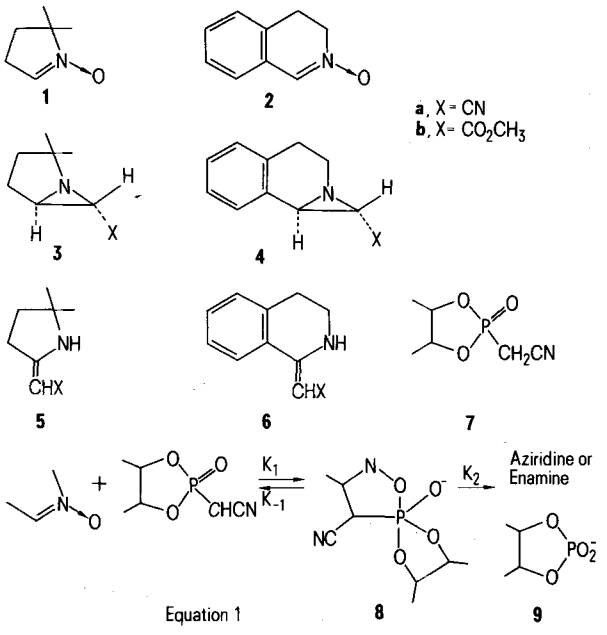
dependent than those of K_{-1} and of K_2. Consequently, raising the temperature will result in an increase of the value of $K_1/K_{-1}+K_2$ and with it the steady-state concentration of *8*.

Indeed, when examining the reaction of *1* with *7* in tetrahydrofuran at 25 °C after 1000 scans, we could observe a signal at +36 ppm (upfield from 85% H_3PO_4) indicating the existence of a pentacoordinated phosphorus derivative, in addition to signals arising from starting materials and product. The same phenomenon could be observed in the following solvents: N,N-dimethylformamide (+34.7 ppm), chlorobenzene (+35.8 ppm) and 1,2-dimethoxyethane (DME) (+38.0 ppm). Thin layer chromatographic examination of the reaction mixtures showed the presence of aziridine *3a* as the sole product of the reactions. Monitoring the reaction of 3,4-dihydroisoquinoline N-oxide (*2*) with *7* in DME using sodium hydride at room temperature, we could also observe a signal at +36.3 ppm. Since, in contrast to the reaction of *1* with *7*, the latter reaction leads only to enaminonitrile (*6a*), our observations described in this communication supply evidence in support of the assumption we have expressed previously, that both types of products, namely aziridines and enamines are formed from a common oxazaphospholidine intermediate.



Equation 1

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Catalase and dehydroascorbate reductase in human polymorphonuclear leukocytes (PMN): Possible functional relationship

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Summary. In leukocytes (PMN) of individuals with Swiss type acatalasemia, the rate of dehydroascorbate reduction is 4 times normal. This observation suggests that the protective function served by catalase in human PMN is supported by dehydroascorbate reductase.

Catalase (E.C.1.11.1.6.) and dehydroascorbate reductase (E.C.1.6.5.4.) may constitute parts of cellular mechanisms which protect against and repair oxidant injury to biomolecules[2-4]. In tissues, H_2O_2 degradation is catalyzed by catalase and glutathione peroxidase. Their relative contribution varies, depending on differences in enzyme concentration among tissues and species, rates of peroxide generation and experimental conditions[5,6]. Only the most profoundly deficient variants of human hereditary acatalasemia (Japanese type) predispose to disease. About half of such individuals develop ulcerative *gingivitis* and *stomatitis*, presumably because of local growth of catalase-negative bacteria producing tissue-damaging amounts of H_2O_2[7]. Acatalasemic red cells accumulate more methemoglobin than do normal erythrocytes during exposure to ionizing irradiation[2]. H_2O_2, one of the oxidant products of radiation-activated water which mediate radiation tissue injury, oxidizes hemoglobin to methemoglobin. Thus, in the human red cell, catalase together with glutathione peroxidase are necessary for efficient removal of hydrogen peroxide.

Reduced ascorbate (AH_2) reacts readily with a variety of oxidants and free radicals. Among human leukocytes, radioresistance correlates with capacity to take up and reduce dehydroascorbate (A)[3,4]. Dehydroascorbate reductase activity, measured in normal granulocyte and lymphocyte homogenates, is directly proportional to measured dehydroascorbate uptake and reduction[8]. Thus, capacity to maintain ascorbate in reduced form may be a determinant of cell capacity to inactivate oxidants and free radicals.

The present study was designed to evaluate the capacity of selected enzymes to promote inactivation of oxidants and free radicals in leukocytes. Granulocytes were isolated from normal subjects and from 1 heterozygous and 2 homozygous Swiss acatalasemic individuals. The data given in the table show that activities of glutathione peroxidase, glutathione reductase and superoxide dismutase were normal.

Enzyme activities in normal and acatalasemic PMN

		Glutathione peroxidase (IU/mg protein)	Glutathione reductase (IU/mg protein)	Superoxide dismutase (U/mg protein)	Catalase (μg/mg protein)	Dehydroascorbate uptake and reduction (nmoles/min/10^8 cells)
Homozygote acatalasemic	1.	0.029	0.034	1.69	0.71	24.3
	2.	0.023	0.055	1.03	0.72	24.6
Heterozygote acatalasemic		0.056	0.051	1.48	2.25	20.6
Normal	1.	0.024	0.044	1.32	2.49	5.0
	2.	0.050	0.075	1.44	2.51	4.8

PMN, 89–95% pure, were isolated from venous blood by dextran sedimentation, suspension of 1 vol. cells in 2 vol. 0.87% NH_4Cl, and 3× washing in Medium 199 pH 7.4 containing glucose 1 mg/ml with 250× g centrifugation, as previously described[4]. Glutathione peroxidase[9], glutathione reductase[9], superoxide dismutase[10], and catalase[11] were assayed in 27,000× g supernatants of 3× freeze-thaw cell lysates, using published methods. Dehydroascorbate uptake and reduction by intact PMN incubated in medium containing 25 mg/ml dehydroascorbate was measured radiochromatographically[4].

The heterozygote and homozygote acatalasemic cells possess 70 and 15%, respectively, of normal catalase activities; both kinds of cells take up and reduce dehydroascorbate at 4 times normal rates. In control experiments, dehydroascorbate uptake and reduction was not significantly changed in normal PMN after inhibition of catalase activity. Catalase was inhibited by incubating cells for 60 min at 37 °C in physiologic buffer containing 3.57 mM 3-NH_2-1,2,4-triazole (Sigma Chemicals, St. Louis, MO). Thus, the higher dehydroascorbate uptake and reduction in acatalasemic cells is not an artifact of assay related to low catalase activity.

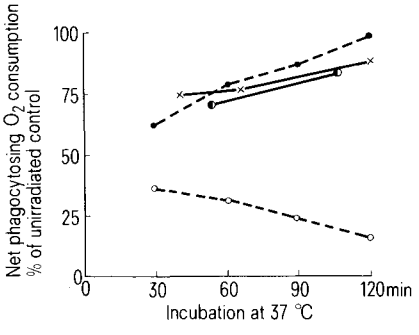
In a previous study[4], granulocytes from 8 of 10 patients with chronic granulocytic leukemia were found to be more radiosensitive than granulocytes from normal donors. The patterns for change in phagocytosing oxygen consumption after 20 krad X-irradiation exhibited by representative radioresistant and relatively radiosensitive granulocytes are illustrated in the figure. Superimposed on this background, measurements of the same parameter following 22.7 krad betatron irradiation show that Swiss acatalasemic PMN exhibit a normal, radioresistant pattern: moderate impairment of phagocytosing O_2 consumption immediately after irradiation, followed by return toward normal values during incubation at 37 °C.

Catalase activity is almost negligible while glutathione peroxidase activity is very high in Sprague-Dawley rat PMN; the obverse relationship is seen in guinea-pig PMN[12]. Granulocytes from a majority of patients with chronic granulocytic leukemia have 1.5–2.5 times higher catalase activity than normal[13,14]; most but not all of such cells take up and reduce dehydroascorbate at approximately half normal rates[4]. The present study shows that the rate of dehydroascorbate uptake and reduction is 4 times normal in mutant granulocytes which possess 15–70% of normal catalase activity; the resistance of these cells to acute injury by ionizing irradiation is normal. Combined, these observations may be taken as indirect evidence that the function served by catalase in intact cells may also be carried out by glutathione peroxidase, as appears to be the case in rats; and that, utilizing the reaction sequence

a) $H_2O_2 + AH_2 \longrightarrow A + 2H_2O$

b) $A + 2GSH \xrightarrow{\text{dehydroascorbate reductase}} AH_2 + GSSG$

the same function may be supported by dehydroascorbate reductase, as in acatalasemic and some chronic leukemic granulocytes. These observations also favor the assumption that cell content of these enzymes is subject to adaptive regulation.



PMN Radioresistance, measured as impairment of phagocytosing O_2 consumption after ionizing irradiation, and subsequent change during incubation at 37 °C. – PMN suspended in Medium 199 containing glucose 1 mg/ml were irradiated with X-ray, 20 krad at 927 rad/min, or with a betatron, 22.7 krad at 1135 rad/min, at 26 °C. They were then kept on ice for 12–25 min, until buffer was changed and incubation at 37 °C was started (t_0). At intervals thereafter, resting and latex-phagocytosing O_2 consumption were measured as previously described[4]. Net phagocytosing O_2 consumption = phagocytosing less resting. ●– – –● normal PMN, 20 krad X-ray; ○– – –○ chronic leukemic granulocytes, 20 krad X-ray; ×——× heterozygote acatalasemic PMN, 22.7 krad betatron; ◐——◐ homozygote acatalasemic PMN, 22.7 krad betatron.

1 This work was aided by grants to L.S. and R.B. from the American Cancer Society (CH-30B), the Grand Bethel of Oregon, International Order of Job's Daughters, and to S.W. and H.A. by grant number 3.384.74 from the Swiss National Science Foundation.

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