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Polymorphonuclear Leukocyte Species Differences in the Disposal of Hydrogen Peroxide (H_2O_2)[1] (40230)

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During phagocytosis by polymorphonuclear leukocytes (PMN), oxygen is reduced to hydrogen peroxide (H_2O_2) and other free radicals. Polymorphonuclear leukocytes utilize the oxygen by-products for the peroxidative dependent killing of ingested bacteria. Previous studies have indicated that the elaboration of H_2O_2 will attenuate phagocytosis and chemotaxis of normal PMN, (1) compared to those activities of PMN maintained in an anaerobic environment. In addition, the H_2O_2 released into the extracellular media from phagocytizing PMNs will rapidly oxidize glutathione in glucose 6-phosphate dehydrogenase deficient red cells leading to a shortening of their *in vivo* survival (2). At least two biochemical defense mechanisms are available in the cytosol of cells to defend against the toxic effects of H_2O_2; these include catalase and the maintenance of adequate GSH levels by enzymes associated with the glutathione pathway. To explore the relative contribution of each of these enzyme systems, we quantitated the activities of enzymes responsible for disposal of H_2O_2 in the PMN obtained from four different mammalian species.

Materials and methods. Human PMN were isolated from peripheral blood as previously described (3). Guinea pig, mouse and rat PMN were collected from the peritoneal cavities of these animals 18 hours after injection of 12% caseinate (4). All PMN samples were then pelleted by a 200*g* 10-min centrifugation. Red cells were lysed from these pellets by a 20 sec exposure to distilled water and isotonicity was then restored. The PMN samples were suspended in Krebs-Ringer phosphate buffer (KRP) pH 7.4 at a cell concentration of 1×10^8 cells/ml. Cell samples were sonicated at an output setting of 3 for 60 sec with a Sonifier Cell Disrupter model W140, Heat Systems-Ultrasonics Inc.

Catalase, glutathione peroxidase (GPX), glutathione reductase (GR) and myeloperoxidase assays were determined on cellular sonicates. The sonicates were clarified by centrifugation at 27,000*g* for 10-min and the supernatant was employed for these enzyme determinations except for myeloperoxidase. The 27,000*g* pellet was resuspended in KRP buffer pH 7.4 and then sonicated an additional 60 sec at an output setting of 5. The sonicated granular fraction was used for the determination of myeloperoxidase activity.

Catalase activity was quantitated by recording the rate of decrease in absorbance of H_2O_2 at 240 nm ($E_{240\,nm} = 43.6\ M^{-1}\ cm^{-1}$) (6). The reactions were performed at 25° in 3.0 ml cuvettes containing 20 m*M* H_2O_2 in 50 m*M* phosphate buffer pH 7.0 and were started with 25–100 μg of sample protein. Glutathione peroxidase activity was measured by observing the rate of oxidation of NADPH at 340 nm ($E_{340\,nm} = 6.22 \times 10^3\ M^{-1}cm^{-1}$) at 25° by the method of Paglia (7). 100–500 μg sample protein was added to a reaction mixture containing 3.0 ml of 50 m*M* potassium phosphate buffer pH 7.0 with 5 mM EDTA and 5 m*M* reduced glutathione, 3.75 m*M* sodium azide, 0.28 m*M* NADPH and 4 units glutathione reductase. The assay was started with 0.1 ml 2.2 m*M* H_2O_2 (final conc. 73 μM) and the change in optical density was recorded for 6 min.

Glutathione reductase activity was measured by observing the rate of oxidation of NADPH at 340 nm at 25° (7). The 3.0 ml reaction mixture contained 50 mM potassium phosphate buffer pH 6.6, 0.4 m*M* NADPH, 1.5 m*M* oxidized glutathione and was started with 100–500 μg sample protein (8).

Reduced glutathione was measured by em-

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ploying the method of Oliver *et al.* (9). 0.2 ml of protein free supernatant from 1.5×10^7 PMN/1.0 ml of KRP buffer pH 7.4 was added to 0.7 ml 0.3 *M* Na_2HPO_4 followed by the addition of 0.1 ml 0.04% 5,5′-dithiobis 2-nitrobenzonic acid (DTNB) in 1% sodium citrate. A blank for each sample was run at the same time by adding 5 μl 0.01 *M* diamide before adding DTNB. Samples were incubated 10 min at 25°, and the optical density was read at 412 nm ($E_{412\,nm} = 1.36 \times 10^4$ $M^{-1}cm^{-1}$). Stimulation of the hexose monophosphate (HMP) shunt during phagocytosis was measured by the method of Root (10).

Results. A reciprocal relationship was observed between catalase activity and GPX-GR activity in the PMN of the animals studied. Catalase activity predominates in human and guinea pig PMN and is almost deficient in rat and mouse PMN (Fig. 1). Catalase units per 10^7 PMN were 139 ± 17, ($\bar{X}$ ± SD), 137 ± 19, <1, <1 for man, guinea pig, mouse and rat respectively. Glutathione peroxidase and glutathione reductase activities were significantly decreased in man and guinea pig relative to those observed in the mouse and rat (Fig. 2, 3). GPX activities expressed as nmoles NADPH oxidized per 10^7 cells min^{-1} were 8.2 ± 1.0, 11.4 ± 1.5, 72.1 ± 6.6, 139 ± 9 and GR activities were 15.9 ± 9, 14.4 ± .7, 61.3 ± 5.5, and 29.4 ± 4.4 nmoles NADPH oxidized per 10^7 cells min^{-1} for man, guinea pig, mouse and rat respectively. Myeloperoxidase activity was greatest in human PMN and decreased progressively in the rat and guinea pig until a one log difference was observed between the mouse and human PMN (Table I).

Reduced glutathione levels were similar

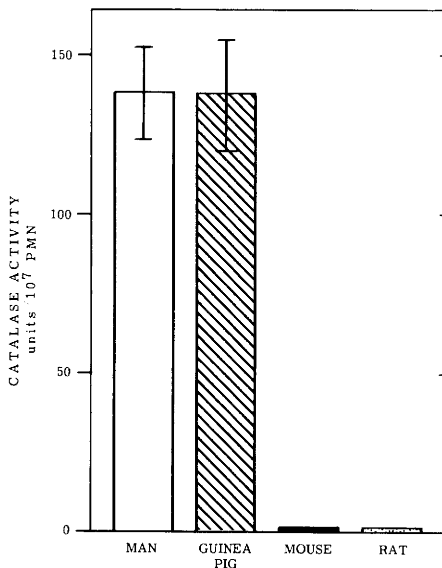

FIG. 1. Catalase activities in PMN cytosol for man, guinea pig, mouse and rat. The differences between man–guinea pig and mouse–rat are significant at $P <$ 0.001 by Student's *t* test.

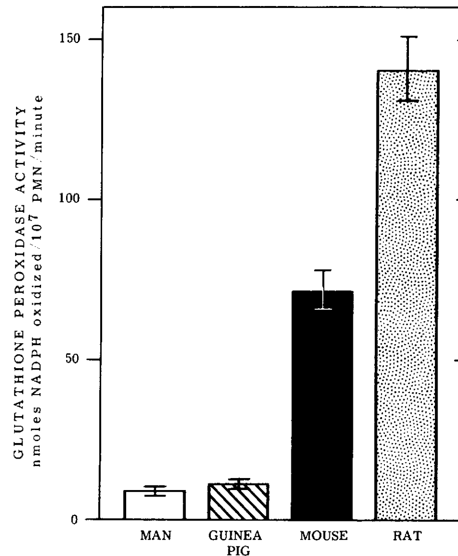

FIG. 2. Glutathione peroxidase activities in PMN cytosol for man, guinea pig, mouse and rat. $P < 0.001$ between man–guinea pig vs. mouse–rat.

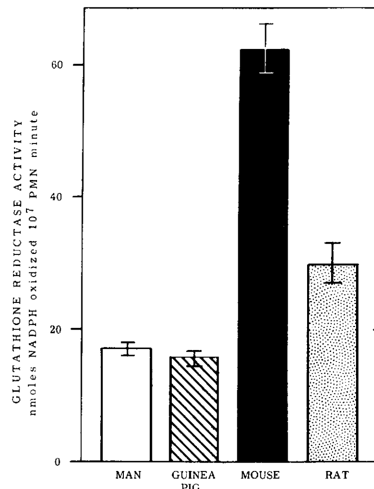

FIG. 3. Glutathione reductase activities in PMN cytosol for man, guinea pig, mouse and rat. $P < 0.001$ between man–guinea pig vs. mouse–rat.

among the four species (Fig. 4) and ranged between 19.9 ± .9 and 24.9 ± 1.3 nmoles/10^7 PMN. Glucose-1-[^{14}C]oxidation through the HMP shunt following phagocytosis of opsonized zymosan was enhanced to a significantly greater extent in those species determined to lack catalase activity as compared to the PMN from mammalian species containing significant catalase (Fig. 5). In the mouse and rat PMN, there was a 5.6 ± .4 and 6.4 ± .4 fold increase in glucose-1-[^{14}C]oxidation compared to a 4.1 ± .6 and 3.5 ± .7-fold increase in man and guinea pig PMN following phagocytosis.

Discussion. A reciprocal relationship between catalase activity and the activity of the glutathione system was found in the cytosol of the PMN among the four species. In the rat and mouse PMN, catalase activity was negligible whereas glutathione peroxidase and glutathione reductase activities were higher than those activities found in PMN obtained from humans and guinea pigs containing substantial catalase activity. Previous studies employing other techniques to assay

TABLE I. MYELOPEROXIDASE ACTIVITIES IN PMN.

Species	Activity (units/10^7 PMN)
Human	0.59 ± .03
Guinea pig	0.18 ± .01
Rat	0.34 ± .02
Mouse	0.06 ± .01

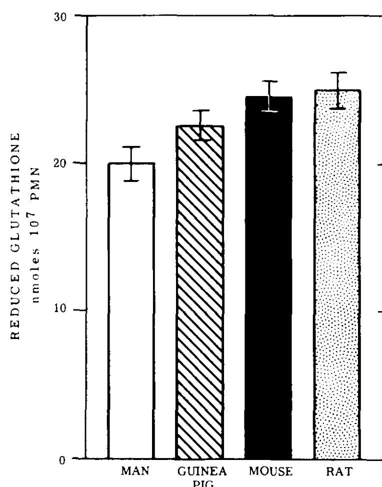

FIG. 4. Reduced glutathione levels in PMN from man, guinea pig, mouse and rat. No difference between the four species.

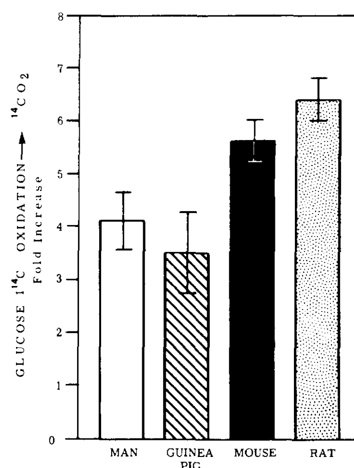

FIG. 5. Fold increase in glucose-1[^{14}C] oxidation through the hexose monophosphate shunt during phagocytosis of opsonized zymosan by PMN from man, guinea pig, mouse and rat. $P < 0.002$ between man–guinea pig vs. mouse–rat.

GPX failed to detect any activity in human and guinea pig PMN (11). Using a more sensitive technique, we found minimal GPX activity in the human and guinea pig PMN. The dramatic differences in catalase and glutathione peroxidase activities among species has been previously noted in red cells (12). Myeloperoxidase activity was substantially different only between human and the mouse as has been previously observed (5).

The functional consequences of these metabolic differences are illustrated in cells deficient in one or the other primary enzyme systems required by that species for disposal of H_2O_2. Glutathione peroxidase deficiency can occur in weanling rats deprived nutritionally of selenium. PMN from those rats have been found to release excessive amounts of H_2O_2 which in turn inflicts damage to the cytoplasmic microtubules leading to alterations in chemotaxis, degranulation and candidacidal activity (13). Human PMNs deficient in glutathione synthetase also manifest functional derangement following phagocytosis indicative of microtubule damage secondary to accumulation of H_2O_2 which would imply that catalase is not the only means of disposing of H_2O_2 in the human PMN (14). Studies of these PMN deficient in the glutathione system indicated that when reduced glutathione levels are not properly main-

tained, oxidation of sulfhydryl groups on critical proteins such as tubulin occurs (9). Further support for a requirement of the glutathione system is found in human cells congenitally lacking catalase activity. Under these conditions, the glutathione system appears inadequate to protect against oxidative damage to hemoglobin protein by H_2O_2 (15). The granular enzyme, myeloperoxidase, does not appear to be a major mechanism for the disposal of H_2O_2 in the PMN but merely employs H_2O_2 for iodination of ingested bacteria as a substrate (16).

This study points out the wide differences between species in activity for those enzymes required for disposal of H_2O_2 in cells. In addition, it calls attention to the fact that one must be aware of these differences to properly interpret metabolic and functional cellular consequences of oxidant challenge. Guinea pig PMN more closely resemble human PMN and may serve as the best animal model to study the interrelationships between oxidative responses and phagocytic function.

Summary. Catalase and the glutathione system containing GPX and GR offer the PMN two mechanisms for disposal of peroxide during phagocytosis. Catalase requires peroxide as substrate whereas GPX requires peroxide and reduced glutathione which is regenerated by GR and NADPH from the HMP shunt. Impairment of either system may lead to accumulation of toxic amounts of peroxide potentially affecting phagocytic function. The activities of catalase compared to GPX and GR were reciprocally related in four mammalian species. Rat and mouse PMNs had negligible catalase activities and enhanced HMP shunt activity reflecting their relative dependence on the more active GPX and GR enzyme systems compared to human and guinea pig PMN which depend more heavily on the cytoplasmic catalase for disposal of peroxide.

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