Serum Zinc in Aging Germ-Free and Conventional Rats (43350)

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Abstract. **Zinc nutritional status appears to decline with age in humans and rodents. Since germ-free rats outlive their conventional counterparts in better health, serum Zn levels were determined in male germ-free and conventional Lobund Wistar rats in samples originating from the Lobund Aging Study. Starting at 5 months of age, germ-free rats showed significantly higher serum Zn levels than did their conventional counterparts. In conventional rats sacrificed up to 30 months of age in apparently good health, serum Zn levels showed no effect of age, while a slight but significant increase with age was observed in the germ-free rats. In healthy germ-free adults (6–24 months of age), serum Zn concentrations were approximately 25% higher than those in comparable conventional animals. In conventional rats 18–30 months of age (average, 24.5 months), sacrificed because of an obvious moribund condition, serum Zn levels were significantly lower than those in rats of the same age range (average, 24.9 months) that were obviously healthy. Results suggest that the often observed higher absorptive capacity of the germ-free gut might have contributed to higher serum Zn levels, and that a decline in serum Zn concentration with age may be a consequence, rather than a causative factor, of declining health.** [P.S.E.B.M. 1992, Vol 199]

Zinc status may affect not only the function of a number of enzyme, receptor, and regulatory systems (1), but also immunocompetence, particularly T cell function (2). The serum Zn compartment is part of a rapid turnover pool that is located in part in bone, liver, and blood. This pool is central to Zn metabolism (3) and may be affected by stressful conditions (3, 4). The generally accepted decline of T cell function with age (5), combined with reports of decline in serum Zn concentration in both older humans (6) and older rodents (7), led us to determine Zn in serum samples of aging germ-free (GF) and conventional (CV) Lobund Wistar (L-W) rats available from the Lobund Aging Study (8). Compared to other rat strains, the CV L-W rat maintained on natural ingredient diet L-485 (see below) has the advantage of a longer than usual life span, apparently due to the virtual absence of kidney pathology (8). Its GF counterpart has the additional advantage of not being exposed, especially later in life, to the potentially stressful implications of the presence of a microbial flora. Presumably, this leads to the somewhat longer life span of the GF rodent (9, 10).

Although the GF rodent has an underdeveloped immune system (as exemplified by lower spleen and lymph node weights [11]), it has long been recognized that its potential to react to exogenous antigenic stimuli is at least comparable to that of its CV counterpart (12–15). Early thymic development in the GF mouse is slightly less than that in the CV mouse, but later in life thymic morphology appears largely comparable (16), although Uno *et al.* (17) found slightly higher thymus weights in older GF rats. The present authors are not aware of any published reports on lymphokinins or thymic hormone in germ-free rodents. Other hormones, as far as studied, do not indicate dramatic differences between the homeostatic control mechanisms of GF and CV rats (18).

The presence of an intestinal microflora appears to have little effect on the B cell IgM repertoire, but it will influence the transition of IgM production to that of other IgG (19, 20). As far as the potentially Zn-affected T cell function is concerned, Yoshikai *et al.* (21) suggested some effect of the microflora on the T cell repertoire of GF mice. Kim and Gilman-Sachs (22),

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analyzing the percentages of T lymphocyte subgroups of the Lobund Wistar rat, found no significant differences in T helper cells, but generally higher amounts of T cytotoxic/suppressor cells in GF than in CV animals. A comprehensive review of the immune potential of the GF animal has been published by Bealmear *et al.* (16).

During the course of this study animals in manifestly healthy condition were sacrificed at various ages. The remaining rats were sacrificed when in obviously moribund condition. The results indicate that, as long as the animals appeared in good health, no decrease in serum Zn concentrations was obvious for at least 25 months in GF rats and 30 months in CV rats. Also, GF rats showed a 20–30% higher serum Zn level than their CV counterparts.

Materials and Methods

All rats were males of the Lobund Wistar strain (Lob:(WI)h). The GF rats were obtained from our closed colony, which is now in its 56th generation. The CV colony was derived from the GF colony, and at regular intervals, GF males and females are introduced into this colony to maintain close genetic proximity. All rats are free of pathogenic microorganisms. Nephrosis is not evident in the L-W rat maintained on diet L-485 until after 30 months of age, and is never severe (8).

The GF rats were housed in plastic and steel isolators and were maintained with routine gnotobiotic procedures (23). The CV rats were housed in plastic isolators that were opened to the local environment for the introduction of feed and water, and weighed outside the isolator . This was done to avoid a possible "locked flora" phenomenon (24). All rats were fed steam-sterilized natural ingredient diet L-485, the Lobund colony diet since 1968 (25). L-485 is composed of ground corn, soybean meal, alfalfa meal, and corn oil to give 20% protein, 5% fat, and 3% fiber. Additional L-lysine, DL-methionine, and vitamins are included in the diet to cover losses due to sterilization. The diet contains 32 mg Zn/kg. The rats were housed four to a cage (commercial plastic boxes), which measured approximately 45 × 24 × 20 cm, with two cages per isolator. All rats were kept on Sani Cell corncob bedding containing 4.4 mg Zn/kg and given sterilized, untreated tap water of which the Zn content was negligible. The rooms containing the isolators were air and humidity controlled with 12:12-hr light:dark cycles. Additional details of the Lobund Aging Study have been reported by Synder *et al.* (8).

Serum samples were available from obviously healthy GF and CV rats up to the age of 25 and 30 months, respectively (Series H). Additional material was available from moribund rats 18 months and older (Series M, average age, 24.5 ± 1.5 months), but no data of a comparable age group of GF rats were available. All rats had been sacrificed between 9:00 and 11:00 AM. Blood was collected by cardiac puncture, and serum was stored at −70°C. Zn was determined after 1/10 dilution with aqua dest. with a Varian AA-175 Atomic Absorption Spectrophotometer. The analytical data have been evaluated with the Statistical Analysis Package (StatPac: Wallonick Associates, Minneapolis, MN).

Results and Discussion

The serum Zn concentrations of Series H are given in Figure 1 and in Table I. They are in the range of values reported by others (3, 26). As long as the rats were healthy according to our criteria, no decrease, but rather a slight increase in serum Zn levels with age could be observed for at least 25 months in the GF rats. At that age, 90% of the GF rats and 70% of the CV rats were alive and healthy. Zn levels of the CV animals showed no effect of age for at least 30 months. Starting at 5 months of age, the GF rats averaged 20–30% higher values than their CV counterparts.

In the aging M series, however, CV animals had substantially lower Zn levels than did H series rats of similar age (18–30 months, Table II). Many of the CV rats in the M series had prostate adenocarcinoma or

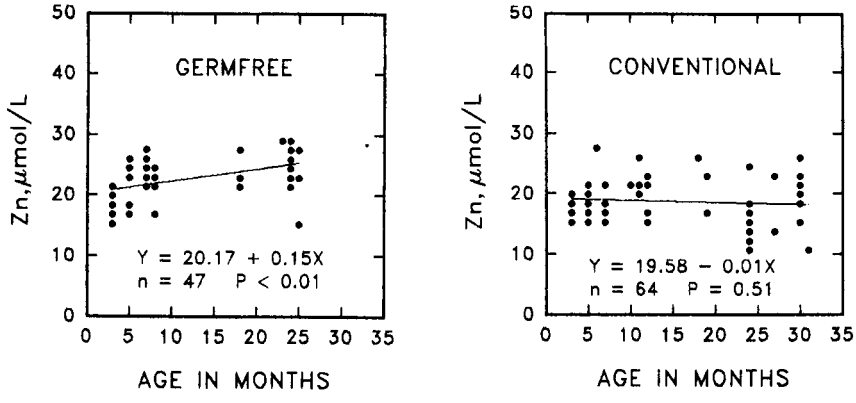

Figure 1. Serum zinc concentrations of germfree and conventional rats (H series, see text). First order regression line shown.

Table I. Serum Zinc Concentrations in Germ-Free and Conventional Male Lobund Wistar Rats Sacrificed in Apparently Good Health (H Series)

Age (mo)	Germ-free[a] (μmol/liter ± SE)	Conventional[a] (μmol/liter ± SE)
3	18.4 ± 0.9 (n = 8)	18.4 ± 0.9 (n = 6)
5	22.6 ± 1.2[b] (n = 8)	18.7 ± 1.5 (n = 8)
6–8	22.8 ± 0.8[b] (n = 13)	18.5 ± 1.1 (n = 8)
10–12	ND	19.6 ± 0.8 (n = 15)
18–24	24.8 ± 0.8[b] (n = 18)	18.1 ± 1.3 (n = 13)
27–30	ND	18.7 ± 1.1 (n = 14)
3–30		18.8 ± 0.5 (n = 64)

[a] Regression (all data points, see Fig. 1) GF, slope: 0.015, P = 0.001; CV, slope: −0.001, P = 0.795.
[b] Significantly different from corresponding CV group, $P < 0.01$.

Table II. Serum Zinc Concentrations of Healthy (H Series) and Moribund (M Series) Conventional Male Lobund Wistar Rats Between 18 and 30 Months of Age

	μmol/liter ± SE	Average age (mo)
H series	18.7 ± 0.8 (n = 26)	24.9
M series	14.9 ± 1.5[a] (n = 10)	24.5

[a] Significantly different from H series, P = 0.03.

prostatitis, which may have lowered their serum Zn levels through an acute phase response (3).

The GF rat intestine appears to have a higher absorptive capacity for a number of nutrients, including Ca, Mg, and xylose (27, 28). Since serum calcium levels are very closely regulated, this results in the heavier and denser skeleton of the GF rat (27). The reported data also suggest that Mg closely follows Ca in this respect, so that with an approximately 50% higher retention of both Ca and Mg, neither serum Ca or Mg are significantly higher in 3- to 4-month-old male GF rats than in CV L-W rats (27). Serum Zn, on the other hand, appears to be less closely controlled than serum Ca. Reddy *et al.* (29) found no significant difference in Zn absorption and retention between GF and CV L-W rats, although data for the GF animals tended to be higher (29). Smith *et al.* (30) obtained results that suggest that GF rats require less Zn than their CV counterparts, as have been demonstrated for a number of other nutrients (28). Thus, with a possibly somewhat higher intestinal absorption and a reduced requirement due to the absence of an intestinal microflora, the higher serum Zn concentration of the GF rat suggests a different Zn homeostasis in the GF animal.

The lower serum Zn levels found in the old CV rats sacrificed in moribund condition (M series, Table II) should explain the overall decline in Zn levels with age under conventional conditions reported by some workers (6, 7), although no consensus appears to have been reached in this matter (31). Results obtained in the H group intimate that the well-established decline in T cell function with age (5) is unrelated to Zn status. Our data suggest that aspects of old age unrelated to the endogenously available Zn pool are involved in the decline and eventual demise of the L-W rat. The lower serum Zn concentrations in the moribund *ad libitum*-fed rats might be a consequence of, rather than a causative factor in, their condition.

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