How relevant are animal models to human ageing?

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Human beings do not crawl around on all fours or possess a long tail. Neither are we worm-like or insect-like; and yet in gerontological laboratories around the world scientists use animals and insects and nematodes in the belief that their ageing mechanisms will resemble those of man. Would the ideal experiment be to keep litters of humans, four or six to a small room with twelve hours' daylight, a diet of cereal pellets, and no contact with the real world? Despite claims that scientists have begun to crack the ageing code, few would insist that findings in any of the existing models apply to man. A more persuasive argument is that study of a wide range of species will identify mechanisms that apply throughout the animal kingdom.

SELECTION OF A MODEL

Model selection, the most important decision for a research team, demands much thought. It should be based upon three criteria: (1) specificity, in this instance, 'does the animal model exhibit a trait of special interest, that is analogous to a particular trait in man?'; (2) generality, 'do the findings from one strain apply to another strain?'; and (3) feasibility, including the cost effectiveness of using a certain strain and the ease of doing so[1].

Rodents

At the time when the US National Institute of Aging (NIA) came into being, the early 1970s, several laboratories were already using rodent models and so the NIA adopted these. Over the next few years these were honed into what are used today. An early question concerned the appropriate maintenance regimens, standardized to allow comparisons within and between laboratories. Thus, the experimental environment was defined in terms of living space, light and dark exposure and diet. The only major change since the early 1980s has been calorie restriction (CR), which is now run in parallel with the *ad libitum* diet regimens.

Animal models have the advantage that tissue can readily be obtained for analysis. In our laboratory we have studied patterns of gene expression during ageing of the brain. Rat brains snap-frozen in liquid nitrogen immediately after killing provide excellent RNA preparations for analysis[2]; human brain taken at necropsy yields degraded RNA whose analysis is much less satisfactory.

With standardization of rodent strains, we now have the possibility of altering the genetic make-up—transgenic manipulation. Initial models for creating transgenic mice involved the introduction of human DNA of interest (the transgene) into the pronuclei of fertilized mouse oocytes or into embryonic stem cells, the result being random integration of various numbers of copies of the injected DNA into a single site on the mouse chromosome; in other words, the dosage for the gene in question is altered[3]. By varying the regulatory region attached to the gene it is also possible to obtain regulated expression of a transgene[4]. Where this methodology works (homologous recombination cannot be obtained with all genes), it is possible to obtain mice carrying null mutations in a gene of interest[5]. Of course, the way in which the model adapts to the human transgene may differ from the way in which a human would adapt to the same mutation, but mice and human beings with a shared gene can show considerable phenotypic overlap in their features of ageing[6,7].

Non-human primates

The use of non-human primates as models of ageing would have clear benefits, to take research beyond rodents and closer to human beings. Ideally the animal should have a short lifespan (10–15 years). The maintenance of such models is very expensive although, in the long term, they might be cost-effective in proving more reliable and worthwhile.

A small number of teams around the world are studying non-human-primate ageing[8,9]. Most use *Macaca mulatta* (rhesus macaques), which being small are simple to house and have a shorter lifespan than other primates (approximately 35 years). One of the main areas of interest is to see whether or not caloric restriction affects longevity in this model. If (as in the rodent models) it does, then we might reasonably conclude that calorie restriction will increase human longevity. Unfortunately, since normal life expectancy is already 35 years, we shall not know the answer for at least 50 years. Even then, the experiments will have to be repeated with other primates.

There are, however, small primates which have the advantages of small size, short lifespan and early sexual

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maturity (usually within 12 months). Three models that are currently being investigated are the *Microcebus murinus* (mouse lemur)[10], *Galagao senegalensis* (lesser bush baby)[11], and *Saimiri scuireus* (squirrel monkey)[12]. The ageing mouse lemur model shares several phenotypic features with humans—whitening of the fur and blindness due to cataracts, and brain lesions similar to those of Alzheimer's disease[10].

Other animal models

One way of increasing the probability that phenomena discovered in rodents are applicable to man would be by detecting the same effects in other phyla. Figure 1 illustrates the approximate times since man diverged from various other species. One model system that might suit this approach is that of the *Marsupialia*. Small marsupials such as the Brazilian grey short-tailed opossum and marsupial mice reach sexual maturity within 6 months, produce large litters of 5–12 and reach menopause within 2 years. Longer lived marsupial models are also being studied but, as with certain non-human primates, data are at present scarce because of their longevity.

Bats are an overlooked but potentially useful ageing model. Certain species of bat can live for over 30 years[13,14], 10–15-fold longer than rodents of the same body mass. Standardization of longevity to body mass makes the bats the longest-lived mammalian order, yet they also have the greatest lifetime energy expenditure per gram of body mass[15]. This would therefore make the bat model ideal for the study of oxidative damage and repair mechanisms, or of antioxidant mechanisms. Unlike rodents, bats produce very few offspring, one pup a year. Like bats, birds are longer lived than mammals of equivalent body mass[16] and would make another suitable model for the study of oxidative stress and repair mechanisms.

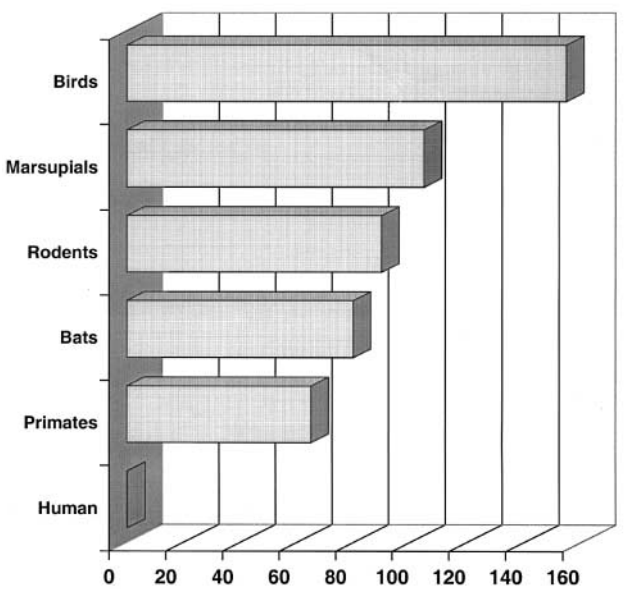

Figure 1 **Divergence (in millions of years) of each animal model, relative to modern-day man**

CONCLUSIONS

Clearly, gerontological research cannot base itself on one or two models. Work on rodents needs to be coupled with studies of animals with longer lifespans, in which the ageing mechanisms may differ. In human beings we already know that genetic factors can be important in determining longevity. The ages of monozygotic twins are more similar than those of dizygotic twins at death, and children from long-lived parents have greater average lifespans than those whose parents die earlier[17]. This begs the question of how results obtained from animal model experiments, which are intended to identify longevity-mediating processes or genes, might be applied to humans. Sprott and Austad[1] wrote, 'A healthy, vigorous animal model research program clearly is gerontology's best insurance against producing a body of research with an overly narrow focus and with questionable relevance to an understanding of human health and function.'

One way in which to protect against this outcome is by the implementation of comparative studies. Several investigators have compared different species in terms of the ways they adapt to extrinsic forces (which are believed to play a central role in the process of ageing) as a factor of chronological age. There are indications that longer-lived species accumulate oxidative damage at a slower rate, with better DNA repair mechanisms and less free radical production[18–22]. Recently Kapahi and co-workers[23] have described a series of carefully controlled experiments to show whether cell survival following different acute stresses varied according to the longevity of the donor species. They compared tissues from a diverse range of phylogenetic species and average lifespans including rodents, lagomorphs, ungulates and primates. After exposure to one of five different stresses for predetermined times and doses, cell cultures were assessed for their ability to synthesize DNA and their mitochondrial function. The results showed that, at lower doses of all of the stressors, cell viability and DNA synthesis were more compromised in the short-lived species than in the long-lived species. These data further support the theory that long-lived species possess superior defences with which to cope with a wide variety of extrinsic cellular stresses. One of the merits of this study is the way in which care was taken to minimize confounding variables.

The best way forward for gerontology is to move away from single-species studies, so as to develop a more detailed picture of underlying mechanisms of ageing and how they integrate with one another. Comparative studies are likely to provide the best insight into ageing. Even if laboratories

choose to work with only one or two models, their data could be available for comparative studies if they subscribed to a code of standard practice and recorded a specific set of variables. Such collaboration would allow comparative research on a scale beyond the capabilities of any single research group. But the answers to such questions as 'Do all species share a central set of ageing mechanisms?' must await data from a much larger range of creatures.

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