Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4337–4341, May 1995
Cell Biology

Oxidative DNA damage and senescence of human diploid fibroblast cells

(8-oxoguanine/protein oxidation/oxygen tension/α-phenyl-*t*-butyl nitrone/replicative life span)

QIN CHEN, ANN FISCHER, JOSHUA D. REAGAN, LIANG-JUN YAN, AND BRUCE N. AMES*

401 Barker Hall, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720

Contributed by Bruce N. Ames, January 23, 1995

ABSTRACT **Human diploid fibroblast cells cease growth in culture after a finite number of population doublings. To address the cause of growth cessation in senescent IMR-90 human fibroblast cells, we determined the level of oxidative DNA damage by using 8-oxoguanine excised from DNA and 8-oxo-2′-deoxyguanosine in DNA as markers. Senescent cells excise from DNA four times more 8-oxoguanine per day than do early-passage young cells. The steady-state level of 8-oxo-2′-deoxyguanosine in DNA is ≈35% higher in senescent cells than in young cells. Measurement of protein carbonyls shows that senescent cells did not appear to have elevated protein oxidation. To reduce the level of oxidative damage, we cultured cells under a more physiological O_2 concentration (3%) and compared the replicative life span to the cells cultured at the O_2 concentration of air (20%). We found that cells grown under 3% O_2 achieved 50% more population doublings during their lifetime. Such an extension of life span resulted from the delayed onset of senescence and elevation of growth rate and saturation density of cells at all passages. The spin-trapping agent α-phenyl-*t*-butyl nitrone (PBN), which can act as an antioxidant, also effectively delayed senescence and rejuvenated near senescent cells. The effect is dose-dependent and is most pronounced for cells at the stage just before entry into senescence. Our data support the hypothesis that oxidative DNA damage contributes to replicative cessation in human diploid fibroblast cells.**

Replicative senescence, induced DNA damage, or differentiation causes cells to irreversibly lose the ability to replicate, though cellular metabolism and integrity remain intact. These cells are unable to respond to mitogenic stimulation, unlike cells whose growth is arrested by nutrient starvation or contact inhibition. In the case of differentiation, failure to proliferate accompanies changes in cell phenotype, a programmed process resulting in part from altered transcriptional regulation. Treatment with DNA-damaging agents causes cells to cease growth. These DNA-damaged cells display a phenotype similar to senescence, including loss of response to growth factors, decreased activity of cell-cycle-dependent enzymes, reduced saturation density, and enlarged size (1). In contrast, it is not known whether natural senescence is associated with DNA damage.

One of the main sources of DNA lesions is endogenous oxidative DNA damage that occurs during aerobic metabolism. 8-Oxoguanine (oxo^{8}Gua), resulting from hydroxyl radical modification of guanine residues in DNA, is a sensitive marker of oxidative DNA damage (2–4). This DNA lesion is repaired by a glycosylase, which removes the base oxo^{8}Gua from DNA, or by an endonuclease, which excises the nucleoside 8-oxo-2′-deoxyguanosine (oxo^{8}dG). The excised oxo^{8}Gua and oxo^{8}dG are excreted into biological fluids (3, 4). Since damage and repair occur continuously, the measurement of excised lesions reflects the balance between DNA damage and repair, while the steady-state level of oxidative damage in DNA reflects the accumulated damages.

Human diploid fibroblast cells have served as an *in vitro* model for studying aging at the cellular level (5–8). Normal cells lose replicative capacity as a function of population doublings (PDs). At the end of their replicative life span, though they maintain the ability to metabolize and to synthesize RNA and protein, the cells arrest growth at the G_1 phase of the cell cycle and will not divide upon any type of stimulation (9). Senescent cells show many changes in gene expression; some genes are down-regulated and some are up-regulated (5). The primary cause(s) of these molecular changes is not known. Here, we examine whether senescence is correlated with an increase in levels of oxidative DNA damage or protein oxidation and determine whether manipulations that reduce the levels of oxidative damage delay senescence.

MATERIALS AND METHODS

Tissue Culture. IMR-90 cells were obtained from the Coriell Institute for Medical Research at population doubling level (PDL) 10.85. The PDs were calculated as $\log_2(D/D_0)$, where D is the density of cells when harvesting and D_0 is the density of cells when seeding. The stock cultures were split weekly and were grown in 100-mm Corning tissue culture dishes containing 10 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) dialyzed fetal bovine serum (Sigma).

To test the effect of ambient oxygen on the life span, cells were cultured in 25-cm^2 Corning flasks with 5 ml of medium. Early-passage cells were seeded at $0.1–0.3 \times 10^6$ cells per flask and late-passage cells were seeded at 0.5×10^6 cells per flask. The flasks were gassed with a mixture of 3% O_2/5% CO_2/92% N_2 or with a mixture of 20% O_2/5% CO_2/75% N_2 for 30 sec, then plug-sealed, and incubated at 37°C. The cultures were split after the cells reached confluence. Early-passage cells usually reached confluence in 5 or 6 days, and late-passage cells, even with increased seeding density, reached saturation density in 10–14 days. At senescence, cells had not doubled for at least 21 days.

To determine the effect of α-phenyl-*t*-butyl nitrone (PBN) on the replicative life span of cells, PBN (stock = 50 mM in phosphate-buffered saline) was added to culture medium at a final concentration of 200–1200 μM after each splitting. If cells were not split on day 7, the cells were fed with fresh medium containing PBN.

Measurement of Free oxo^{8}Gua and Steady-State Level of oxo^{8}dG in DNA. Immunoaffinity columns made from monoclonal antibodies against oxo^{8}dG were used to isolate free oxo^{8}Gua from conditioned (spent) medium (10). Confluent

Abbreviations: PD, population doubling; PDL, PD level; oxo^{8}Gua, 8-oxoguanine; oxo^{8}dG, 8-oxo-2′-deoxyguanosine; PBN, α-phenyl-*t*-butyl nitrone.
*To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

cultures were washed two times with phosphate-buffered saline and then incubated 3 days in 10 ml of fresh DMEM containing 2% (wt/vol) bovine serum albumin and 1% dialyzed serum that had been pretreated with anti-oxo^{8}dG antibody-conjugated Sepharose (10). Such a treatment reduces levels of background free oxo^{8}Gua and oxo^{8}dG in serum. Medium from six dishes, each containing 10 ml (young cells), or from 12 dishes, each containing 5 ml (senescent cells), was pooled and passed through an anti-oxo^{8}dG antibody-based immunoaffinity column. The adducts were eluted with methanol (10). The solvent was evaporated under nitrogen gas at 50°C and the adducts were dissolved in 0.1 M Tris/20 mM EDTA, pH 8.0, containing 1 mM deferoxamine for analysis with HPLC with electrochemical detection (4).

For determination of steady-state levels of oxo^{8}dG in DNA, cells were harvested by trypsinization and cell pellets (5×10^6 cells per sample) were resuspended in extraction buffer (0.1 M Tris·HCl, pH 8.0/0.1 M NaCl/20 mM EDTA/0.1% Triton X-100/2 mM butylated hydroxytoluene/1 mM deferoxamine). DNA was extracted by phenol/chloroform after RNase A/RNase T and proteinase K digestion and was then precipitated with ethanol. DNA was digested with nuclease P1 and alkaline phosphatase for HPLC analysis with electrochemical detection (4).

Measurement of Carbonyl Contents in Cells. Postconfluent cells were lysed in 100-mm dishes with phosphate-buffered saline containing 0.1% deoxycholic acid, 20 mM EDTA, 1 mM deferoxamine, and protease inhibitors (11) at 1×10^6 cells per ml. Carbonyls in trichloroacetic acid-precipitable macromolecules were determined by using 2,4-dinitrophenylhydrazine as described (11). Though the method predominately measures oxidized protein, carbohydrate could contribute to a small extent to the content.

RESULTS

Oxidative DNA damage in IMR-90 cells was measured by analyzing the levels of oxo^{8}Gua excised from DNA per day and excreted into the medium and by analyzing steady-state levels of oxo^{8}dG in DNA. IMR-90 cells senesce at PDL 56.3 ± 4.5 (n = 9 experiments). Before PDL 45, the doubling time was 0.8–1.0 day and the cells did not show obvious differences in the rate of proliferation. Under most circumstances, the rate of cell proliferation declines when cells approach PDL 50. Levels of oxidative DNA damage were determined with young cells (PDL <30; doubling time <1 day) and near senescent cells (PDL >50; doubling time >1.5 days). Senescent cells (PDL 51.1–54.2), compared to early-passage cells (PDL 16.7–26.5), excised per day ≈4 times more oxo^{8}Gua, the major repair glycosylase excision product of oxidative DNA damage (Fig. 1). The excision rate of free oxo^{8}G, a product of oxygen radical attack on RNA, was 11.0 ± 3.8 pmol per 10^9 cells per day in young cells and 30.3 ± 8.3 pmol per 10^9 cells per day in near senescent or senescent cells. The level of free oxo^{8}dG excised was below the detection limit in the experiments, indicating excision by endonuclease is not significant. Measurement of the steady-state level of oxo^{8}dG in DNA showed that senescent cells have 35% more oxo^{8}dG in DNA than at earlier passage (Fig. 1). Therefore, senescent cells produce higher levels of excised oxo^{8}Gua and have higher steady-state levels of oxo^{8}dG in their DNA.

The level of protein oxidation was also measured to determine whether it could contribute to cellular senescence. Protein oxidation is correlated with an increased level of carbonyls in cellular macromolecules (12, 13). We compared the protein carbonyl content in young (PDL 29.7–35.2) and senescent (PDL 48.1–56.2) cells. A summary of five experiments showed that the average of protein carbonyls in young cells was 4.5 ± 2.9 nmol per mg of protein whereas that in senescent cells was 2.9 ± 1.0 nmol per mg. Therefore, we did not observe any increase in oxidized protein in the senescent cells based on the measurement of carbonyl content.

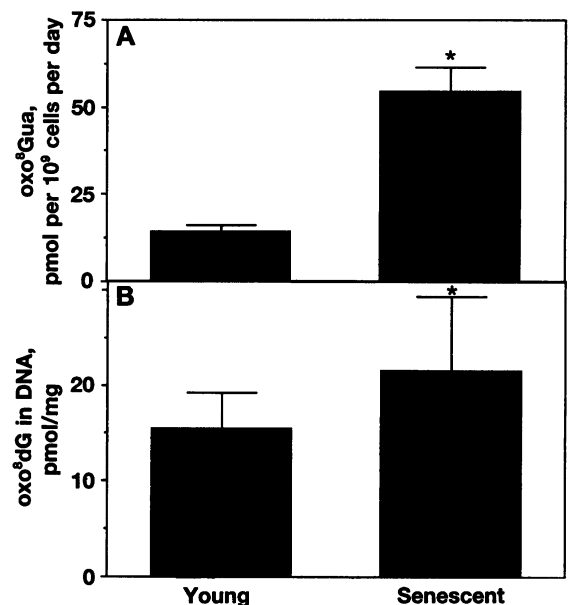

FIG. 1. Levels of oxidative DNA damage in young and senescent IMR-90 cells. The rate of free oxo^{8}Gua production (*A*) and the steady-state levels of oxo^{8}dG (*B*) in DNA of young (PDL 16.7–26.5) or senescent cells (PDL 51.1–54.2) were determined. Data are the summary of three to six experiments. An asterisk indicates a significant difference between young and senescent cells by a standard t test ($P \leq 0.01$) or by the Wilcoxon paired nonparametric test ($P \leq 0.05$).

Oxidative DNA damage may result from oxidant byproducts produced during aerobic metabolism of cells. Oxygen radicals are derived from mitochondrial respiration and the activity of various oxidases. Under standard tissue culture conditions, cells are exposed to 20% O_2, which is much higher than the physiological level. By reducing the level of oxygen of cells to more physiological levels of ≈3%, the production of oxidants is reduced. To test the possibility that high levels of oxygen are a cause of oxidative DNA damage and cellular senescence, we compared the replicative life span of cells grown under reduced O_2 concentration to cells grown at standard oxygen concentrations (Fig. 2). During continuous culture of IMR-90 cells under 20% or 3% O_2, we found that the cells grown under 3% O_2 achieved 40–50% more PDs than

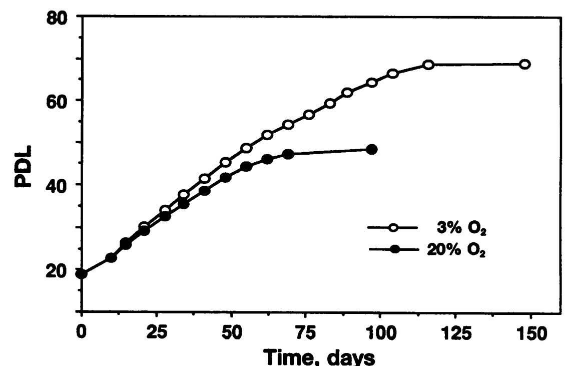

FIG. 2. Cumulative growth curve of IMR-90 cells cultured under 3% or 20% O_2. IMR-90 cells at PDL 18.8 were cultured under 20% O_2/5% CO_2/75% N_2 or under 3% O_2/5% CO_2/92% N_2 until they became senescent. Cells were seeded at a density per 25-cm^2 flask of 0.2×10^6 cells for the first two passages, 0.25×10^6 cells for the third to eighth passages, 0.3×10^6 cells at the ninth passage, and 0.4×10^6 cells for the remaining passages. Data show the average of triplicate samples and variations are smaller than symbols.

did the cells grown under 20% O_2 (Fig. 2). In experiment 1, cells at PDL 20.2 were cultured under 3% or 20% O_2. The cells cultured under 20% O_2 senesced at PDL 40.4, whereas the cells cultured under 3% O_2 did not senesce until PDL 63.5. In experiment 2, cells at PDL 18.8 were cultured under either 3% or 20% O_2. The cells cultured under 20% O_2 senesced at PDL 48.5, whereas the cells cultured under 3% O_2 senesced at PDL 69.0 (Fig. 2). To exclude the possibility that gassing may abbreviate the replicative life span of cells, we compared the life span of cells cultured under standard tissue culture conditions (5% CO_2/95% air) with cells plug-sealed with 20% O_2. The results were similar (PDL 50.4 for standard tissue culture and PDL 48.5 for 20% O_2 gas plug-sealed culture). Nevertheless, the cells grown under 3% O_2 achieved more PDs than cells grown under 20% O_2.

The increased replicative life span in 3% O_2 was associated with increased rates of cell proliferation and increased saturation density. The cells cultured under 3% O_2 divided faster; their doubling time was 4–20% shorter than that of cells cultured under 20% O_2 (Table 1). In addition, cells at all PDLs cultured at 3% O_2 tension reached a higher saturation density.

To further examine the role of oxidative damage in cellular senescence, we sought to extend the replicative life span with reagents that prevent oxidative DNA damage. We did not observe an extension of the life span with α-tocopherol acetate (200 μM), *N*-acetylcysteine (2 mM), butylated hydroxytoluene (100 μM), salicylic acid (200 μM), or *N,N'*-diphenyl-1,4-phenylenediamine (40 μM). 3,3,5,5-Tetramethyl-1-pyrroline *N*-oxide showed minimal increase of replicative life span at 500 μM (a gain of 1 PD before senescence). However, the spin-trapping agent and lipophilic free-radical scavenger PBN (14) was capable of extending the replicative life span of IMR-90 cells (Fig. 3 and Table 2).

PBN was most effective when added to cells just before they reduced their growth rate. Under optimum conditions, the saturation density of cells cultured with PBN doubled from that of cells cultured without PBN. We added PBN to cells at different PDLs. The initial treatment with PBN caused a small inhibition of cell growth. In all nine experiments, we observed that the cell number in the first passage treated with 200 μM PBN, compared to the untreated cells, was reduced by 50% in one experiment, 30–35% in two experiments, 12–17% in five experiments, and 7% in one experiment. After one to three passages, such inhibition of cell growth had diminished and PBN-treated cells showed either a comparable or faster growth rate than untreated cells, depending on the PDL. Cell growth stimulation was observed at later stages of the replicative life span. This effect was not influenced by the PDL when PBN was included in culture medium (Fig. 3 and Table 2). On average, untreated cells senesced at PDL 56.8 ± 5.1, while the cells treated with PBN became senescent at PDL 60.5 ± 5.4. Fig. 3 shows a cumulative growth curve and the effect of PBN over the culture lifetime. The effect of PBN is dose-dependent (Fig. 4 and Table 3).

Table 1. Growth rate and saturation density of IMR-90 cells cultured under 20% or 3% O_2

PDL	Doubling time, days		Saturation density, cells per 25-cm² flask	
	20% O_2	3% O_2	20% O_2	3% O_2
20.3	0.82	0.78	3.4×10^6	4.3×10^6
27.1	0.79	0.76	3.3×10^6	3.9×10^6
34.8	0.92	0.76	1.4×10^6	2.3×10^6
42.7	1.01	0.92	1.8×10^6	2.5×10^6
46.1	1.18	1.08	1.0×10^6	2.0×10^6
52.4	1.16	1.07	1.6×10^6	2.0×10^6
55.1	1.78	1.45	0.8×10^6	1.1×10^6

Cells at the PDLs indicated were seeded at a density of 0.12×10^6 cells per 25-cm² flask (PDL 27.1 and 46.1), 0.125×10^6 cells per flask (PDLs 20.3, 34.8, and 42.7), or 0.2×10^6 cells per flask (PDL 52.4 and 55.1). Cells were cultured for 7–9 days under 20% O_2/5% CO_2/75% N_2 or under 3% O_2/5% CO_2/92% N_2. Data are the average of duplicate samples.

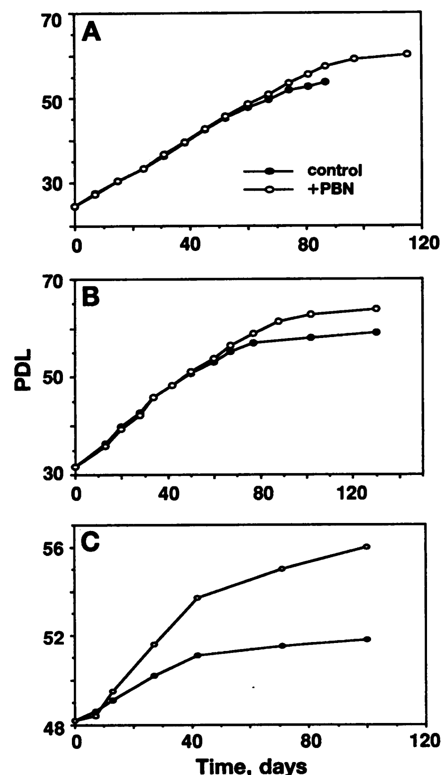

FIG. 3. Cumulative growth curve of IMR-90 cells cultured with or without 200 μM PBN. (*A*) Cells at PDL 24.5 were seeded at a density per 25-cm² flask of 0.3×10^6 cells for the first to eleventh passages and 0.4×10^6 for the remaining passages. (*B*) Cells at PDL 31.5 were seeded at a density per 25-cm² flask of 0.1×10^6 for the first passage, 0.25×10^6 for the second and third passages, 0.3×10^6 for the fourth to eleventh (without PBN) or twelfth (with PBN) passages, and 0.4×10^6 for the remaining passages. (*C*) Cells at PDL 48.7 were seeded at a density per 25-cm² flask of 0.3×10^6 for all passages. Data show the average of triplicate samples.

DISCUSSION

Our experiments suggest that oxidative DNA damage is associated with the senescence of human diploid fibroblast cells. The steady-state level of oxo^{8}dG in DNA is higher in senescent cells than in young cells. Senescent cells excise from DNA and excrete higher levels of oxo^{8}Gua than young cells, suggesting that the oxidant "hit rate" is higher.

Table 2. Effect of PBN on the life span of IMR-90 cells

Starting PDL	PDL at senescence		Gain of PDs, no.
	− PBN	+ PBN	
21.1	57.3	58.2	0.9
24.5	53.7	57.5	3.8
31.5	58.1	62.8	4.7
35.6	65.7	72.3	6.6
42.2	48.6	55.7	7.1
44.7	57.6	61.3	3.7
48.2	51.8	56.0	4.2

Cells at the PDL indicated were cultured in medium with or without 200 μM PBN until they became senescent. Cells were split weekly or every 2 weeks at which time they reached saturation density, and the number of PDs was calculated. Data are the average of triplicate samples.

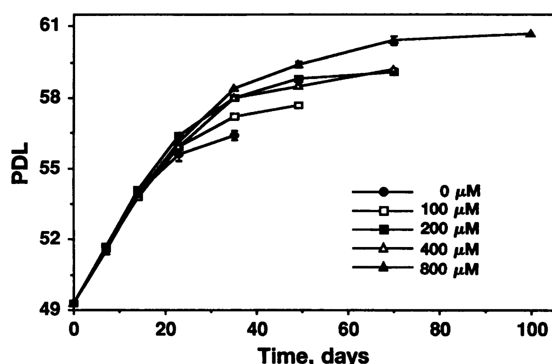

FIG. 4. Effect of various concentrations of PBN on the replicative life span of IMR-90 cells. IMR-90 cells at PDL 49.3 were cultured in medium with or without PBN at the indicated concentrations until they became senescent. Cells were seeded at a density per 25-cm^2 flask of 0.3×10^6 cells for the first three passages and 0.4×10^6 for the remaining passages. Cells were fed with fresh medium containing PBN if they had not reached their saturation density at 7 days. Data show the average of triplicate samples and variations are indicated as error bars or are smaller than symbols.

One cause of increased levels of oxidative DNA damage may be defective mitochondria in senescent cells. Oxidants are produced continuously during aerobic metabolism, mainly from mitochondrial respiration (15). Although mitochondrial mass and the rate of O_2 consumption do not decrease with replicative age in cultured human diploid fibroblast cells (16, 17), the increased glucose consumption, lactate production, and transmembrane potential suggest that oxidative phosphorylation is inefficient in senescent cells (17, 18). Such inefficiency may result from imbalances in the mitochondrial electron transport chain and, therefore, may also result in an increase in superoxide and hydrogen peroxide production (15, 19). For example, reduced cytochrome *c* oxidase activity results in direct electron flow to molecular oxygen and a corresponding increased production of superoxide in human fibroblast cells from aged individuals (20). A deficiency of reduced components in the electron transport chain would also increase production of superoxide in aged cells. In addition to increased production of oxidants, it is also possible that cellular defense systems are less functional, causing an accumulation of reactive oxygen species in senescent cells.

Structural changes may also contribute to the increased levels of oxidative DNA damage in senescent cells. Senescent cells are characterized by increased cell volume and increased organization of the cytoskeleton (21). The DNA may be less protected by chromatin, since senescent cells have less condensed chromatin (22). Therefore, DNA in senescent cells may be relatively more exposed and more susceptible to oxidative damage. The fact that senescent cells produce higher levels of free oxo^{8}Gua suggests that glycosylase, the enzyme that excises the damaged base, is functional in senescent cells.

The increased level of oxidative DNA damage correlates with growth cessation in senescent cells. The argument that DNA damage, rather than damage to protein, is a cause of growth cessation is supported by our data that senescent cells do not contain an increased amount of oxidized protein. It has been reported, however, that oxidized protein accumulated with age in rats and humans but not with senescence of cells in culture (12, 13). The fact that cellular senescence in tissue culture did not correlate with an increase in oxidized protein may be due to increased activity of proteases (23–25). The damaged DNA may serve as a signal to activate the expression of genes that negatively regulate the growth of cells. We have previously discussed a plausible mechanism based on the work of Hanawalt and Botchan whereby the transcription apparatus senses DNA lesions when these lesions block transcription and initiates DNA repair with the replication protein A (RPA) and other proteins. The tumor suppressor gene product p53 is then released from the RPA–p53 complex, thereby activating the cyclin kinase inhibitor p21$^{(WAF1/Cip1/sdi1)}$ and turning off cell division at G_1 phase (1). Senescent cells express higher levels of the negative growth regulatory gene p21 (26). Both p53 and p21 are directly induced by DNA damaging agents (27, 28). Introducing p21$^{(sdi1)}$ into young cells inhibits DNA synthesis (26). Although it seems that an increase of oxidative DNA damage may trigger the cascade of responses leading to growth cessation, we cannot exclude the possibility that other factors independent of DNA damage are also involved in the responses. For example, UV-irradiation causes activation of transcription factors AP-1 and NF-κB through a Ras-dependent pathway, independently of DNA damage (29).

Reducing O_2 concentration from the standard 20% to the more physiological 3% extended the replicative life span of cells by ≈50% more PDs. Reduced O_2 tension delayed the onset of senescence and allowed cells to divide faster and reach a higher saturation density, resulting in an increased number of accumulated PDs. Similar results on the beneficial effect of lower and thus more physiological O_2 level have been described (30–33). Reduced O_2 concentration enhances cell response to serum and growth factors (34) and increases epidermal growth factor-induced proliferation by enhancing the binding of epidermal growth factor to its receptor (35). In addition, synthesis and secretion of transforming growth factor β_1 are increased by reducing O_2 concentration, resulting in paracrine stimulation of fibroblast cell growth (36). The effects of O_2 concentration are cell-density-dependent (33). Reducing O_2 tension would be expected to prevent the production of reactive oxygen species and, therefore, reduce the level of oxidative damage.

PBN, a spin-trapping agent and lipophilic radical trap, delayed senescence and extended the replicative life span of cells. PBN provides a useful tool to extend the replicative life span of human diploid fibroblast cells. In contrast, a number of other antioxidants, such as α-tocopherol, butylated hydroxytoluene, *N,N′*-diphenyl-1,4-phenylenediamine, salicylic acid, and *N*-acetylcysteine, failed to extend the life span of the cells. As a spin-trapping agent, PBN traps radicals and forms a stable adduct preventing the interaction of the radical with cellular macromolecules (37). Though PBN is a hydrophobic compound, it also has a fairly aqueous solubility and partitions in both membrane and other cellular compartments (14, 38). The nuclear and mitochondrial distribution of PBN (38) and its radical-trapping efficiency may account for its superiority to the other antioxidants. Animal studies have shown that PBN reduces the level of protein oxidation and reverses some neurological aging processes in gerbils (39). Though it is plausible that PBN extended the life span of IMR-90 cells by reducing the level of oxidative DNA damage, we cannot exclude the possibility that PBN acted by an alternate mechanism, for example, by activating a signal transduction pathway (41). PBN has been reported to decompose to yield some nitric oxide under UV-irradiation and activate guanylate cyclase, which could serve as signals to regulate cell growth (40). The

Table 3. Effect of various concentrations of PBN on the life span of cells

Starting PDL	PDL at senescence					
	0	100 μM	200 μM	400 μM	800 μM	1200 μM
45.2	51.2	51.5	51.5	52.4	52.1	53.1
45.6	60.9	63.5	64.3	64.8	65.3	64.4
49.3	59.2	60.5	61.9	62.0	63.5	

Cells at the PDL indicated were cultured in medium with the indicated concentration of PBN until they became senescent. Cells were split weekly or every 2 weeks at which time they reached saturation density. Data are the average of triplicate samples.

latter explanation seems less plausible because the rate of decomposition is very low under standard conditions (≈0.1% in 2 weeks) (40) and is much lower than the rate of trapping radicals. Thus several lines of evidence support the hypothesis that oxidative DNA damage contributes to replicative senescence of human diploid fibroblast cells.

We thank Drs. J. Campisi, R. Floyd, L. Packer, and E. Stadtman for suggestions and reviewing the manuscript. This work was supported by the National Cancer Institute Outstanding Investigator Grant CA39910, the National Institute of Environmental Health Science Center Grant ESO1896 (B.N.A.), and the Colgate–Palmolive Postdoctoral Fellowship Grant M1597 from the Society of Toxicology (Q.C.).

1. Chen, Q. & Ames, B. N. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4130–4134.
2. Ames, B. N. (1991) *Jpn. J. Cancer Res.* **82,** 1460–1461.
3. Shigenaga, M. K. & Ames, B. N. (1991) *Free Radical Biol. Med.* **10,** 211–216.
4. Shigenaga, M. K., Aboujaoude, E. N., Chen, Q. & Ames, B. N. (1994) in *Methods in Enzymology: Oxygen Radicals in Biological Systems, Part D*, ed. Packer, L. (Academic, San Diego), pp. 16–33.
5. Goldstein, S. (1990) *Science* **249,** 1129–1133.
6. Holliday, R. (1990) *J. Gerontol.* **45,** 41.
7. Peacocke, M. & Campisi, J. (1991) *J. Cell. Biochem.* **45,** 147–155.
8. Smith, J. R. & Lincoln, D. W. I. (1984) *Int. Rev. Cytol.* **89,** 151–176.
9. Sherwood, S. W., Rush, D., Ellsworth, J. L. & Schimke, R. T. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9086–9090.
10. Park, E. M., Shigenaga, M. K., Degan, P., Korn, T. S., Kitzler, J. W., Wehr, C. M., Kolachana, P. & Ames, B. N. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3375–3379.
11. Reznick, A. Z. & Packer, L. (1994) *Methods Enzymol.* **233,** 357–363.
12. Oliver, C. N., Ahn, B. W., Moerman, E. J., Goldstein, S. & Stadtman, E. R. (1987) *J. Biol. Chem.* **262,** 5488–5491.
13. Stadtman, E. R. (1992) *Science* **257,** 1220–1224.
14. Carney, J. M. & Floyd, R. A. (1991) *J. Mol. Neurosci.* **3,** 47–57.
15. Shigenaga, M. K., Hagen, T. M. & Ames, B. N. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10771–10778.
16. Goldstein, S., Moerman, E. J. & Porter, K. (1984) *Exp. Cell Res.* **154,** 101–111.
17. Goldstein, S., Ballantyne, S. R., Robson, A. L. & Moerman, E. J. (1982) *J. Cell. Physiol.* **112,** 419–424.
18. Martinez, A. O., Over, D., Armstrong, L. S., Manzano, L., Taylor, R. & Chambers, J. (1991) *Growth Dev. Aging* **55,** 185–191.
19. Sohal, R. S. & Brunk, U. T. (1992) *Mutat. Res.* **275,** 295–304.
20. Hayashi, J., Ohta, S., Kagawa, Y., Kondo, H., Kaneda, H., Yonekawa, H., Takai, D. & Miyabayashi, S. (1994) *J. Biol. Chem.* **269,** 6878–6883.
21. Angello, J. C., Pendergrass, W. R., Norwood, T. H. & Prothero, J. (1989) *J. Cell. Physiol.* **140,** 288–294.
22. Macieira-Coelho, A. (1991) *Mutat. Res.* **256,** 81–104.
23. Carlin, C., Phillips, P. D., Brooks, F. K., Knowles, B. B. & Cristofalo, V. J. (1994) *J. Cell. Physiol.* **160,** 427–434.
24. Blumenthal, E. J., Miller, A. C., Stein, G. H. & Malkinson, A. M. (1993) *Mech. Ageing Dev.* **72,** 13–24.
25. Okada, A. A. & Dice, J. F. (1984) *Mech. Ageing Dev.* **26,** 341–356.
26. Noda, A., Ning, Y., Venable, S. F., Pereira, S. O. & Smith, J. R. (1994) *Exp. Cell Res.* **211,** 90–98.
27. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. & Craig, R. (1991) *Cancer Res.* **51,** 6304–6311.
28. Johnson, M., Dimitrov, D., Vojta, P. J., Barrett, J. C., Noda, A., Pereira-Smith, O. M. & Smith, J. R. (1994) *Mol. Carcinog.* **11,** 59–64.
29. Devary, Y., Rosette, C., DiDonato, J. A. & Karin, M. (1993) *Science* **261,** 1442–1445.
30. Packer, L. & Fuehr, K. (1977) *Nature (London)* **267,** 423–425.
31. Balin, A. K., Goodman, B. P., Rasmussen, H. & Cristofalo, V. J. (1976) *J. Cell. Physiol.* **89,** 235–249.
32. Honda, S. & Matsuo, M. (1983) *Exp. Gerontol.* **18,** 339–345.
33. Balin, A. K., Fisher, A. J. & Carter, D. M. (1984) *J. Exp. Med.* **160,** 152–166.
34. Storch, T. G. & Talley, G. D. (1988) *Exp. Cell Res.* **175,** 317–325.
35. Wing, D. A., Talley, G. D. & Storch, T. G. (1988) *Biochem. Biophys. Res. Commun.* **153,** 952–958.
36. Falanga, V., Qian, S. W., Danielpour, D., Katz, M. H., Roberts, A. B. & Sporn, M. B. (1991) *J. Invest. Dermatol.* **97,** 634–637.
37. Sen, S. & Phillis, J. W. (1993) *Free Radical Res. Commun.* **19,** 255–265.
38. Cova, D., De, A. L., Monti, E. & Piccinini, F. (1992) *Free Radical Res. Commun.* **15,** 353–360.
39. Carney, J. M., Starke, R. P., Oliver, C. N., Landum, R. W., Cheng, M. S., Wu, J. F. & Floyd, R. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3633–3636.
40. Chamulitrat, W., Jordan, S. J., Mason, R. P., Saito, K. & Cutler, R. G. (1993) *J. Biol. Chem.* **268,** 11520–11527.
41. Joseph, J. A., Villalobos-Molina, R., Erat, S. & Strain, J. (1994) *Age (Chester, Pa)* **17,** 173 (abstr. 64).