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The effect of metal ions on neutrophil degranulation

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This study examined neutrophil activation, after incubation of these cells with metal ions. Activation was examined using lysozyme and chemiluminescence assays. Cobalt ions (0.5–30 p.p.m.), chromium ions (0.5–20 p.p.m.), nickel ions (1–50 p.p.m.) and aluminium ions (0.05–7.5 p.p.m.) did not stimulate neutrophils to release lysozyme and did not stimulate a respiratory burst. Copper ions (0.25–7.5 p.p.m.) stimulated neutrophils to release lysozyme, a result which was significant at the 5% level. This was not as a result of copper ions causing neutrophils to lyse. Lysozyme secretion was inhibited when neutrophils were pre-incubated with cytochalasin B, although cytochalasin B had no effect on the enzyme secretion due to stimulation by opsonized zymosan and zymosan-activated serum. This suggests that the mechanism by which copper ions stimulate neutrophil degranulation is different from that of opsonized zymosan and zymosan-activated serum. Enzyme secretion was not accompanied by a respiratory burst.

1. Introduction

Metals are commonly used components in implant surgery. With these implantable devices, the interaction of metals with tissue is important in determining their overall performance. There has been much interest in the mechanisms by which implanted metals react with tissues including studies of soft-tissue and hard-tissue responses to metals [1, 2], metal–cell interactions in culture [3] and the influence of metal ions on chemotaxis [4]. Results from these studies have shown that metal ions potentially derived from implants inhibit chemotaxis and may be toxic to macrophages and fibroblasts [5, 6]. Such observations have been placed in the context of metal toxicology in general; it is known, for example, that cadmium ions inhibit phagocytosis by macrophages [7] and zinc ions inhibit macrophage migration by inducing cells to spread [8].

One of the features of the tissue response to implanted materials is the infiltration of inflammatory cells such as neutrophils. Neutrophils play a fundamental role in host defence, their primary function being to phagocytose and to digest invading micro-organisms. During phagocytosis, neutrophils may secrete lysosomal enzymes into the tissue. Degradative enzymes are also released during the process of frustrated phagocytosis, where the particle is too big to be phagocytosed. A mature neutrophil contains two types of enzyme-containing lysosome, azurophil granules and specific granules. The specific granules contain the neutral proteases and 50% of the lysozyme. The azurophil granules contain acid hydrolases and other enzymes as well as the remaining lysozyme.

Lysosomal enzymes have been shown to provoke acute inflammation and tissue injury. Some lysosomal enzymes have acid pH optima and probably cannot operate outside the lysosome. The primary sources of enzyme activity from human polymorphs that is responsible for tissue degradation at physiological pH are the neutral proteases which have an optimal activity at neutral pH [9]. The two best known are elastase and collagenase [3]. Elastase can digest blood vessel walls and collagenase is an enzyme which initiates the degradation of collagen.

Degranulation may be accompanied by a respiratory burst, providing a battery of oxidizing agents (high-energy free radicals) which can be used by the neutrophil for the destruction of micro-organisms. When these free radicals are released into tissue they can injure other cells or membranes, e.g. superoxide anion can damage connective tissue, such as the hyaluronate of synovial fluid [10].

In the present study, a lysozyme assay, not previously used in the biomaterials field, was adapted to examine enzyme secretion from human neutrophils incubated with metal ions. A chemiluminescence assay, which detects light emitted from high-energy oxygen radicals, was used to investigate the respiratory burst. A small range of metals were selected, with different characteristics. Some have distinct and clear relevance to current implantable alloys, e.g. Co and Cr in Co–Cr and stainless steel and Ni in stainless steel. Others have more limited relevance, for example Cu in IUDs and dental casting alloys and Al in certain ceramics, both of these being included to extend the range to metals with interesting biological properties.

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2. Experimental procedure

2.1. Materials

The following materials were used: copper chloride, aluminium chloride, cobalt chloride, nickel chloride, chromic chloride, glutaraldehyde and Triton X 100, obtained from BDH. Ficoll-Hypaque was obtained from Pharmacia, and cytochalasin B from Aldrich Chemicals. Hanks balanced salt solution (HBSS) and phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS) were obtained from Gibco. Zymosan, N-formyl-methionine–leucine–phenylalanine (FMLP), calcium ionophore, luminol, dimethylsulphoxide, *Micrococcus lysodeikticus* and morpholinopropane sulphonic acids (MOPS) were all obtained from Sigma Ltd. MOPS was used at a final concentration of 10 mM in HBSS. Luminol was diluted to 10^{-2} M in dimethyl sulphoxide and diluted to a final concentration of 10^{-4} M in PBS. β-glucuronidase diagnostic kit No. 325 and lactate dehydrogenase diagnostic kit No. 500 were obtained from Sigma Ltd.

2.2. Neutrophil isolation

Neutrophils were isolated from fresh heparinized human blood obtained from healthy volunteers after mixing 2 volumes blood with 1 volume 6% dextran. After 25–30 min, the supernatant was layered on to lymphocyte separation mixture (Ficoll-Hypaque) and centrifuged at 1200 r.p.m. for 25 min. The pellet was transferred to a fresh conical bottomed test tube and washed once in HBSS–MOPS. The remaining red blood cells were lysed by resuspending the pellet in 1 ml sterile distilled water for 30 s, after which 10 ml HBSS–MOPS was added. This provided a population of > 95% round neutrophils.

2.3. Enzyme assays

1 ml of neutrophils at 2×10^6 cells ml^{-1} were incubated for 30 min at 37 °C with 1 ml of either PBS (negative control), opsonized zymosan (1 mg ml^{-1}) (positive control), or one of the following metal chlorides: copper, aluminium, cobalt, chromium or nickel, at the required concentration. In some experiments, neutrophils were pre-incubated with cytochalasin B (5 μg ml^{-1}) for 15 min at 37 °C, and then added to the test material and incubated for 1 h.

After incubation the tubes were centrifuged at 1200 r.p.m. for 5 min. 1 ml of supernatant was added to 1 ml of *M. lysodeikticus* (1.5 mg ml^{-1}) and incubated for 30 min at 37 °C. Lysozyme breaks down the cell wall of *M. lysodeikticus*. Lysozyme activity was assessed by the amount of light passing through the *M. lysodeikticus* suspension. Absorbance readings were examined using a spectrophotometer at a wavelength of 541 nm. In order to quantify lysozyme secretion, a standard curve was prepared with dilutions of neutrophil lysate versus absorbance readings. Lysozyme release from test samples was calculated as a percentage of the total amount of lysozyme present. Neutrophil supernatant was also examined for β-glucuronidase using the β-glucuronidase diagnostic kit.

2.4. Cell viability

Examination for release of the cytoplasmic enzyme LDH was used to determine cell viability. LDH release was determined using the LDH diagnostic kit No. 500. A suspension of neutrophil lysate was used as a positive control.

A polarization assay [4] was also used to examine cell viability. After prior incubation with metal ions for 30 min at 37 °C, neutrophils were incubated with 10^{-8} M FMLP for 30 min at 37 °C. The neutrophils were fixed with 2.5% glutaraldehyde and examined using light microscopy. For viable neutrophil populations, > 90% of neutrophils polarized.

2.5. Chemiluminescence

0.5 ml of cell suspension (5×10^6 cells ml^{-1}) was added to 0.5 ml of reaction mixture, i.e. metal ions, PBS (negative control) or opsonized zymosan (positive control). 0.1 ml of luminol was added at a concentration of 10^{-4} M. The mixture was placed on a Spiramix in a 37 °C incubator and removed from the incubator at time intervals up to 1 h. Light output was measured in mV using a luminometer (luminometer 1250, LKB Wallace).

Statistical analysis of data was performed using a paired *t* test (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Lysozyme

The quantity of lysozyme in human neutrophils varied from subject to subject (Fig. 1); an individual calibration curve was therefore used for each experiment. It was found, however, that for a specific subject the total lysozyme content did not vary greatly from day to day.

Opsonized zymosan stimulated neutrophils to secrete between 10 and 20% of their lysozyme compared to < 6% in the negative control (Tables I–V). Neutrophils incubated with nickel or aluminium ions, at all concentrations examined, did not secrete lysozyme above negative control levels (Tables II and III). Cobalt ions at 30.0 p.p.m. (expt 2, Table IV) and chromium ions at 10 p.p.m. (expt 1, Table I) did stimulate lysozyme release, but at these concentrations the metal ions precipitated. This precipitate may be responsible for neutrophil aggregation and inhibition of chemotaxis under agarose [4] and may also be responsible for causing neutrophils to release lysozyme in the present study. At lower concentrations of cobalt ions and chromium ions, no lysozyme release was detected. In these experiments, opsonized zymosan was used as a positive control.

Neutrophils incubated with copper ions at concentrations ranging from 0.25 to 7.5 p.p.m. were stimulated to secrete lysozyme (Tables V and VI). This increase above negative control values is significant at the 5% level. At increasing concentrations of copper ions there is an increase in the quantity of lysozyme secreted (Tables V and VI). Lysozyme secretion from neutrophils stimulated with copper, but not opsonized

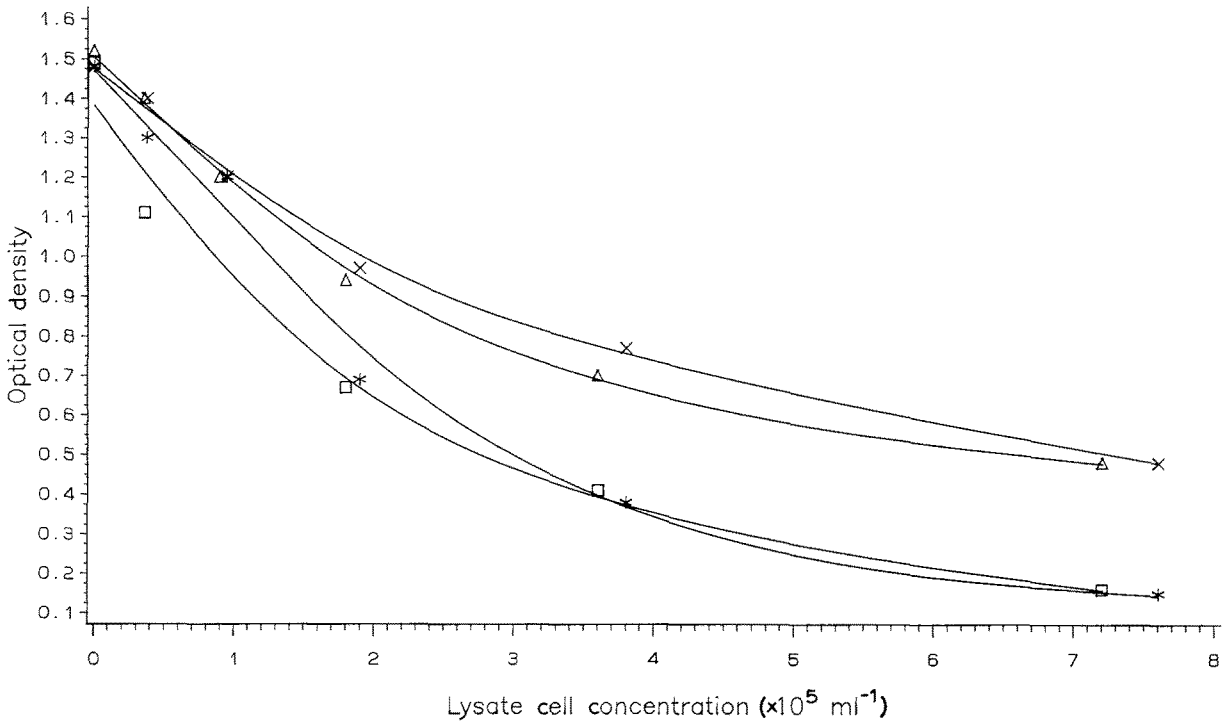

Figure 1 Plots of lysate cell concentration against optical density: (×) subject 1, (*) subject 2, (△) subject 3, (□) subject 4.

TABLE I Lysozyme release from neutrophils after incubation with chromium ions

Material	Lysozyme release (%)	
	Expt 1	Expt 2
PBS	3.3	3.9
Op. zymosan	17.0	17.7
10.0 p.p.m.	10.2	3.9
5.0 p.p.m.	3.6	3.9
2.5 p.p.m.	3.6	5.5
1.25 p.p.m.	3.9	–
0.5 p.p.m.	3.9	–
0.25 p.p.m.	4.6	–

TABLE II Lysozyme release from neutrophils after incubation with nickel ions

Material	Lysozyme release (%)[a]
PBS	2.8 ± 2.3
Op. zymosan	18.4 ± 1.1
50.0 p.p.m.	4.0 ± 1.00
40.0 p.p.m.	4.0 ± 1.00
20.0 p.p.m.	4.26 ± 1.1
10.0 p.p.m.	4.16 ± 0.85
5.0 p.p.m.	4.17 ± 1.10
0.1 p.p.m.	5.1 ± 2.2

[a] Results expressed as the mean ± standard deviation of three experiments.

TABLE III Lysozyme release from neutrophils after incubation with aluminium ions

Material	Lysozyme release (%)[a]
PBS	4.1 ± 2.53
Op. zymosan	17.90 ± 8.41[b]
7.5 p.p.m.	3.23 ± 2.14
2.5 p.p.m.	3.63 ± 1.77

[a] Results expressed as the mean ± S.D. of 19 experiments.
[b] There is a significant difference between these results and those for PBS, at the 95% confidence limit using the paired Student's *t* test.

TABLE IV Lysozyme release from neutrophils after incubation with cobalt ions

Material	Lysozyme release (%)	
	Expt 1	Expt 2
PBS	6.1	4.9
Op. zymosan	14.2	12.0
30.0 p.p.m.	8.3	23.3
20.0 p.p.m.	5.9	–
10.0 p.p.m.	4.9	5.5
5.0 p.p.m.	5.2	–
2.5 p.p.m.	5.7	4.6
0.5 p.p.m.	–	4.9
0.05 p.p.m.	6.1	5.1
0.005 p.p.m.	–	5.1

zymosan nor zymosan-activated serum, was inhibited by pre-incubating neutrophils with cytochalasin B (Table VII). For copper at 7.5 and 5 p.p.m., the inhibition was significant at the 1% and 6% levels, respectively. There was no enhancement of the release of lysozyme due to opsonized zymosan or zymosan-activated serum after pre-incubation with cytochalasin B. Copper ions did not influence the activity of neutrophil lysozyme to lyse *M. lysodeikticus* (Table VIII).

3.2. β-Glucuronidase

Neutrophils contained detectable levels of β-glucuronidase as shown in experiments with lysed neutrophil suspensions (Table IX). Copper (0.05–7.5 p.p.m.), aluminium (0.05–7.5 p.p.m.), cobalt (0.5–30 p.p.m.),

chromium (0.5–20 p.p.m.) and nickel (1–50 p.p.m.) ions did not stimulate the release of detectable levels of β-glucuronidase from the azurophil granules of neutrophils. Other agents, e.g. opsonized zymosan, FMLP, calcium ionophore and ZAS, which have previously been reported to stimulate [11–13] or not to stimulate [14, 15] neutrophils to secrete β-glucuronidase, did not do so in this study. Pre-incubation of neutrophils with cytochalasin B (5 $\mu g\,ml^{-1}$) had no effect on the activity of neutrophils to secrete β-glucuronidase.

3.3. Cell viability

Neutrophil viability was assessed using a neutrophil polarization assay and an LDH assay. Copper and aluminium ions did not inhibit polarization of human neutrophils in the presence of the known chemotactic factor FMLP (Tables X and XI), indicating that the neutrophils were viable. A previous study [4] has also shown that cobalt, chromium and nickel ions do not inhibit the neutrophil polarization response to FMLP. LDH activity was inhibited by copper ions (Table XII), and this is therefore not a good test for cell viability in this case.

TABLE V Lysozyme release from neutrophils after incubation with copper ions

Material	Lysozyme release (%)[a]
PBS	3.46 ± 2.51
Op. zymosan	18.86 ± 10.32[b]
7.5 p.p.m.	15.65 ± 9.01[b]
5.0 p.p.m.	11.38 ± 5.04[b]
2.5 p.p.m.	9.45 ± 4.83[b]
0.5 p.p.m.	7.61 ± 4.58[b]
0.25 p.p.m.	6.52 ± 4.34[b]
0.05 p.p.m.	4.97 ± 3.52
0.025 p.p.m.	4.18 ± 3.26
0.005 p.p.m.	4.36 ± 3.12
0.0025 p.p.m.	2.97 ± 1.36
0.0005 p.p.m.	3.90 ± 4.60

[a] Results expressed as the mean ± S.D. of 19 experiments.
[b] There is a significant difference between these results and those for PBS, at the 95% confidence limit using the paired Student's *t* test.

Aluminium ions did not stimulate neutrophils to polarize (Table XIII). Copper ions stimulated neutrophils isolated from subject 1 (Table XIV) to polarize. The polarization response of neutrophils isolated from other volunteers was also examined. In some cases, copper ions did not stimulate neutrophils to polarize (subjects 2 and 4, Table XIV). These results suggest that the interaction of neutrophils with copper ions varies between neutrophil populations isolated from different subjects.

3.4. Chemiluminescence

Cobalt, chromium, nickel [4], aluminium and copper ions did not stimulate a neutrophil respiratory burst (Fig. 2).

4. Discussion

It has been suggested that neutrophils and macrophages are responsible for tissue damage adjacent to metallic implants, and ions from metal salts and from corrosion products of stainless steel have been reported to bind to white cells *in vivo* [16, 17]. Since neutrophils are associated with a wide range of inflammatory conditions [18], owing to their ability to produce tissue-degrading enzymes and toxic oxygen metabolites upon activation, the present study examined whether metal ions activate neutrophils. In particular, cobalt, chromium, nickel, aluminium and copper ions, which are important constituents of metallic implants, were examined.

A lysozyme assay was adapted to examine enzyme secretion from human neutrophils incubated with metal ions. Lysozyme release from neutrophils was used as a marker for enzyme secretion and lysozyme was detected through its activity to lyse the bacteria *M. lysodeikticus*. This resulted in a decrease in the optical density of the *M. lysodeikticus* suspension. A standard curve of neutrophil lysozyme activity versus optical density was included in each experiment (Fig. 1). After stimulation of a neutrophil population, the percentage lysozyme release was determined by extrapolation from the standard curve.

TABLE VI Lysozyme release from neutrophils after incubation with copper ions

Test material	Mean lysozyme release (%)		
	Subject 1[a]	Subject 2[b]	Subject 3[c]
PBS	5.54 ± 2.08	1.62 ± 0.43	4.2
Op. zymosan	21.62 ± 2.20[d]	12.25 ± 3.71[d]	31.7
7.5 p.p.m.	15.20 ± 2.95[d]	8.53 ± 0.81[d]	28.1
5.0 p.p.m.	15.73 ± 1.42[d]	8.13 ± 3.45[d]	13.5
2.5 p.p.m.	14.30 ± 2.96[d]	7.35 ± 3.00[d]	8.4
0.5 p.p.m.	12.47 ± 2.21[d]	–	6.4
0.25 p.p.m.	11.20 ± 3.16[d]	–	5.9
0.05 p.p.m.	7.87 ± 3.53	2.87 ± 0.81	5.6
0.025 p.p.m.	8.50 ± 4.03	2.62 ± 0.88	–
0.005 p.p.m.	–	3.27 ± 1.10	5.0

[a] Results expressed as the mean ± S.D. of four experiments.
[b] Results expressed as the mean ± S.D. of three experiments.
[c] Results expressed as the mean of two experiments.
[d] There is a significant difference between these results and those for PBS, at the 95% confidence limit using the paired Student's *t* test.

TABLE VII Lysozyme release from neutrophils stimulated with copper ions after pre-incubation with cytochalasin B

Test material	Lysozyme release (%)[a]
PBS	5.74 ± 5.24
PBS + cyto. B	3.46 ± 2.10
Op. zymosan	25.23 ± 15.80
Op. zymosan + cyto. B	26.69 ± 14.97
ZAS	14.20 ± 11.53
ZAS + cyto. B	15.94 ± 11.24
7.5 p.p.m.	24.88 ± 15.85[b]
7.5 p.p.m. + cyto. B	16.75 ± 12.84[b]
5.0 p.p.m.	18.96 ± 11.89[c]
5.0 p.p.m. + cyto. B	12.28 ± 11.25[c]

[a] Results expressed as the mean ± S.D. of 12 experiments.
[b] There is a significant difference between these results at the 99% confidence limit using the Student's paired *t* test.
[c] There is a significant difference between these results at the 94% confidence limit using the paired Student's *t* test.

TABLE VIII Effect of copper ions on the activity of lysozyme

Lysate dilution	Incubation medium	Lysozyme activity[a]
9/10	PBS	91
	100 p.p.m. Cu	90
4/5	PBS	88
	7.5 p.p.m. Cu	83
3/5	PBS	79
	7.5 p.p.m. Cu	83
2/5	PBS	57
	7.5 p.p.m. Cu	62
1/5	PBS	43
	7.5 p.p.m. Cu	40
1/10	PBS	32
	7.5 p.p.m. Cu	30
1/20	PBS	14
	7.5 p.p.m. Cu	12
1/40	PBS	5
	7.5 p.p.m. Cu	4

[a] Activity expressed as a percentage of the total enzyme activity present in the undiluted lysate.

TABLE IX β-Glucuronidase activity in neutrophil lysate[a]

Lysate dilution	Equivalent cell conc. ($\times 10^4$)	β-Glucuronidase (Sigma units/ml)[b]
1/40	3.06	12
1/20	6.12	6
1/10	12.25	21
1/5	24.50	36
1/2	61.25	60
0	122.50	108
PBS	0.0	0

[a] After incubation of neutrophils with copper ions (0.05–7.5 p.p.m.), aluminium ions (0.05–7.5 p.p.m), cobalt ions (0.5–30 p.p.m.), chromium ions (0.5–20 p.p.m.) and nickel (1–50 p.p.m.), no β-glucuronidase activity was detected in the supernatant.
[b] One Sigma unit of β-glucuronidase activity will liberate 1 μg of phenolphthalein glucuronic acid per hour at 56 °C.

TABLE X Neutrophil polarization response to FMLP after pre-incubation of neutrophils with copper ions

Test material	Polarization (%)[a]
PBS	1.5
FMLP	82.5
7.5 p.p.m.	93.0
5.0 p.p.m.	93.5
2.5 p.p.m.	93.5
0.5 p.p.m.	90.5
0.025 p.p.m.	89.5

[a] Results expressed as the mean of two experiments.

TABLE XI Neutrophil polarization response to FMLP after pre-incubation of neutrophils with aluminium ions

Test material	Polarization (%)[a]
PBS	1.5
FMLP	82.5
7.5 p.p.m.	94.0
5.0 p.p.m.	93.0
2.5 p.p.m.	93.0
0.5 p.p.m.	91.5
0.025 p.p.m.	85.5

[a] Results expressed as the mean of two experiments.

TABLE XII The effect of copper ions on the activity of lactate dehydrogenase

Lysate dilution	Incubation medium	LDH (Sigma units/ml)[a]
0	PBS	< 2000
	100 p.p.m. Cu	< 2000
1/2	PBS	< 2000
	7.5 p.p.m. Cu	195
1/4	PBS	160
	7.5 p.p.m. Cu	90
1/10	PBS	410
	7.5 p.p.m. Cu	90
1/20	PBS	290
	7.5 p.p.m. Cu	90
1/40	PBS	160
	7.5 p.p.m. Cu	0
1/100	PBS	50
	7.5 p.p.m. Cu	0

[a] One Sigma unit is defined as the amount of LDH that will reduce 4.8×10^{-4} μmol of pyruvate per minute at 25 °C.

TABLE XIII Polarization response of neutrophils incubated with aluminium ions

Test material	Polarization (%)[a]
PBS	4.00 ± 3.21
FMLP	98.00 ± 1.00
7.5 p.p.m.	4.53 ± 2.52
5.0 p.p.m.	3.60 ± 1.53
2.5 p.p.m.	2.50 ± 1.00
0.5 p.p.m.	1.75 ± 1.53
0.025 p.p.m.	3.10 ± 1.15

[a] Results expressed as the mean ± S.D. of three experiments.

In this study, opsonized zymosan was used as a positive control for neutrophil activation. In 19 experiments examined (Table V), opsonized zymosan stimulated neutrophils to secrete 15.5% of their total lysozyme above negative control levels. This is in agreement with previous studies where neutrophils have been reported to release a relatively small quantity of lysosomal enzymes, after incubation with secretagogues [15]. Henson [19] has previously remarked that the maximum percentage of lysosomal enzymes

TABLE XIV Polarization response of neutrophils incubated with copper ions

Test material	Polarization (%)			
	Subject 1[a]	Subject 2	Subject 3	Subject 4
PBS	3.3 ± 3.2	1.0	1.5	1.5
FMLP	98.0 ± 1.0	98.0	94.0	98.0
7.5 p.p.m.	88.3 ± 4.9	6.0	21.0[b]	6.0
5.0 p.p.m.	81.3 ± 10.2	9.0	15.0[b]	3.0
2.5 p.p.m.	63.3 ± 18.9	3.0	10.0[b]	3.0
0.5 p.p.m.	21.3 ± 5.0	2.0	1.0	2.0
0.025 p.p.m.	1.6 ± 1.1	2.0	2.0	3.0

[a] Results expressed as the mean and standard deviation of three experiments.
[b] Neutrophils changed shape but were not truly polarized.

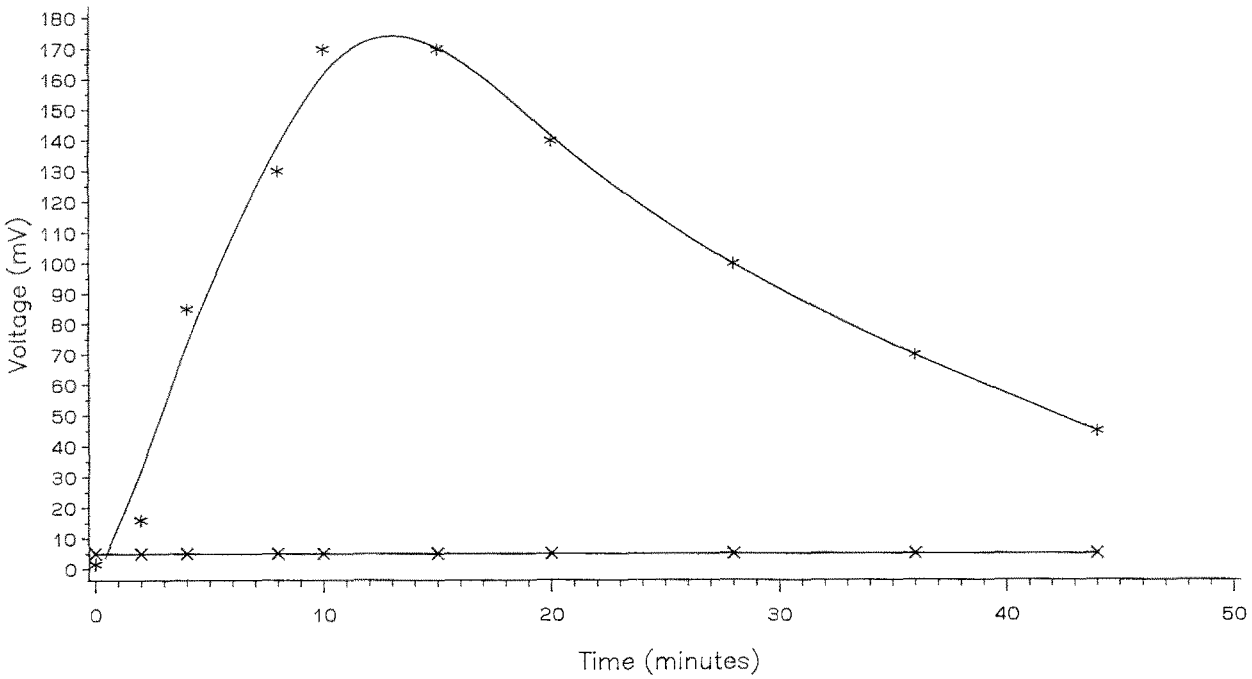

Figure 2 Chemiluminescence plots of voltage against time: neutrophils incubated with (×) PBS, 15.0 p.p.m., 10.0 p.p.m. or 5.0 p.p.m. Cu ions, or (*) opsonized zymosan.

secreted by neutrophils *in vitro* varies from individual to individual but seldom exceeds 30% of the total available.

The metal ions cobalt (0.05–20 p.p.m.), chromium (0.25–10 p.p.m.) and nickel (0.5–50 p.p.m.) did not stimulate neutrophils to secrete lysozyme (Tables I, II and IV) and did not stimulate a neutrophil respiratory burst (Fig. 2). Thus this study suggests that the tissue response to metallic implants, such as the reported inflammatory response around cobalt and nickel discs, implanted intramuscularly into rats [1], does not result from tissue damage due to metal ions activating neutrophils. However, for intramuscularly implanted copper discs, histochemical observations indicated a massive release of lysosomal enzymes and an inflammatory response occurred in tissue adjacent to the implant [1]. These results correlate well with results from this study which show that copper ions stimulate neutrophils to degranulate (Tables V, VI and VII). At increasing concentrations of copper (0.5–7.5 p.p.m.), the quantity of lysozyme secreted increased. The polarization experiments confirm that the lysozyme released is not due to any toxic effects from the copper ions (Table X). Enzyme secretion is not accompanied by a respiratory burst (Fig. 2).

Neither copper ions nor zymosan stimulated neutrophils to secrete detectable levels of the acid hydrolase β-glucuronidase (Table IX). β-glucuronidase is found only in the azurophil lysosomal granules. These results suggest that the neutrophils may not be stimulated to secrete the contents of their azurophil granules, or that the quantity of β-glucuronidase released is insufficient to be detected using this assay. This result is in accord with a previous study where neutrophils were stimulated to secrete lysozyme but not β-glucuronidase [14, 15], but differs from other studies where stimulatory agents caused the secretion of both lysozyme and β-glucuronidase [11–13]. The reason for conflicting reports about the ability of different agents to stimulate secretion of β-glucuronidase from neutrophils is not clear, but may be due to differences in the neutrophil isolation procedures.

The mechanism of neutrophil stimulation by copper ions is not known. It seems to be different to the mechanism(s) by which opsonized zymosan and ZAS stimulate neutrophil degranulation. Unlike the latter

agents, neutrophil degranulation in response to copper ions is inhibited if the neutrophils are pre-incubated with cytochalasin B (Table VII). Cytochalasin B has previously been described as an enhancer of enzyme secretion from neutrophils incubated with the aforementioned secretagogues [12, 20].

In the case of neutrophil populations from some individuals, copper ions stimulate neutrophils to polarize (Table XIV). This is comparable to the response of neutrophils incubated with nickel ions, where nickel ions stimulated neutrophils to polarize, and the number of polarized neutrophils increased as the concentration of nickel increased [4]. In these experiments, nickel ions inhibited chemotaxis under agarose. This was considered to be a possible result of the activity of nickel to act as a calcium ion agonist, and inhibit calcium ion-dependent contractile events during chemotaxis.

Calcium ions are also an important part of the signal transduction mechanism which is involved in the stimulation of degranulation of neutrophil lysosomes [13]. It may be the case that divalent copper ions stimulate neutrophils to degranulate as a result of their similarities to calcium ions.

5. Conclusions

In the present study a lysozyme assay and chemiluminescence assay have been used to examine neutrophil activation, after incubation of neutrophils with metal ions. Cobalt, chromium, aluminium and nickel ions did not stimulate either enzyme secretion or a respiratory burst. Copper ions did stimulate enzyme secretion, but not a respiratory burst. The mechanism of secretion appears to be different from that of opsonized zymosan and zymosan-activated serum.

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