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Research Article

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Myeloperoxidase-dependent Effect of Amines on Functions of Isolated Neutrophils

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ABSTRACT Isolated neutrophilic leukocytes were incubated with primary amines and related nitrogenous compounds. Stimulation of neutrophil oxygen (O_2) metabolism with phorbol myristate acetate or opsonized zymosan resulted in production of hydrogen peroxide (H_2O_2), myeloperoxidase-catalyzed oxidation of chloride (Cl^-) to hypochlorous acid (HOCl), and the reaction of HOCl with the added compounds to yield nitrogen-chlorine (N-Cl) derivatives. Formation of N-Cl derivatives of low lipid solubility resulted in accumulation of the derivatives in the extracellular medium. These oxidizing agents were identified and measured on the basis of their absorption spectra and their ability to oxidize 5-thio-2-nitrobenzoic acid to the disulfide form. The yield of N-Cl derivatives was in the order: taurine > Tris > spermidine > spermine > glucosamine > putrescine > guanidinoacetate. Accumulation of N-Cl derivatives was also observed in the absence of added amines, owing to the reaction of HOCl with endogenous taurine and other amines that were released from the cells into the medium.

In the presence of compounds that yield lipophilic N-Cl derivatives, little or no accumulation of oxidizing agents was observed. Instead, these compounds inhibited the accumulation of N-Cl derivatives that was obtained with taurine, and their effect was competitive with taurine. Inhibition was in the order: methylamine > ethanolamine > phenylethylamine > *p*-toluenesulfonamide > ammonia > guanidine. Formation of lipophilic N-Cl derivatives also resulted in inhibition of O_2 uptake and glucose metabolism. Inhibition was prevented by adding catalase to eliminate H_2O_2 , dapsone to inhibit myeloperoxidase, taurine to compete for reaction with HOCl, or compounds that are rapidly oxidized by HOCl or N-Cl derivatives, to reduce these

oxidizing agents. The results indicate that: (a) formation of N-Cl derivatives that do not penetrate biological membranes can protect leukocytes against the cytotoxicity of HOCl and lipophilic N-Cl derivatives, and (b) formation of membrane-permeable N-Cl derivatives in the absence of target cells or readily oxidized substances results in oxidative attack by the N-Cl derivatives on leukocyte components and inhibition of leukocyte functions.

INTRODUCTION

Myeloperoxidase (MPO)¹ and other antimicrobial enzymes and proteins are stored in cytoplasmic granules of human neutrophilic polymorphonuclear leukocytes (neutrophils). These phagocytic cells take up microorganisms into intracellular membrane-bound compartments formed by invagination of the leukocyte cell membrane (1). The granules fuse with these phagocytic vesicles, releasing their contents and forming phagolysosomes. The granule contents contribute to microbicidal activity and digestion of microbial components (1-3). Fusion of granules with the leukocyte cell membrane can also result in release of granule contents to the cell exterior (4, 5). The granule contents may contribute to extracellular microbicidal (6) or tumoricidal (7-9) activity, but may also attack host tissues and contribute to inflammatory tissue destruction (5, 10).

Phagocytosis or the interaction of certain agents with membrane receptors results in greatly enhanced rates of leukocyte oxygen (O_2) metabolism and pro-

¹ *Abbreviations used in this paper:* dapsone, 4,4'-diaminodiphenyl sulfone; fluorescamine, 4-phenylspiro[furan-2(3H)-1'-phthalan]-3,3'-dione; HRP, horseradish peroxidase; MPO, myeloperoxidase; Nbs, 5-thio-2-nitrobenzoic acid; PMA, phorbol 12-myristate 13-acetate; SOD, superoxide dismutase.

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duction of superoxide (O_2^-), which rapidly dismutates to yield hydrogen peroxide (H_2O_2) (8, 11, 12). Purified MPO catalyzes H_2O_2 -dependent oxidation of Cl^- to hypochlorous acid (HOCl) (13–17), and several studies have shown that stimulated neutrophils produce HOCl (18–22). Therefore, HOCl may contribute to intracellular microbicidal activity, to attack on extracellular targets, and to inactivation of leukocyte components.

A recent study demonstrated leukocyte HOCl production by trapping HOCl with high concentrations of taurine (21). HOCl reacts rapidly with primary amines (RNH_2) such as taurine and with a wide variety of other nitrogenous compounds (*N*-compounds) to yield derivatives containing the nitrogen-chlorine (*N*-Cl) bond (23, 24). The *N*-Cl derivative of taurine (taurine monochloramine) accumulates in the extracellular medium during incubation of stimulated neutrophils with taurine (21).

N-Cl derivatives are powerful oxidizing agents, similar to HOCl in their ability to oxidize biological materials. The *N*-Cl derivatives or HOCl can be considered to contain the oxidized Cl atom, Cl^+ . For example, both monochloramines ($RNHCl$) and HOCl contain two oxidizing equivalents and will oxidize 2 mol of a sulfhydryl compound ($R'SH$) to the disulfide ($R'SSR'$).



Some *N*-Cl derivatives decompose rapidly (14, 17, 25), but most are at least as stable as HOCl (23, 24, 26).

The attack of *N*-Cl derivatives on intact cells differs from that of HOCl, depending on the nature of the *R* moiety. For example, taurine monochloramine is predominantly in the anionic form, $^-SO_3(CH_2)_2NHCl$, at biologically relevant pH values. In general, biological membranes are impermeable to anions, except for anions that interact with specific transport systems. Adding HOCl to bacteria in the presence of taurine results in only very slow-acting bactericidal action, and Cl^+ remains unreacted in the extracellular medium as taurine monochloramine (27). Similarly, *N*-compounds that yield highly polar or high-molecular weight *N*-Cl derivatives block killing. When HOCl is added to bacteria in the absence of exogenous *N*-compounds, a significant fraction of the HOCl reacts with nitrogenous cell-envelope components, yielding *N*-Cl derivatives that do not readily penetrate biological membranes (28). The *N*-Cl derivatives remain on the bacterial surface or are shed into the medium and contribute little to killing. In contrast, exogenous *N*-compounds such as ammonia (ammonium ion; NH_4^+) compete with bacterial surface compounds for reaction with HOCl and yield lipophilic *N*-Cl derivatives such

as NH_2Cl (monochloramine) (27). These *N*-compounds increase killing because Cl^+ is not wasted in formation of *N*-Cl derivatives of cell-surface components and because the lipophilic *N*-Cl derivatives can penetrate cell membranes and attack membrane-associated and intracellular components.

These observations suggest that the biological activity of the $MPO-H_2O_2-Cl^-$ system may be regulated by endogenous *N*-compounds of the leukocytes (2, 27). As a step toward elucidating such regulation, stimulated neutrophils were incubated with *N*-compounds differing in reactivity toward HOCl and in the lipophilicity of their *N*-Cl derivatives. Accumulation of *N*-Cl derivatives in the medium was measured, and O_2 uptake and glucose metabolism were studied as measures of leukocyte function.

METHODS

Materials. MPO purified from human leukemic leukocytes was provided by M. Morrison and J. Naskalski. MPO concentration was calculated assuming a millimolar extinction coefficient of 89 at 430 nm (29). Sodium hypochlorite ($NaOCl$) and ethyl acetate (spectrophotometric grade) were from Fisher Scientific Co., Fair Lawn, NJ. Dapsone (4,4'-diaminodiphenyl sulfone; ICI Pharmaceutical Division, Macclesfield, England) and PMA (phorbol 12-myristate 13-acetate; Consolidated Midland Corp., Brewster, NY) were dissolved in dimethyl sulfoxide. $D-[U-^{14}C]$ glucose (Amersham Corp., Arlington Heights, IL) was diluted to 12.5 Ci/mol with unlabeled *D*-glucose. Catalase crystals (Boehringer Mannheim Biochemicals, Indianapolis, IN) were washed by centrifugation in water and dissolved in isotonic 0.15 M sodium chloride ($NaCl$), 7 mM potassium phosphate, pH 7.4. Dextran (5×10^5 mol wt), horseradish peroxidase (HRP), superoxide dismutase (SOD), zymosan, scopoletin, and 5,5'-dithiobis(2-nitrobenzoic acid) were from Sigma Chemical Co., St. Louis, MO. Zymosan was boiled in 0.1 M $NaOH$, washed by centrifugation, and suspended to 50 mg/ml in the isotonic medium, opsonized (30) by incubation for 2 h at 37°C with 3 vol of human serum, and then washed and suspended to 50 mg/ml in the medium. Scopoletin was dissolved in 50 mM sodium carbonate and immediately diluted to 0.4 mM in the medium. Reduction of 5,5'-dithiobis(2-nitrobenzoic acid) to 5-thio-2-nitrobenzoic acid (Nbs) was as described previously (31). Concentration of Nbs was calculated assuming a molar extinction coefficient of 13,600 at 412 nm (32).

Partitioning of *N*-Cl derivatives. *N*-Cl derivatives were synthesized by adding $NaOCl$ in 0.01 M $NaOH$ to solutions of the *N*-compounds at 25°C in 0.1 M $NaCl$ containing either 0.01 M sodium hydroxide ($NaOH$) or 0.1 M phosphate, pH 5 (20). Mono-*N*-chloramine, -chlorosulfonamide, or -chloroguanidino derivatives ($RNHCl$) were obtained at 1:100 molar ratio of $NaOCl$ to the *N*-compound, at high pH. Di-*N*-chloramines ($RNCl_2$) were obtained at a 2:1 ratio of $NaOCl$ to the amine, at pH 5. Di-*N,N'*-monochloramines, $R(NHCl)_2$, were obtained at a 1:1 ratio of $NaOCl$ to primary amino groups of the polyamines, at high pH. Derivatives of primary amines were at least 95% in the specified form, as determined from the ratio of absorbance at 252 and 300 nm (20, 26).

Solutions of N-Cl derivatives were diluted with 0.2 M phosphate, pH 5 or 7, to yield 0.1 mM RNHCl or 0.05 mM RNCl₂ or R(NHCl)₂. Ethyl acetate was treated with sodium borohydride and 0.1 M NaOH to remove oxidizing agents and acids (33), and equilibrated with 0.1 M NaCl. Extraction was performed at a 1:1 volume ratio of ethyl acetate to aqueous volume. Partitioning of oxidizing equivalents was determined by reacting portions of the organic and aqueous phases with Nbs at pH 7 as described previously (33).

Neutrophils. Whole blood (50 ml) from adult volunteers was collected in acid-citrate-dextrose anticoagulant solution. The cells were washed by centrifugation in the isotonic medium, and all steps below were in this medium. Cells were suspended to 50 ml in 0.1% (wt/vol) gelatin, mixed with 50 ml 3% (wt/vol) dextran, and allowed to settle for 20 min at 37°C. Cells in the supernatant fraction were fractionated by a modification of the method of Harbeck et al. (34). Cells were collected by centrifugation, suspended to 18 ml in 40% Percol (Pharmacia Fine Chemicals, Piscataway, NJ), and 9-ml portions layered on discontinuous gradients composed of 9 ml each of 50, 60, 70, and 80% Percol. After centrifugation at 250 g for 30 min at 20°C, cells at the 60:70% and 70:80% interfaces were pooled, washed, suspended, and subjected again to density-gradient centrifugation. Cells at the 60:70% and 70:80% interfaces were pooled, washed, and suspended to 2 × 10⁷ cells/ml. The cells were at least 90% neutrophils and free of erythrocytes.

Accumulation of N-Cl derivatives. Reaction mixtures of 0.5 ml total volume in siliconized glass centrifuge tubes contained 4 × 10⁶ cells/ml in the isotonic medium with 10 mM glucose and 1 mM magnesium sulfate (MgSO₄). The N-compounds were added as solutions of the free bases or hydrochloride (HCl) salts in the medium adjusted to pH 7.4. When added, PMA was 20 nM and opsonized zymosan was 5 mg/ml. Incubations were for 1 h at 37°C with continuous mixing. Mixtures were cooled to 4°C, and then 1 μg catalase, 0.25–0.5 ml Nbs in the medium, and 2 ml medium with 1 mM MgSO₄ were added. The mixtures were centrifuged at 7,000 g for 10 min at 4°C, and absorbance of the supernatants at 412 nm was measured. Concentration of N-Cl derivatives was calculated from one-half the difference between the amount of Nbs added and the amount remaining (27, 28, 31), assuming 1 Cl⁺ per derivative.

Release of neutrophil components into the incubation medium. Reaction mixtures and incubation conditions were as described above. Mixtures were centrifuged at 7,000 g for 10 min at 4°C, and 0.1 mM dithiothreitol was added to the supernatants. Protein was determined by the dye-binding method (35), with reagents from Bio-Rad Laboratories, Inc., Richmond, CA. Total primary amines were determined by reaction with 4-phenylspiro[furan-2(3H)-1'-patalan]-3,3'-dione (fluorescamine) (36), with the fluorescamine derivative of taurine as the standard. Taurine was determined by thin-layer chromatography on silica gel G plates (Analtech, Inc., Newark, DE) in *n*-butanol/acetic acid/water, 4:1:1. The area corresponding to taurine (R_f, 0.25) was scraped from the plate and extracted twice with 1 ml 0.05 M sodium acetate, pH 7. The extracts were pooled and reacted with 0.5 vol of 0.3% (wt/vol) fluorescamine in acetone, diluted with 1.5 ml of acetone/water, 2:1, and clarified by centrifugation. Taurine concentration was calculated from fluorescence relative to standard curve prepared by chromatography of known amounts of taurine.

Glucose metabolism. Reaction mixtures and incubation conditions were as described above except that 0.5 mM D-[U-¹⁴C]glucose replaced the 10 mM glucose. Mixtures were centrifuged at 7,000 g for 10 min at 4°C, supernatant and

cell pellets were separated, and the cells were washed by centrifugation in 5 ml medium with MgSO₄ and suspended to 0.5 ml. Cell-associated label was measured by adding portions of resuspended cells to scintillation fluid (37) and measuring radioactivity in a liquid scintillation spectrometer. Nonvolatile label in the supernatants was determined by acidifying portions with 0.2 M HCl, drying under N₂, and then dissolving in water and scintillation fluid. The ratio of lactate to glucose in supernatants was determined by thin-layer chromatography on silica gel G plates in ethanol/ammonium hydroxide/water 20:1:4. Areas corresponding to lactate (R_f, 0.7) and glucose (R_f, 0.4) were scraped from the plates, and label determined as above. No other nonvolatile substances were detected. Volatile label was calculated as label in the supernatant of a mixture that was not incubated minus the sum of nonvolatile and cell-associated label in the incubated mixtures. The amount of glucose metabolized was calculated as the sum of label in lactate and in cell-associated and volatile forms.

O₂ uptake. Reaction mixtures of 2.5 ml total volume contained 4 × 10⁶ cells/ml in the medium with 10 mM glucose, 1 mM MgSO₄, 100 U/ml SOD, and N-compounds. O₂ uptake was initiated by adding PMA or opsonized zymosan, and was measured in a stirred chamber at 37°C with a Clarke-type O₂ electrode.

RESULTS

Comparison of N-Cl derivatives. Lipid solubility or membrane permeability of N-Cl derivatives was evaluated from the partitioning (38) of oxidizing equivalents between the organic and aqueous phase upon extraction with ethyl acetate. Extraction was performed at acid and neutral pH, to mimic conditions that may exist in the phagolysosome (39) and the blood or interstitial fluid. In Table I, the compounds tested are grouped into four classes. Class 1 consisted of primary amines with charged groups or multiple polar groups in addition to the reactive N-moiety. The N-Cl derivatives of these compounds were not extracted, and extraction was not influenced by pH or the extent of chlorination. For example, neither the RNHCl nor RNCl₂ derivative of taurine was extracted.

Class 2 consisted of polyamines, which can be chlorinated at more than one N-moiety. Extraction depended primarily on the extent of chlorination, but was also influenced by pH. With putrescine for example, the RNHCl derivative was less lipophilic than R(NHCl)₂. These derivatives would be predominantly in the charged (+NH₃[CH₂]₄NHCl) and neutral (NHCl[CH₂]₄NHCl) forms. Similarly, derivatives of spermine and spermidine were less extractable at low pH, owing to their secondary amino groups, which would be predominantly in the charged form at low pH; e.g., NHCl(CH₂)₃N⁺H₂(CH₂)₄NHCl.

Class 3 consisted of primary amines with no charged groups aside from the single reactive N-moiety, and included ethanolamine with one polar group. The N-Cl derivatives had high solubility in the organic phase.

TABLE I
Partitioning of N-Cl Derivatives into the Organic Phase

Class	N-compound	N-Cl derivative	Percentage extracted*	
			pH 5	pH 7
1	Taurine	RNHCl	0	1
		RNCl ₂	0	2
	Tris	RNHCl	1	1
	Glucosamine	RNHCl	5	1
2	Spermine	RNHCl	3	0
		R(NHCl) ₂	3	10
	Spermidine	RNHCl	2	1
		R(NHCl) ₂	30	45
Putrescine	RNHCl	20	23	
	R(NHCl) ₂	99	97	
3	Ethanolamine	RNHCl	35	33
		RNCl ₂	88	89
	Methylamine	RNHCl	82	85
	Phenylethylamine	RNHCl	81	86
4	Guanidinoacetate	RNHCl	4	4
	Guanidine	RNHCl	24	23
	Ammonia	NH ₂ Cl	60	65
	<i>p</i> -Toluenesulfonamide	RNHCl	100	67

* Aqueous solutions of the N-Cl derivatives at pH 5 or 7 were extracted with ethyl acetate at a 1:1 volume ratio. The percentage of total oxidizing equivalents extracted into the organic phase was measured. Variation between duplicate determinations was $\pm 4\%$.

The RNCl₂ derivative of ethanolamine was extracted to a greater extent than RNHCl, indicating that the -NCl₂ moiety is less polar.

Class 4 consisted of compounds with N-moieties other than primary amino groups, and included NH₄⁺. These compounds were selected to yield N-Cl derivatives with a wide range of lipid solubilities. Guanidinoacetate is an analogue of taurine, whereas N-Cl derivatives of the other compounds had significant lipid solubility. Less of the derivative of *p*-toluenesulfonamide was extracted at the higher pH, where it may exist partly as the N-chloro-sulfonamidate, CH₃C₆H₄SO₂NCl⁻ (40).

Fig. 1 illustrates competition between N-compounds for reaction with HOCl. Compounds were mixed at pH 7, HOCl was added, and partitioning of the mixture of N-Cl derivatives was measured. In Fig. 1 (left),

enough NH₄⁺ or guanidine was present to react with all the HOCl, resulting in formation of lipophilic derivatives. When increasing taurine was mixed with NH₄⁺ or increasing guanidinoacetate mixed with guanidine, these compounds competed for reaction with HOCl, yielding mixtures with decreasing lipid solubility. Fig. 1 (right) shows the converse experiment, which yielded mixtures of increasing lipid-solubility.

These results also indicated that HOCl reacted more readily with taurine than with NH₄⁺. With 1 mM NH₄⁺ alone, the percentage extracted was 67%, whereas with 1 mM NH₄⁺ and 0.2 mM taurine the percentage extracted was 29%, suggesting that at a 5:1 ratio of NH₄⁺ to taurine about half the oxidizing equivalents were in NH₂Cl and half in taurine monochloramine. In other experiments at pH 7, there was a 20-fold preference for reaction with taurine as compared with guanidine, and fivefold preference for reaction with NH₄⁺ as compared with guanidinoacetate. Taurine and other primary amines were similar in their reactivity, and reacted preferentially as compared with NH₄⁺ or sulfonamide, secondary amino, amide, or other N-moieties. Therefore, compounds in class 4 of Table I are less reactive toward HOCl than compounds in classes 1-3.

In another variation on these experiments, HOCl was added to a compound from class 1 or classes 3-4,

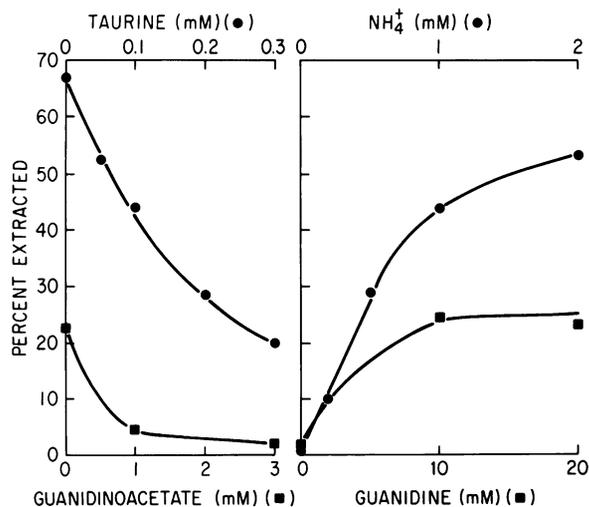


FIGURE 1 Competition for reaction with HOCl. HOCl (0.1 mM) was generated by adding NaOCl into solutions buffered at pH 7. Solutions contained 1 mM NH₄⁺ and the indicated concentrations of taurine (left, ●), 1 mM guanidine and the indicated concentrations of guanidinoacetate (left, ■), 0.1 mM taurine and the indicated concentrations of NH₄⁺ (right, ●), or 1 mM guanidinoacetate and the indicated concentrations of guanidine (right, ■). The mixtures were extracted and the percentage of total oxidizing equivalents partitioning into the organic phase was measured.

followed by addition of a compound from classes 3–4 or class 1, and then the mixture was extracted. The percentage extracted was determined by the compound that was present when HOCl was added. Therefore, there was no exchange of Cl^+ between the *N*-compounds within the time required for these experiments (5–10 min). Moreover, these experiments indicated that extraction of oxidizing equivalents was not due to hydrolysis of *N*-Cl derivatives to yield free HOCl. Adding excess taurine as a trap for HOCl had no effect on the percentage extracted. Similarly, formation of lipophilic *N*-Cl derivatives of radioactively labeled compounds resulted in enhanced extraction of the label, indicating that the R moiety and the oxidizing equivalents were extracted together as the *N*-Cl derivative.

Accumulation of *N*-Cl derivatives. Table II shows results of incubating stimulated neutrophils with the *N*-compounds listed in Table I. With taurine or other compounds that yield charged or highly polar *N*-Cl derivatives (class 1), oxidizing equivalents accumulated in the extracellular medium and could be measured by adding excess Nbs at the end of the incubation. The oxidizing agents that accumulated under these conditions were identified as *N*-Cl derivatives as described in a later section.

Qualitatively similar results were obtained with PMA or opsonized zymosan as the stimulus for O_2 reduction and Cl^- oxidation, although the yield of *N*-Cl derivatives was about twice as high with zymosan. In other experiments, maximum stimulation was obtained with 20 nM PMA, whereas the yield of *N*-Cl derivatives increased with zymosan concentrations up to at least 5 mg/ml. No oxidizing agent accumulated in the absence of PMA or zymosan, and the *N*-compounds did not stimulate O_2 uptake.

Table II shows that the yield of *N*-Cl derivatives was increased by raising the taurine concentration and by supplementing the incubation medium with exogenous MPO. Therefore, the amounts of *N*-compound and endogenous MPO activity limited the yield with either PMA or zymosan. In other experiments with PMA and 10 mM taurine, the yield was proportional to time and the number of cells up to at least 1 h and 5×10^6 cells/ml.

Accumulation of *N*-Cl derivatives was also obtained with polyamines (class 2), although the yield was lower. At the high amine concentrations of these experiments, the charged monochloramines (RNHCl) would be the major products. With amines that yield lipophilic *N*-Cl derivatives (class 3), little or no accumulation of oxidizing equivalents was observed. In-

TABLE II
Accumulation of *N*-Cl Derivatives

Class	<i>N</i> -compound	Concentration	<i>N</i> -Cl derivatives*			
			PMA	PMA + MPO	Zymosan	Zymosan + MPO
		mM			μM	
—	None	—	35±36	32±3	31±8	23±9
1	Taurine	0.3	64±32	158±16	97±63	179±77
	Taurine	10	83±33	177±70	203±54	383±23
	Tris	10	69±13	—	135±46	—
	Glucosamine	10	43±17	—	85±29	—
2	Spermine	5	61±21	—	126±44	—
	Spermidine	5	61±20	—	141±51	—
	Putrescine	5	44±13	—	59±21	—
3	Ethanolamine	10	0±0	—	0	—
	Methylamine	10	1±0	—	0	—
	Phenylethylamine	10	0±0	—	0	—
4	Guanidinoacetate	10	49±14	—	26±15	—
	Guanidine	10	15±9	—	11	—
	Ammonia	10	6±6	—	0	—
	<i>p</i> -Toluenesulfonamide	10	9±4	—	0	—

* Neutrophils were incubated with PMA or opsonized zymosan and the indicated *N*-compounds, with or without exogenous 0.1 μM MPO. After 1 h, *N*-Cl derivatives in the medium were measured with Nbs. Values are the mean±SD from a minimum of four experiments with separate neutrophil preparations.

stead, the yield was lower than that observed without added *N*-compounds. With *N*-compounds that do not contain amino-groups (class 4), low yields were obtained with guanidinoacetate, and the yield decreased with increasing lipophilicity of the *N*-Cl derivatives.

Table II also shows that in the absence of exogenous taurine or other *N*-compounds, a low yield of *N*-Cl derivatives was consistently observed. Exogenous MPO did not increase the yield, but in other experiments a fourfold increase in cells (to 1.6×10^7 /ml) resulted in a threefold increase in yield. These results suggested that the supply of endogenous *N*-compounds was limiting.

In other experiments, accumulation of *N*-Cl derivatives was blocked by catalase (10 μ g/ml) and by compounds that were proposed to act as MPO inhibitors. Methimazole or diethyldithiocarbamate (30) at 0.2–0.3 mM were required to block accumulation of 0.1 mM *N*-Cl derivatives. The requirement for high concentrations suggested that these compounds acted as reducing agents for HOCl or *N*-Cl derivatives rather than as MPO inhibitors. Methimazole and diethyldithiocarbamate were found to rapidly reduce taurine monochloramine with a \sim 2:1 and 1:1 stoichiometry. Similarly, other readily oxidized compounds such as dithiothreitol, serotonin, or methionine (20) prevented accumulation of the oxidizing agents. In contrast, 30 μ M dapsone (41) was sufficient to inhibit by 60–90%, and 0.1 mM dapsone did not reduce 0.1 mM HOCl or taurine monochloramine within 1 h at 37°C. Dapsone at 30 μ M did not inhibit release of proteins, glucose metabolism, or O₂ uptake by stimulated cells. Therefore, dapsone appeared to prevent accumulation of *N*-Cl derivatives by inhibiting MPO activity.

Identification of *N*-Cl derivatives. In experiments similar to those described above, the oxidizing agent that accumulated in the medium during incubation of stimulated neutrophils with taurine was identified as taurine monochloramine, based in part on a shoulder of absorbance at 250 nm (21). In our hands, both stimulated and unstimulated cells released material that absorbed at 250 nm and that was unrelated to formation of *N*-Cl derivatives. It was possible to distinguish between absorbance of the *N*-Cl derivative and the spurious 250-nm absorbance by reducing the *N*-Cl derivative with sodium hydrosulfite (dithionite). Dithionite absorbance was negligible when dithionite was in slight excess of the amount required for complete reduction. Fig. 2 (left) shows that after incubation with PMA and taurine only a small part of the absorbance was due to the *N*-Cl derivative.

Fig. 2 (left) also shows that a large part of the spurious 250-nm absorbance was removed by ultrafiltration through a UM-2 filter (Amicon Corp., Lexington, MA) to remove protein and other high molecular

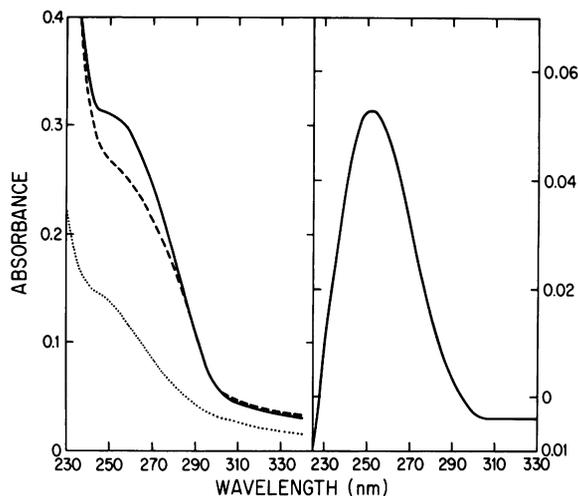


FIGURE 2 Absorption spectra of the supernatant and *N*-Cl derivative. Neutrophils were incubated 1 h with PMA and 10 mM taurine, and then centrifuged. (Left) Absorption of the supernatant was measured before (—) and after (---) reduction with 0.2 mM dithionite. Also, absorption was measured after ultrafiltration of the reduced supernatant (···). The reference cuvette contained the medium with PMA and taurine. (Right) Absorption of the filtered, non-reduced supernatant was measured. The reference cuvette contained the filtered, reduced supernatant.

weight material. Filtration lowered 230 and 250 nm absorbance by 67 and 50%. In contrast, *N*-Cl derivatives were not removed by filtration. Fig. 2 (right) shows that the spectrum of the *N*-Cl derivative was obtained by filtering the nonreduced supernatant, dividing the filtrate into two portions, reducing one portion with dithionite, and measuring the difference in absorbance between the nonreduced and reduced portions. The spectrum obtained was similar to that of monochloramines (26), and the absorbance of 0.052 at 252 nm corresponds to concentration of 120 μ M RNHCl, assuming a molar extinction coefficient of 429 (20). This concentration was similar to that determined with Nbs.

In other experiments, similar results were obtained with other *N*-compounds. With PMA and exogenous MPO, yields of 230, 210, and 110 μ M RNHCl were obtained with 10 mM taurine, Tris, and glucosamine, respectively. Significant dichloramine formation was observed only with the polyamines (class 2). With 5 mM spermine, the yield was 70 μ M RNHCl and 30 μ M RNCl₂, assuming a molar extinction coefficient for RNCl₂ of 370 at 300 nm (20). Without added *N*-compounds and with increased cell density (1.6×10^7 cells/ml), the yield was 90 μ M RNHCl, with or without exogenous MPO.

Release of amines and proteins. Table III shows that endogenous amines were released from the cells

TABLE III
Release of Endogenous Amines and Proteins

	Primary amino groups*	Protein
	μM	$\mu g/ml$
4°C	20	20
No additions	80±50	40±10
Taurine	ND†	50
Ammonia	ND	50
PMA	140±40	70±10
PMA + taurine	ND	60±20
PMA + ammonia	130±40	50±20
Zymosan	130±50	100±30
Zymosan + taurine	ND	100±30
Zymosan + ammonia	140±60	90±30
Zymosan - cells	10	20±10

* Unless otherwise indicated, neutrophils were incubated at 37°C with or without PMA or opsonized zymosan. When added, taurine or NH_4^+ was 10 mM. After 1 h, cells were removed by centrifugation, N-Cl derivatives were reduced, and primary amines and protein were measured in the supernatant fractions. Values are the mean±SD from a minimum of three experiments with separate neutrophil preparations.

† ND, not done.

into the medium, as determined by reacting portions of the supernatant with fluorescamine, which is specific for primary amines (36). Release of amines was sufficient to account for the yield of N-Cl derivatives that was observed in the absence of added N-compounds (Table II).

About 30 to 40% of the amines detected consisted of endogenous taurine, as determined by thin-layer chromatography. Leukocytes contain high concentrations of taurine (42). Smaller amounts of other low molecular weight amines were detected, and the remainder consisted of protein amino groups. Table III shows that higher levels of released protein were obtained with zymosan than with PMA as the stimulus. Stimulation resulted in a small increase in the amount of released amines, owing to increased release of protein amino groups.

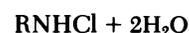
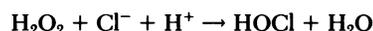
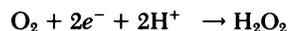
In other experiments, the identity of the proteins released depended on the stimulus, as determined by gel electrophoresis (43). After incubation with PMA, the major polypeptides in the supernatant had apparent molecular weights of 80,000 and 14,000. With zymosan, a number of polypeptides were obtained in addition to the 14,000-mol wt polypeptide, but less of the 80,000-mol wt polypeptide was observed. Lactoferrin (80,000 mol wt) is contained in the specific granules, lysozyme (14,000 mol wt) in the specific and

azurophil granules, and MPO and other proteins in the azurophil granules (4, 44). Therefore, the results were consistent with reports that PMA stimulates secretion principally of specific-granule components, and that zymosan stimulates secretion of both specific and azurophil-granule components (4, 45-47).

Table III and the results of electrophoresis showed that taurine or NH_4^+ had little effect on the amount or identity of proteins released into the medium. Therefore, the high yield of N-Cl derivatives obtained with taurine was not due to enhanced secretion of MPO. Moreover, the low yield obtained with NH_4^+ was not due to inhibition of secretion or to cell lysis.

O₂ uptake and accumulation of N-Cl derivatives. Rates of O₂ uptake upon stimulation with zymosan were only 30-40% of the rates with PMA, despite the observation that a higher yield of N-Cl derivatives was obtained with zymosan (Table II). To evaluate the relation between O₂ uptake and accumulation of N-Cl derivatives, cells were incubated with compounds from classes 1-2, O₂ uptake was allowed to proceed for a period of time sufficient for uptake of 150 μM O₂ (from 0.2 to 0.05 mM O₂), and then N-Cl derivatives were measured.

Table IV shows that with PMA as the stimulus, the efficiency of trapping was 31-55%, calculated as moles of N-Cl per mole of O₂ taken up. If O₂ uptake was limiting, the efficiency would be 100%:



With exogenous MPO, the efficiency was higher (59-85%).

This observation suggested that accumulation of N-Cl derivatives was limited by the release of MPO from a cryptic, granule-associated state (48) into an environment where oxidation of Cl^- took place. The higher yield of N-Cl derivatives obtained with opsonized zymosan as the stimulus (Tables II and IV) may be due to enhanced mobilization of the granules containing MPO.

Inhibition of accumulation. Those compounds that yield lipophilic N-Cl derivatives inhibited the accumulation of N-Cl derivatives that was obtained with endogenous or exogenous amines. Fig. 3 shows that inhibition by NH_4^+ was competitive with taurine. With increasing taurine, higher concentrations of NH_4^+ were required to inhibit. These results suggested that taurine and NH_4^+ competed for reaction with HOCl, and

TABLE IV
Yield of N-Cl Derivatives as a Percentage of O₂ Uptake

Class	N-compound	Stimulus	-MPO*		+MPO	
			N-Cl derivatives	Yield	N-Cl derivatives	Yield
			μM	%	μM	%
1	Taurine	Zymosan	108	72	112	75
	Taurine	PMA	62	41	127	85
	Tris	PMA	46	31	97	65
	Glucosamine	PMA	81	54	102	68
2	Spermine	PMA	82	55	107	71
	Spermidine	PMA	72	48	102	68
	Putrescine	PMA	47	31	89	59

* Neutrophils stimulated with opsonized zymosan or PMA were incubated with class 1 (10 mM) or class 2 (5 mM) compounds, without or with 0.1 μM exogenous MPO. O₂ uptake was monitored with the O₂ electrode, and the yield of N-Cl derivatives was measured after 150 μM O₂ had been taken up.

that the inhibition observed with NH₄⁺ was due to formation of the N-Cl derivative of NH₄⁺, which did not accumulate.

To evaluate competition, the concentration of NH₄⁺ required to inhibit by 50% (one-half effective dose; ED₅₀) was calculated at 0, 0.3, and 10 mM exogenous taurine. Table V summarizes results obtained with NH₄⁺ and other inhibitors, calculating ED₅₀ values from plots as in Fig. 3. With all the compounds, inhibition was competitive with taurine. The change in ED₅₀ with increasing taurine concentration was greater for NH₄⁺ and the other compounds that competed

poorly with taurine for reaction with HOCl (class 4). Therefore, the relative efficacy of the inhibitors was determined primarily by their ability to compete for reaction with HOCl, and to a lesser extent by the lipid solubility of their N-Cl derivatives.

Inhibition of glucose metabolism. Leukocyte glucose metabolism was studied to determine whether the cells were damaged by N-compounds or N-Cl derivatives as indicated by a decreased rate of metabolism. Because unstimulated and stimulated leukocytes metabolize glucose at similar rates (49), it was possible to determine whether the N-compounds had an effect on both unstimulated and stimulated cells, which would indicate that the N-compounds were toxic, or whether only stimulated cells were inhibited, which could indicate that inhibition depended on formation of N-Cl derivatives.

To facilitate measurements of glucose metabolism, a low glucose concentration (0.5 mM) was used. At this concentration, the rate of glucose metabolism was only slightly less than that obtained with 10 mM glucose, and the yield of N-Cl derivatives was ~80% of that obtained with 10 mM glucose (Table II).

Table VI shows that the N-compounds had little or no effect on metabolism of unstimulated cells (-PMA). Average results are shown for compounds in each class. Glucosamine at 10 mM did interfere with metabolism of 0.5 mM [¹⁴C]glucose, by competing for transport or metabolism (50), and was excluded from this study. In the unstimulated cells, glucose was converted primarily to lactate, consistent with their dependence on glycolysis (51). Moreover, a major portion was converted to cell-associated forms. In other experiments, most of the cell-associated label from [¹⁴C]glucose was

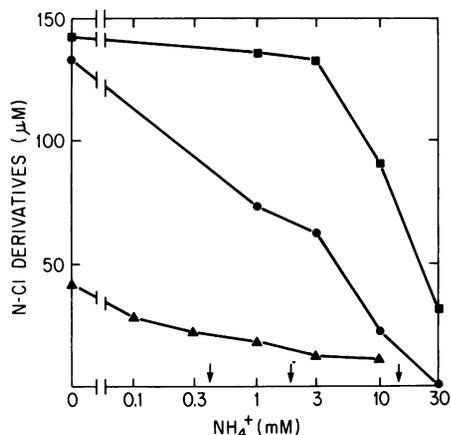


FIGURE 3 Inhibition of accumulation of N-Cl derivatives by NH₄⁺. Neutrophils were incubated with PMA, the indicated concentrations of NH₄⁺, and 10 mM (■) or 0.3 mM (●) taurine, or no exogenous taurine (▲). After 1 h, N-Cl derivatives were measured with Nbs. Arrows indicate NH₄⁺ concentrations that inhibited by 50% (ED₅₀).

TABLE V
Inhibition of Accumulation of N-Cl Derivatives

Class	N-compound	ED ₅₀ *		
		0 Taurine	0.3 mM Taurine	10 mM Taurine
		<i>mM</i>		
3	Ethanolamine	0.8	1.2	3.3
	Methylamine	0.1	0.8	2.2
	Phenylethylamine	0.1	0.6	1.7
4	Guanidine	ND†	10-20	>20 mM
	Ammonia	0.4	1.8	15
	<i>p</i> -Toluenesulfonamide	ND	5-10	>20 mM

* Neutrophils were incubated with PMA and 0, 0.3, or 10 mM taurine, with varying concentrations of the indicated N-compounds. After 1 h, N-Cl derivatives were measured with Nbs. The concentration of the N-compound required to inhibit by 50% (ED₅₀) was measured.

† ND, not done.

found in acid-precipitable forms, consistent with glycogen synthesis (51, 52).

Stimulation by PMA resulted in decreased glycogen and lactate production and increased production of volatile substances, consistent with increased CO₂ pro-

duction by way of the hexose-monophosphate pathway (51). Compounds in classes 1-2 had little or no effect. Compounds in class 3 inhibited glucose metabolism and also caused a qualitative change in metabolism. That is, the distribution of label between cell-asso-

TABLE VI
Effect of N-Compounds on Glucose Metabolism

Class	N-compounds	PMA	Glucose metabolized (percent of control)	Percent cell associated	Percent lactate	Percent volatile
—	None	—	(100)*	21±5	49±2	31±6
1	All (10 mM)†	—	93±5	19±1	51±4	29±2
2	All (5 mM)	—	87±1	27±4	61±11	12±15
3	All (3 mM)	—	91±15	18±6	63±3	20±6
4	All (10 mM)	—	87±18	16±6	54±5	27±7
—	None	+	92±14	4±1	40±8	56±8
1	All (10 mM)	+	88±10	4±1	42±6	54±7
2	All (5 mM)	+	85±4	7±5	46±12	47±17
3	Ethanolamine (3 mM)	+	51	11	74	15
	Methylamine (3 mM)	+	51	16	75	9
	Phenylethylamine (3 mM)	+	62	20	63	18
4	Guanidinoacetate (10 mM)	+	75	6	53	41
	Guanidine (10 mM)	+	66	6	49	45
	Ammonia (10 mM)	+	50±11	13±4	68±7	19±11
	<i>p</i> -Toluenesulfonamide (10 mM)	+	29	3	98	-1

* Unstimulated (-PMA) or stimulated (+PMA) neutrophils were incubated with [U-¹⁴C]glucose and the indicated N-compounds. After 1 h, the amount of glucose metabolized and the distribution of metabolized ¹⁴C label was measured. Standard deviation is shown where a minimum of four values were averaged. The mean glucose utilization by unstimulated cells in five experiments was 180±59 μM.

† All. Average results are shown for all the compounds in this class, excluding glucosamine.

ciated forms, lactate, and volatile forms was more like that of unstimulated cells. Inhibition was also observed with compounds in class 4. Relative inhibition by compounds in classes 3–4 were correlated at least in part with lipid solubility of their N-Cl derivatives.

Inhibition was prevented by agents that would interfere with formation of N-Cl derivatives or with the reaction of N-Cl derivatives with leukocyte components. In an experiment with 10 mM NH_4^+ , glucose metabolism as a percentage of the control was raised from 44% to 66, 70, 82, or 87% by 10 mM taurine, 10 $\mu\text{g/ml}$ catalase, 30 μM dapsone, or 0.2 mM dithiothreitol, respectively.

Inhibition of O_2 uptake. To determine whether inhibition of accumulation of N-Cl derivatives was due to inhibition of H_2O_2 production, O_2 uptake by stimulated leukocytes was measured in the presence of the N-compounds. Fig. 4 shows plots of O_2 concentration vs. time upon stimulation with PMA.

The rate of O_2 uptake in the presence of taurine (curve 1) was nearly constant down to O_2 concentrations of 10–20 μM . A rate of 20 $\mu\text{M/min}$ for the suspension containing 4×10^6 cells/ml was calculated from the initial, linear portion of the plot. The slower rate at low O_2 concentrations was not due to inhibition of O_2 uptake. Instead, the form of the curve is close to that predicted for substrate concentration vs. time for a saturable process with a Michaelis constant (K_m) much lower than the initial substrate concentration. A K_m for O_2 of 4–5 μM was calculated from the O_2

concentration at which the slope was one-half the initial slope (53). This K_m is consistent with that determined by other methods (54).

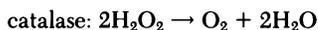
Without added N-compounds (curve 2), the rate of O_2 uptake was slower, with an initial rate, or V_0 , of 19 $\mu\text{M/min}$. There was little inhibition of O_2 uptake during the time required to consume all the O_2 .

In contrast, O_2 uptake in the presence of NH_4^+ (curve 3) was initially rapid and linear with a $V_0 = 22 \mu\text{M/min}$ and then decreased after several minutes to a slow inhibited rate, or V_i , of 5 $\mu\text{M/min}$. In other experiments, the rate did not increase upon reaerating the suspension, indicating that the slow rate was due to inhibition of O_2 uptake, rather than to a change in the O_2 concentration dependence. In prolonged incubations (15–30 min) with high concentrations of NH_4^+ or other inhibitors, O_2 uptake usually stopped completely.

NH_4^+ did not interfere with stimulation by PMA, in that V_0 was the same with or without NH_4^+ . Moreover, similar inhibition was observed if NH_4^+ was added several minutes after the addition of PMA. Adding more PMA did not prevent or reverse the effect of NH_4^+ .

To evaluate inhibition by NH_4^+ or other compounds, V_0 and V_i were calculated, and the time required to observe inhibition, t_i , was calculated from the intercept of the two linear portions of the plot. In Fig. 4 (curve 3), $t_i = 4$ min. That is, inhibition was observed within 4 min after adding PMA, after $\sim 60 \mu\text{M}$ O_2 had been taken up. In other experiments, the lowest NH_4^+ concentration at which inhibition was observed was 1–2 mM. Lowering the NH_4^+ concentration increased t_i , but had little effect on V_i .

O_2 uptake was also measured with HRP and 0.1 mM scopoletin (curve 4) or catalase (curve 6) as traps for H_2O_2 . The observed rate with HRP-scopoletin was faster than with catalase (34 and 17 $\mu\text{M/min}$, respectively, owing to the stoichiometry of H_2O_2 dismutation by catalase (55), which yields 0.5 mol O_2 /mol of O_2 reduced to H_2O_2).



The approximate twofold difference in rates indicated that nearly all the O_2 taken up was converted to H_2O_2 (53, 56).

The rate obtained with HRP-scopoletin was also faster than that observed with taurine, indicating that a portion of the O_2 taken up in the presence of taurine was recycled to O_2 by cellular catalase. Therefore, the endogenous MPO did not utilize all of the H_2O_2 produced, accounting for the 31–55% efficiency of conversion of H_2O_2 to N-Cl derivatives (Table IV).

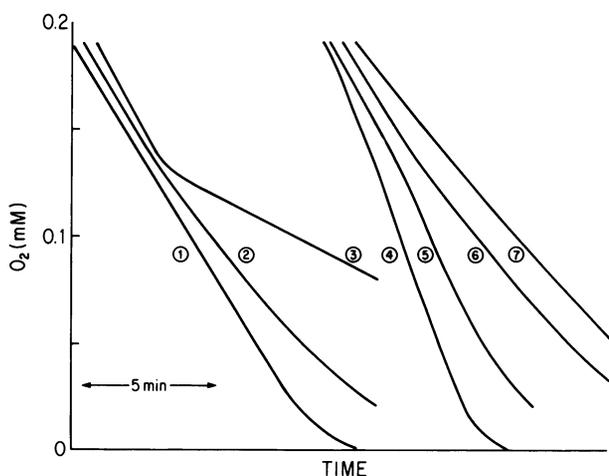


FIGURE 4 Effect of taurine or NH_4^+ on O_2 uptake. Neutrophils were incubated with PMA and (1) 10 mM taurine, (2) no N-compound, (3) 10 mM NH_4^+ , (4) HRP (10 $\mu\text{g/ml}$)-scopoletin (0.1 mM), (5) NH_4^+ and HRP-scopoletin, (6) catalase (30 $\mu\text{g/ml}$), and (7) NH_4^+ and catalase. The spacing of the curves is arbitrary. For each curve, the first point shown is 15–30 s after addition of PMA.

Inhibition by NH_4^+ was almost completely prevented by HRP-scopoletin (curve 5) or catalase (curve 7). Calculated V_0 values were 26 and 15 $\mu\text{M}/\text{min}$. No discontinuity was observed in plots of O_2 concentration vs. time, so that V_i and t_i were not calculated. Similarly, inhibition by 10 mM NH_4^+ was almost completely prevented by 10 mM taurine, serotonin, or methionine ($V_0 = 21, 18, \text{ or } 15 \mu\text{M}/\text{min}$), or by 0.2 mM dithiothreitol, methimazole, or diethyldithiocarbamate ($V_0 = 17, 19, \text{ or } 18 \mu\text{M}/\text{min}$). Therefore, inhibition was prevented by agents that would interfere with formation of the lipophilic N-Cl derivative, or which would reduce the derivative.

Although inhibition could be prevented, it could not be reversed. Taurine or the other agents did not increase the rate of O_2 uptake when added after the rate became inhibited. Moreover, when cells were incubated with PMA and 10 mM NH_4^+ for 5 min, removed from the chamber and washed to remove NH_4^+ , and then suspended and incubated with PMA and taurine, inhibition was not reversed.

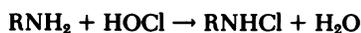
Table VII summarizes results of O_2 uptake studies. Compounds that yield charged or polar N-Cl derivatives (class 1) did not inhibit. The polyamines (class 2) did not inhibit within the time required to consume all the O_2 (8–12 min). Compounds that yielded lipophilic N-Cl derivatives (class 3) inhibited O_2 uptake. In other experiments, inhibition was obtained with amine concentrations as low as 0.1 mM, and a 10:1 molar ratio of taurine to the inhibitor was required to

prevent inhibition. With class 4 compounds, inhibition was correlated with lipid solubility of their N-Cl derivatives. Higher concentrations of these compounds were required to inhibit, and a 1:1 ratio of taurine to the inhibitor was sufficient to prevent inhibition.

DISCUSSION

The results indicate that stimulated neutrophils produce HOCl, and that the HOCl reacts rapidly with endogenous or exogenous N-compounds to yield N-Cl derivatives. In particular, the reaction with primary amines is favored. The fate of the N-Cl derivatives and their effect on leukocyte functions can be predicted on the basis of their relative lipophilicity.

Primary amines are predominantly in the charged form at biologically relevant pH values, but N-chlorination raises the dissociation constant by as much as 10 orders of magnitude (26), so that RNHCl rather than RNH_2Cl^+ is the predominant form.



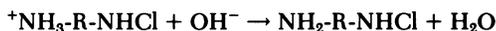
For compounds that contain other charged or highly polar groups, N-chlorination has little effect on solubility properties. For polyamines, the effect on solubility depends on the extent of N-chlorination and on pH. The charged groups of polyamines can be eliminated by N-chlorination of all the amino groups, or

TABLE VII
Effect of N-Compounds on O_2 Uptake

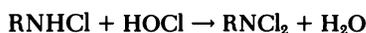
Class	N-compounds	Concentration	O_2 uptake*		
			V_0	V_i	t_i
		mM	$\mu\text{M}/\text{min}$		min
—	None	—	19±2	—	—
1	All	10	18±1	—	—
2	All	5	20±2	—	—
3	Ethanolamine	1	23	11	4
	Methylamine	1	21	6	4
	Phenylethylamine	1	15	9	4
4	Guanidinoacetate	10	21	—	—
	Guanidine	10	14	7	6
	Ammonia	10	17±2	6±2	4±1
	p-Toluenesulfonamide	10	13	2	6

* The PMA-stimulated initial rate of neutrophil O_2 uptake (V_0), the inhibited rate (V_i), and the time required for inhibition (t_i) were measured. Standard deviation is shown where at least four values were averaged.

by raising pH so as to favor deprotonation of unreacted groups.



For compounds with no charged or polar groups aside from the *N*-moiety, chlorination yields lipophilic oxidizing agents. Their lipophilic character is further enhanced by formation of RNCl_2 derivatives.



These lipophilic agents may readily penetrate biological membranes, oxidizing and inactivating membrane and cytoplasmic components.

Fig. 5 presents a diagrammatic summary of results of incubating stimulated neutrophils with exogenous *N*-compounds, using taurine and NH_4^+ as examples. Metabolism of glucose resulted in production of lactate and volatile products including CO_2 . Metabolism also served as the source of reducing equivalents (NADPH) for reduction of O_2 to O_2^- . Dismutation of O_2^- to yield H_2O_2 occurred spontaneously or was catalyzed by SOD. MPO catalyzed the oxidation of Cl^- by H_2O_2 to yield HOCl, which reacted with compounds such as taurine, $^-\text{SO}_3(\text{CH}_2)_2\text{NH}_3^+$, to yield highly polar or charged *N*-Cl derivatives that accumulated in the extracellular medium. These *N*-Cl derivatives did not inhibit leukocyte metabolism. On the other hand, reaction of HOCl with compounds such as NH_4^+ yielded lipophilic *N*-Cl derivatives that reacted with and in-

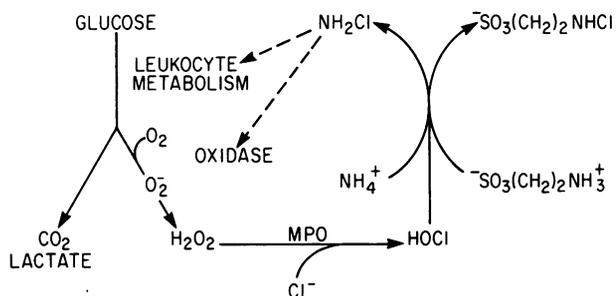


FIGURE 5 MPO-dependent effect of *N*-compounds. Solid arrows represent (a) steps in leukocyte metabolism that convert glucose to CO_2 and lactate and which yield reducing equivalents for reduction of O_2 to O_2^- by the oxidase enzyme, (b) dismutation of O_2^- to yield H_2O_2 , (c) MPO-catalyzed oxidation of Cl^- by H_2O_2 to yield HOCl, and (d) the competition between *N*-compounds for reaction with HOCl. Dashed arrows represent reactions of lipophilic *N*-Cl derivatives such as NH_2Cl with leukocyte components resulting in inhibition of metabolism and O_2 uptake. Charged or highly polar *N*-Cl derivatives such as taurine monochloramine, $^-\text{SO}_3(\text{CH}_2)_2\text{NHCl}$, accumulate in the medium and do not inhibit.

activated leukocyte components. Evidence for the reaction of these *N*-Cl derivatives with leukocyte components was the absence of accumulation of the *N*-Cl derivatives, and the inhibition of glucose metabolism and O_2 uptake.

Amines and other *N*-compounds can have a number of effects on phagocytic cells, including inhibition of acidification of the lysosomal contents and perhaps inhibition of membrane fusion (57). However, it was possible to distinguish between effects of the *N*-compounds and the toxicity of lipophilic *N*-Cl derivatives by blocking toxicity with traps for H_2O_2 , with an inhibitor of MPO activity, and with readily oxidized compounds that competed with leukocyte components for reaction with HOCl or the *N*-Cl derivatives. Moreover, inhibition was prevented by taurine as a competing amine that did not yield toxic *N*-Cl derivatives.

Although inhibition could be prevented, it could not be reversed, indicating that the chemical modifications resulting in inactivation of leukocyte components were irreversible. Inhibition of O_2 uptake could be due to inactivation of enzymes involved in glucose metabolism and NADPH production, or to direct inhibition of the oxidase. MPO-dependent progressive inactivation of the NADPH-oxidase of stimulated neutrophils has been reported (30). The leukocyte components that are inactivated and the nature of the chemical modifications that result in inactivation remain to be determined.

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