PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 113–117 (1987)

# Correction of a Glutathione Deficiency in the Aging Mosquito Increases Its Longevity (42454)

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*Abstract.* The decrease of tissue glutathione (GSH) concentrations in different senescent organisms gave rise to our hypothesis that a glutathione deficiency is a biochemical cause of the aging process. A rigorous test of this notion would be the correction of the deficiency and concomitant increase in life span. To this end, adult mosquitoes were fed magnesium thiazolidine-4-carboxylic acid, and their GSH levels and life spans were determined. The GSH levels increased 50–100% ($P < 0.005$) regardless of the age when feeding was initiated or whether the feeding period extended over 2 days or the entire life span. Also the median life spans increased 30–38% over control values ($P < 0.005$). The responses were specific for the thiazolidine carboxylate moiety, because $MgCl_2$ had no effect. These findings confirm the GSH deficiency hypothesis and demonstrate a specific biochemical mechanism of aging that can be nutritionally modified.


A general phenomenon of aging tissues appears to be a decrease in glutathione (GSH), the most abundant cellular reducing agent. This decrease, which occurred in a variety of tissues from different organisms such as mosquito, mouse, and man (1–5), supports our earlier findings of aging-specific decreases in reducing capacity, such as lower levels of several $NADP^+$-linked enzyme activities, $NADPH/NADP^+$ ratios, protein biosynthesis, and DNA replication *in vivo* (6–10).

These results led to our hypothesis that a GSH deficiency is a biochemical cause of the aging process which could have broad metabolic implications considering its many well-demonstrated, biochemical roles (11–13). In brief, the importance of GSH stems from its high concentrations (0.5–8.0 m*M*) as an intracellular component in nearly all organisms and its many biological functions such as the maintenance of cell membranes, destruction of metabolic peroxides and free radicals, detoxification of xenobiotics, maintenance of sulfhydryl groups of enzymes and proteins, control of redox status and disulfide exchange reactions, and translocation of amino acids and peptides across membranes.

The objectives of this study were to test our hypothesis by determining if correction of the GSH deficiency is accompanied by a concomitant increase in longevity. A GSH precursor was administered to mosquitoes to raise the tissue GSH levels. The choice of precursor was limited as the most obvious compounds, cysteine (Cys) and GSH, were not effective in preliminary experiments, most likely due to their rapid metabolism or autooxidation to inactive compounds (14). Thus, a Cys precursor, magnesium thiazolidine-4-carboxylic acid (MgTC), was selected. Previously others have shown in mice that thiazolidine-4-carboxylic acid (TC) derivatives can increase tissue GSH content (15, 16), as these amino acid precursors are readily transported into cells and converted to Cys, which is utilized for GSH biosynthesis. Further, in other studies administration of MgTC increased the life span of fruit flies (17, 18), although its effect on GSH status was not determined.

Our experimental aging model was the yellow fever mosquito (*Aedes aegypti*) because of its unique advantages. First, it is a multicellular, eukaryotic organism that resembles mammals in its biochemical composition and larval nutritional requirements (19). Second, the mosquito has been validated as an aging model by extensive biochemical and biological characterization (20). Third, techniques for its laboratory culture and aging have been standardized and used routinely for over three decades (21). Finally, the mosquito has a short adult life span of only 30 days.

**Materials and Methods.** Our standard culture procedures were followed to produce adult mosquitoes of all ages of the life span (21). From 25–35 female adults were placed



0037-9727/87 $1.50

in cages consisting of glass lantern chimneys fitted with nylon net tops and screened petri dish bottoms. The mosquitoes were fed diets provided on cotton pads placed on the tops of the cages. The experimental diets consisted of the control diet of 10% (w/v) sucrose solution supplemented with MgTC (0.637–6.37 m*M* final concentration). The cotton pads were replaced every other day. An environmental temperature of 29 ± 1°C and a 12-hr light cycle were maintained throughout the investigation.

At given times, pooled samples of 10–20 mosquitoes were cold-inactivated and immediately homogenized (10%, w/v) in ice-cold 5% (w/v) metaphosphoric acid using an all-glass Ten Broeck homogenizer. After centrifugation at 14,000*g* for 15 min, the supernatants were collected and diluted with 0.1 *M* sodium phosphate/0.005 *M* EDTA, pH 7.5.

Total glutathione was assayed by a modification of the enzymic cycling method of Tietze (22) in which the rate of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) reduction is proportional to the amount of total glutathione (reduced plus oxidized glutathione) (5). Tissue extracts (volume 25–100 μl) were added to an assay medium containing 0.5 EC unit of yeast glutathione reductase, 0.5 μmol of DTNB, and 0.2 *M* sodium phosphate/0.005 *M* EDTA, pH 7.5, in a volume of 0.9 ml. This reaction mixture was preincubated for 2 min at 20–22°C to allow thiol components in the tissue extract to interact with DTNB. Then the enzymic cycling reaction was initiated by the addition of 0.1 ml of NADPH equivalent to 0.2 μmol, and the rate of DTNB reduction was determined from the increase in $A_{412}$ during the next 3–5 min. This initial rate was corrected for the reaction of DTNB with glutathione reductase without tissue sample. Routinely, two or more concentrations of each sample were analyzed to ensure that the initial rates were proportional to sample size. Also every daily assay included standard curves using known amounts of GSH.

**Results.** The results indicated that administration of either 0.637 or 3.18 m*M* MgTC for 8 days to mature, 12-day-old adults caused a 39–45% increase of GSH levels occur the controls (Table I). This increase was specific for TC and not $Mg^{2+}$, for 3.18 m*M* $MgCl_2$ had no effect on GSH levels.

This MgTC effect could be elicited in adult mosquitoes regardless of age. After 2 days of 3.18 m*M* MgTC feeding, GSH levels increased 50–100% in mature (11 and 15 days), old (26 days), and very old (41 days) adult mosquitoes (Fig. 1). Thus, the capability to increase GSH levels was retained even in the very old mosquito.

In long-term feeding experiments, 3.18 m*M* MgTC was fed to 1-day-old adults for their entire life span of about 30 days. The GSH levels increased 36% after 2 days and 62% after 10 and 33 days of supplementation (Fig. 2). More importantly, the median life span increased to 40 days which was 38% over the control group value of 29 days ($P < 0.005$). A lower dose of 0.637 m*M* MgTC also increased the median survival time 29% over the control ($P < 0.01$) (Table II). In these experiments the

TABLE I. INCREASE IN GLUTATHIONE LEVELS IN ADULT MOSQUITOES FED MAGNESIUM THIAZOLIDINE CARBOXYLIC ACID

| Group | Experiment No. | Total glutathione (nequiv. GSH/mg tissue)[a] | % of control |
|---|---|---|---|
| Control | I | 0.604 ± 0.0402 | (100) |
| | II | 0.651 ± 0.0311 | (100) |
| 0.637 m*M* MgTC | I | 0.878 ± 0.0577** | 145 |
| 318 m*M* MgTC | I | 0.842 ± 0.0496** | 139 |
| | II | 0.869 ± 0.0428* | 133 |
| 3.18 m*M* $MgCl_2$ | II | 0.632 ± 0.0683 | 97 |

*Note.* Experiments were carried out with adult, 12-day-old female mosquitoes fed a control, MgTC-, or $MgCl_2$-supplemented diet for 8 days.

[a] Results are shown as means ± SEM of four samples.

* Difference from control is statistically significant, $P < 0.01$.

** Difference from control is statistically significant, $P < 0.001$.

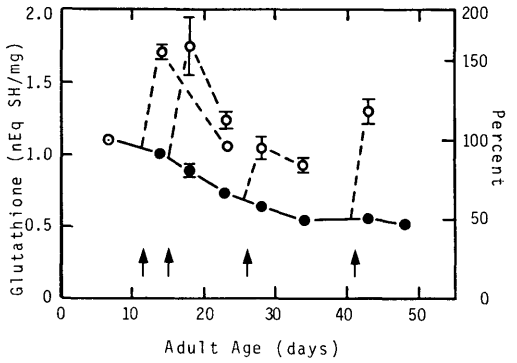


FIG. 1. MgTC feeding increases glutathione content in mosquitoes of different ages. GSH content was determined in control (●) and MgTC-fed (○) mosquitoes. MgTC (3.18 m*M*) feeding was initiated in adults of different ages, indicated by the arrows, and continued for 2–12 days. Each point with bar represents the mean ± SEM of three to six samples. Bars were omitted if SEM was less than the size of the point. Each sample consisted of 8–15 mosquitoes. Details are given in text.

specificity for TC was verified, for 3.18 m*M* $MgCl_2$ had no effect on survival. Finally, the GSH and longevity effects were not due to starvation or restricted intake because the mosquito weights were constant in all groups. In contrast, a control group of starved mosquitoes lost weight and died within 2 days.

**Discussion.** This enhancement of GSH levels and concomitant increase in life span confirmed our hypothesis that a GSH deficiency is a key biochemical cause of the aging process. We believe this is the first direct evidence of a specific molecular mechanism of aging, and the involvement of GSH is of special interest because of its well-established central role in various metabolic pathways. Also these results showed that this deficiency can be corrected by nutritional intervention.

Our earlier results demonstrated that the cause of the GSH deficiency in the aging mosquito was a decrease in overall GSH biosynthesis (23). The present findings indicated specifically that a lack of the GSH precursor, Cys, rather than a decrease in biosynthetic enzyme activities is responsible for the deficiency.

A major consequence of a GSH deficiency of aging is an increased susceptibility to endogenous and environmental insults. This is consistent with the classic definition by Alex Comfort who described aging as an "increase in vulnerability occurring with advanced age" (24). Thus a decrease in GSH could lead to a loss of detoxification capacity against many compounds including peroxides, free radicals, toxicants, and carcinogens, all of which have been shown to be detoxified by GSH (11, 12). Indeed, we found that acetaminophen is seven times more toxic in the very old mosquito than in the mature adult and that the median lethal dose was highly correlated ($r^2$ = 0.97) with GSH concentration (25).

Our GSH hypothesis is consistent with other aging theories involving the impairment of protective mechanisms or detoxification capacities such as the free radical theory (26) and the immunological theory (29). Indeed, GSH may be involved in many other biological aging theories.

The TC effect on GSH levels and longevity was specific, because dietary supplements of

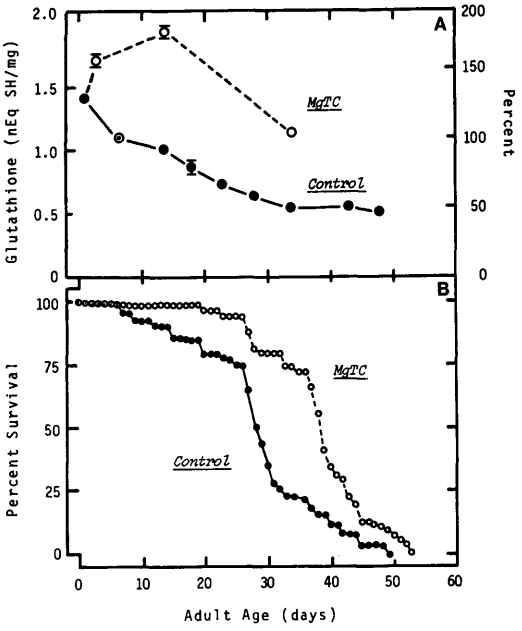


FIG. 2. MgTC feeding increases both glutathione and longevity. MgTC (3.18 m*M*) feeding was initiated in 1-day-old adult mosquitoes and continued through the life span. (A) GSH content was determined in control (●) and MgTC-fed (○) mosquitoes. Each point with bar represents the mean ± SEM of three to five samples. Bars were omitted if SEM was less than the size of the point. Each sample consisted of 8–15 mosquitoes. Details are given in text. (B) Survival was determined in control and MgTC-fed adults, as described in text.

TABLE II. INCREASED LONGEVITY OF ADULT MOSQUITOES FED MAGNESIUM THIAZOLIDINE CARBOXYLIC ACID THROUGHOUT THE LIFE SPAN

| Group | Experiment No. | No. of mosquitoes | Median survival time | |
|---|---|---|---|---|
| | | | Days | % of control |
| Control | I | 30 | 29.7 | (100) |
| | II | 32 | 28.2 | (100) |
| 0.637 m*M* MgTC | II | 38 | 36.3* | 129 |
| 3.18 m*M* MgTC | I | 27 | 40.2* | 135 |
| | II | 40 | 38.0** | 135 |
| 6.37 m*M* MgTC | I | 31 | 31.5 | 106 |
| 3.18 m*M* $MgCl_2$ | II | 31 | 24.9 | 88 |

*Note.* Experiments were carried out with 1-day-old adult female mosquitoes fed control, MgTC-, or $MgCl_2$-supplemented diets throughout their entire life span.

* Difference from control is statistically significant, $P < 0.01$.

** Difference from control is statistically significant, $P < 0.005$.

GSH, GSSG, Cys, cystine, Glu, and Gly had no effect in our preliminary experiments. Further it should be pointed out that the 10% sucrose solution simulates the normal diet of plant and fruit nectars for adult mosquitoes. Blood meals, which can be taken only by females, are necessary solely for ovarian development (28). Indeed, results comparing the life span of blood-fed vs sucrose-fed adults indicated a much shorter life span for blood-fed mosquitoes (29).

The biochemical mechanism for the TC enhancement of GSH levels is probably through the formation of the GSH precursor, Cys, a conversion *in vitro* demonstrated by others (30). Also, TC can replace Cys as a nutrient in the rat (31). Further *in vivo* evidence is that the 2-oxo and 2-methyl derivatives of TC are precursors of Cys in the mouse (15, 16).

These results may have clinical significance, since TC has been used as a drug (Hepalidine) for the treatment of hepatic and biliary disorders. Its reported effects resemble those shown for GSH and include hepatoprotection, anti-cancer activity, and radioprotection (32). Also the 2-oxo and 2-methyl derivatives of TC protected against toxicants such as acetaminophen (31, 33). Thus, this enhancement of GSH content for the treatment of a variety of disorders and the increase in longevity in humans deserves further study.

The authors thank Dr. J. Miquel for his generous gift of MgTC and Marcia Liu for her technical assistance.

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Received May 28, 1986. P.S.E.B.M. 1987, Vol. 184.
Accepted October 13, 1986.