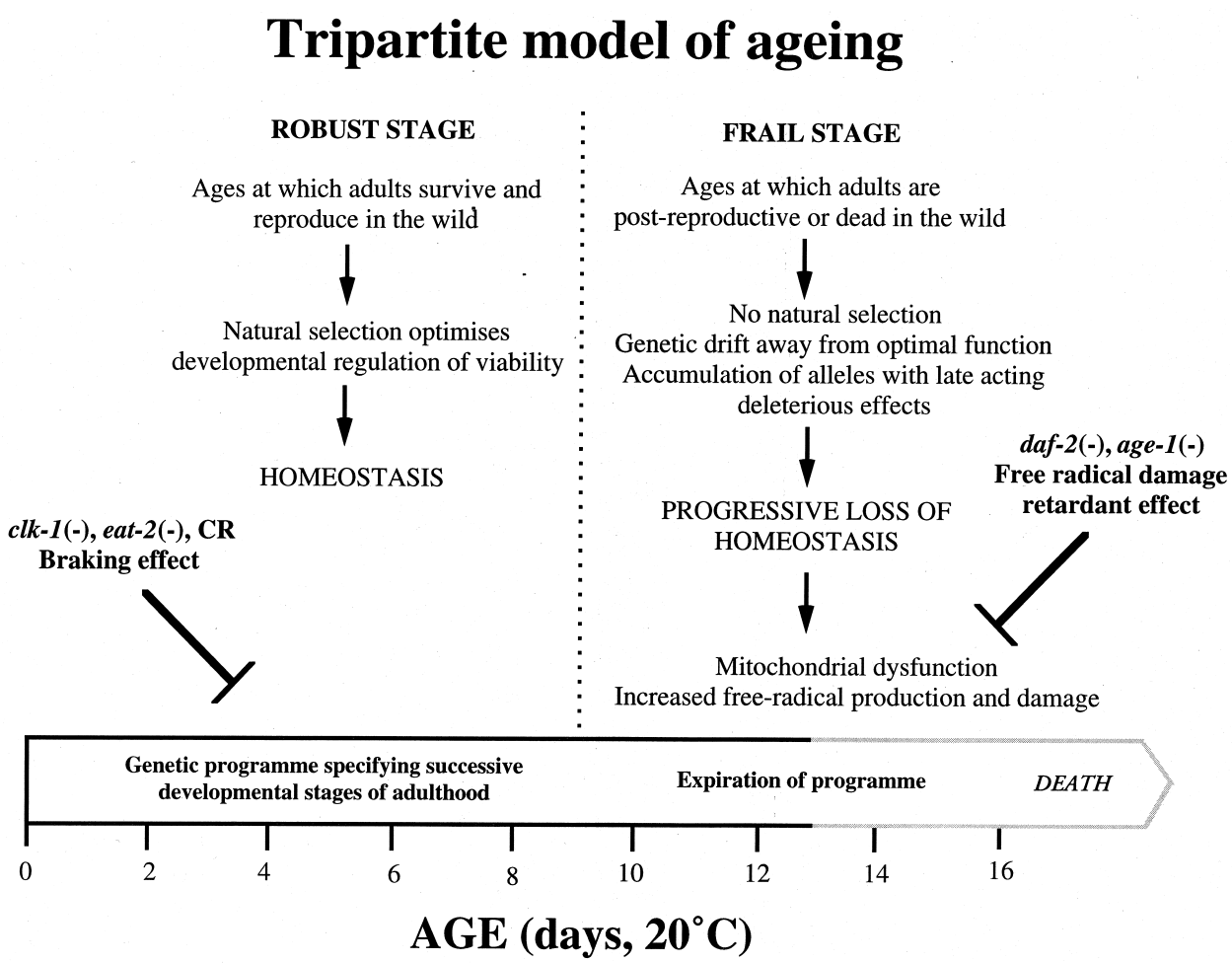

Fig. 1. Tripartite model of ageing (see text for explanation). T bars represent inhibitory effects of mutations or environmental manipulations on different elements of ageing. Vertical dotted line depicts the approximate time of the transition from robust to frail stage. One difference between the free radical hypothesis of ageing and the tripartite model is that in the former, dyshomeostasis is viewed as the consequence of free-radical damage. In the latter, mitochondrial dysfunction is viewed as initially a consequence of a more proximal, developmentally determined dyshomeostasis. CR, caloric restriction.

EVIDENCE FOR DEVELOPMENTAL CHANGES DURING ADULTHOOD

Several approaches have been taken to look for changes in gene expression during adulthood in nematodes. In one, animals were fed with radiolabelled *E. coli*, and proteins from young and old animals separated by 2-dimensional gel electrophoresis and compared (Johnson & McCaffrey, 1985). Over 700 newly synthesised proteins were resolved, but no new major proteins were observed during the adult phase, nor did any abundant proteins cease to be made. However, in later studies of silver-stained protein extracts, variation in relative levels of certain proteins with age was seen (Meheus et al. 1987; Vanfleteren & De Vreese, 1994). Another approach taken was to probe replicate *C. elegans* cDNA libraries with labelled cDNA prepared from young and old nematodes (Fabian & Johnson, 1995). From a screen of 3000 cDNAs, 9 transcripts were identified which decreased in abundance with age, 2 that increased slightly with age, and 1 that peaked in abundance in mid-adulthood. Among the 9 transcripts that decreased in abundance were 3 that encoded vitellogenins (yolk proteins) (Fabian & Johnson, 1995).

Overall, this work suggests that gene regulatory changes do occur during ageing, but are very subtle by comparison with those occurring during embryogenesis and larval development. However, this does not rule out the possible occurrence of nonadaptive programmed ageing. The failure of natural selection to maintain fitness at advanced ages could result in a loss of the fine tuning of gene regulatory networks, resulting in a loss of homeostasis. Such changes might be difficult to detect at the level of protein or RNA abundance. The approaches described are unlikely to be sufficiently sensitive to detect subtle changes in transcriptional levels, for example in genes expressed

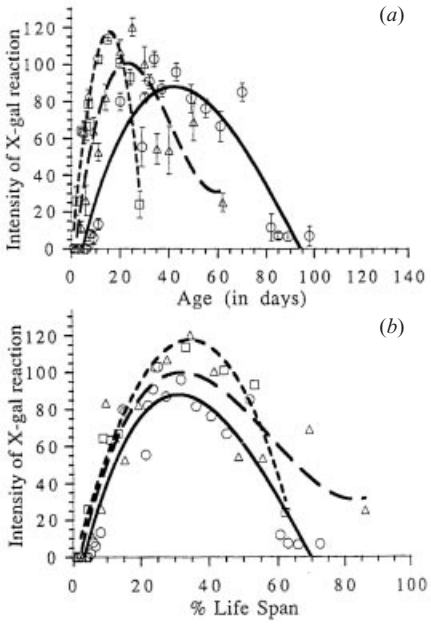

Fig. 2. Effect of altering life span by means of ambient temperature on temporal pattern of expression of β-galactosidase in the antennae of transgenic *D. melanogaster* (line 1085). Squares, 29 °C; triangles, 25 °C; circles, 18 °C. (*a*) Changes plotted as a function of time (chronological age). (*b*) Changes plotted as a function of percentage of maximum life span (biological age). (Reproduced with permission from Helfand et al. 1995).

at low levels or in small numbers of cells, and cannot detect spatial changes in gene expression. For example, differential screening usually only allows detection of relatively abundant transcripts (more than 0.05–0.1% of total mRNA).

A more sensitive and powerful method for studying gene expression during ageing is the visualisation of reporter gene expression during ageing in transgenic animals. In *D. melanogaster* this approach has revealed the occurrence of complex changes in intensity and spatial distribution of gene expression during the traversal of adulthood (Helfand et al. 1995; Rogina & Helfand, 1996; Rogina et al. 1997). Particularly striking is the complexity of the spatio-temporal changes in expression seen in some cases, which are more consistent with the sort of complex gene regulatory changes that drive development than responses to age-associated stochastic changes. Furthermore, these changes were not accompanied by increased variability with age in expression, arguing against a dysdifferentiative interpretation, where dysfunction occurs in gene regulatory mechanisms (Rogina et al. 1998). Interestingly, when life span was altered by changing ambient temperature, the changes in gene expression changed also, such that the overall pattern of change scaled with life span (Fig. 2) (Helfand et al. 1995).

C. elegans LIFE SPAN MUTANTS AND THE TRIPARTITE MODEL

The genetic determination of life span in *C. elegans* has been studied by means of mutations that alter life span (reviewed in Kenyon, 1997; Gems, 2000). The proposed model suggests specific interpretations of key findings from this work. For example, the tripartite model suggests that the reduction in life span resulting from mutations in *mev-1* may not represent an acceleration of the primary ageing process (the expiration of programme), but rather of the secondary process of mitochondrial dysfunction which normally ensues as the adult developmental programme ensuring full homeostasis starts to expire.

Studies of *C. elegans* life span mutants have predominantly focused on those with increased life span. Many of these life span mutants fall into 1 of 2 types. The first are those which affect dauer larva development. The dauer larva is a developmentally arrested, stress-resistant alternative third stage larva which forms under conditions of low food and high population density (Riddle & Albert, 1997). While adults survive 2–3 wk, dauer larvae can survive up to 70 d (Klass & Hirsh, 1976). Mutations in the genes *age-1* and *daf-2* can increase adult life span up to threefold, possibly by inducing heterochronic expression of dauer longevity in the adult. Both dauer larvae and *age-1* and *daf-2* adults show elevated resistance to oxidative damage. Another group of life span genes are the clock (*clk*) genes. Unlike *daf-2* and *age-1*, *clk* mutants exhibit an overall slowdown in biological processes, including development, movement, feeding and egg-laying (Hekimi et al. 1998). Mutant interaction studies suggest that the mechanism of life extension by these 2 groups are different (Lakowski & Hekimi, 1996). They also suggest that in *eat* mutants, which eat less and live longer (probably due to caloric restriction), the underlying mechanism of life extension is the same as that of the *clk* mutants (Lakowski & Hekimi, 1998).

One interpretation of these findings is that in *clk* and *eat* mutants the primary, developmental ageing process is affected, and in *daf-2* and *age-1* mutants retardation of secondary ageing (free radical damage) is occurring (Fig. 1). Thus, in *clk* mutants the time taken until the expiration of the developmental programme specifying healthy adulthood is increased, and the subsequent loss of homeostasis is delayed. In the case of *age-1*, evidence suggests that increased longevity results from a retardation of free radical damage (Larsen, 1993; Vanfleteren, 1993; Adachi et al. 1998).

This interpretation suggests that if age-related changes in gene expression were studied in *C. elegans*, the effect of *clk* mutants would be similar to that of temperature as in Figure 2, changing the pattern of change to scale to life span. By contrast, *daf-2* and *age-1* would not be expected to alter the timing of age-related changes in gene expression. In fact, the effect of mutation of *age-1* on changes in the expression levels of 6 genes has been examined by Fabian & Johnson (1995), and no clear slowing effect by *age-1*(-) was seen, supporting this interpretation.

It has been proposed that increased longevity evolves via the generation of fit, healthy developmental genetic identities for advanced ages. The properties of *clk* mutants suggests an alternative possibility: that the pace of expression of the overall programme may also be slowed down. While the properties of *clk* mutants may provide insight into the evolution of life span, the proposed model suggests that major differences in longevity in animal species, such as that between *C. elegans* and *O. volvulus*, are unlikely to be due to retardation of secondary ageing, as proposed to occur in *age-1* mutants. This is because increased free radical damage is merely a secondary consequence of loss of selective pressure to maintain homeostasis at advanced ages.

One prediction of this interpretation is that *clk* and *eat* mutants will not show elevated resistance to oxidative damage. However, this may not be so, since *clk-1* mutants show increased resistance to UV-radiation, a free radical generator (Murakami & Johnson, 1996), and mutation of *ctl-1*, which encodes a cytosolic catalase, suppresses *clk-1* life extension (Taub et al. 1999). The relation between oxidative stress resistance and increased longevity in *clk* and *eat* mutants needs to be investigated further.

ROBUST AND FRAIL STAGES OF ADULTHOOD

There is further evidence supporting the view of oxidative damage as a secondary element of ageing that is retarded in *age-1* mutants. Consider the effect of hyperoxia on life span in wild-type, *age-1* and *mev-1* strains (Fig. 3) (Adachi et al. 1998). At oxygen concentrations of 80% the life spans of the 3 strains are not statistically different from one another. One interpretation of this result in the light of the tripartite model is that the span of *C. elegans* adulthood comprises 2 stages: a robust stage, where a developmental genetic programme ensures full homeostasis, lasting until around d 8–10 (20°), followed by a frail stage during which animals are increasingly susceptible to the breakdown of mitochondrial function, and the resulting downward spiral of free radical damage and further mitochondrial dysfunction (Fig. 1). The existence of robust and frail stages of adulthood is also suggested by the effects of mating with males on hermaphrodite life span. Increasing the ratio of males to hermaphrodites up to around 1.5 decreases hermaphrodite life span, but at higher ratios no further decrease of hermaphrodite median life span below 8–9 d is seen (Gems & Riddle, 1996). By contrast, the tripartite model predicts that the robust stage in *clk* or *eat-2* mutants should be extended.

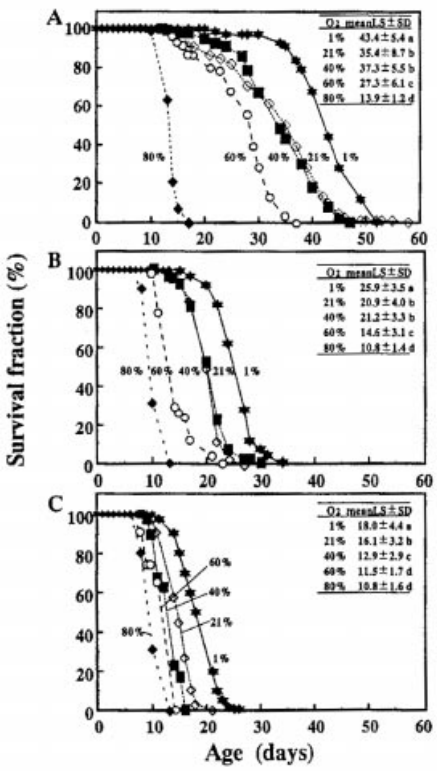

Fig. 3. Survival curves of *age-1*, wild-type N2, and *mev-1* strains under various concentrations of oxygen continuously present later than 4 days after hatching. (*A*) *age-1*, (*B*) wild-type N2, (*C*) *mev-1*. Oxygen concentrations: stars, 1%; open diamonds, 21%; filled squares, 40%; open circles, 60%; and filled diamonds, 80%. Mean life span values with different letters at each strain are significantly different ($P < 0.01$) according to Duncan's multiple range test. (Reproduced with permission from Adachi et al. 1998.)

ORGANISMS THAT LACK BIOLOGICAL TIME?

The proposed nonadaptive programmed ageing model may explain recent and surprising findings about ageing in the Cnidarian *Hydra vulgaris*. Based on evolutionary considerations, and its age at first reproduction, the predicted life span of hydra is 1.2–2.6 mo (Martínez, 1998). In fact, hydra appears to be nonageing and potentially immortal. Hydras maintained for 4 y showed negligible age-specific mortality (let alone any increase). The explanation for

this may lie with the fact that all of hydra's 20 or so cells types are continually replaced, including its neurons, and it was estimated that each hydra body was entirely replaced at least 60 times during the 4 y trial (Martínez, 1998). Hydra's failure to evolve senescence may be accounted for if (1) a succession of age-specific, developmental genetic identities are required for the evolution of ageing, and (2) such marking of biological age requires the continuous existence of at least a subset of cells, i.e. is not regulated at a level of organisation above that of the cell. By this view, hydra has no mechanism for marking biological time. It is, as it were, off the clock. A prediction of this hypothesis is that the evolution of senescence will not occur in species where all the cells in the body are turned over.

The determinants of longevity in the long-lived dauer larva of *C. elegans* may also be considered in the context of the tripartite model. Dauer larvae can retain the capacity to resume development for more than 70 d, after which they probably die of starvation, since they are nonfeeding and survive on stored nutrients (Klass & Hirsh, 1976). Possible contributory factors to dauer longevity are increased stress-resistance and reduced metabolism; by contrast, in *age-1* adults, while stress-resistance is increased, metabolism is not reduced (Vanfleteren & De Vreese, 1995; Riddle & Albert, 1997). A remaining question is whether the span of dauer larva survival involves a succession of age-specific developmental identities, or whether in the state of developmental arrest, as in hydra, there is no marking of biological time. Possibly if dauer larvae were able to feed they would survive indefinitely. In this context it is interesting to consider the developmentally arrested L2 stage of the parasitic Ascarid nematode *Toxocara canis*. Like the dauer larva, this form has a sealed buccal cavity, and is detergent-resistant (D. Gems, unpublished). However, *T. canis* L2s are able to absorb nutrients via the cuticle, and have been shown to survive in paratenic hosts for up to 9 ys (Beaver, 1966). This supports the view that *T. canis* L2s are, like hydra, running off the biological clock.

CONCLUSIONS

A new integrated model of ageing has been proposed, composed of 3 subtheories of ageing: the evolutionary theory, the oxidative damage theory, and a non-adaptive programmed ageing theory. It might be argued that a surfeit of ageing theories already exist. In response it is emphasised that the ideas put forward in this discussion are primarily intended as exploratory hypotheses, and the basis for future experimental design.

ACKNOWLEDGEMENTS

The author thanks Thomas Johnson, Diana McCulloch and Linda Partridge for reading the manuscript. The work was supported by the Royal Society.

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