Response: The analysis by Vaupel et al. allows the extension of our data in ways that we (1) did not originally envision and provides a much better fit of the raw data. The slope of the Gompertz curve varies at 8 days of age, but mortality still continues to increase exponentially until the end of life. However, large errors in the estimation of mortality rates late in life, which result from the small number of deaths at this period, prevent us from determining the accuracy of this statement with a high degree of precision.

I find the statements from Curtisger et al. to be convincing, and these precautions seem to adequately rule out the possibility that there was significant contamination by progeny in the aging cohorts displayed in their earlier report (2). Nevertheless, any significant amount of progeny contamination can produce a huge artifact when only a small proportion of the starting population is being examined as is done in examining the oldest old. Great care must be taken to avoid it.

The analyses conducted by Wang et al. purport to show (i) that a "nonparametric locally weighted least squares method" provides a better fit (3) and detects a decrease in mortality rates at "between 8 and 10 days" and (ii) that each of the 79 genotypes analyzed in our report (1) themselves appear to be composed of two slopes. We completely concur on the first aspect of their analysis (4). However, we are uncertain that the deviations from the exponential curve are biologically significant. It remains to be demonstrated that the details of these deviations from the Gompertz will be replicated in further analyses. It is our opinion that experimental replication, not extensive mathematical analyses, should be the critical basis for testing theories in this area.

We disagree on the second aspect of the comment by Wang et al. The analyses have a high inherent inaccuracy because the analysis is based on total cohorts of 30 animals or less. Many of the individual estimates of age-specific rates of mortality are based on none or only a single death in a given time period. However, too few details are presented to allow us to determine the accuracy of the methodology. We do agree that there was censoring of the data (this was stated in the original report (1)) and that this censoring has resulted in apparently higher mortality rates early in life. In analyzing this data, Wang et al. suggest that the four quartiles behave synchronously; because the division into quartiles was based on mean life span (1), it is not unexpected that there is a fairly uniform distribution of mean life span in each quartile. However, the mean life spans of these RI's are distributed essentially normally (5, 6) and all RI's are derived from crosses between the Bristol and Bergerac wild-type strains of C. elegans (5, 6, 7).

The principal comment in the analyses put forth by Wang et al. is twofold. First, the small population size results in considerable inaccuracy in the estimates of mortality rates and the rates estimated could be off by several orders of magnitude. Second, Wang et al. found absolute mortality rates early in life to range from 10^{-8} to 10^{-6}, which can be compared with our estimates of 10^{-3} or less (1, 5, 8) in populations of about 200 worms. Error estimates in the latter two studies (5, 8) were obtained directly by analysis of the four component populations, each of 50 worms, and direct estimation of error. The standard error of the mortality rate at 3 days of age in these estimates (8) was 15 to 40% of the mean. Also, the log mortality rate at 3 days of age in two different estimates for the wild type (N2) were \(-2.09 (5)\) and \(-2.70 (8)\), which suggests considerable variation between experiments. In contrast, Wang et al. suggest that less than one worm out of 100 million is dying per day early in life; surely an estimate such as this cannot be made on a cohort of population size 30.

Before the methods proposed by Wang et al. are accepted for the analysis of mortality in small cohorts, they should show that their procedure allows the accurate reconstruction of the original mortality rates with the use of small, simulated data sets. One could simulate a population of organisms dying with exponential kinetics to see if their analytic method would generate an exponential model after sampling populations of size 30. Indeed, one of the principal arguments put forth in an earlier study "is that it may not be possible to determine the mortality pattern of a species from data on 100 or even fewer individuals . . ." (3, p. 460).

Thomas E. Johnson
Institute for Behavioral Genetics,
University of Colorado,
Boulder, CO 80309–0447, USA

REFERENCES

Identification of Calcium Channels That Control Neurosecretion

The report by David B. Wheeler et al. (1) addresses the important question of which Ca^{2+} channel types control synaptic transmission in the mammalian central nervous system. Wheeler et al. studied glutamatergic transmission between Schaffer collateral fibers and CA1 pyramidal neurons in the rat hippocampus and used synthetic toxins that target high voltage-activated Ca^{2+} channels in an effort to identify which types trigger glutamate release at this synapse. Wheeler et al. argue for the primary involvement of a novel class of Ca^{2+} channel, which they have labeled "Q." In pharmacological experiments such as these, three criteria should be met before conclusions can be drawn with confidence: (i) the concentration of antagonists at the synaptic site must be known, (ii) estimates of potency must be made at or near equilibrium for antagonist binding, and (iii) the antagonists employed should be specific. These fundamental criteria have not been consistently met in the report by Wheeler et al.

Wheeler et al., measuring the field excitatory postsynaptic potential (fEPSP), demonstrate a slow onset for action of omega-agatoxin IVA [IVA, a P channel antagonist (2)]; 30 nM toxin produced no effect in 20 min and 200 nM produced inhibition at a rate of about 1% per minute. They use this to argue for the relative inefficacy of IVA on Schaffer collateral Ca^{2+} channels (and for the lack of P channels in Schaffer collateral nerve terminals). Our recent results suggest that the rate of onset for toxin action in a tissue slice is largely a function of the rate of toxin delivery to the synaptic region rather than the on-rate for toxin binding. The application times that Wheeler et al. used in their experiments with 30 nM IVA, therefore, are insufficient to achieve a steady-state concentration in the synaptic region. With the use of whole cell recording from superficial CA1 neurons in the slice, we found that 100 to 200 nM IVA
Response
Thomas E Johnson

Science 266 (5186), 828.
DOI: 10.1126/science.7973643