Antioxidant Capacity in Different Tissues of Young and Old Rats (43981)

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Abstract. **The antioxidant capacity in heart, liver, lung, and kidney was studied in young (6 months) and old (22 months) male Fischer 344 rats, using the oxygen radical absorbance capacity (ORAC) assay system with two different reactive oxygen species (ROS) generators. The results indicated that liver in old rats had significantly lower peroxyl radical absorbance capacity ($ORAC_{ROO \cdot}$, units/g wet wt), but higher hydroxyl radical absorbance capacity ($ORAC_{OH \cdot}$, units/mg protein) than in young rats. The decreased liver $ORAC_{ROO \cdot}$ in the old rats was mainly due to the loss of cytosol protein, while the increased liver $ORAC_{OH \cdot}$ in the old rats was a result of an increased resistance of cytosol proteins to the attack of ROS. This conclusion was further supported by the finding that the contribution of nonprotein fraction of liver cytosol to the $ORAC_{OH \cdot}$ of the cytosol decreased with age. No effect of age was found on either $ORAC_{ROO \cdot}$ or $ORAC_{OH \cdot}$ in other tissues. The antioxidant capacity for both $ORAC_{ROO \cdot}$ and $ORAC_{OH \cdot}$, was usually high in liver and kidney but low in lung and heart.**

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Reactive oxygen species (ROS)–mediated events in biochemical processes may lie at the heart of the etiology and natural history of a number of human diseases, including cancer and atherosclerosis, as well as the aging process itself. The antioxidant mechanisms that exist for the control of the generation of ROS and of their proliferation, therefore, present a major line of defense regulating general health status. All oxygen-consuming organisms are endowed with well-integrated antioxidant systems, which include enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase; macromolecules such as albumin, ceruloplasmin, and ferritin, and an array of low–molecular mass antioxidants, including ascorbic acid, α-tocopherol, β-carotene, ubiquinol-10, glutathione, methionine, uric acid, and bilirubin (1, 2).

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Because of the many different antioxidant components (including the known and unknown) in the body and the relative difficulty of measuring each known antioxidant separately, several methods (3–8) were developed in recent years to evaluate the antioxidant capacity of biological samples. However, most of the work was done in buffer, plasma or serum, and other extracellular fluids. The primary antioxidant activity of blood plasma or serum and other extracellular fluids is mainly derived from low–molecular mass antioxidants and some macromolecules; the antioxidant enzymes usually have very low activities in serum or other extracellular fluids (9). Therefore, the antioxidant activity of plasma or serum may not be representative of the antioxidant capacity in tissues that contain these important antioxidant enzymes. In addition, the antioxidant activity depends on which ROS is generated, how it is generated, where it is generated, and what target of damage is measured (10). Using a different ROS generator and a different biological target for the ROS can yield different results. We have previously demonstrated *in vitro*, using the oxygen radical absorbance capacity (ORAC) assay system (7), that the ORAC of antioxidants, such as Trolox (a water-soluble vitamin E analog) and uric acid, decreased at high concentrations when Cu^{2+}-H_2O_2 was used as a ROS generator (11). This was not the case however

when ABTS$^+$ (8), peroxyl (3, 7), or luminol radicals (6) were used.

Since aging, according to the free radical theory (12), may be accompanied by an increased susceptibility to damage from ROS, it is of interest to examine antioxidant capacity in the tissues of both young and old animals. The objectives of this study were to determine the antioxidant capacity in heart, liver, lung, and kidney of young (6 months) and old (22 months) rats using the ORAC assay system with two different ROS generators (7, 11). The two ROS generators were 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), a peroxyl radical generator, and Cu^{2+}-H_2O_2, mainly a hydroxyl radical generator (11).

Materials and Methods

Chemicals. Porphyridium cruentum β-phycoerythrin (β-PE) was purchased from Sigma Chemical Co. (St. Louis, MO). The β-PE used in this study usually loses more than 90% of its fluorescence within 30 min in the presence of 4 m*M* AAPH. We have found that different manufacturing lots behave differently. R-phycoerythrin (R-PE) can also be used in place of the β-PE in the ORAC assay. AAPH was obtained from Polyscience (Warrington, PA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from Aldrich (Milwaukee, WI).

Animals. Fischer 344 rats were purchased from Harlan, Sprague & Dawley / NIA. Young (6 months) and old (22 months) male rats were used in this study. The use of animals was conducted in compliance with all applicable laws and regulations as well as the principles expressed in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health, USPHS. Rats were sacrificed by decapitation. After perfusion with normal saline, tissues (heart, lung, liver, and kidney) were collected and stored at −80°C until analyzed.

Preparation of samples. The cytosolic samples from heart, lung, liver, and kidney were prepared by homogenizing tissues in 75 m*M* phosphate buffer and separating the soluble fractions by two centrifugation steps (12,000*g* for 10 min, and 100,000*g* for 15 min at 4°C). The resulting cytosol and its nonprotein fraction were used in the antioxidant capacity assay. For preparation of a nonprotein fraction, the cytosol was diluted with 100% saturated ammonium sulfate (1:4, v/v) and kept in ice water for 10 min. The sample was then centrifuged at 16,000*g* for 30 min at 4°C, and the supernatant was removed as the nonprotein fraction of the cytosol for the measurement of antioxidant capacity. The protein content of a cytosolic sample was determined according to the method of Bradford (Bio-Rad Laboratories, Richmond, CA).

ORAC assay. The antioxidant capacity was determined by the ORAC assay using a peroxyl radical (ROO·) or hydroxyl radical (OH·) generator (7, 11). Briefly, in the final assay mixture (2 ml total volume), β-PE (3.34×10^{-8} *M*) was used as a target for free radical attack, AAPH (4 m*M*) as a peroxyl radical generator or Cu^{2+}-H_2O_2 (Cu^{2+}, 9 μ*M*; H_2O_2, 0.3%) as a hydroxyl radical generator, and Trolox as a control standard. Following addition of AAPH or Cu^{2+}-H_2O_2, the assay mixture was incubated at 37°C. Fluorescence of β-PE was measured every 5 min at the emission of 565 nm and excitation of 540 nm, using a fluorescence spectrophotometer, until zero fluorescence occurred, which usually was within 70 min. When the nonprotein fraction of a cytosolic sample was analyzed, 80% saturated ammonium sulfate in phosphate buffer instead of just the buffer itself was used as a blank. Final results were expressed as peroxyl radical absorbance capacity ($ORAC_{ROO\cdot}$) or hydroxyl radical absorbance capacity ($ORAC_{OH\cdot}$) units. One $ORAC_{ROO\cdot}$ unit is equal to the peroxyl radical absorbance capacity of 1 μ*M* (final concentration) Trolox in the ORAC assay system. One $ORAC_{OH\cdot}$ unit is equal to the hydroxyl radical absorbance capacity of 10 μM (final concentration) Trolox in the ORAC assay system. The ORAC of the protein fraction of a cytosolic sample was calculated by subtracting the ORAC of the nonprotein fraction from the ORAC of the total cytosol.

Statistical analysis. The effects of age on the ORAC and protein content of tissue cytosol from different organs were analyzed by analysis of variance (ANOVA) using SYSTAT (SYSTAT, Inc., Evanston, IL). Pairwise multiple comparisons were evaluated by Tukey's honestly significant difference (HSD) test used in SYSTAT. The effect of age on the protein content or ORAC of a single organ (e.g., liver) was also evaluated with Student's two-sample *t* test. Differences at $P \leq 0.05$ were considered significant.

Results

The protein content in cytosolic samples from different tissues of young and old rats are shown in Table I. The results of ANOVA showed that the protein content is significantly different among different tissues (heart and lung < kidney < liver). However, a significant effect of aging was observed only in liver and kidney; the cytosolic protein contents of these two organs in old rats were significantly lower than those in young animals.

The $ORAC_{ROO\cdot}$ of cytosolic samples from different tissues of young and old rats are shown in Figure 1 and 2. Based on the protein content of the cytosolic samples, no aging effect was observed on the $ORAC_{ROO\cdot}$ in any of the tissues examined (Fig. 1). The $ORAC_{ROO\cdot}$ of the cytosol (Fig. 1A) and the cytosol protein fraction (Fig. 1B) was significantly lower in both heart and liver tissue than that in lung and kidney

Table I. Cytosolic Protein Content of Different Tissues from Young and Old Rats (mg/g wet wt)

	Heart	Liver	Lung	Kidney
Young (6 months)	31.3 ± 0.8 (7)[a]	62.8 ± 1.7 (8)[b,*]	34.4 ± 3.6 (8)[a]	44.1 ± 1.2 (8)[c,†]
Old (22 months)	30.3 ± 1.7 (8)[a]	56.6 ± 1.9 (8)[b]	32.9 ± 1.9 (8)[a]	38.5 ± 1.1 (8)[c]

Note. Data are presented as mean ± SEM (*n*). Student's two-sample *t* test: young versus old, $^*P < 0.05$; $^\dagger P < 0.01$.
[a,b,c] Values not sharing a common superscript letter differ significantly, $P < 0.05$.

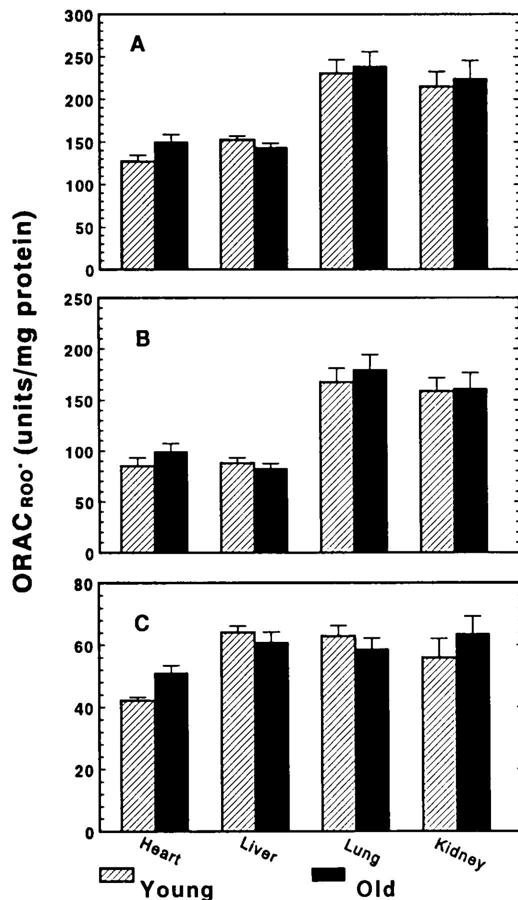

Figure 1. The peroxyl radical absorbance capacity ($ORAC_{ROO\cdot}$) of heart, liver, lung, and kidney tissues of young and old rats. The $ORAC_{ROO\cdot}$ was based on cytosol protein content. The data were presented as mean ± SEM of seven to eight samples. (A) Cytosol. ANOVA: effect of tissue, $F(3,55) = 24.7$, $P < 0.001$; heart or liver versus lung or kidney, $P < 0.001$. (B) Protein fraction of the cytosol. ANOVA: effect of tissue, $F(3,55) = 32.6$, $P < 0.001$; heart or liver versus lung or kidney, $P < 0.001$. (C) Nonprotein fraction of the cytosol. ANOVA: effect of tissue, $F(3,55) = 6.7$, $P < 0.001$; heart versus liver, lung or kidney, $P < 0.001$.

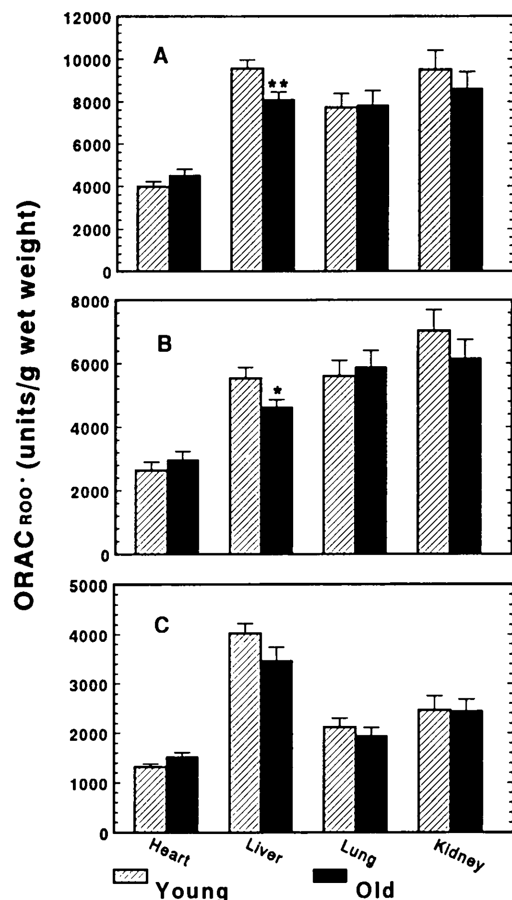

Figure 2. The peroxyl radical absorbance capacity ($ORAC_{ROO\cdot}$) of heart, liver, lung, and kidney tissues of young and old rats. The $ORAC_{ROO\cdot}$ was based on the wet weight of tissues. The data were presented as mean ± SEM of seven to eight samples. (A) Cytosol. Two-sample t test: **old versus young, $P = 0.02$. ANOVA: effect of tissue, $F(3,55) = 27.0$, $P < 0.001$; heart versus liver, lung, or kidney, $P < 0.001$. (B) Protein fraction of the cytosol. Two-sample t test: *old versus young, $P = 0.05$. ANOVA: effect of tissue, $F(3,55) = 24.6$, $P < 0.001$; heart versus liver, lung, or kidney, $P < 0.001$; liver versus kidney, $P < 0.01$. (C) Nonprotein fraction of the cytosol. ANOVA: effect of tissue, $F(3,55) = 46.2$, $P < 0.001$; liver versus heart, lung, or kidney, $P < 0.001$; heart versus lung or kidney, $P < 0.05$.

($P < 0.0001$). The $ORAC_{ROO\cdot}$ of the nonprotein cytosol fraction of heart tissue was significantly lower than that of any other organs examined (Fig. 1C).

However, based on the wet weight of the tissue used for the cytosolic sample preparation, a significant age effect was found on the $ORAC_{ROO\cdot}$ of cytosol from liver (Fig. 2A), which was significantly lower in old rats than in young rats ($P = 0.02$). This observed age effect was mainly due to the decrease of protein content in the liver cytosol (Table I). The protein fraction $ORAC_{ROO\cdot}$ of the liver cytosol in old rats was also significantly lower than in young rats ($P = 0.05$, Fig. 2B), while the nonprotein fraction $ORAC_{ROO\cdot}$ of the liver cytosol in old rats was not significantly different from that in young animals ($P = 0.13$; Fig. 2C). Of the different tissues analyzed, heart tissue had the lowest $ORAC_{ROO\cdot}$ in its cytosol (heart < lung, liver, and kidney; Fig. 2 A–C), and liver tissue had the highest nonprotein cytosolic $ORAC_{ROO\cdot}$ (heart < lung and kidney < liver; Fig. 2C).

The $ORAC_{OH\cdot}$ of cytosolic samples from different tissues of young and old rats are shown in Figure 3 and 4. Based on the cytosolic protein content, the results of the ANOVA demonstrated a significant main effect of age in cytosol ($F[1,54] = 3.91$; $P = 0.05$) (Fig. 3A) but not in the nonprotein or protein fraction of the cytosol. The old rats had a higher $ORAC_{OH}$ in cytosol than the young rats (Fig. 3A). However, the two-sample t test showed a significant age effect only in liver (for cytosol and the cytosol protein fraction, $P = 0.02$ and 0.04, respectively; Fig. 3A and B). Heart tissue had the lowest $ORAC_{OH\cdot}$ in cytosol and cytosolic fractions (heart < lung, liver, and kidney; Fig. 3 A–C). Kidney had the highest $ORAC_{OH\cdot}$ in nonprotein cytosolic fraction (heart < lung and liver < kidney; Fig. 3C).

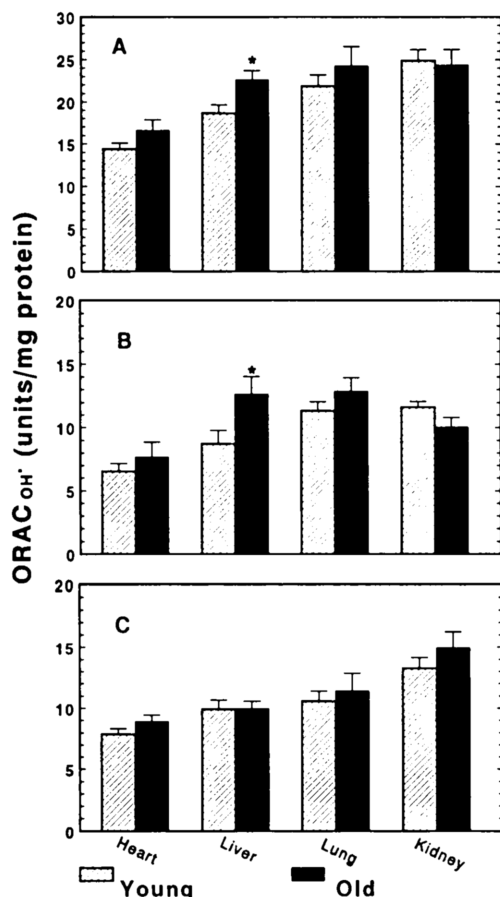

Figure 3. The hydroxyl radical absorbance capacity ($ORAC_{OH\cdot}$) of heart, liver, lung, and kidney tissues of young and old rats. The $ORAC_{OH\cdot}$ was based on the cytosol protein content. The data were presented as mean ± SEM of seven to eight samples. (A) Cytosol. Two-sample t test: *old versus young, $P < 0.05$. ANOVA: effect of age, $F(1,54) = 3.91$, $P = 0.05$; effect of tissue, $F(3,54) = 15.2$, $P < 0.001$; heart versus liver, lung, or kidney, $P < 0.001$; liver versus kidney, $P < 0.05$. (B) Protein fraction of the cytosol. Two-sample t test: *old versus young, $P < 0.05$. ANOVA: effect of tissue, $F(3,54) = 9.5$, $P < 0.001$; heart versus liver, lung, or kidney, $P < 0.01$. (C) Nonprotein fraction of the cytosol. ANOVA: effect of tissue, $F(3,55) = 46.2$, $P < 0.001$; kidney versus heart, lung, or liver, $P < 0.001$; heart versus lung, $P < 0.05$.

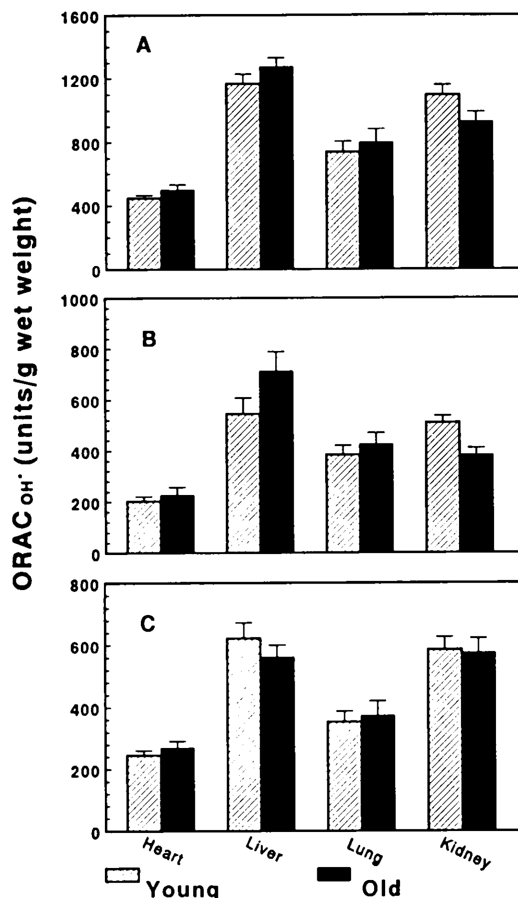

Figure 4. The hydroxyl radical absorbance capacity ($ORAC_{OH\cdot}$) of heart, liver, lung, and kidney tissues of young and old rats. The $ORAC_{OH\cdot}$ was based on the wet weight of tissues. The data were presented as means ± SEM of seven to eight samples (A) Cytosol. ANOVA: effect of tissue, $F(3,54) = 56.9$, $P < 0.001$; heart versus lung, kidney, or liver, $P < 0.001$; lung versus kidney or liver, $P < 0.01$; kidney versus liver, $P < 0.01$. (B) Protein fraction of the cytosol ANOVA: effect of tissue, $F(3,54) = 26.9$, $P < 0.001$; heart versus lung, kidney, or liver, $P < 0.01$; lung or kidney versus liver, $P < 0.01$. (C) Nonprotein fraction of the cytosol. ANOVA: effect of tissue, $F(3,55) = 34.2$, $P < 0.001$; heart or lung versus liver or kidney, $P < 0.001$.

When the $ORAC_{OH\cdot}$ was expressed on the basis of the wet weight of the tissue used for the cytosolic sample preparation, no effects of age were found by ANOVA in those tissues examined. The $ORAC_{OH\cdot}$ was also significantly different among different tissues in cytosol (heart < lung < kidney < liver; Fig. 4A), the protein fraction (heart < lung and kidney < liver; Fig. 4B) and nonprotein fraction (heart and lung < kidney and liver; Fig. 4C) of the cytosol.

The cytosolic samples were separated into two fractions, the protein fraction and non-protein fraction; the low-molecular mass antioxidants would be found mainly in the latter. The contribution of non-protein fraction to the antioxidant capacity ($ORAC_{ROO\cdot}$ and $ORAC_{OH\cdot}$) of cytosol from different tissues of young and old rats is shown in Table II. The

Table II. The Contribution of the Nonprotein Fraction to the Antioxidant Capacity of Cytosol of Different Tissues of Young and Old Rats

	Heart	Liver	Lung	Kidney
$ORAC_{ROO·}$				
Young	0.341 ± 0.028 (7)[a]	0.424 ± 0.019 (8)[b]	0.276 ± 0.011 (8)[c]	0.260 ± 0.018 (8)[c]
Old	0.346 ± 0.021 (8)[a]	0.426 ± 0.024 (8)[b]	0.249 ± 0.008 (8)[c]	0.285 ± 0.013 (8)[d]
$ORAC_{OH·}$				
Young	0.551 ± 0.034 (7)	0.540 ± 0.042 (8)	0.481 ± 0.019 (8)	0.531 ± 0.012 (8)
Old	0.553 ± 0.045 (8)[a,b]	0.412 ± 0.020 (7)[c,*]	0.465 ± 0.021 (8)[a,c]	0.586 ± 0.022 (7)[b]

Note. The contribution of nonprotein fraction to the antioxidant capacity of a cytosolic sample = ORAC measured in the nonprotein fraction of a cytosolic sample/ORAC measured in the cytosolic sample. Data are presented as mean ± SEM (*n*). Student's two-sample *t* test, young versus old, $^*p < 0.05$.
[a,b,c] Values, within an age group, not sharing a common superscript letter differ significantly, $P < 0.05$.

contribution of the nonprotein fraction to the $ORAC_{ROO·}$ of cytosol was 25%–43% (kidney and lung < heart < liver; ANOVA: effect of tissue, $P < 0.001$; effect of age, $P > 0.05$; age × tissue, $P > 0.05$) and for $ORAC_{OH·}$ was 41%–59% (for old rats: liver < heart and kidney; lung < kidney; ANOVA: effect of tissue, $P < 0.01$; effect of age, $P > 0.05$; age × tissue, $P < 0.05$), respectively. A significant effect of age ($ORAC_{OH·}$ related) was observed also only in liver cytosol. The contribution of nonprotein fraction to the $ORAC_{OH·}$ of liver cytosol was significantly lower in old rats than in young rats ($P = 0.02$).

Discussion

In the reported experiments, two different ROS generators were used (i.e., AAPH and Cu^{2+}-H_2O_2) to measure the antioxidant capacity of cytosolic samples from rat heart, liver, lung, and kidney. AAPH is a peroxyl radical (ROO·) generator, and Cu^{2+}-H_2O_2 is usually considered to be a hydroxyl radical (OH·) generator, although other ROS, such as $O_2^{·-}$ and $HO_2·$, are also produced in this system (11). $O_2^{·-}$ and $HO_2·$ themselves are weak antioxidants, while OH· is the most reactive and harmful ROS found in the body. ROO· is less reactive than OH· and thus possesses an "extended" half-life of seconds instead of nanoseconds.

In this study, the cytosolic sample was separated into two fractions (i.e., nonprotein and protein). The ORAC was measured in cytosol and in the nonprotein fraction of the cytosol. The ORAC of the protein fraction of the cytosol was computed by difference. The antioxidant activity of the cytosol is from all components in the cytosol, while the antioxidant activity of the nonprotein fraction of the cytosol is derived from low–molecular mass antioxidants, such as ascorbic acid, α-tocopherol and uric acid in the $ORAC_{ROO·}$ assay, and glucose, uric acid, and mannitol in the $ORAC_{OH·}$ assay. The antioxidant activity of the protein fraction of the cytosol is mainly from proteins. However, in general, the ORAC assay does not measure directly the specific activities of antioxidant enzymes.

The $ORAC_{ROO·}$, or the peroxyl radical absorbance capacity, expressed as units per milligram of cytosolic protein or per gram of tissue (wet weight), of cytosol from heart, lung, and kidney of the old rats was not significantly different from that of the young rats. The only age effect observed on the $ORAC_{ROO·}$ (units/g wet wt) was in liver tissue. In old rats, the $ORAC_{ROO·}$ of liver cytosol was significantly lower than that in young rats. The $ORAC_{ROO·}$ of the protein fraction of the liver cytosol of the old rats was also significantly lower than that of the young rats ($P = 0.05$). The $ORAC_{ROO·}$ of the nonprotein fraction of the liver cytosol of the old rats was not significantly lower ($P = 0.13$) than that of the young rats. The lower $ORAC_{ROO·}$ (units/g wet wt) found in liver cytosol and the protein fraction of the liver cytosol in old rats can be largely attributed to the loss of liver cytosol protein in these old animals. Our results indicated clearly that, compared with the young rats, the old rats had significantly less cytosol protein in both liver and kidney. The $ORAC_{ROO·}$ (units/g wet wt) of kidney also tended to decrease with aging, although this difference was not significant.

When the data are expressed on the basis of the cytosol protein content of a tissue, the hydroxyl radical absorbance capacity ($ORAC_{OH·}$) of cytosol generally increased with aging, especially in liver. This aging effect found in liver cytosol was also due to changes in the protein fraction of the cytosol, since the $ORAC_{OH·}$ of the nonprotein fraction of the cytosol was not affected by aging. However, the increased $ORAC_{OH·}$ (units/mg protein) in the protein fraction of the liver cytosol in old rats appeared to be a result of change in quality, not quantity of the cytosol protein. The resistance of proteins to the attack of ROS, especially OH·, may increase with aging.

Glycation of proteins, which can be mediated synergistically by ROS, is one of the possible mechanisms underlying the increased resistance of soluble protein

to the attack of OH· and other ROS. Glucose is an effective OH· quencher with a rate constant comparable to that of mannitol (9). The evidence for the glycation hypothesis of aging was demonstrated in the food-restricted rodent model (13). Recently, an emerging hypothesis of aging, the free radical–glycation/Maillard reaction theory of aging, was also proposed (14).

Our results show that 25%–43% of the peroxyl radical absorbance capacity in cytosol was due to its nonprotein fraction; while the hydroxyl radical absorbance capacity in cytosol was almost equally due to its protein and nonprotein fractions. The significant decrease with age in the contribution of nonprotein fraction, or stated in other terms, the significant increase with age in the contribution of the protein fraction to the $ORAC_{OH\cdot}$ of liver cytosol supports the finding that increased protein fraction $ORAC_{OH\cdot}$ (units/mg protein) of liver cytosol in old rats was due to an increased resistance of protein to the attack of ROS.

The two different ROS generators, AAPH (a ROO· generator) and Cu^{2+}-H_2O_2 (mainly a OH· generator), used in ORAC assay produced differing results in these experiments. Thus, the antioxidant capacity measured depends upon which ROS are generated (10). $ORAC_{ROO\cdot}$ measures all common antioxidants including ascorbate, α-tocopherol, β-carotene, glutathione, billirubin, uric acid (7, 15), and proteins; while the $ORAC_{OH\cdot}$, measured using Fenton reagents, measures glucose, mannitol, uric acid (at physiological concentrations), proteins, and transition metal chelators, but not ascorbate (11). The effects of glycation of proteins, as happens with aging, may be detected by the $ORAC_{OH\cdot}$, but not by the $ORAC_{ROO\cdot}$ assay.

The influence of aging on the concentrations of some low–molecular mass antioxidants was previously investigated systematically in male Fischer 344 rats by Rikans and Moore (16). They found that aqueous-phase antioxidant levels in different tissues were not uniformly affected by the aging process (16). For example, based on the wet weight of tissues, ascorbic acid levels decreased with aging in liver and lung, and were unaffected in heart and kidney. Glutathione levels increased with aging in kidney, and were unaffected in liver, heart, and lung tissues. Uric acid levels showed a decrease with aging in liver, no change in lung, and an increase in heart and kidney tissues (16). In our study, no significant age effect on the cytosol nonprotein fraction $ORAC_{ROO\cdot}$ or $ORAC_{OH\cdot}$ was observed in any examined tissues, whether the results were expressed as units per milligram of protein or units per gram of wet weight. These results indicate a similarity in antioxidant capacity of low–molecular mass antioxidants between the young and old rats, and further suggest that aging processes have no significant effect on the overall antioxidant status provided by these low–molecular mass antioxidants.

Glutathione appears to be the main contributor to the antioxidant capacity of nonprotein fraction in these tissues because of its high concentration in tissues (16). The concentration of ascorbic acid, glutathione, and uric acid in liver tissues of male Fischer rats was reported to be 0.88–1.86, 7.42–8.19, and 0.19–0.26 μmol/g wet wt, respectively (16), which equals 207–437 (7), 2282–2518 (15), and 88–122 (7) $ORAC_{ROO\cdot}$ units/g wet wt. The $ORAC_{ROO\cdot}$ of the nonprotein fraction of liver cytosol measured in this study was 3470–4027 units/g wet wt. Therefore, if we assume that most of liver low–molecular mass antioxidants were in the nonprotein fraction, the contribution of ascorbic acid, glutathione, and uric acid to the $ORAC_{ROO\cdot}$ of the nonprotein fraction of liver cytosol would be around 9%, 64%, and 3%, respectively. The concentrations of α-tocopherol and β-carotene in rat liver tissue are usually less than 0.1 and 1 μmol/g wet wt, respectively (17–20). Their contribution to the $ORAC_{ROO\cdot}$ of the nonprotein fraction of liver cytosol would also be less than 10%.

The antioxidant capacity differed among the various tissues. Generally, liver and kidney had higher $ORAC_{ROO\cdot}$ and $ORAC_{OH\cdot}$ (units/g wet wt) than did heart and lung. This finding can be partially explained by the higher cytosol protein content measured in these two tissues. The highest nonprotein fraction $ORAC_{ROO\cdot}$ (units/g wet wt) was found in rat liver tissue as well, which can be further attributed to the high concentration of glutathione measured in the liver (16). Liver is the major organ for *de novo* glutathione synthesis and supplies 90% of the circulating glutathione (20). The relative higher nonprotein $ORAC_{ROO\cdot}$ and $ORAC_{OH\cdot}$ found in kidney may be explained by the high urate content in this organ. The lowest antioxidant capacity was found in heart tissue. However, other experimental results from our laboratory (21) indicated that the antioxidant capacity ($ORAC_{ROO\cdot}$ and $ORAC_{OH\cdot}$) in rat brain, including brain cortex and cerebellum, was even lower than that in heart. Thus, brain and heart may be more susceptible to oxidative stressors than other tissues.

In summary, the effect of age on the antioxidant capacity of different tissues was investigated in this study. The results demonstrated that old rats had significantly lower $ORAC_{ROO\cdot}$ (units/g wet wt), but higher $ORAC_{OH\cdot}$ (units/mg protein) in liver tissue than young rats. The decreased $ORAC_{ROO\cdot}$ (units/g wet wt) in the liver tissue of old rats was mainly due to the loss of cytosol protein, while the increased $ORAC_{OH\cdot}$ (units/mg protein) in the old rats could be attributed to an increased resistance of cytosol proteins to the attack of ROS. The aging processes have no significant effect

on the overall antioxidant status provided by nonprotein antioxidants. The antioxidant capacity was usually high in liver and kidney but low in lung and heart tissues.

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