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ALTERATIONS OF THE OXIDATIVE METABOLISM AND OTHER MICROBICIDAL ACTIVITIES OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES BY ZINC

BRIGITTE BAGINSKI

Institute of Hygiene, University of Düsseldorf, Düsseldorf, FRG

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Zinc is a physiological constituent of many human enzymes and also involved in an optimal immune response. Zinc deficiency as well as excessive zinc supplementation lead to disturbed functions of immune cells. In this study with isolated human polymorphonuclear leukocytes the toxic oxygen species generated during the oxidative metabolism were enhanced in presence of zinc ions. However, when the generation of superoxide anion was measured alone it was decreased by zinc. The phagocytic capacity was diminished in presence of zinc ions, too. The release of lysosomal enzymes was not influenced (lysozyme) or weakly inhibited (β-glucuronidase). Our results may indicate an impairment of the microbicidal capacity due to the diminished phagocytosis, but a promotion of inflammatory reactions due to an increase of toxic oxygen species in the presence of zinc ions.

KEY WORDS: Human PMNL, zinc, chemiluminescence, phagocytosis, lysosomal enzymes, cytotoxicity.

ABBREVIATIONS: PMNL, polymorphonuclear leukocytes; DMEM, Dulbecco's minimum essential medium; PMA, phorbol myristate acetate; MPO, myeloperoxidase; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (biological buffer); TCA, trichloroacetic acid.

INTRODUCTION

Zinc is an important trace element for humans and other species as a physiological and essential constituent of many enzymes.[1,2] Due to zinc deficiency developmental abnormalities[1,3,4] and inhibitions of immunocompetence were observed.[5,6,7] Whereas zinc deficiency and the resulting abnormalities are well investigated, only little is known about the effects of excessive substitution of zinc. Human adults with an excessive intake of zinc over three months showed an inhibition of phagocytosis and chemotaxis in their isolated phagocytes.[8] The same effects were found in animals following intratracheal or intraperitoneal zinc administration[9,10] and also the generation of superoxide anion was decreased by zinc supply.[9] *In vitro* addition of zinc to polymorphonuclear leukocytes (PMNL) of dogs resulted in similar effects: inhibition of the phagocytic capacity and of the bactericidal activity of PMNL.[11] In few studies the production of superoxide anion by PMNL was tested which is generated during the non-mitochondrial oxidative metabolism. The latter is started following phagocytosis

Correspondence: Brigitte Baginski, M.D., Institute of Hygiene, University of Düsseldorf, Auf'm Hennekamp 50, D-4000 Düsseldorf 1, W. Germany.

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or contact with soluble stimuli leading to the formation of toxic oxygen species such as superoxide anion, hydrogen peroxide and the hydroxyl radical.[12,13] The effect of zinc on the oxidative metabolism was not uniform in several studies. The generation of superoxide anion in human PMNL was decreased or enhanced by zinc depending on the experimental conditions[14] and on the stimulus applied.[15,16]

These divergent results prompted us to investigate *in vitro* the effects of zinc (as zinc chloride) on the microbicidal activities of isolated human PMNL under various experimental conditions. The oxidative metabolism quantified by means of chemiluminescence measurements was tested when the cells were stimulated (1) by phagocytosis of zymosan particles, (2) without phagocytosis by binding of heat-aggregated IgG to Fc-receptors or (3) by activation with the soluble stimulus phorbol myristate acetate. Furthermore we tested the phagocytic capacity and the release of lysosomal enzymes of the different granula[17] in presence of zinc chloride.

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MATERIALS AND METHODS

$ZnCl_2$, analytical grade, was obtained from Merck, Darmstadt, FRG. A stock solution (10 mM) in double distilled water was freshly diluted for each experiment in Dulbecco's Minimum Essential Medium with Hepes (DMEM, Boehringer Mannheim, FRG), the medium used for all experiments.

Preparation of Human PMNL

Human PMNL were isolated from veneous heparinized blood of healthy adults by separation on Ficoll-Paque (Pharmacia, Freiburg, FRG).[18] The PMNL were collected from the pellet as previously described,[19] suspended in DMEM and adjusted to the cell number needed for the respective experiments.

Immune-Phagocytosis

The phagocytic capacity was assessed by counting of ingested yeast cells (Saccharomyces cerevisiae) as described earlier.[19,20] Briefly, yeast cells were opsonized with pooled human serum. After a preincubation of PMNL with $ZnCl_2$ for 30 min the PMNL were incubated with the opsonized yeast cells in a ratio yeast cell: PMNL as 10:1 for further 30 min and then the number of ingested yeast cells per 200 PMNL was counted.

Receptor-independent Phagocytosis

This assay was performed according the method above described for immune-phagocytosis. Instead opsonized yeast cells unopsonized latex beads (2.16 μm, Coulter Electronics Krefeld) were added in a ratio 30:1 (beads:PMNL) and incubated and counted as above described.

Chemiluminescence

Luminol, lucigenin, zymosan and phorbol myristate acetate (PMA) were purchased from Sigma Chemical Co., St. Louis, USA. Human standard immunoglobulin was

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obtained from Behringwerke, Marburg, FRG. Chemiluminescence was induced by three different stimuli: 1. Zymosan particles, a preparation of yeast cell walls, in a concentration of 50 μg/ml. 2. Heat aggregated IgG (human Standard Immunoglobulin was shaken for 30 min in a waterbath at 63°C)[21] in a concentration of 60 μg/ml. 3. Phorbol myristate acetate (PMA) in a concentration of 10 ng/ml.

The chemiluminescence measurements were performed in presence of luminol (0.05 mM) or lucigenin (0.1 mM) as described earlier.[22] Chemiluminescence curves in presence of $ZnCl_2$ were only compared to the daily control curves.

Measurements of Hydrogen Peroxide

The measurements of H_2O_2 were performed according to the method described by Hildebrandt and Roots[23] depending on the formation of $Fe(SCN)_3$ from ferroammonium sulfate and potassium thiocyanate by oxidation of Fe^{2+} to Fe^{3+} by H_2O_2. 100 μl cell suspension with 10^6 PMNL were stimulated with zymosan (100 μg/ml), heat aggregated IgG (250 μg/ml) or PMA (10 ng/ml) for 60 min, then the reaction was stopped by addition of 1 mM NaN_3 (50 μl) and 1 ml 3% TCA. Following a centrifugation with 10000 × g for 1 min aliquots of 1 ml of each supernatant was mixed with 0.1 ml ferroammonium sulfate (10 mM) and 0.05 ml potassium thiocyacate (2.5 M). The optical density of the colour given by $Fe(SCN)_3$ was measured spectrophotometrically at 492 nm. Hydrogen peroxide solutions were prepared from Perhydrol (Merck) diluted to 10^{-2} M standardized spectrophotometrically at 240 nm using a molar extinction coefficient $\varepsilon = 43.6\,M^{-1}cm^{-1}$.

Measurements of Lysomal Enzymes

Lysozyme (EC 3.2.1.17), micrococcus luteus, β-glucuronidase (EC 3.2.1.31) and 4-nitrophenylglucuronide were obtained from Boehringer Mannheim, FRG. The measurements were performed as described earlier[22] in supernatants of PMNL stimulated with zymosan (200 μg/ml), IgG aggregated as described above (250 μg/ml) or PMA (10 ng/ml) and incubated with or without $ZnCl_2$ for 30 min. The amount of lysozyme was estimated by lysis of *micrococcus luteus* suspended in phosphate buffer (0.05 M, pH 7.0), measured as decrease of the optical density at 450 nm over a period of 2 min. The activity of β-glucuronidase was assessed using 4-nitrophenylglucuronide as substrate following an incubation period of 18 h at 37°C in acetate buffer (0.1 M, pH 4.5). The optical density of the reaction mixture was read vs. acetate buffer at 405 nm.

Cell viability

Cell viability was tested using dye exclusion test with trypan-blue as previously described.[19]

RESULTS

$ZnCl_2$ inhibited the phagocytic capacity of human PMNL in a concentration-dependent manner. The effect with unopsonized latex particles was more evident than with opsonized yeasts when phagocytosis was receptor-mediated (Figure 1).

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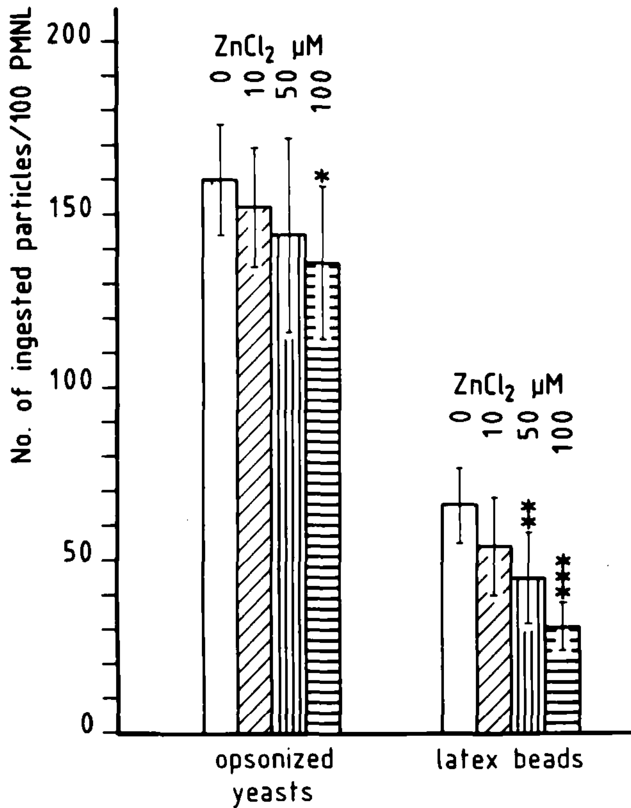

FIGURE 1 Effect of $ZnCl_2$ on the phagocytic capacity of human PMNL. Following 30 min of exposure to $ZnCl_2$ PMNL were incubated for further 30 min with opsonized yeast cells or with unopsonized latex beads (2.16 μm). Then the number of ingested yeast cells or latex beads resp. per 100 PMNL was counted. Values are means $\pm$ S.D., $n = 7$. Significant differences to the control values: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, (students t-test).

The oxidative metabolism of PMNL quantified by measuring chemiluminescence was influenced in a different manner when measured in presence of luminol or lucigenin. With luminol the toxic oxygen products such as H_2O_2, hydroxyl radical and the products of the H_2O_2-MPO-system were detected,[24,25] whereas lucigenin indicated the production of superoxide anion.[26,27] In Figure 2 one of at least four experiments with cells from different donors is shown for each experimental condition. The intensity (counts/10 sec) and the kinetics of chemiluminescence in PMNL without $ZnCl_2$ are different for the various stimuli, but in the normal range observed in all our earlier experiments. For technical limitations of our apparatus we could not compare within different probes the very fast increase of chemiluminescence intensity in the first minutes. Therefore the measurements were beginning 10 min after the addition of the stimulus. So we cannot judge the influence of $ZnCl_2$ on the oxidative metabolism in the first minutes but the later effect over a period up to two hours. However, the chemiluminescence maximum in some experiments was reached before as shown in Figure 2.

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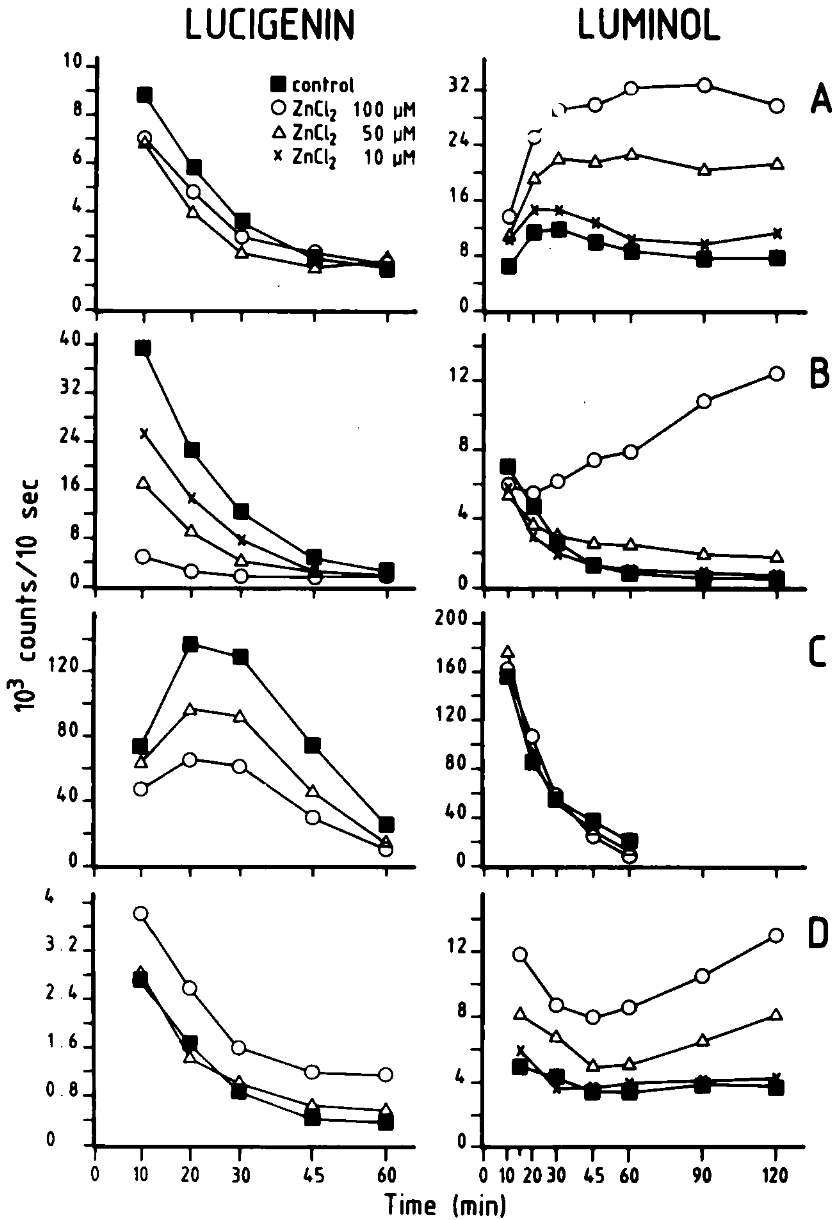

FIGURE 2 Effect of $ZnCl_2$ on the chemiluminescence of human PMNL measured with lucigenin and with luminol. $ZnCl_2$ was added together with the stimuli. A: zymosan (50 µg/ml), B: heat aggregated IgG (60 µg/ml), C: PMA (10 ng/ml), D: resting PMNL. Chemiluminescence was measured at time points indicated. Values are means of triplicates, S.D. less than 10%.

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The results in detail: The generation of superoxide anion measured with lucigenin, was decreased by $ZnCl_2$ in stimulated cells. The lowest effect was shown with unopsonized zymosan particles despite their inhibition of phagocytosis by zinc. In unstimulated resting cells, however, the production of superoxide anion was slightly increased. Measuring the oxygen products with luminol, these products were remarkably increased by $ZnCl_2$. Only when the PMNL were stimulated by PMA no influence of $ZnCl_2$ was seen. Whereas the chemiluminescence induced by the stimuli used in this study has passed its maximum intensity within one hour in presence of $ZnCl_2$ it was increased longer than this period, in resting cells it was stable up to three hours (not shown). The amounts of H_2O_2 measured following 1 h of incubation were not altered in resting cells and slightly decreased in stimulated PMNL in the presence of $ZnCl_2$ compared to the controls (Table I).

Lysosomal enzymes were only measured when they were released into the surrounding medium, for technical reasons we could not measure the release into the phagocytic vacuoles. The release of lysozyme was not influenced in presence of $ZnCl_2$ neither in resting PMNL (tested over a period of 2 h, data not shown) nor in stimulated PMNL. The reduction of lysozyme release by $ZnCl_2$ observed in PMNL stimulated by phagocytosis of zymosan seems to be due to the zinc induced inhibition of phagocytosis itself. The release of β-glucuronidase was weakly decreased in stimulated cells (Table II).

The cytotoxic effect of zinc for the human PMNL was rather weak. Within 3 h of

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TABLE I
Effect of $ZnCl_2$ on the amount of H_2O_2 in human PMNL

	nM $H_2O_2/10^6$ PMNL	
	Control	100 μM $ZnCl_2$
Resting	27.0 ± 9.5	26.6 ± 8.5
IgG	45.2 ± 12.9	41.3 ± 11.1
PMA	65.9 ± 14.9	58.6 ± 13.5
Zymosan	40.4 ± 4.6	35.6 ± 7.6

The amount of H_2O_2 in suspension with 10^6 PMNL was determined after 60 min of incubation with $ZnCl_2$ when the PMNL were in the resting state or stimulated with zymosan (100 μg/ml), heat aggregatred IgG (250 μg/ml) or PMA (10 ng/ml). Values are means ± S.D., $n = 8$.

TABLE II
Effect of $ZnCl_2$ on the release of lysosomal enzymes in human PMNL

	IgG	PMA	Zymosan
Lysozyme (μg)			
Control	0.38 ± 0.07	0.55 ± 0.03	0.42 ± 0.03
$ZnCl_2$ 100 μM	0.40 ± 0.06	0.55 ± 0.06	0.34 ± 0.03
β-Glucuronidase (IU)			
Control	139.9 ± 14.0	85.8 ± 6.6	81.3 ± 17.1
$ZnCl_2$ 100 μM	127.4 ± 16.8	73.0 ± 8.8	71.1 ± 15.8

The amount of lysozyme and the activity of β-glucuronidase were measured in supernatants of 5×10^5 PMNL after an incubation time of 30 min with $ZnCl_2$ when the PMNL were stimulated with zymosan (100 μg/ml), heat aggregated IgG (250 μg/ml) or PMA (10 ng/ml). $ZnCl_2$ was added together with the stimuli. Values are means ± S.D., $n = 6$.

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TABLE III
Effect of $ZnCl_2$ on viability of human PMNL

	No. of trypan-blue-stained cells/100 PMNL	
	3 h exposure	20 h exposure
Control	3.1 ± 1.2	3.0 ± 0.9
$ZnCl_2$ 10 μM	2.9 ± 1.1	4.3 ± 2.1
50 μM	5.2 ± 1.8	7.1 ± 0.6
100 μM	7.5 ± 2.7	10.7 ± 2.1

The number of dead trypan-blue stained cells was determined in 100 PMNL following 3 h and 20 h of preincubation with $ZnCl_2$ Values are means ± S.D., $n = 6$.

exposure to 100 μM $ZnCl_2$ about 5% cells over the control range were stained with trypan-blue indicating cell death (Table III).

DISCUSSION

Whereas many studies dealt with the effect of zinc deficiency on human blood cells, only few studies were performed with enhanced levels of zinc. This study should contribute to the question of zinc effects on the functions of human PMNL, especially on their oxidative metabolism. We tested the effect *in vitro* on isolated PMNL of healthy adults in concentrations up to about the tenfold of the normal zinc plasma level (14 μM ± 2.3 = 94 μg ± 15/dl).[28]

Our experiments showed a concentration-dependent inhibition of the phagocytic capacity by zinc chloride independent of receptor mediation by opsonisation. This effect is similar to earlier reports about *in vitro* and *in vivo* experiments.[8,11] The inhibition of phagocytosis suggests that the precondition for intracellular killing of microbes is disturbed by zinc.

The effects of zinc on the oxidative metabolism showed the same tendency when the PMNL were stimulated with the different agents. Our chemiluminescence measurements in presence of lucigenin showed a decrease of superoxide anion in stimulated cells in presence of zinc, the amount of H_2O_2 in these cells was slightly diminished, but the oxygen products measured in presence of luminol such as hydroxyl radical, singlet oxygen and H_2O_2-MPO derived products generated during the oxidative metabolism were increased by $ZnCl_2$ or, with PMA, at least higher then expected from measurements with lucigenin.

The decrease of superoxide anion was similar to the effects always reported.[14,15] The enhanced generation of the oxygen products during the oxidative metabolism measured in presence of luminol is not yet described. Although it is known that luminol-chemiluminescence is strongly dependent on the activity of MPO[25] it is not to assume that the increase of chemiluminescence observed in our study is due to an enhanced release of MPO. This enzyme is located together with β-glucuronidase in the azurophilic granula, and we found the release of β-glucuronidase slightly decreased. Studies of Venge,[29] too described an inhibition of the release of several enzymes by zinc in human PMNL, and especially the release of MPO was rather unaffected by zinc. Another reason for the finding of enhanced toxic oxygen products may be an altered activity of detoxicating systems such as hydroperoxidases, but with our experiments this question can not be clarified.

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The quantitative differences of zinc effects on the oxidative metabolism when it was induced by the various stimuli such as zymosan, aggregated IgG or PMA may be explained by different mechanisms of the respective stimulus to activate the oxidative metabolism,[30-33] but it is not to derive from our experiments in which way zinc influences these mechanisms. In this context it should be emphasized that we did not find a correlation between the phagocytic capacity and the generation of toxic oxygen species in presence of zinc ions. A similar discrepancy was shown with lead[34] and clearly indicates an interfering mechanism of the heavy metals independent of phagocytosis.

An important biological aspect of our results is the following: Normally the toxic oxygen species are emitted into the phagocytic vacuoles or, when activation occurs without phagocytosis, into the surroundings of the PMNL. In the latter case the toxic oxygen species can damage adjacent tissues leading to inflammatory reactions.[35,36] These inflammatory effects could be enhanced in presence of zinc due to the reduction of phagocytosis and due to the increase of toxic oxygen species.

In summary, we conclude that enhanced zinc supply could have inhibitory effects on microbicidal activities of human PMNL by the way of diminished phagocytosis. But whereas the data published until now suggest a general inhibition of phagocytes by zinc, the data about the oxidative metabolism presented in this study lead to the assumption that enhanced zinc supply also may induce inflammatory reactions due to enhanced release of toxic oxygen products.

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References

1. C.F. Mills (1981) Biochemical roles of trace elements. *Progression in Clinical Biology Research*, **77**, 179–188.
2. H.W. Macartney and H. Tschesche (1981) The metal ion requirement for activation of latent collagenase from human polymorpho-nuclear leukocytes. *Hoppe-Seylers Z. Physiol. Chem.*, **362**, 1523–1531.
3. J.H. Gainer (1977) Effects of heavy metals and of deficiency of zinc on mortality rates in mice infected with encephalomyocarditis virus. *American Journal of Veterinary Research*, **38**, 869–872.
4. G.W. Evans (1986) Zinc and its deficiency diseases. *Clinical Physiology of Biochemistry*, **4**, 94–98.
5. D.C. Haynes, M.E. Gershwin, M.S. Golub, A. Cheung, L.S. Hurley and A. Hendrickx (1985) Studies of marginal zinc deprivation in rhesus monkeys: VI. Influence on the immunohematology of infants in the first year. *American Journal of Clinical Nutrition*, **42**, 252–262.
6. R.K. Chandra and D.H. Dayton (1982) Trace element regulation of immunity and infection. *Nutrition Research*, **2**, 721–733.
7. L.D. Koller (1980) Immunotoxicology of heavy metals. *Internation Journal of Immunopharmacology*, **2**, 269–279.
8. R.K. Chandra (1984) Excessive intake of zinc impairs immune responses. *JAMA*, **252**, 1443–1446.
9. J. Yatsuyanagi, K. Iwai and T. Ogiso (1987) Suppressive effect of zinc on some functions of neutrophils: Studies with carrageenan-induced inflammation in rats. *Chemistry and Pharmacology* Bulletin, **35**, 699–704.
10. W.A. Skornik and J.D. Brain (1983) Relative toxicity of inhaled metal sulfate salts for pulmonary macrophages. *American Review of Respiratory Disease*, **128**, 297–303.
11. M. Chvapil, L. Stankova, Ch. Zukoski IV and Ch. Zukoski III (1977) Inhibition of some functions of polymorphonuclear leukocytes by *in vitro* zinc. *Journal of Laboratory Clinical Medicine*, **89**, 135–146.
12. B.M. Babior (1978) Oxygen-dependent microbial killing by phagocytes. *New England Journal of Medicine*, **298**, 659–668.
13. I. Fridovich (1974) Superoxide radical and the bactericidal action of phagocytes. *New England Journal Medicine*, **290**, 624–625.
14. P.H. Beswick, P.C. Brannen and S.S. Hurles (1986) The effects of smoking and zinc on the oxidative reactions of human neutrophils. *Journal of Clinical Laboratory Immunology*, **21**, 71–75.
15. M.A. Amoruso, G. Witz and B.D. Goldstein (1982) Enhancement of rat and human phagocyte superoxide anion radical production by cadmium *in vitro*. *Toxicology Letters*, **10**, 133–138.

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16. D. Malamud, A. Scott, D. Shapiro and I.M. Shapiro (1985) Low levels of mercury inhibit the respiratory burst in human polymorphonuclear leukocytes. *Biochemistry and Biophysics Research Communications*, **128**, 1145–1151.
17. D.F. Bainton (1973) Sequential degranulation of the two types of polymorphonuclear leukocyte granules during phagocytosis of microorganisms. *Journal of Cell Biology*, **58**, 249–264.
18. A. Böyum (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scandanavian Journal of Clinical Laboratory Investigation*, **21**, 77–89.
19. B. Baginski (1985) Einfluß von Blei und Cadmium auf die Vitalität und Phagozytosefähigkeit humaner polymorphkerniger Leukozyten. *Zbl. Bakt. Hyg., I.Abt. Orig.* **B 181**, 461–468.
20. L. Schmid and K. Brune (1974) Assessment of phagocytic and antimicrobial activity of human granulocytes. *Infection and Immunity*, **10**, 1120–1126.
21. I.M. Goldstein, D. Roos, H.B. Kaplan and G.J. Weissmann (1975) Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytosis. *Clinical Investigation*, **56**, 1155–1163.
22. B. Baginski (1988) Effect of mercuric chloride on microbicidal activities of human polymorphonuclear leukocytes. *Toxicology*, **50**, 247–256.
23. A.G. Hildebrandt and I. Roots (1975) Reduced Nicotinamide adenine dinucleotide phosphate (NADPH)-dependent formation and breakdown of hydrogen peroxide during mixed function oxidation reactions in liver microsomes. *Archives of Biochemistry and Biophysics*, **171**, 385–397.
24. R.C. Allen and L.D. Loose (1976) Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. *Biochemistry and Biophysics Research Communications*, **69**, 245–252.
25. C. Dahlgren and O. Stendahl (1983) Role of myeloperoxidase in luminol-dependent chemiluminescence of polymorphonuclear leukocytes. *Infection and Immunity*, **39**, 736–741.
26. R.C. Allen (1981) Lucigenin chemiluminescence: A new approach to the study of polymorphonuclear leukocyte redox activity, in M.A. DeLuca and W.D. McElroy (Eds.) Bioluminescence and Chemiluminescence, Academic Press, New York, pp. 63–72.
27. H. Gyllenhammar (1987) Lucigenin chemiluminescence in the assessment of neutrophil superoxide production. *Journal of Immunology Methods*, **97**, 209–213.
28. J. Versieck, F. Barbier, A. Speecke and J. Hoste (1974) Plasma-zinc levels. *The Lancet*, **682**.
29. P. Venge, R. Hällgren, G. Stalenheim and I. Olsson (1979) Effects of serum and cations on the selective release of granular proteins from human neutrophils during phagocytosis. *Scandanavian Journal of Haematology*, **22**, 317–326.
30. L.R. de Chatelet, C.J. Lees, C.E. Walsh, G.D. Long and P.S. Shirley (1982) Comparison of the calcium ionophore and phorbol myristate acetate on the initiation of the respiratory burst in human neutrophils. *Infection and Immunity*, **38**, 969–974.
31. J.G. Bender, D.E. van Epps (1985) Stimulus interactions in release of superoxide anion (O_2^-) from human neutrophils. Further evidence for multiple pathways of activation. *Inflammation*, **9**, 67–79.
32. N.O. Christiansen, C.S. Larsen, H. Juhl and V. Esmann (1988) Membrane-associated protein kinase C activity in superoxide-producing human polymorphonuclear leukocytes. *Journal of Leukocyte Biology*, **44**, 33–40.
33. J. Nishihira and J.T. O'Flaherty (1985) Phorbol myristate acetate receptors in human polymorphonuclear neutrophils. *Journal of Immunology*, **135**, 3439–3447.
34. B. Baginski, D. Holzwig and R. Helmrich (1988) Lead effects on human polymorphonuclear leukocytes – promotion of inflammatory reactions? in Heavy metals in the hydrological cycle (M. Astruc and J.N. Lester, eds.) Selper Ltd., London, pp. 35–40.
35. T. Sacks, Ch.F. Moldow, Ph.R. Craddock, T.K. Bowers and H.S. Jakob (1978) Oxygen radicals mediate endothelial cell damage by complement- stimulated granulocytes. *Journal of Clinical Investigation*, **61**, 1161–1167.
36. P.A. Ward (1983) Role of toxic oxygen products from phagocytic cells in tissue injury. *Advances in Shock Research*, **10**, 27–34.

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