

The Impact of Glutathione on Health and Longevity

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ABSTRACT

Glutathione has been called the master antioxidant because of its critical roles in metabolic mechanisms and pathways. Uniquely, this simple tripeptide is ubiquitous and found in virtually all animal, plant, and microbial cells. In this brief review some of the strategy in our experiments in aging models and humans are presented. Hopefully, others may become interested in the experiences.

INTRODUCTION

FOR MANY YEARS, my research has been on biological aging and life span changes involving glutathione (GSH). Currently, this field has expanded and now includes oxidative stress, free radicals, and antioxidants. Thus, this review on GSH and longevity is an attempt to describe the background and integrate the findings of our research.

GSH is a unique and well-known biochemical that plays vital roles in life stages from birth to senescence. It was discovered in 1888 by J. de Rey-Pailhade of France in animal, plant, and yeast cells who named it *philothione*. Not until 1921–1941 were the structures and metabolism of GSH elucidated by F. Gowland Hopkins, “the Father of Biochemistry” of Cambridge, England who renamed it *glutathione*. World War II intervened, and for the next 40 years GSH was virtually forgotten. However, a renaissance occurred in the 1980s, led by Alton Meister and his collaborators at Cornell University, on the functional and metabolic aspects

of GSH. Many publications by outstanding investigators appeared including excellent reviews on different aspects of GSH.^{1–5}

What is GSH? Briefly, it is a tripeptide, L- γ -glutamyl-L-cysteinyl-glycine with a critical sulfhydryl group. The unique feature is its ubiquitous distribution in almost all living cells, and thus GSH is the most abundant cellular antioxidant. It plays many key roles in the transport of amino acids, biosynthesis and activity of proteins, enzymes, and hormones, and defense against toxic compounds and oxidative stress.

My experience as a graduate student of Bacon Field Chow at Johns Hopkins University was on biochemical aspects of newly discovered vitamin B₁₂, including redox effects, links with GSH in diabetes, and the development of tolerance tests with aged rats and humans. These fascinating topics shaped my future research.

My first independent study was to quantify enzyme activities during rat growth and frog embryogenesis and to measure biochemical pa-

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rameters of the life span.⁶⁻⁸ This broad experience with growth, development, and aging of different animal systems covered too many areas to be effective. Thus, I focused my research on biosynthesis during the life span.

OF MOSQUITOES, MICE, AND MEN

Aging research was rare in the 1940s. A major reason was the lack of an experimental organism that is readily cultured and is easy to dissect and analyze. Although humans are the ultimate beneficiaries of research findings, they have disadvantages such as a wide diversity of subjects, a long life span and experimental time, and many moral, legal, and fiscal constraints.

The most common laboratory animals to consider as aging models were rats and mice, which had been used extensively by many investigators for biomedical research. The C57BL/6J mouse and the Fischer 344 rat are two aging strains that are relatively tumor-free and healthy in their old age. Also the National Institute on Aging now provides these rodents for pilot studies and doctoral dissertation projects. However, for research in 1963 it was necessary to establish our own aging colony of C57BL/6J mice by ordering a batch of 200 mice every 4 months and maintaining them for several years. Our median survival time determined over a 10-year span was about 31 months, similar to that of other colonies.⁹

While seeking a future job after graduation, I sought the advice of Elmer V. McCollum, my mentor who founded the Department of Biochemistry at the Johns Hopkins School of Hygiene and Public Health. He asked whether I had considered insects as model organisms. Relatively little was known about their biochemistry and nutrition, and he felt insects would be good experimental organisms as *Escherichia coli* was in ongoing molecular studies. In retrospect, this was a logical outgrowth of his own experience, for he pioneered the use of rats as convenient and better animals than cows to study nutrition in his first job in a dairy department. This use of the laboratory rat opened up future research in nutrition, biochemistry, and many other medical sciences.

His suggestion prompted my study of the mosquito and its biochemistry with Lloyd E. Rozeboom, the medical entomologist in the Department of Parasitology at Johns Hopkins. Mosquito problems were intriguing, and I studied them my entire research career and trained all of my students with them. Although other mosquitoes were occasionally studied, *Aedes aegypti* (Linnaeus) was the main species because of its extensive laboratory and field history as a vector of yellow fever, the major obstacle to the construction of the Panama Canal.

There were many advantages of using *A. aegypti* as an aging model. First, it was easy to grow under standard controlled conditions or in axenic (germ-free) culture.¹⁰ It is safe, for neither yellow fever virus nor HIV in infected mosquitoes can be transmitted to other mosquitoes. Second, its nutritional requirements and intermediary metabolism were similar to those of humans and mammals, and many findings such as the essential amino acids, carbohydrates, lipids, vitamins, and minerals were readily applicable. Third, it is a poikilotherm, so the metabolism and life span could be manipulated and were inversely proportional to ambient temperature.¹¹ A unique advantage is that the mosquito, unlike some insects and many mammals, is a heteromorphic organism, and thus its morphologically different stages are readily identified. Furthermore, growth occurs only in the larval stages, whereas aging occurs only in the adult. Finally, the total life span is only about 6 weeks long at the standard rearing temperature of 29°C,¹⁰ considerably shorter and less expensive than the 27 months needed for the Fischer 344 rat or 30 months for the C57BL/6 mouse.

An initial project was to grow the mosquito axenically on a chemically defined medium. Existing procedures employed semipurified and crude media, which resulted in subnormal growth rates. Our method was the first to quantitatively grow axenic mosquitoes at normal rates for *A. aegypti* and a number of other mosquito species.¹⁰

A. aegypti is a practical experimental organism in many ways. It is large enough to analyze individually with simple equipment. Also a single adult mosquito can be accurately in-

jected with 1 μ l of test solution in less than 30 sec. In large numbers, they are easy to culture, because a 1 cubic foot cage will hold several hundred mosquitoes, an advantage in obtaining sufficient numbers for statistical analysis. They are about 10 times larger than *Drosophila*, because the adult female weighs about 4 mg and the male 2 mg. Thus mosquitoes are sufficiently large for analysis, since many biochemical measurements are in the nano- and picogram range.

The concepts of gerontology were not clear in the 1950s. A popular myth was that the aging process was a linear decline in body processes from birth until death. This view was not supported with evidence but was based on conjecture or on two-point curves. Thus, we devised a metabolism scheme for the life span¹² (Fig. 1).

Metabolism was expressed as the ratio of overall anabolism to catabolism and was plotted against the stages of the life span, growth, mature, and aging. The ratio is >1 and is predominately anabolic during growth; it decreases to 1.0 or slightly less during maturity, when anabolism essentially equals catabolism. This is supported by data on human physiological functions and basal metabolic rates during the life span. Finally, aging or senescence—the last stage—shows a sharp decline to a ratio of <1 and is mainly catabolic. Thus metabolism changes from anabolism at the beginning to catabolism at the end of life. Marked aging phenomena take place during the life span decline from maturity to senescence. Our later work on mosquitoes and mice verified this pattern. Unfortunately, a great deal of earlier aging research concentrated on only changes between

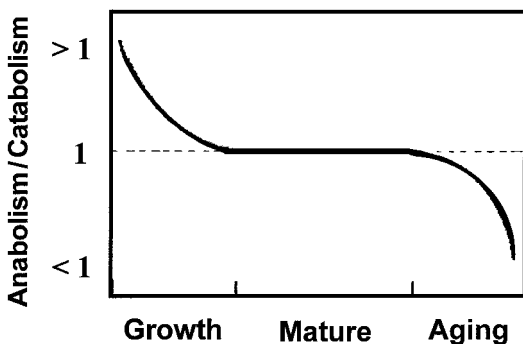


FIG. 1. Metabolic balance during the life span.

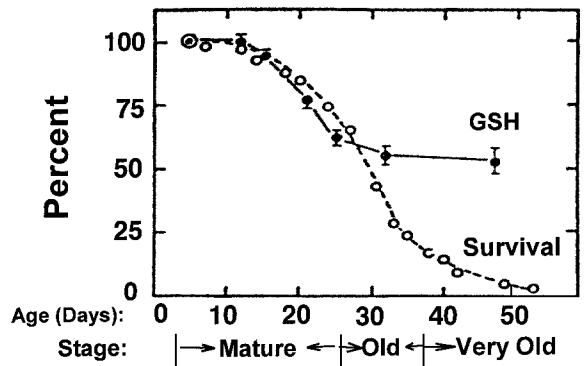


FIG. 2. Glutathione and survival during the adult life span of the mosquito.

growth and maturity that do not refer to senescence and have unknown significance.

THE AGING MOSQUITO

There were many intriguing projects to pursue, but we began with a systematic analysis of reduced glutathione (GSH) and oxidized glutathione (GSSG) of the life span of the mosquito, which had never been reported previously.¹³ Marked changes occurred, for larval growth and pupal metamorphosis had much higher GSH levels and biosynthesis than adults ($p < 0.001$). The GSH levels decreased in the early adult and then dropped 46% in the old and very old adult ($p < 0.001$). This was a general phenomenon, for it occurred in the, head, thorax, and abdomen of both sexes. Starvation of up to 3 days did not affect the GSH levels.

A provocative finding was the decrease of GSH level with aging in the adult mosquito (Fig. 2). Percent survival was superimposed on the GSH content up to the 32nd day of adult age—the mean survival time.

From this time, the GSH level was near constant to 47 days, a very old age and end of the study. Thus, in the last half of the life span, the relative GSH concentration was 50% of the earliest adult level. This finding suggested that about 50% is required for survival and gave strong evidence for the importance of GSH for longevity.

Could the GSH decrease of aging be arrested or delayed? Magnesium thiazolidine carboxylate (MTC), a cyclic precursor of cysteine, was

found previously to increase the life span when fed to *Drosophila*, but the effect on GSH status was not determined.^{14,15} Others showed that mice given thiazolidine derivatives increased their tissue GSH content.^{16,17} Thus, we reasoned and found that feeding MTC to mosquitoes would both increase their GSH content and enhance longevity.¹⁸ Figure 3 shows that both GSH and longevity were increased by MTC fed continuously throughout the life span.

The controls were 5-day-old mosquitoes who were fed their customary 10% sucrose solution. Their GSH contents are indicated by the closed symbols and solid line. Subgroups were fed 3.18 mM MTC for 2–12 days as indicated by arrows at 11–41 days. Their GSH levels are indicated by open circles and dashed lines. Each point represents three to six samples. The results demonstrate that the short-term feeding of MTC increased the GSH contents as high as 60% above the beginning adult level. Later, regardless of adult age, GSH dropped to contents still above the beginning level.

In Figure 4A, the upper, dashed line curves signify the MTC-fed groups, and the lower, solid line curve, the control mosquitoes. The 35-day-old MTC group had the same GSH content as the 7-day controls. In Figure 4B, the percentage survival curves of the MTC and control groups are presented, and the differences between median age and maximal age values of the two groups are clearly seen.

These results demonstrate that induced al-

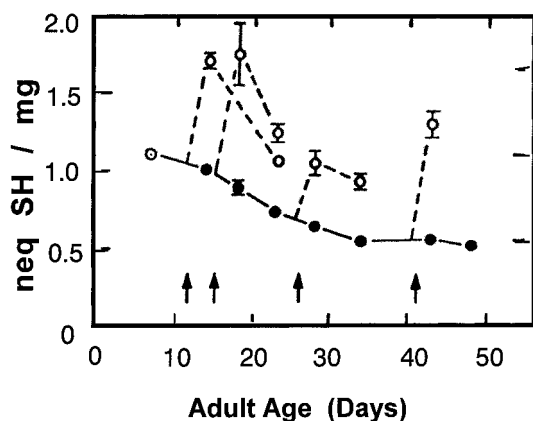


FIG. 3. Single dose magnesium thiocarboxylate increases glutathione in different ages.

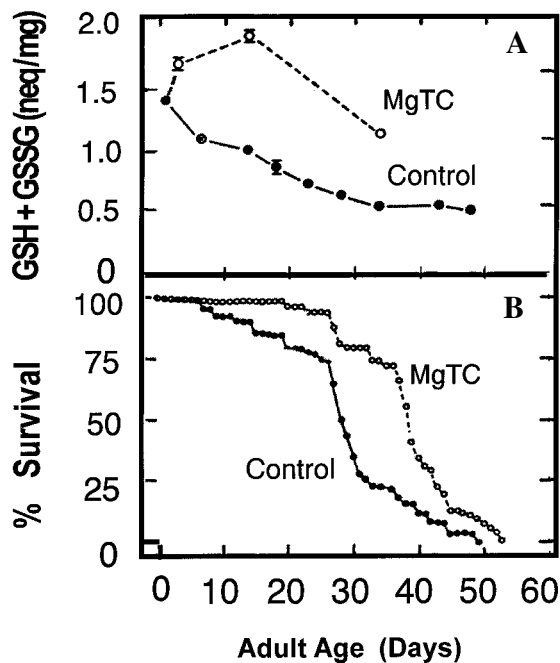


FIG. 4. Magnesium thiocarboxylate increases glutathione and longevity.

teration in GSH metabolism has important functional consequences. The MTC treatment increased the GSH levels 50–100% ($p < 0.005$) regardless of the age when the MTC was started or how long the treatment lasted. The median life span increased from 29 to 40 days, an increase of 38% ($p < 0.005$). At the time this study was done (1987), this constituted the first time that a biochemical intervention proved capable of significantly increasing the median life span. There was also an increase of 4 days in the maximum life span from 49 to 53 days.

This experiment demonstrated that the enzymatic machinery to synthesize GSH from Cys (and MTC) was active even in the very old mosquito. Also, this finding suggested a rationale for the possible nutritional enhancement of GSH in elderly humans.

THE AGING MOUSE

Our original study of GSH status and erythrocyte age as well as mouse age¹⁹ was later repeated with different organs.^{20,21} The age profiles, normalized at 17 months, of percent GSH in mouse liver, kidney, and heart paral-

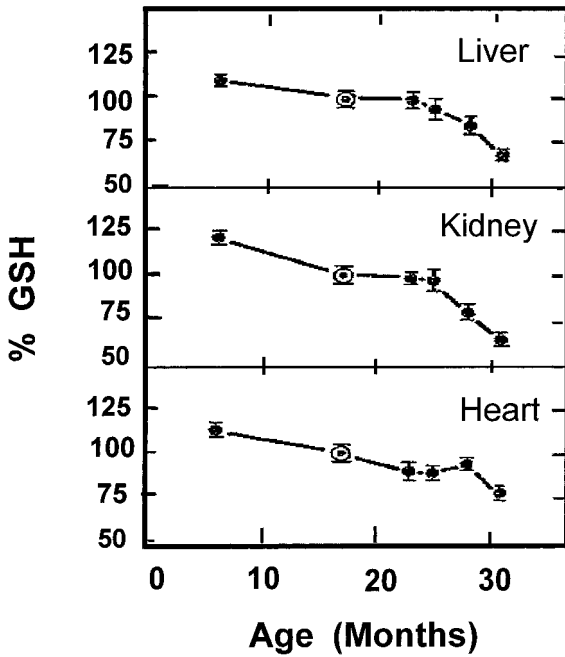


FIG. 5. Glutathione levels of mouse tissues during the life span.

months of age, and the SEM bars were omitted for clarity (Fig. 6).

All curves were flat or rose slightly. At about 27 months (the onset of senescence), the GSH contents of all organs dropped. These data showed that the blood GSH level indicated by the bold curve paralleled the GSH levels of other organs. Thus, the blood GSH content was considered an index of whole body GSH during adult aging.

leled each other in the mature adult (Fig. 5). However, at the onset of senescence at about 27 months, the percent GSH content markedly decreased.

A decade later aging profiles were repeated for mouse blood, spleen, lung, brain, liver, heart, and kidney. Age patterns similar to those discovered before were found,²² suggesting that the analyses were robustly repeatable.

To integrate the various organ-age curves, each was adjusted to 100% relative GSH at 12

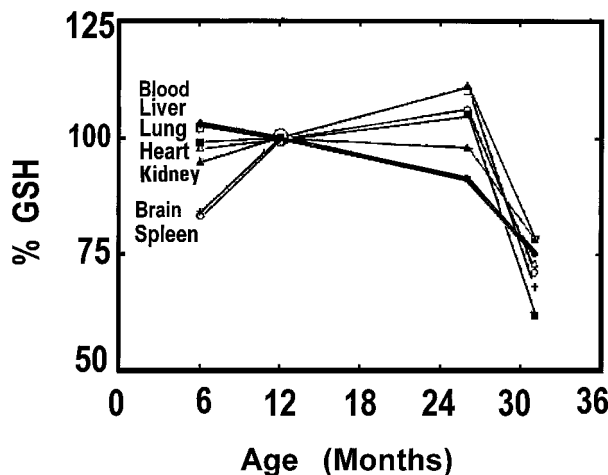


FIG. 6. Glutathione levels of blood reflects other tissues.

GSH ANALYSIS BY HPLC-DEC

Our early experiments on GSH used the 5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB spectrophotometric analysis²³ and later a modification that was coupled with GSSG reductase.^{24,25} Other thiols and disulfides needed to be investigated, so we developed the HPLC-DEC (dual electrochemical) method that simultaneously measures reduced and oxidized forms of glutathione, cysteine (GSH, GSSG, Cys, CSSC), and other thiols and disulfides.²⁶ The HPLC-DEC method is highly specific and regarded as the best for determining GSH status. A typical pattern is shown (Fig. 7).

OF AGING MEN

The deficient blood GSH levels found in aging mosquitoes and mice suggested the possibility that the same state may occur in aging men. This study was comprised of community residents and university colleagues who were

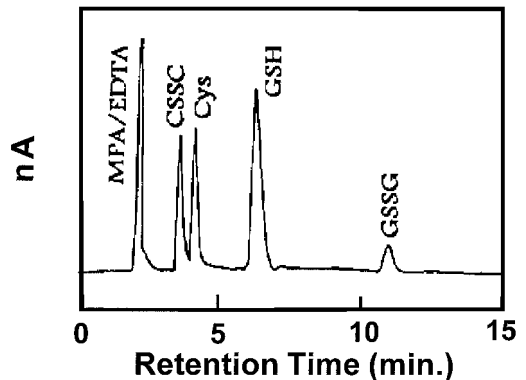


FIG. 7. HPLC-DEC chromatogram of standards.

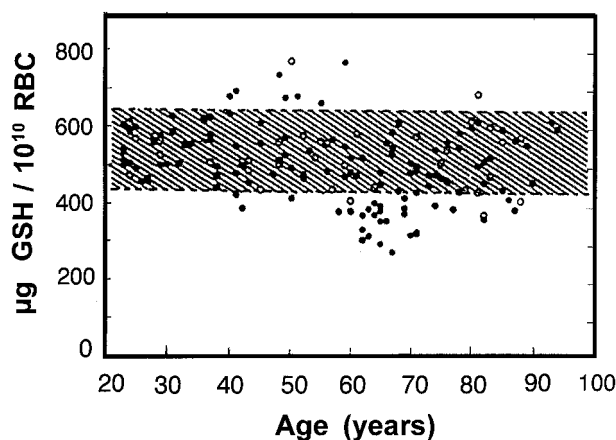


FIG. 8. Blood glutathione levels of healthy subjects.

clinically healthy adults of ages 20–94 years. These subjects were from different socioeconomic and environmental backgrounds²⁷ (Fig. 8).

The GSH results were expressed as mg of GSH per 10^{10} erythrocytes. This was done because over 99.5% of the GSH of blood is localized in the erythrocytes. Our subsequent data demonstrating blood GSH deficiencies in humans with chronic disease are based on this expression.

Results from the 20 to 39-year-old subjects served as the normal reference range (the shaded area) because maximal levels of many physiological functions are attained at those ages. By comparison, a large number of the

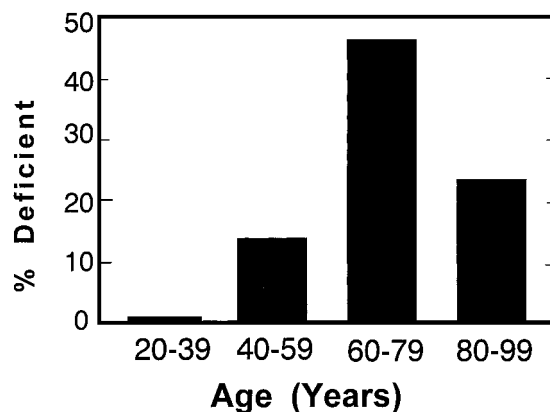


FIG. 9. Glutathione-deficient subjects increase with age.

40–94-year-old subjects were GSH deficient, with values below normal (Fig. 9).

The number of GSH-deficient subjects increased with age, and almost half of the 60 to 79-year-olds subjects were deficient. Surprisingly, there were fewer in the oldest, 80+ year group, which we believe is due to the selective death of frailer members of this cohort in earlier years. Confirmation of this will require future longitudinal studies.

After the discovery of GSH deficiency in healthy, elderly subjects, what is the status of unhealthy persons with chronic disease? Our pilot study suggested that some self-assessed elderly were deficient.²⁸ The present strategy was to study a larger sample who need medical care, so newly admitted hospital patients were recruited. In a double-blind design of 74

TABLE 1. BLOOD GLUTATHIONE CONTENT IN CHRONIC DISEASES^a

Disease category	GSH eq $\mu\text{mol}/10^{10}$ RBC	n	p value ^b
Control group	2.30 ± 0.070	32	—
Genitourinary	1.83 ± 0.0995	10	0.0013
Gastrointestinal	1.75 ± 0.073	18	<0.001
Cancer	1.71 ± 0.051	17	<0.001
Cardiovascular	1.71 ± 0.010	10	<0.001
Musculoskeletal	1.66 ± 0.212	6	0.0013
Lung	1.94 ± 0.155	4	0.086
Gallbladder	1.87 ± 0.076	3	0.070
Diabetes	1.31, 1.18	2	—
Kidney	1.37, 1.31	2	—
Endocrine	1.92	1	—
Blood	1.64	1	—
Total patients	1.73 ± 0.036	74	<0.001

^aValues are expressed as mean \pm SEM except for individual values for diabetes, kidney, endocrine, and blood categories.

^bAs compared with control group.

patients who were diagnosed by physicians, blood GSH deficiencies were found in 36% of the patients ($p < 0.001$; Table 1). This was due to lower GSH levels and not to GSSG levels, which were the same as in healthy controls.

These data suggest that different chronic diseases may have a common deficiency for GSH. Another suggestion was that GSH enhancement may be a new therapy for chronic diseases.

IN THE FUTURE

There are many more interesting GSH and aging questions to explore. What other diseases are GSH deficient? Can GSH enhancement be accomplished by administration of other antioxidants? Which single antioxidant or combination of antioxidants is best? Does an increase in GSH provide a new therapeutic intervention? These and other questions can now be evaluated in humans and laboratory animals using a blood GSH assay.

GSH research offers many possibilities, but as stated in this review, the fundamentals of analytical methodology are especially critical for redox compounds. For example, if blood GSH samples are chilled immediately after collected, and then acid denatured soon there will be fewer problems. But validation is still necessary such as recovery of spiked samples.

Animal models are particularly needed in gerontological studies to provide more rapid experimental systems. Also, the results reported in this paper with the mosquito has been due to its unusually close metabolic relationships with humans. Future studies with other models may be as fruitful and gratifying.

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