TOXICOLOGY AND APPLIED PHARMACOLOGY 53, 14–23 (1980)

Effects of Heavy Metal Ions on Selected Oxidative Metabolic Processes in Rat Alveolar Macrophages

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Received August 21, 1979; accepted November 21, 1979

Effects of Heavy Metal Ions on Selected Oxidative Metabolic Processes in Rat Alveolar Macrophages. CASTRANOVA, V., BOWMAN, L., REASOR, M. J., AND MILES, P. R. (1980). *Toxicol. Appl. Pharmacol.* **53**, 14–23. The effects of four heavy metal cations, Cd^{2+}, Hg^{2+}, Ni^{2+}, and Pb^{2+}, on oxygen consumption, glucose metabolism, and the release of active oxygen species (as measured by chemiluminescence) were studied in rat alveolar macrophages at rest (no phagocytosis) and during phagocytosis. All four heavy metals depress the oxygen consumption and glucose metabolism which occurs in alveolar macrophages at rest by about 60–95%. During phagocytosis there is release of reactive forms of oxygen from the cells, a two- to threefold increase in oxygen consumption, but no change in glucose metabolism from that which occurs in resting cells. The metals inhibit the release of active oxygen from the cells and the oxygen consumption which occurs during phagocytosis by 75–85%. The ED_{50} values, i.e., the concentrations of metals which produce one-half of the maximal effects, indicate that the mechanism for release of active oxygen is affected by much lower concentrations of metals than is oxygen consumption. Also, experiments with trypan blue provide evidence that the metals can affect oxidative metabolism without causing gross membrane damage. The results of these experiments indicate that heavy metals inhibit oxidative metabolic processes in alveolar macrophages and, thus, may diminish the antibacterial activity of these cells.

Many heavy metals have been found to be associated with airborne particles which are derived from combustion sources, such as fossil-fueled power plants, metallurgical smelters, and blast furnaces, and also from automobiles and municipal incinerators (Lee *et al.*, 1972; Lee and Von Lehmden, 1973; Natusch *et al.*, 1973). Some of the metals, such as cadmium, lead, zinc, nickel, manganese, and copper, are found in very high concentrations in some of the smallest particles collected from ambient air (Lee *et al.*, 1972; Natusch *et al.*, 1973). Many of these airborne particles are able to reach the alveoli where they may affect alveolar macrophages. Indeed, many *in vivo* and *in vitro* studies dealing with the effects of heavy metals on alveolar macrophages have been done. For example, vanadium, nickel, manganese, cadmium, and chromium cause decreases in viability, phagocytic activity, and lysosomal enzyme activity in alveolar macrophages (Waters *et al.*, 1974; Waters and Gardner, 1975; Loose *et al.*, 1978). Other *in vitro* studies have shown that various metal oxides which have been adsorbed on fly ash also produce decreases in cell viability and phagocytic activity (Aranyi *et al.*, 1977). Thus, alveolar macrophage function can be influenced by various heavy metals.

When alveolar macrophages are exposed

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to foreign particles, phagocytosis of the particles occurs. In addition to engulfment of the material, exposure of the cells to particles leads to the release of antibacterial substances from the alveolar macrophages. These antibacterial substances are various reactive forms of oxygen. For example, exposure of pulmonary macrophages to foreign particles leads to the production of superoxide anion (Drath and Karnovsky, 1975), hydrogen peroxide (Gee *et al.*, 1970; Klebanoff and Hamon, 1976), and possibly singlet oxygen and hydroxyl radical (Miles *et al.*, 1978). The release of antibacterial substances in response to zymosan particles can be measured as chemiluminescence (Miles *et al.*, 1978). In addition to the release of these substances, other changes in the metabolic activity of the cells occur. Increases in oxygen consumption and glucose metabolism have been shown to occur with exposure to particles in cells from some animals (Ouchi *et al.*, 1965; Myrvik and Evan, 1967). Therefore, it appears as though oxygen metabolism plays an important role in the antibacterial activity of pulmonary macrophages.

The objective of this investigation was to study the effects of four heavy metal cations, Cd^{2+}, Hg^{2+}, Ni^{2+}, and Pb^{2+}, on some of the oxidative metabolic events which occur in rat alveolar macrophages. The experiments were done by measuring oxygen consumption, glucose metabolism, and the release of reactive forms of oxygen (measured as chemiluminescence) in alveolar macrophages at rest and following exposure to zymosan particles. A preliminary report of these results has appeared previously (Castranova *et al.*, 1979).

METHODS

Alveolar macrophages were obtained from male Long–Evans hooded rats (250–300 g) by tracheal lavage according to the method of Myrvik *et al.* (1961). The animals were anesthetized with sodium pentobarbital (0.2 g/kg body wt) and exsanguinated by cutting the abdominal aorta. The lungs from each rat were "lavaged" 12 times with a total of 80 ml of an ice-cold Ca^{2+}-free solution which contained 145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, and 5 mM glucose (pH = 7.4). The cells were separated from the lavage fluid by centrifugation at 500*g* for 5 min. Then the alveolar macrophages from several animals were pooled and washed twice by alternate centrifugation and resuspension in a Hepes-buffered medium of the following composition: 140 mM NaCl, 5 mM KCl, 10 mM Na-Hepes (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid), and 5 mM glucose (pH = 7.4). The cells were then resuspended in the Hepes-buffered medium in which all measurements were made. Hepes buffer was used because it does not bind to heavy metals in solution (Good *et al.*, 1966). The metals used in these studies were added to the cell suspensions as either the chloride or acetate salts.

The measurements were made with the cells in suspension at rest (no exposure to particles) and following exposure to foreign particles. The particle used in all experiments was zymosan, an extract from the cell walls of *Saccharomyces cerevisiae* yeast (Sigma Chemical Co., St. Louis, Mo.). The zymosan was prepared by boiling 20–50 mg in 2–3 ml of Hepes-buffered solution for 15 min. Then the zymosan was separated from the solution by centrifugation and resuspended in fresh Hepes-buffered solution. This procedure seemed to prevent the zymosan particles from clumping during the course of the experiments. The ratio of zymosan to cells used in these studies was 20 mg/10^7 alveolar macrophages.

Oxygen consumption was measured with a Gilson K-1C oxygraph which was equipped with a Clark electrode (Gilson Medical Electronics; Middleton, Wisc.). The oxygraph was calibrated by measuring the levels of oxygen in various Hepes-buffered media which had been bubbled with gases of known oxygen concentrations until saturation occurred. In order to measure the amount of oxygen consumed by alveolar macrophages, the cells were preincubated at 37°C for 15 min either in the presence or absence of the heavy metals. Then 3×10^6 cells in 1.65 ml of Hepes-buffered medium were transferred to the chamber of the oxygraph which was also maintained at 37°C, and oxygen consumption was measured over a period of 10 min. When the effects of particle exposure were studied, zymosan was added to the cell suspension at the time when the cells were transferred to the oxygraph (zero time). Oxygen consumption was expressed as nanomoles of oxygen consumed per 10^7 alveolar macrophages per minute.

Glucose metabolism was studied by measuring the amount of $^{14}CO_2$ produced during incubation of the alveolar macrophages in medium containing [1-^{14}C]-glucose. The experiments were performed by using a

slight modification of the method of Myrvik and Evan (1967). The cells were preincubated at 37°C for 15 min either in the presence or absence of the heavy metals. Then aliquots of the cell suspension (each aliquot is 6×10^6 cells in 4 ml of Hepes-buffered medium) were transferred to the main compartments of Warburg flasks and 2 μCi of [1-^{14}C]glucose (New England Nuclear Corp., Boston, Mass.) was added to each flask. The center well of each flask contained 0.5 ml of 2 M KOH. When the effects of particle exposure were to be studied, zymosan was added to the cell suspension at the time of the addition of radioactive isotope. Then the flasks were stoppered and incubated at 37°C for 15 min. After this incubation, 0.4 ml of 3 M H_2SO_4 was added to each cell suspension and the flasks were incubated for an additional 30 min at 37°C. Then 0.4 ml of the KOH was removed from each flask, added to 0.4 ml of 2 M HCl, and placed in a liquid scintillation vial with 10 ml of Aquasol (New England Nuclear Corp.). These samples were counted in a liquid scintillation counter and the disintegrations per minute determined. Glucose metabolism was expressed as nanomoles of glucose utilized per 10^7 cells per minute.

Chemiluminescence (CL) was measured according to a method we published previously (Miles *et al.*, 1978). Briefly, 5×10^6 alveolar macrophages in 5 ml of Hepes-buffered medium were preincubated at 37°C for 15 min either in the presence or absence of the heavy metals. All incubations were performed in dark-adapted plastic liquid scintillation vials. After the period of preincubation, zymosan was added to each vial and CL was measured immediately (zero time measurement). The CL was measured as counts per minute in the tritium channel of a liquid scintillation counter (Model LS-345; Beckman Instrument Co., Fullerton, Calif.) operated in the out-of-coincidence mode. After the zero time determination, the vials were returned to the water bath where they were maintained at 37°C. Measurements of CL were then made at various later times. The background level of chemiluminescence, i.e., CL measured in the presence of zymosan and buffer, was also measured. Background was approximately 15,000–20,000 cpm. In this paper chemiluminescence is expressed as counts per minute minus the background level.

The chemiluminescence which occurs when cells are exposed to zymosan is a result of the reaction between reactive forms of oxygen and zymosan (Miles *et al.*, 1978). In some experiments, the effects of heavy metals on the CL generated by the reaction between sodium superoxide (NaO_2) or hydrogen peroxide (H_2O_2) and zymosan were measured. Zymosan (2 mg) was added to 5 ml of Hepes-buffered medium and incubated at 37°C for 15 min either in the presence or absence of the heavy metals. After the incubation period, NaO_2 (final concentration = 1 mM) was added as a solid and H_2O_2 (1 ml of a 30% solution) as a liquid to the appropriate samples. CL is maximal immediately after the addition of NaO_2 or H_2O_2 to the samples containing zymosan. CL was measured immediately after these additions as described above.

The ability of the cells to exclude trypan blue dye was measured according to the method of Phillips (1973). In these experiments the cells were incubated in Hepes-buffered medium at 37°C for 30 min either in the presence or absence of the heavy metals. Then the cells were separated from the incubation medium and resuspended in fresh Hepes-buffered medium. An aliquot of a 0.4% solution of trypan blue dye was added to this suspension so that the final concentration was 0.1 ml of dye per milliliter of suspension. After 4 min in the dye, the cells were observed with a light microscope. Viable cells were taken as those which excluded dye and which were not broken.

The concentrations of heavy metals which produced 50% inhibition, i.e., the ED50 values, were determined for oxygen consumption and chemiluminescence measurements. These values were obtained from double reciprocal plots. The reciprocal of the percentage inhibition produced by a given concentration of metal was plotted on the y axis against the reciprocal of that metal concentration on the x axis. The straight line of best fit was drawn through these points by using linear regression analysis. The ED50 value was obtained as the negative reciprocal of the x intercept of this line.

RESULTS

Oxygen Consumption, Glucose Metabolism, and Chemiluminescence in the Absence of Heavy Metals

In order to determine the levels of cellular activity in the absence of heavy metals, oxygen consumption, glucose metabolism, and chemiluminescence were measured in resting alveolar macrophages and in cells exposed to zymosan. The results of the oxygen consumption and the glucose metabolism measurements are shown in Table 1. Oxygen consumption in resting rat alveolar macrophages occurs at a rate of approximately 7.7 nmol/10^7 cells/min. When the cells are exposed to zymosan, the level of oxygen consumption increases by about two- to threefold. Other investigators have also reported increases in oxygen consump-

TABLE 1

OXYGEN CONSUMPTION AND GLUCOSE METABOLISM IN RAT ALVEOLAR MACROPHAGES[a]

Condition	Oxygen consumption		Glucose metabolism	
	nmol/10^7 cells/min	Percentage control	nmol glucose/ 10^7 cells/min	Percentage control
Rest (no particle exposure)	7.7 (±0.6)	100	0.86 (±0.35)	100
Exposure to zymosan	20.4 (±2.4)	272 (±27)	0.95 (±0.34)	125 (±25)

[a] The cells were preincubated at 37°C for 15 min before the measurements were made. Oxygen consumption was measured over a 10-min period and glucose metabolism was measured over a 15-min period. Zymosan was added to the cells just prior to the time during which the measurements were made. The numbers shown are mean values for 6 to 19 experiments ± the SEM.

tion during phagocytosis (Ouchi *et al.*, 1965; Myrvik and Evan, 1967; Drath and Karnovsky, 1975). Glucose metabolism occurs in resting cells at a rate of approximately 0.9 nmol of glucose utilized/10^7 cells/min. However, the level of glucose metabolism does not change when the cells are exposed to zymosan. In previous studies it has been shown that glucose metabolism increases during phagocytosis in alveolar macrophages from some animals and decreases in cells from other species (Ouchi *et al.*, 1965; Myrvik and Evan, 1967; Gee *et al.*, 1970).

The time course of the chemiluminescence (CL) which is produced when rat alveolar macrophages are exposed to zymosan is shown in Fig. 1. The peak response occurs approximately 5 min after exposure of the cells to zymosan and then CL gradually diminishes over the next 90 min. This time course is slightly different from that obtained with rabbit alveolar macrophages in which the peak response occurs about 15 min after exposure to zymosan (Miles *et al.*, 1978). In this previous study we found that the chemiluminescence produced when rabbit alveolar macrophages are exposed to

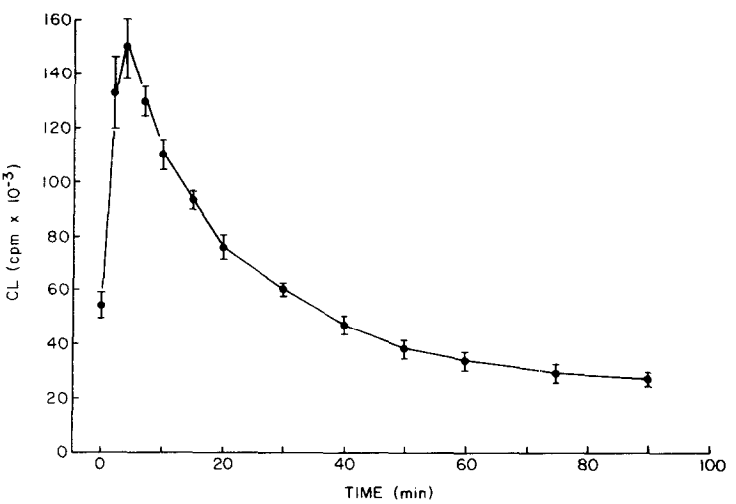

FIG. 1. Time course of the chemiluminescence produced when rat alveolar macrophages are exposed to zymosan. Zymosan was added to the cell suspension at zero time. The points are mean values for six experiments and the bars are the SE of the means.

zymosan is due, at least in part, to an extracellular reaction between reactive forms of oxygen, which are released by the cells, and zymosan (Miles *et al.*, 1978). Chemiluminescence has also been shown to be associated with release of active oxygen in other phagocytic cells (Johnston, 1978). In addition, the CL reported in this study, which is produced by exposure of rat alveolar macrophages to zymosan, is inhibited by superoxide dismutase. Thus, these results indicate that rat alveolar macrophages release reactive forms of oxygen when exposed to zymosan and that the release of these substances can be measured as chemiluminescence.

Effects of Heavy Metals on Oxygen Consumption, Glucose Metabolism, and Chemiluminescence

The effects of heavy metal cations on oxygen consumption and glucose metabolism were studied in resting cells and in cells exposed to particles. The results of the measurements of oxygen consumption are shown in Table 2. All of the metals inhibit oxygen consumption. The maximal inhibition, which is 70 to 90% inhibition, is not much different in resting cells from that which occurs in cells exposed to particles. The ED50 values, i.e., the concentrations of metals which produce 50% of the maximal inhibition, are slightly different in resting and in phagocytizing cells. The order of effectiveness of the metals in inhibiting oxygen consumption in resting cells (based on the ED50 values) is $Hg^{2+} > Cd^{2+} = Pb^{2+} > Ni^{2+}$. The order in phagocytizing cells is $Hg^{2+} > Ni^{2+} = Cd^{2+} > Pb^{2+}$. The effects of the heavy metals on glucose metabolism are shown in Table 3. All of the metals inhibit glucose metabolism in both resting and phagocytizing cells by at least 60%. Thus, all four heavy metal cations inhibit both oxygen consumption and glucose metabolism in resting alveolar macrophages and in cells exposed to particles.

The chemiluminescence which is produced when rat alveolar macrophages are exposed to zymosan particles is inhibited in a dose-dependent manner by the heavy metals. A dose–response curve for one of the metals, Ni^{2+}, is shown in Fig. 2. Similar curves were obtained for the other three heavy metal cations. The results of the experiments with all of the metals are summarized in Table 4. The maximal in-

TABLE 2

EFFECTS OF HEAVY METALS ON OXYGEN CONSUMPTION IN RAT ALVEOLAR MACROPHAGES[a]

Metal	Rest		Exposure to zymosan	
	Maximal inhibition (%)	ED50 (M)	Maximal inhibition (%)	ED50 (M)
Cd^{2+}	69 (±3)	$3.6\ (\pm0.9) \times 10^{-4}$	76 (±1)	$4.1\ (\pm0.8) \times 10^{-4}$
Hg^{2+}	76 (±2)	$2.5\ (\pm1.1) \times 10^{-5}$	82 (±4)	$1.4\ (\pm0.3) \times 10^{-5}$
Ni^{2+}	73 (±3)	$3.9\ (\pm2.0) \times 10^{-3}$	87 (±2)	$3.8\ (\pm2.0) \times 10^{-4}$
Pb^{2+}	79 (±4)	$5.6\ (\pm1.3) \times 10^{-4}$	80 (±3)	$2.6\ (\pm1.4) \times 10^{-3}$

[a] The cells were incubated in the presence of the metal cations for 15 min before the measurements were made. Oxygen consumption was measured over a 10-min period. Zymosan was added to the cells just prior to the time during which oxygen consumption was measured. The ED50 is the concentration of metal which produces 50% of the maximal inhibition. Maximal inhibition was produced by the following metal concentrations: $Cd^{2+} = 10^{-3}$ M, $Hg^{2+} = 10^{-4}$ M, $Ni^{2+} = 10^{-2}$ M, and $Pb^{2+} = 10^{-2}$ M. The numbers shown are mean values for six experiments ± the SEM.

TABLE 3

EFFECTS OF HEAVY METALS ON GLUCOSE METABOLISM IN RAT ALVEOLAR MACROPHAGES[a]

Metal (concentration)	Maximal inhibition (%)	
	Rest	Exposure to zymosan
Cd^{2+} (10^{-3} M)	75 (±8)	83 (±7)
Hg^{2+} (10^{-4} M)	80 (±8)	84 (±6)
Ni^{2+} (10^{-2} M)	77 (±9)	82 (±7)
Pb^{2+} (10^{-2} M)	61 (±9)	69 (±13)

[a] The cells were incubated in the presence of the metals for 15 min before the measurements were made. Glucose metabolism was measured over a 15-min period. Zymosan was added to the cells just prior to the time during which the measurements were made. The numbers shown are mean values for six experiments ± the SEM.

hibition of CL, i.e., 70–85%, is similar to the maximal inhibition of oxygen consumption which is produced by these same metals. In addition, the order of effectiveness of the heavy metals in inhibiting CL (based on ED50 values), i.e., $Hg^{2+} > Ni^{2+} = Cd^{2+} > Pb^{2+}$, is similar to that obtained for inhibition of oxygen consumption in phagocytizing cells. However, the effects on CL occur at lower heavy metal concentrations than do the effects on oxygen consumption; i.e., the ED50 values for CL are lower by about an order of magnitude than the ED50 values obtained for oxygen consumption. These results suggest that the heavy metal cations inhibit the release of active forms of oxygen from alveolar macrophages during exposure to particles.

Since chemiluminescence occurs as a result of the reaction between active forms of oxygen and zymosan (Miles *et al.*, 1978), the effects of heavy metals on this reaction were tested. The results are shown in Table 5. None of the metals causes a decrease in the CL generated by the addition of either superoxide anion or hydrogen peroxide to zymosan. In fact, Pb^{2+} and Hg^{2+} actually

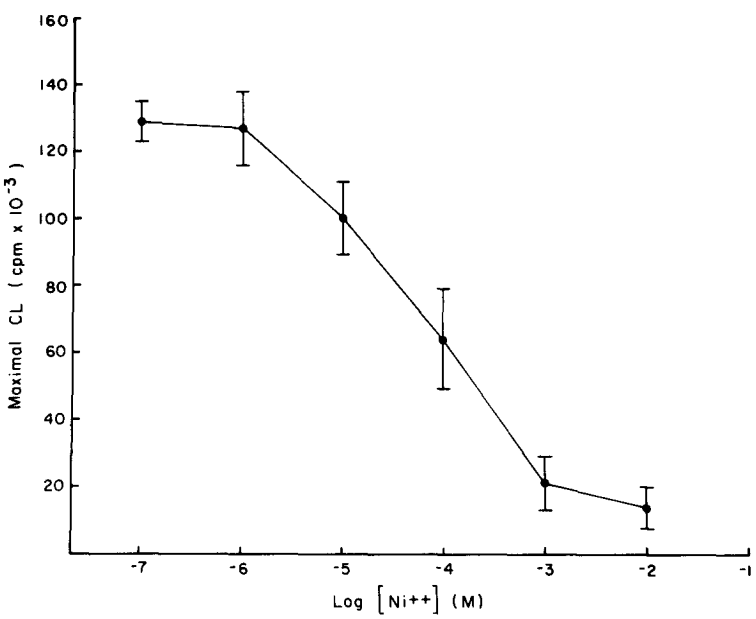

FIG. 2. Dose–response curve for the effect of Ni^{2+} on maximal chemiluminescence (CL). The cells were preincubated at 37°C for 15 min in the presence of Ni^{2+} before the measurements were made. The effects of various concentrations of Ni^{2+} on the maximal CL generated following the addition of zymosan to the cells were determined. The points are mean values for six experiments and the bars are the SEM.

TABLE 4

EFFECTS OF HEAVY METALS ON MAXIMAL CHEMILUMINESCENCE ON RAT ALVEOLAR MACROPHAGES[a]

Metal	Maximal inhibition (%)	ED50 (M)
Cd^{2+}	73 (±1)	$7.6 (\pm 2.8) \times 10^{-5}$
Hg^{2+}	80 (±2)	$2.3 (\pm 0.8) \times 10^{-6}$
Ni^{2+}	84 (±3)	$6.6 (\pm 3.1) \times 10^{-5}$
Pb^{2+}	75 (±1)	$1.2 (\pm 0.5) \times 10^{-4}$

[a] The cells were incubated in the presence of the metals for 15 min before the measurements were made. The ED50 is the concentration of the metal which produces 50% of the maximal inhibition. Maximal inhibition was produced by the following metal concentrations; $Cd^{2+} = 10^{-3}$ M, $Hg^{2+} = 10^{-4}$ M, $Ni^{2+} = 10^{-2}$ M, and $Pb^{2+} = 10^{-2}$ M. The numbers shown are mean values for six experiments ± the SEM.

seem to enhance the CL generated by the addition of H_2O_2 to zymosan. These results indicate that the metals exert their effects on alveolar macrophages by inhibiting the release of active oxygen from the cells.

Finally, the effects of the heavy metals on trypan blue exclusion from the cells were tested. The results of these experiments indicate that cell viability is greater than 90% in the presence of each of the metals, i.e., the metals do not affect the ability of the cells to exclude trypan blue. These results indicate that there is no great change in cell membrane integrity during incubation with the metal cations. Thus, the metals are affecting the oxygen metabolism of the cells specifically and not through a loss of membrane integrity.

DISCUSSION

We have shown that the four heavy metal cations, Cd^{2+}, Hg^{2+}, Ni^{2+}, and Pb^{2+}, affect oxygen metabolism of rat alveolar macrophages at rest and during phagocytosis. In resting alveolar macrophages the metals produce a decrease in the level of oxygen consumption and in glucose metabolism. During phagocytosis the heavy metals also depress oxygen consumption and glucose metabolism and, in addition, they inhibit the release of active forms of oxygen from the cells. The release of active oxygen seems to be most sensitive to heavy metals since the ED50 values for chemiluminescence are lower by an order of magnitude than the ED50 values for metal effects on oxygen consumption. Since oxygen consumption is often used as a measure of cell viability, this result also indicates that heavy metals specifically affect the release of active oxygen; i.e., metals do not inhibit release of active oxygen due to a general deterioration in cell viability. The results of the experiments dealing with trypan blue exclusion also support this conclusion.

In this study chemiluminescence was employed to measure the release of active oxygen species from alveolar macrophages. We also attempted to directly measure the

TABLE 5

EFFECTS OF HEAVY METALS ON MAXIMAL CHEMILUMINESCENCE GENERATED BY SODIUM SUPEROXIDE OR HYDROGEN PEROXIDE AND ZYMOSAN ON RAT ALVEOLAR MACROPHAGES[a]

Metal (concentration)	CL (cpm × 10^{-3})	
	NaO_2	H_2O_2
Control	449 (±24)	1138 (±13)
Cd^{2+} (10^{-3} M)	472 (±31)	1267 (±32)
Hg^{2+} (10^{-2} M)	388 (±14)	2954 (±274)
Ni^{2+} (10^{-2} M)	404 (±20)	1673 (±29)
Pb^{2+} (10^{-3} M)	566 (±29)	3979 (±91)

[a] Sodium superoxide (final concentration = 1 mM) was added as a solid and H_2O_2 (1 ml of a 30% solution) as a liquid to 2 mg of zymosan in 5 ml of Hepes-buffered medium. When metals were used, they were added to the medium containing zymosan 15 min before the addition of NaO_2 or H_2O_2. The CL values shown are maximal values which occur immediately after the addition of the NaO_2 or H_2O_2. The experiments were performed at 37°C. The appropriate background level of CL (i.e., no NaO_2 or H_2O_2 was present) was determined and subtracted from each response. The numbers shown are mean values for six experiments ± the SEM.

effects of metals on the release of one form of active oxygen, superoxide anion, from the cells. Superoxide anion production can be followed directly in phagocytic cells by measuring the reduction of cytochrome *c* caused by the superoxide (Babior *et al.*, 1973). It was found that the metals do not inhibit superoxide-dependent reduction of cytochrome *c*. We were concerned that the metals were binding to cytochrome *c* and, thus, being effectively taken out of solution. In order to test whether the metals could be bound by proteins, the metal effects on chemiluminescence were tested in the presence of bovine serum albumin (an amount equimolar to the cytochrome *c*). Bovine serum albumin was used because cytochrome *c* produces color quenching of the chemiluminescence. In the presence of protein the metals do not inhibit chemiluminescence. Thus, direct measurement of superoxide release in the presence of heavy metals is not possible since the metals probably bind to cytochrome *c* in solution. We have shown in this paper that the metals do not interfere with the light-producing reaction, i.e., the reaction between active forms of oxygen and zymosan. Therefore, chemiluminescence is the method of choice for measurement of the effects of heavy metals, or other substances which may react with cytochrome *c*, on the release of active oxygen species from phagocytic cells.

There is evidence to indicate that the release of active oxygen species is associated with an increase in oxygen consumption and that some superoxide anion is produced at the cell membrane in phagocytic cells. For example, during phagocytosis in neutrophils from peripheral blood there is a "respiratory burst" which consists of an increase in oxygen consumption (Sbarra and Karnovsky, 1959), an increase in glucose metabolism (Sbarra *et al.*, 1972), and production of reactive forms of oxygen (Babior *et al.*, 1973; Root and Metcalf, 1977). A similar relationship between oxygen consumption and superoxide anion release exists for macrophages (Johnston, 1978). The superoxide anion is thought to be produced by a reaction which involves a superoxide-forming enzyme and NADPH as follows:

$$2O_2 + \text{NADPH} \xrightarrow{O_2^{\cdot-}\text{ - forming enzyme}} O_2^{\cdot-} + \text{NADP}^+ + \text{H}^+.$$

In neutrophils this enzyme is thought to be located in the plasma membrane (Dewald *et al.*, 1979; McPhail *et al.*, 1979). It is well known that heavy metal ions bind to cell membranes (Vincent and Blackburn, 1958; Weed *et al.*, 1962). In fact, there is evidence that heavy metal ions inhibit plasma membrane bound enzymes in alveolar macrophages (Mustafa and Cross, 1971; Mustafa *et al.*, 1971). Thus, one possible mechanism by which metals can influence oxygen metabolism in alveolar macrophages is by affecting the activity of membrane-bound enzymes which are involved in the "respiratory burst."

If a large part of the oxygen consumed by alveolar macrophages during phagocytosis is converted to superoxide anion and if the metals do exert their effects on membrane-bound enzymes, one might expect the ED50 values for oxygen consumption and chemiluminescence to be identical. Yet, they are different by an order of magnitude. Drath *et al.* (1976) have reported that much of the oxygen consumed by rat alveolar macrophages is not utilized for formation of superoxide anion. Data from our laboratory indicate that as little as 5% of the oxygen consumed during phagocytosis is involved in superoxide formation (Sweeney, 1979). Therefore, it is not surprising that the ED50 values for metal effects on oxygen consumption and chemiluminescence differ.

Finally, it is possible that heavy metal ions can decrease the antibacterial activity of alveolar macrophages via an effect on oxygen metabolism. The "respiratory burst" which occurs in neutrophils has been found to be closely associated with the anti-

bacterial activity of these cells (Johnston, 1978). Although similar changes in oxygen metabolism occur in alveolar macrophages during phagocytosis, its relationship with the microbicidal activity of the cells has not been established. However, there is some evidence to support a relationship between oxygen metabolism and microbicidal activity in alveolar macrophages (Johnston, 1978). If such a relationship exists, heavy metal cations may affect the antibacterial activity of these cells.

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