Oxidative Status in Senescence-Accelerated Mice

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OXYGEN radicals attack cell structure and impair cell functions. If this oxidative stress exceeds defending capacity, it leads to a number of pathological conditions (Halliwell and Gutteridge, 1989; Yu, 1994). Aging is included as one of these conditions proposed by Harman (1956), who saw the chronic accumulation of oxidative damage in cells as part of the aging process.

Many lines of evidence supporting this free radical theory of aging were extensively reviewed by Yu (1993, 1994). These include an increase with age in the content of lipid peroxides in tissues of insects (Sohal et al., 1981) and rats (Laganiere and Yu, 1989), a positive correlation between life span and several major free radical scavengers (Cutler, 1985), a decrease of oxidative DNA damage in food-restricted rats that live longer than rats fed ad libitum (Chung et al., 1992), and so forth. This free radical theory is further supported by experiments showing the extension of life span by enhancing the ability to remove oxygen radicals as observed in mice fed with antioxidant-supplemented diets (Heicklen and Brown, 1987) and in transgenic flies carrying elevated levels of Cu,Zn-superoxide dismutase (SOD) and catalase genes (Orr and Sohal, 1994).

Takeda et al. (1981, 1991) developed a murine model of senescence-accelerated mice (SAM) which they believed would be useful in a variety of gerontological studies. The senescence-prone strain (SAM-P/1) has a mean life span of 9 months, in comparison to 13 months in the senescence-resistant strain (SAM-R/1). SAM-P/1 also manifests a number of aging signs, both in gross appearance and histology, earlier than SAM-R/1, even though both substrains were developed from the same ancestor mice.

Kohno et al. (1985) observed that SAM-P/1 on food restriction live as long as SAM-R/1. It was also observed that SAM-P/1 show a higher level of lipid peroxides in brain than SAM-R/1 (Choi et al., 1994), and SAM-P/8 (a sub-strain of SAM showing accelerated deterioration of learning and memory) show a higher level of lipid peroxides and a lower level of glutathione in tissues than ddY mice exhibiting normal aging (Liu and Mori, 1993). These findings led us to assume that increased oxidative stress may be one of the factors contributing to accelerated senescence observed in SAM-P/1. In an attempt to test this hypothesis, this study was designed to compare age-dependency differences in the oxidative status between the two SAM strains by examining lipid peroxidation and the activities of antioxidant enzymes in tissues.

In this experiment, we found that at all ages, the livers of SAM-P/1 show a higher level of lipid peroxidation and lowered specific activity and amount of Cu,Zn-SOD in mitochondria of this organ, but no apparent difference in the mRNA levels. These results indicate that impaired transport of Cu,Zn-SOD into mitochondria after cytosolic synthesis reduces defense against oxidative stress. That reduction may be one of the factors contributing to accelerated senescence in SAM-P/1.

METHODS

Animals
SAM-P/1 and SAM-R/1 mice were obtained from Prof. T. Takeda of Kyoto University. They were reared under conventional conditions at 24 ± 2 °C, fed with a commercial diet (CE-2; Nihon CLEA, Tokyo, Japan) and ad libitum tap water, and were bred up to the 20th generation.

Materials
Glutathione reductase, Denhardt's solution, and oligo(dT) cellulose were purchased from Sigma Chemical (St. Louis, MO). BamHI and HindIII endonucleases were purchased from Boehringer Mannheim (Sandhofer Strasse, Germany). Other chemicals used were of reagent grade. Polyclonal sheep anti-human Cu,Zn-SOD and anti-human Mn-SOD antibodies were purchased from Biodesign (Kennebunk, ME). The Cu,Zn-SOD probe for Northern blot analysis was pre-
pared from pUC118 containing human Cu,Zn-SOD cDNA, which was obtained from Dr. Haruka Toyoda (Kitasato University). *Escherichia coli* IM103 cells were transfected with this recombinant plasmid according to Sambrook et al. (1989). Recombinant plasmids were isolated by the alkali lysis method after the chloramphenicol-amplified culture of an ampicillin-resistant colony in the presence of 5-bromo-4-chloro-3-indolyl (X-Gal) and isopropyl β-D-thiogalactoside (IPTG). A DNA probe (0.56 kb), obtained by the digestion of *Bam*II and *Hind*III, was prepared by electrophoresis on a 1% agarose gel followed by electroelution. The DNA probe was labeled by a random priming technique by using [α-32P]dATP (Feinberg and Vogelstein, 1983). A Mn-SOD probe for Northern blot analysis, 30-mer oligonucleotide (5'-GACCTGCCTT ACG ACT ATGGGCGCTGG AG-3', positions 38–168; Hallweil et al., 1986) was synthesized by using the phosphotriester approach. The synthetic oligonucleotides were detritylated using an oligonucleotide purification cartridge. The oligonucleotides were further purified by polyacrylamide gel electrophoresis and labeled by kination with [γ-32P]dATP (Maxam and Gilbert, 1977).

**Measurement of Fluorescent Age Pigment and Conjugated Dienes**

Fluorescent age pigment (FAP) and conjugated dienes (CDs) were measured as markers for lipid peroxidation. FAP was measured according to the method described by Sheldahl and Tappel (1974), with slight modification. Fresh livers were homogenized briefly in 10 volumes (vol/wt) of chloroform-methanol mixture (2:1 vol/vol). An aliquot (6 ml) of the homogenate was mixed with 3 ml of deionized water and centrifuged at 3000 × g for 20 min. After washing with 3 ml of deionized water, 3 ml of the chloroform extract was assayed for FAP at excitation 366 nm and emission 435 nm using a spectrofluorometer (model SFM 25; Kontron, Zurich, Switzerland). A 0.001% quinine sulfate solution was used for the standard on a scale of 100. CDs were measured according to Steenken and Glende’s method (1984). Two milliliters of the chloroform extract of liver homogenates obtained from the above procedure were dried at 50 °C; after dissolving the dried product in 3 ml of cyclohexane, the solution was assayed for CDs at 250 nm using a spectrophotometer (model 8452A; Hewlett-Packard).

**Enzyme Assays**

The two fractions, cytosolic and mitochondrial, were assayed for four antioxidant enzymes: SOD, catalase, glutathione peroxidase, and glutathione reductase.

**Tissue fractions.** — Both cytosolic and mitochondrial fractions were prepared for the assays of antioxidant enzymes. In the present experiments, we emphasized mitochondrial enzymes because of the organelle’s role in generating oxygen radicals. To prepare this cytosolic fraction, freshly isolated livers were excised and homogenized in 10 volumes of cold isotonic buffer (10 mM potassium phosphate, pH 7.4/0.25 M sucrose/1 mM EDTA). We centrifuged the homogenate at 1000 × g for 10 min, then filtered the supernatant through four layers of cheesecloth. It was again centrifuged at 10,000 × g for 15 min to obtain mitochondria. After centrifuging the supernatant at 100,000 × g for 60 min, the resulting supernatant was used as a cytosolic fraction. The mitochondrial fraction was prepared from the pellet precipitated by the above centrifugation at 10,000 × g. The mitochondrial pellet was washed three times with the previously described isotonic buffer by suspension-centrifugation, then stored at −70 °C. The frozen pellet was suspended in cold hypotonic buffer (1 mM EDTA/50 mM potassium phosphate, pH 7.4). After thawing the pellet, we used a sonicator (model W-385; Heat System/Ultrasonics) at 180 W for 3 min to disrupt mitochondria. The sonicate was clarified by centrifugation at 100,000 × g for 30 min and used for the mitochondrial fraction. All the procedures were performed at 4 °C, and protein amounts were determined by the method of Lowry et al. (1951).

**SOD (spectrophotometric method).** — The method described by Misra and Fridovich (1972) was used, which measures the inhibition of adrenochrome formation from epinephrine by SOD. A reaction medium (1 ml) containing 50 mM NaHCO3-Na2CO3 buffer (pH 10.2), 10 mM epineph- rine, and 1 mM EDTA was monitored at 30 °C for the autoxidation rate of epinephrine to adrenochrome at 325 nm with a UV-spectrophotometer (model 8452A; Hewlett-Packard). Each of the fractions was added to this medium to observe the autoxidation inhibition. One unit of SOD is defined as the activity required for 50% inhibition under this condition.

**SOD (activity band method).** — For two purposes — as a check for the spectrophotometric method and to determine SOD type — SOD was assayed by the activity band method as described by Beauchamp and Fridovich (1971), using nondenaturing gel electrophoresis according to Davis’ method (1964). The tissue fractions obtained from six livers of each 12-month-old SAM strain were pooled and then applied to the slab gel (10% and 4% of polyacrylamide for separating and stacking gels, respectively) and electrophoresed at a constant current of 1 mA per well. The activity bands of SOD were made visible by soaking the gel in a staining solution containing 20 mM potassium phosphate (pH 7.0), 1 mM nitro blue tetrazolium, 20 mM tetramethyl ethylenediamine, and 30 μM riboflavin for 15 min. When SOD type was determined, the gel was treated with 2 mM potassium cyanide solution for 15 min before the staining procedure. After a brief rinse with deionized water, the SOD bands were visualized by illumination with a fluorescent lamp (20 W), and band density was determined by using a densitometer (model GS-670; Bio-Rad, Richmond, CA).

**Catalase.** — We measured catalase activity by monitoring the initial rate of hydrogen peroxide disappearance as described by Claiborne (1986). The tissue fractions were added to a reaction medium (3 ml) containing 50 mM potassium phosphate (pH 7.0), 12 mM hydrogen peroxide, and 1 mM EDTA. The initial absorbance decrease rate at 240 nm was monitored at 30 °C. One unit of this enzyme is defined as the activity to consume 1 μmol of hydrogen peroxide per minute.
Glutathione peroxidase. — This assay was performed according to DelMaestro and McDonald’s method (1985), using t-butylhydroperoxide as a substrate. The tissue fractions were added to a reaction medium (1 ml) containing 50 mM potassium phosphate (pH 7.0), 1.2 mM t-butylhydroperoxide, 1 mM reduced glutathione, 0.15 mM reduced nicotinamide-adenine dinucleotide phosphate (NADPH), 0.24 unit glutathione reductase, and 1 mM EDTA. Enzyme activity was monitored as a rate of NADPH oxidation at 340 nm at 30 °C. One unit of this enzyme is defined as the activity to consume 0.5 µmol of NADPH per minute.

Glutathione reductase. — We determined glutathione reductase activity by monitoring the oxidation of NADPH at 340 nm in a reaction mixture (1 ml) containing 50 mM potassium phosphate (pH 7.0), 2.2 mM oxidized glutathione, 0.15 mM NADPH, 1 mM EDTA, and a tissue fraction at 37 °C (Goldberg and Spooner, 1983). One unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 0.5 µmol of NADPH per minute.

Immunoblot Analysis
The amounts of Cu,Zn-SOD and Mn-SOD in cytosolic and mitochondrial fractions were determined with immunoblot analysis by using polyclonal sheep anti-human Cu,Zn-SOD (no. K90077C; Biodesign) and sheep anti-human Mn-SOD (no. K90066C; Biodesign). Aliquots of pooled cytosolic and mitochondrial fractions obtained from six livers of each 12-month-old SAM substrain were electrophoresed on discontinuous SDS/polyacrylamide gels (12.5%), and the separated proteins in the gels were electrotransferred onto nitrocellulose membranes with a current of 100 mA for 6 h. Nitrocellulose membranes were saturated with a solution of 3% non-fat dry milk and cut into strips. The nitrocellulose strips were then incubated with alkaline phosphatase conjugated goat anti-sheep IgG immunoglobulin. To detect SOD bands, the strips were treated with a solution of 0.1 M Tris-HCl (pH 9.5), 0.24 unit glutathione reductase, and 1 mM EDTA. Enzyme activity was monitored as a rate of NADPH oxidation at 340 nm at 30 °C. One unit of this enzyme is defined as the amount of enzyme that catalyzes the oxidation of 0.5 µmol of NADPH per minute.

Northern Blot Analysis
Total cellular RNA was isolated from four livers of each 12-month-old SAM substrain by using the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski et al., 1987). The poly(A) + RNA was purified from the pooled cellular RNA using oligo(dT)-cellulose chromatography (Aviv et al., 1972). We electrophoresed the poly(A) + RNAs on 1.2% agarose/6.6% formaldehyde gels, then transferred them to nitrocellulose membranes. The membranes were incubated at 37 °C in a prehybridization solution (25 mM potassium phosphate, pH 7.4/5 × saline sodium citrate (SSC); 0.075 M sodium citrate, pH 7.0/0.75 M NaCl)/5 × Denhardt’s solution (0.1% Ficoll, polyvinylpyrrolidone, and bovine serum albumin)/50 µg/ml salmon sperm DNA/50% formamide]. We finished the process by placing the membranes in a hybridization solution (prehybridization solution containing 5% dextan sulfate) for 24 hr at 42 °C. The membranes were washed twice in 1 x SSC/0.1% sodium dodecyl sulfate (SDS) and twice in 0.5 x SSC/0.1% SDS for 15 min at room temperature, and then autoradiographed.

Statistics
The results are expressed as means ± SE. The age-related increasing rates of FAP and CDs were compared between two substrains by using a linear regression analysis and t-test. A Student’s t-test and an analysis of variance (ANOVA) followed by Duncan’s multiple range test were performed in comparing the SOD activity at one age and at various ages, respectively. Differences were considered significant when p < .05.

RESULTS

Content of FAP and CDs. — Levels of FAP and CDs in the tissues of two SAM substrains were compared with the function of aging to examine differences in oxidative stress. Figure 1A illustrates the result of FAP in livers. FAP content increased gradually with age in the livers of both SAM

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**Figure 1.** Comparison of lipid peroxidation in livers between SAM-R/1 (R/1) and SAM-P/1 (P/1). Lipid peroxidation at various ages was determined by measuring fluorescent age pigment (A) and conjugated dienes (B), as described in Methods. Fluorescent intensity on ordinate in A is in arbitrary units. Each point in both panels represents the mean ± SE of six experiments. The slopes (mean ± SE per month) for R/1 and P/1 in A were 12.59 ± 1.47 and 26.50 ± 2.43, respectively, and those for R/1 and P/1 in B were 0.0181 ± 0.0014 and 0.0335 ± 0.0025, respectively. The slopes for R/1 and P/1 in each panel were significantly different (p < .01; linear regression analysis and t-test).
substrains. However, at every stage after 4 months, the FAP content in SAM-P/1 was higher than that in SAM-R/1. After 8 months, differences between the two substrains were significant. In Figure 1B, CD content shows a similar result. Other organs tested, such as the brain, kidney, heart, and lung, did not show such significant differences as observed in liver (data not shown). These results indicate that the liver of SAM-P/1 is subject to more oxidative insult than that of SAM-R/1, and that differences in oxidative insult increase with age. At 16 months of age, we only have the data from the SAM-R/1 sample, as most of the SAM-P/1 died by that time.

Activities of antioxidant enzymes. — To assess overall oxidative status of the livers from the two SAM substrains, we monitored the activity of four antioxidant enzymes: SOD, glutathione peroxidase, catalase, and glutathione reductase. Before comparing the influence of age, preliminary examination was conducted on the substrains at 12 months of age (Figure 2). In the cytosolic fraction, none of the four enzymes showed significant differences in activity between SAM-P/1 and SAM-R/1. In the mitochondrial fraction, the activities of all four enzymes were lower in SAM-P/1 than in SAM-R/1, but the difference was statistically significant (p < .01) only in SOD. The SOD in SAM-P/1 showed only 60% the activity of SAM-R/1. The significant difference in SOD activity at 12 months prompted us to compare the SOD activity between the substrains at other ages. As shown in Figure 3, the activity in SAM-P/1 was only 50–60% that of SAM-R/1 at all ages examined. It is important to note that the difference in SOD activity was measured after birth and remained virtually unchanged through life. The other three enzymes were also examined at different ages, but no differences were apparent (Figure 4).

The SOD activity observed in Figures 2 and 3 may not accurately reflect the total activity contributed by both Cu,Zn-SOD and Mn-SOD in mitochondria because the assay was done at pH 10.2 where Cu,Zn-SOD is approximately 4 times more active than Mn-SOD (Misra, 1985). Therefore, the difference in mitochondrial SOD activity was checked again in liver mitochondrial fractions obtained from the 12-month-old SAM substrains. As expected, mitochondrial fractions showed two different activity bands (indicated by arrows 1 and 2 in lanes 3–6 in Figure 5A). On densitometry, the total density of the two bands of SAM-P/1 (lane 6 in Figure 5A) was 68% that of SAM-R/1 (lane 5 in Figure 5A; Table 1). Thus, we still found the difference in mitochondrial SOD activity between two substrains in the activity band assay. But the activity difference decreased a little compared to the result in Figure 3. This is due to the accurate measurement of Mn-SOD activity by the band assay method.

Determination of the type of mitochondrial SOD showing decreased activity in SAM-P/1. — The activity band method was further used to determine which type of SOD showed decreased activity in mitochondria of SAM-P/1. The cytosolic fractions obtained from two 12-month-old SAM substrains showed only one band (arrow 2 in lanes 1 and 2 in Figure 5A). These bands indicate Cu,Zn-SOD in the cytosols of mammalian cells. The two activity bands had nearly identical densities. This result is consistent with the finding in Figure 2, which showed no significant differences in the cytosolic fractions of SAM-P/1 and SAM-R/1. On the other hand, mitochondrial fractions show two bands (arrows 1 and 2 in lanes 3–6 in Figure 5A). Lower bands indicated by arrow 2 appeared at the same position as the cytosolic enzyme, indicating it is Cu,Zn-SOD. In contrast to the cytosolic enzyme, however, the density of this band of SAM-P/1 (lane 6 in Figure 5A) was 49% that of SAM-R/1.
The upper bands indicated by arrow 1 in lanes 3–6 in Figure 5A are Mn-SOD, since the bands were still visible in the staining solution containing KCN (lanes 3–6 in Figure 5B). The density of the upper bands in SAM-P/1 (lane 6 in Figure 5A) was almost the same as that in SAM-R/1 (lane 5 in Figure 5A; Table 1). In contrast to the upper bands, the lower bands of mitochondrial fractions, as well as the bands of cytosolic fractions, did not develop during KCN treatment (Figure 5B). We concluded from these results that the lower bands of mitochondrial fractions illustrating differences in band density are Cu,Zn-SOD and that the activity difference of mitochondrial SOD between the two SAM substrains originates from the difference of Cu,Zn-SOD activity.

Immunoblot and Northern blot analysis. — The type of SOD in each fraction of the substrains was compared for the amount of protein and mRNA by using blot analyses. On the immunoblot analysis, Cu,Zn-SOD in cytosol did not show a difference in its amount between SAM-P/1 and SAM-R/1 (lanes 1 and 2 in Figure 6A). Conversely, a significant difference was seen in mitochondrial Cu,Zn-SOD between two SAM substrains (lanes 3–4 in Figure 6B). But, as seen in Figure 6C, no differences were found in the Mn-SOD of the mitochondrial fractions from the two SAM substrains (lanes 1 and 2 in Figure 6C). The results of Northern blot analyses of mRNAs are shown in Figure 7. It was found that both Cu,Zn-SOD and Mn-SOD showed no differences in the amounts of mRNA in the two SAM substrains.

DISCUSSION

The results of the experiments presented here show that senescence-prone mice, SAM-P/1, are more susceptible to oxidative stress than senescence-resistant mice, SAM-R/1, and that mitochondria of SAM-P/1 livers are less capable of removing oxygen radicals than those of SAM-R/1 livers. The evidence for the increased susceptibility in SAM-P/1 livers is that the levels of lipid peroxidation products are higher in the livers of SAM-P/1 than those of SAM-R/1 (Figure 1). The lower defense capacity against oxygen free radical of mitochondria in SAM-P/1 livers is supported by the finding that the mitochondrial SOD activity in SAM-P/1 livers is much lower than that in SAM-R/1 livers (Table 1 and Figure 3). The other antioxidant enzymes in mitochondria show no activity differences in both substrains (Figure 2). The lower activity of mitochondrial SOD in SAM-P/1 is attributed to the reduction in the activity and amount of Cu,Zn-SOD, because in the activity band assay (Table 1 and Figure 5) and in the amount analysis by immunoblot (Figure 6), only the densities of Cu,Zn-SOD bands in SAM-P/1 are reduced to half those in SAM-R/1, whereas the densities of Mn-SOD bands are almost identical in both substrains. In view of the fact that mitochondria serve as a major source of cellular oxygen free radicals, the lower capacity of mitochondria in removing oxygen free radicals may be one of the factors contributing to increased oxidative stress observed in SAM-P/1.

Since Harman (1956) introduced the free radical hypothesis of aging, the involvement of oxidative stress in the aging process has been confirmed by increases of lipid peroxidation in tissues with age. For example, in houseflies, lipid peroxidation products increase in the tissues of aged flies (Sohal et al., 1981). Similarly, in older rats, peroxidation products increase in the brain (Yoshikawa and Hirari, 1967) and in expired gas (Sagai and Ichinose, 1980). When milk-

![Figure 3. SOD activity in mitochondrial fraction of SAM livers at various ages. Mitochondrial fractions were obtained from livers of SAM-R/1 (R/1) and SAM-P/1 (P/1) at various ages and assayed for SOD, as described in Methods. Each point represents the mean ± SE of six experiments. *Significant difference between groups with same age (p < .01; Duncan’s multiple range test).](https://academic.oup.com/biomedgerontology/article/51A/5/B337/578709)

![Figure 4. Activities of antioxidant enzymes in mitochondrial fraction of SAM livers at various ages. Mitochondrial fractions were obtained from livers of SAM-R/1 (R/1) and SAM-P/1 (P/1) at various ages and assayed for catalase, glutathione (GSH) peroxidase, and glutathione (GSSG) reductase as described in Methods. Each point indicates the mean ± SE of six experiments.](https://academic.oup.com/biomedgerontology/article/51A/5/B337/578709)
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Figure 5. Activity band assay of superoxide dismutase in SAM livers. Cytosolic and mitochondrial fractions obtained from livers of 12-month-old SAM-R/1 (R/1) and SAM-P/1 (P/1) were applied to nondenaturing polyacrylamide gels. After electrophoresis, gels were stained to observe the activity band of superoxide dismutase (A) or stained after the gel was treated with 2 mM KCN (B). Lanes 1 and 2, 0.25 μg protein of cytosolic fractions; lanes 3 and 4, 3 μg protein of mitochondrial fraction; lanes 5 and 6, 6 μg protein of mitochondrial fraction. The details are described in Methods.

Table 1. Densitometric Comparison of Activity Bands of Mitochondrial SOD on Assay Gel

<table>
<thead>
<tr>
<th>Type of SOD</th>
<th>SAM-R/1</th>
<th>SAM-P/1</th>
<th>SAM-P/1: SAM-R/1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn-SOD</td>
<td>0.51</td>
<td>0.46</td>
<td>90</td>
</tr>
<tr>
<td>Cu,Zn-SOD</td>
<td>0.59</td>
<td>0.29</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>1.10</td>
<td>0.75</td>
<td>68</td>
</tr>
</tbody>
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Note: Densitometry was performed on lanes 5 and 6 in Figure 5A. The numbers are the integral values of peaks obtained from scanning the bands in the gel. Mn-SOD and Cu,Zn-SOD are the bands indicated by arrows 1 and 2, respectively.

weed bugs' metabolic rate is increased by raising the ambient temperature, survival rate drops with age, and at the same time, the amount of lipid peroxidation in tissues increases (McArthur and Sohal, 1982). Considering these reported results, it is quite possible that the aging acceleration observed in SAM-P/1 is related to increases of oxidative stress demonstrated in our study. In this sense, data we obtained from the animal model of aging may also support the free radical theory of aging.

Several groups have already examined oxidative status in SAM substrains. Choi et al. (1994) observed that SAM-P/1 show an age-associated decrease in SOD activity. Liu and Mori (1993) found that when compared with ddY mice, SAM-P/8 show a lower glutathione level and higher lipid peroxidation, but show higher SOD activity. In the former experiment, however, the data of SAM-P/1 were presented without complete comparison with SAM-R/1 data as a control. In the latter, the data appear to be inconsistent in explaining free radical roles in aging. Therefore, these...
findings do not seem to clearly support the finding that increased oxidative stress plays a contributing role in the accelerated aging in SAM.

In our experiments, we demonstrated that the SOD activity in mitochondrial fraction of SAM-P/1 livers is only about half that of SAM-R/1 livers throughout all ages, even immediately after birth (Table 1 and Figure 3). This result may explain the enhanced lipid peroxidation observed in livers of SAM-P/1 (Figure 1). Mitochondria consume over 90% of the total oxygen utilized in mammalian tissues and are equipped with antioxidant enzymes as a defense against normal metabolism (Takeshige and Minakami, 1979; Sohal et al., 1990). For this reason, this organelle should be well equipped with antioxidant enzymes as a defense against oxygen radicals. If any of these antioxidant enzymes are impaired, this organelle is threatened by oxygen radical attacks, and the resulting damage accumulates with age. The age dependency of lipid peroxidation differences in the livers of SAM-P/1 and SAM-R/1 support this theory. As shown in Figure 1, the difference is small at younger ages but becomes larger as the animal ages.

According to the oxidative stress hypothesis of aging, a primary factor influencing the aging process is the net balance between oxygen radical production and deleterious free radical species removal (Yu, 1994). Various conditions known to influence mitochondrial metabolism can thus affect the oxidative status. For example, the oxidative processes expected to be accelerated by increased physical activity (Powers et al., 1993; Meydani and Evans, 1994) or higher ambient temperature (Strehler, 1961). However, as mentioned, increased oxidative stress can be counterbalanced by a well-developed defense system. The anti-aging action of dietary restriction with the enhancement of anti-oxidant capacity is an excellent example of the mode of attenuation of oxidative stress (Luhtala et al., 1994). This effectiveness of such a paradigm can be shown even with senescence-prone SAM-P/1 mice. When placed on dietary restriction, they lived as long as SAM-R/1 (Kohno et al., 1985) with less oxidative damage in mitochondria (Chung et al., 1992). This hypothesis can be also supported by an experiment (Heck and Brown, 1987) reporting that a diet supplement of diethylhydroxylamine as an antioxidant shows an anti-aging effect. Thus, our data on senescence-prone SAM-P/1 with lower mitochondrial SOD activity than its senescence-resistant counterpart can partly explain the accelerated aging of SAM-P/1. This interpretation seems to be consistent with the analysis of Cutler (1985), which shows a strong correlation between defense capacity against oxidative stress and the maximum life span potential.

Superoxide radicals are generated in mitochondria, in the electron transport system located in the inner membrane of this organelle (Turrens and Boveris, 1980). The superoxides formed in the inner membrane diffuse into two compartments, the matrix space and the intermembranous space (Forman and Boveris, 1982). Mitochondria need SOD in both spaces to remove superoxides efficiently. In eukaryotic cells, Cu,Zn-SOD is present in the intermembranous space and in cytosol (Weisiger and Fridovich, 1973; Lippman, 1989) and Mn-SOD in the matrix of mitochondria (Weisiger and Fridovich, 1973). The less-active SOD in mitochondria of SAM-P/1 livers was identified in this study as Cu,Zn-SOD (Table 1 and Figure 5). The Mn-SOD found in mitochondria and Cu,Zn-SOD in cytosol were nearly identical in both SAM substrains (Figure 6). In the Northern blot analysis, however, there was no difference in either the mRNA level of Cu,Zn-SOD or Mn-SOD (Figure 7). These results indicate that the reduction in the amount of Cu,Zn-SOD in liver mitochondria of SAM-P/1 does not originate from transcription or from translation, because Cu,Zn-SOD in cytosol shows no difference in activity (Figure 2), amounts of protein (Figure 5), or mRNA level (Figure 7). The gene encoding the Cu,Zn-SOD is located in nuclear DNA, not in mitochondrial DNA (McKusick and Ruddle, 1977). This enzyme, after synthesis in cytosol, should be transported into the intermembranous space. Thus, transport impairment is one possible reason for lower levels of this enzyme in
mitochondria. Details of the process involving the transport of Cu,Zn-SOD are scarce. Therefore, efforts to find differences in the transport process between the two SAM strains is another approach to elucidate the mechanism of accelerated senescence in this mouse.

In summary, we have shown that the liver of SAM-P/1 is subject to more oxidative stress than that of SAM-R/1, and that mitochondria of SAM-P/1 livers have much less capacity to remove oxygen radicals than those of SAM-R/1 throughout life. Therefore, we expect that mitochondria of SAM-P/1 livers are more vulnerable to oxygen radical attack, thus leading to further functional and structural deterioration than the mitochondria of SAM-R/1. In view of the involvement of reactive species in the aging and disease processes (Yu, 1993, 1994), these findings provide one possible explanation for the accelerated senescence observed in SAM-P/1.

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This overview session will introduce students and new members of GSA to the structure and function of the Society and its four sections. It will provide information about current research and professional activities.
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This session is designed to offer students, new investigators and others interested in the biological processes that influence the rates of aging, an overview of some areas of ongoing biogerontological research. Underlying concepts and various aspects of current biological research will be discussed along with the relationship of processes of aging and the etiology of disease. Aspects of public policy, funding of aging research, and the interests of science will also be discussed.
Speakers: G. Baker, III, PhD (Shock Aging Research Foundation and Gerontology Research Center, NIA/NIH); R. Adelman, PhD (University of MI); R. Arking, PhD (Wayne State University); A. Passaniti, PhD (Gerontology Research Center, NIA/NIH).