Linus Pauling and the Riddle of Aging

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Early Observations

Linus Pauling's first contribution to the aging literature came, unwittingly, in 1931. In that year he predicted, on theoretical grounds, the existence of the superoxide radical.[1] His first deliberate foray into the field of aging was his 1958 paper on "The Relation Between Longevity And Obesity in Human Beings", read before the National Academy of Sciences.[2] Pauling appears then to have been more interested in curve-fitting than in obesity *per se*; the thrust of the paper is that a quadratic function, with its minimum at normal weight, fit the observed relation between weight and life-shortening better than did the commonly-used linear term. Deviation to either side of the optimum (normal) weight is associated with decreased longevity, as Pauling's curve predicts. Pauling's approach here is consistent with his later insistence on optimum concentrations of biomolecules in orthomolecular medicine.

Pauling's next effort, "Observations on Aging and Death", came two years later in Caltech's *Engineering and Science* magazine.[3] In it, he evaluated the effects of various health risks on the well-known Gompertz curve. Gompertz, an English insurance actuary, discovered in the 19th century that the age-specific death rates of adults are an exponential function of the age. From mid-life onward, the age-specific mortality (percentage of the cohort that dies) increases exponentially with a doubling time of 8.5 ± 0.5 years. It is possible, of course, to do Gompertz plots for mortality from individual causes. Pauling used this type of analysis to estimate the benefits from eliminating various conditions that shorten life. According to his calculations, if all cancer were eliminated, the average American would live 2.8 years longer. Eliminating all smoking (arguably the principal cause of life-shortening in Americans) would increase mean life expectancy by 4 years. Medical radiology accounts for 100 days of decreased longevity (if one does not factor-in the benefits of X-rays), and air travel shortens the average life by 1 day. Pauling estimated the mean decrease in life expectancy from all-out nuclear war at 35 years per American.

In the 1960s, Pauling served as a faculty advisor to the Veterans Administration Satellite Laboratory Program for aging research. He advised his old friend and former student, Arthur Cherkin, whose research laboratory was located at the Sepulveda, California VA Hospital. Cherkin worked mostly on the molecular biology of memory, and although the two men indubitably communicated with one another, no joint publication resulted from this interaction. In some fashion, that may have been related to the strong objection of the hospital's director, a retired military man, to Pauling's pacifist views.

Vitamin C

By 1974, Pauling was well established in his advocacy of vitamin C. He contributed a chapter, "The Process of Aging," to Leon Pomeroy's *New Dynamics of Preventive Medicine*.[4] In this, he briefly reviewed a few theories on aging (somatic mutation, cross-linking, free radicals, and Arthur Robinson's theory related to the deamidation of certain protein constituents). After discussing the value of vitamin C, he boldly asserted his conclusion that "an increased intake of vitamin C might well decrease the morbidity and mortality from all diseases by 50%." On the Gompertz curve, that would correspond to an increase of about 8 years in life expectancy. He ventured further to estimate that the combination of proper vitamin intake, decreasing sucrose, and abolition of ciga-

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rette smoking would probably increase the average American's length of life and period of well-being by 15 to 20 years.

Pauling's last paper on aging, although not his final thought on the subject, was presented in Mexico at a symposium on metabolic dysfunctions.[5] Critical of the research establishment's priorities in the field of cardiovascular diseases, he suggested that an insufficient intake of vitamin C might be directly responsible for heart disease in many cases, and responsible as a cofactor in other cases. He also discussed his work in collaboration with Ewan Cameron on cancer, and predicted that vitamin C would have great value in controlling the problems (e.g., cancer and heart disease) associated with advancing age.

As Arthur Cherkin once explained to me, "Genius is the ability to hit very large nails, not quite squarely on the head." So it is with Pauling's predictions about vitamin C. Two papers published last year address this point. In the first, Kromhour et al. reported that, at the population level, saturated fat, vitamin C status, and smoking are important determinants of all-cause mortality.[6] A 5% reduction in saturated fat calories, a mere 20 mg/day increase in vitamin C, and a 10% decrease in the prevalence of smokers were calculated to decrease the 25-year all-cause population mortality rate by 12.4%, at an average all-cause rate of 45%. In the other paper, Loria et al. evaluated the association between serum ascorbate concentrations and mortality, utilizing data from the second NHANES study.[7] Among men, those in the lowest quartile with respect to ascorbate had a 57% greater risk of dying from any cause and a 62% higher risk of dying from cancer, compared to those in the highest quartile. Curiously, no such association was seen in women. There was no increased risk of cardiovascular disease with low vitamin C status. Whether or not the subjects smoked did not affect the conclusions.

Eight years previously, Enstrom et al. had reported their analysis of data from the first NHANES study, in relation to vitamin C status and mortality. Vitamin C intake was inversely correlated with the standardized mortality ratio (SMR) in both sexes, strongly so in males and weakly in females. (SMR is defined as 1.00 for all U.S. Caucasians). Among those with the highest intake, SMR was 0.78 and 0.86 for all cancers, 0.58 and 0.75 for all cardiovascular diseases, and 0.65 and 0.90 for all causes, for males and females respectively.[49]

Pauling's last word on aging came in the form of a popular book, *How to Live Longer and Feel Better,* published when its author was 85 years old. Biographer Barbara Marinacci describes the book as partially a recapitulation of Pauling's prior thoughts on the subject, "full of information both fascinating and readable."[8] Pauling's "Regimen for Better Health" included a specific vitamin and mineral program, activity and exercise, stress avoidance, choosing enjoyable work, and avoidance of harmful substances such as tobacco and sucrose. Pauling–the very picture of health on the book's cover–seems to have successfully resolved the issues that concerned him at age 59, when he had written, "At my age, when the ills that the flesh is heir to begin to make themselves increasingly evident, one begins to appreciate one's youthful period of good health and vigor. As I grow older I must expect to suffer more and more from physical frailty and disease."[3]

Despite these intimations of mortality from recollections of early Pauling, the great man seems to have approached the scientific problem of aging with neither the zeal nor the rigor that he applied to matters like molecular structure. It seems odd that the greatest theoretical chemist of our time–perhaps of all times–should have exhibited so little curiosity about the underlying mechanisms of aging, and should instead have contented himself with advocating what, in the final analysis, are public

health measures to reduce morbidity and postpone mortality. Perhaps, like most in his generation, he simply took aging as a "given", and perhaps he may have considered it trivial in the light of other problems (including the nuclear threat) that define the human condition in our time.

Certain it is that not all of his comments seem to have been well thought-out. For instance, the somatic-mutation theory cannot be true, at least in its pure form, because of several lines of evidence related to ionizing radiation.[9] In general, under conditions that cause an increased rate of mutation, there should be a corresponding increase in the rate of aging; however, this is not true experimentally. Moreover, Pauling uncritically cites the notion of his associate, Robinson, that evolution made it beneficial to the species for older organisms to die after they have fulfilled the function of procreation.[4] Of course, it is impossible that natural selection of genetically-determined traits will occur in the case that genes cannot be transmitted, as in post-reproductive individuals. In the final analysis, Pauling's contribution to the aging conundrum should be understood as the foundations of molecular biology and molecular medicine, but not a direct attack on the problem.

Theories of Aging

Some of the most cogent scientific thoughts on aging were also the earliest, penned 10 years before Pauling's birth by August Weismann.[10] It was he who proposed the now-accepted conceptual division of the metazoan organism into immortal germ cells and expendable somatic cells. In his time the idea seems to have been controversial, as it should well have been: like Copernicus's heliocentric cosmology, and Darwin's evolution from the apes, it was an affront to human self-importance. To imagine that all of the human qualities we treasure in our offspring exist merely to assure the propagation of some insentient germ cells!

Weismann is sometimes identified as a proponent of programmed aging. However, a careful reading of *Essays Upon Heredity* will reveal his view that aging results from the loss of functions (i.e., program elements) in the somatic cell line (under the principle that every faculty must disappear as soon as it ceases to be necessary); those same elements are maintained by natural selection in the immortal germ line (or, as we would say nowadays, they are "highly evolutionarily conserved."

Weismann wrote a great deal about both the reproduction and the immortality of protozoa. Among these creatures, the micronuclei represent the germ line. Micronuclei give rise to reproductive nuclei through reduction divisions, and those come together when two individuals fuse in a fertilization process called conjugation. Without conjugation (or a self-fertilizing equivalent called autogamy), a protozoan will grow senescent and die of old age. Weismann, however, was uncomfortable with the notion of fertilization as a rejuvenating process, believing rejuvenation implied unscientifically postulating a "vital force", and pointing to parthenogenesis as an exception to the rule.

Others have studied senescence in protozoa since Weismann's day. It appears that defective micronuclei can arise from the action of an aged macronucleus (the separate, metabolic nucleus)[11] and/or cytoplasmic influences; aged but undamaged micronuclei, on the other hand, function normally in young cytoplasm.[12] Mutations in micronuclei of Paramecium are repaired less well with advancing age of a clone (number of mitotic divisions since the last cross- or self-fertilization).[13]

The immortality of the germ line was reconsidered by Kirkwood and Holliday in 1979 and by Medvedev in 1981. The former authors considered that high energy expenditure is necessary for accurate synthesis of macromolecules; such expenditure is

of course necessary for germ cells, but not for the "expendable soma", which may have evolved that way as an energy-conserving strategy.[14] Holliday has further written that a key function of meiosis is the removal of epigenetic defects via recombination.[15] Medvedev noted that differentiated somatic cells are unable to carry out certain rejuvenating events that occur during meiosis: elimination or reversal of DNA damage through recombination and haploidization, regeneration of transcriptional and translational systems during gametogenesis, and selection of stable genotypes.[16]

In humans and other mammals, the first meiotic division of the ova has already occurred prior to sexual maturity. The second, held in abeyance by the c-mos protooncogene,[17] finally occurs when the ovum is fertilized. Since an infant born of a middle-aged woman is every bit as young as that of a teen-aged mother, showing no imprint of decades of difference in maternal age, one may assume that a rejuvenative process occurs early in development, perhaps (in view of the preceding statements) in relation to the second meiotic division. Whatever the "reset" mechanism is, it is powerful and far-reaching; cloning experiments (in which nuclei are transferred to ova that are then activated) underscore this point. In 6 cloned calves derived by nuclear transfer from senescent fibroblasts, their cells expressed 3.5 to 5 times more of an age-dependent gene, EPC-1, and had longer telomeres, than age-matched controls; thus the cloned animals were phenotypically younger than "natural" calves of the same age.[18] Egg cytoplasm causes dedifferentiation of somatic-cell nuclei largely through action of the ISWI enzyme, which erases the TATA binding protein from association with the nuclear matrix.[19]

Is regeneration of telomere length the key rejuvenative event of meiosis? Much interest has been generated by the telomere theory of senescence since Olovnikov first proposed a version of it in 1971.[20] In brief, telomeres are specialized DNA-protein complexes at the ends of linear chromosomes, essential for maintenance of the chromosomes. These repetitious structures decrease in length with successive cell divisions (clonal age of cells), in systems as diverse as mutant yeast[21] and human skin,[22] until, according to the theory, they are so depleted that cell division ceases–unless the regenerative enzyme telomerase is present, as it is in germ cells, cancer cells, and "immortalized" cultured cells.[23] (Telomerase is normally suppressed by gene(s) on human chromosome 3.[24]) The theory is attractive because it provides an explanation for the Hayflick limit (limited division potential of somatic cells). However, there are a few problems with it. It does not account for senescence in non-dividing cells, which observably occurs. Moreover, telomeres remain of constant length during aging of brewers' yeast cells[25] and they do not shorten during senescence in *Paramecium*.[26] Age-related changes in expression of yeast genes located near telomeres occur without any telomere attrition.[27] Immortalization of hamster cell lines is unrelated to telomere maintenance.[28]

Does the reset mechanism select healthy mitochondria or otherwise restore mitochondrial vigor? These organelles do age; their DNA mutates, their morphology changes,[29] and their function arguably is impaired (depending on methodology used to study them).[30] The mitochondria in human cells derive exclusively from those of the mother's ovum, which in the case of a middle-aged mother, has been quietly aging for a significant period. Perhaps the aging of the ovum's energy-producing mitochondria explains decreased fertility as well as cytogenetic anomalies (e.g., trisomy-21) that rise exponentially with maternal age. In any case, whatever DNA mutations may have occurred in a meiotically-arrested ovum over a span of many decades, they do not seem to be passed to the offspring;

passive failure-to-thrive of defective zygotes, or an active reset mechanism, are plausible explanations.

Mitochondria possess their own DNA (mtDNA), in the form of a single-stranded circle, which codes for nearly a quarter of the polypeptides of the mitochondrial respiratory complexes, plus mitochondrial ribosomal RNA and transfer RNAs. The mtDNA is peculiarly subject to mutational damage from free radicals, owing both to its single-strandedness and its proximity to sites of active oxidation. Mitochondria are the chief source of endogenous oxidants. Indeed, a major reason for the observed prolongation of life in caloric-restriction experiments is postulated to be reduced production or leak of free radicals from the mitochondria.[31] Damage to mtDNA may be more repairable than hitherto believed, according to Kopsidas et al.[32] The latter authors propose a schema of stochastic mtDNA replication and repair, in which increasing mutagen production with age progressively overwhelms repair pathways, so that nonfunctional mtDNA accumulates and a relatively smaller pool of intact mtDNA remains for transcription to restore mitochondrial number, which is set by nuclear control. The model is consistent with some observations that I made a number of years ago. In brief, I observed that turnover of mitochondrial constituents (DNA and various lipids) did not change with age in the mouse brain. However, relative to the concentration of the lipids, the concentration of mtDNA was about 40% higher in the old brains. Moreover, twice as much of the "old" mitochondrial DNA was found to be newly synthesized, by ^{3}H labeling.[33] Another prediction of the model of Kopsidas et al. is the accumulation of point mutations affecting mtDNA replication. Recently, Attardi's group at Caltech found, in normal aged fibroblasts, high copy point mutations in the control region for replication of mtDNA.[34] The authors also reported finding a specific mutation in up to 50% of the mtDNA molecules in over half of the human subjects above age 65. Such a finding implies that the environment in aged cells is conducive to the retention of mutant mitochondria.

The finding of high prevalence of a mutation among mitochondria is somewhat surprising, since deletions and related lesions typically affect less than 1% of the aging mtDNA, thought to be too small a percentage to inflict serious metabolic damage. In fact, intercellular nuclear–and mitochondrial–transfer experiments have indicated that recessive nuclear–DNA mutations, not mitochondrial mutations, are responsible for decreased mitochondrial protein synthesis and cytochrome C activity in aged cells.[35] However, mitochondrial mutations are suspected etiologically in senescent diseases like Alzheimer's and Parkinson's disease. Moreover, Attardi's group was able to demonstrate a very significant age-related decrease in respiratory rates of cultured human mtDNA-less cells, into which mitochondria had been transplanted from donors of various ages;[36] thus mutational damage to these organelles must be regarded seriously.

Utilizing intact mitochondria in intact cells, Tory Hagen of the Linus Pauling Institute showed significantly lower mitochondrial membrane potential in two-thirds of the cells from aged rats' livers; the other liver cells showed less or no depolarization. Hagen also was able to improve mitochondrial function by dietary supplementation of rats with acetyl-L-carnitine plus alpha-lipoic acid; the latter was necessary to control oxidant production, which increased when ALC alone was used.[37] It is plausible (although it does not necessarily follow) that improving the intracellular environment might select for healthier mitochondria, to the detriment of mutant types. In yeast cells, mitochondria are evidently able to improve their own intracellular environment to a degree by signaling

the nucleus to activate genes for metabolic enzymes; this extends the cells' lifespan.[38]

Does the reset mechanism restore redundant DNA other than that in telomeres? Strehler postulated that deletion of tandemly duplicated genes, such as those coding for ribosomal RNA (and so referred to as rDNA), may occur through mispairing of the two DNA strands.[39] He and his coworkers found a substantial age-related decrease in rDNA in brain, heart, and skeletal muscle of dogs, and in human heart muscle.[40,41,42] (The approximately 1:7 ratio between dog-years and human years is quite apparent from Strehler's data.) The decrement was far less in the animals' mitotically active tissues. Along with the measurable decrease of rDNA in postmitotic tissues, there occurs a parallel loss of nucleolar organizing regions (stainably visible locations of rDNA) on 6 pairs of chromosomes. Strehler refers to the process as "the most egregious, substantial and gradual loss of the most key kind of DNA–the DNA that is necessary for any cell to manufacture any kind of protein."[43]

In *Paramecium,* significantly less of the macronucleus (metabolic nucleus) is occupied by nucleoli as clonal age increases, suggesting an age-related loss of rRNA synthesizing capacity[44] (and, by inference, unavailability of the rDNA template). A significant gain in nucleolar density occurs when cells undergo autogamy, however. In yeast, as can be shown by deletion of control genes, silencing of the rDNA locus will extend lifespan;[38] seemingly that is a paradox, but it is further evidence that rDNA is somehow involved in aging. Also in yeast, caloric restriction will extend lifespan, just as it will in rodents. In the latter, the mechanism is unknown, but in yeast, the mechanism involves silencing of rDNA chromatin, via a regulatory protein called Sir2p that is activated by NAD.[45] The authors of this recent report believe that caloric restriction thus extends lifespan by increasing rDNA stability, reducing rDNA recombination and consequent formation of toxic rDNA circles. Because a homologous mammalian gene, mSira, has similar functional characteristics to the yeast gene, the authors speculate that maintenance of silencing is also critical to longevity in metazoans.

Intriguingly, it is possible to completely replace the rDNA genes of the ciliate *Tetrahymena thermophila,* which exist as free linear molecules that contain replication origins and telomeres. Microinjection of antibiotic-resistant rDNA into individuals of a normal strain results (under the appropriate conditions) in the recovery of antibiotic-resistant cells that breed true.[46] Perhaps a way can be developed to accomplish a similar feat in mammalian cells.

Conclusion

The future of aging research seems bright with promise. The Human Genome Project will contribute greatly to research in this field, as the interrelated sequential functions and phylogenetic homologies for our many genes become elucidated. The steps in physiologic rejuvenation could someday be schematized in the manner of computer code, making it easier to discern potential interventional strategies (for instance, calling a genetic subroutine from a different point in the code script). Improved vectors[47] will permit efficient transfer of cloned or *de novo*-synthesized genetic material to our cells.

If Linus Pauling were alive today, he could not help but be fascinated by all the new ideas and the activity. He might even be interested in contributing to this line of research. Certainly he would be awed by the progress that has occurred since he first pondered the nature of life more than 7 decades ago, and embarked on the first-ever molecular biology research a scant few years afterward.[48]

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