*Biochemical Pharmacology*, Vol. 44, No. 3, pp. 553–563, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00


# INHIBITION OF THE HUMAN LEUKOCYTE ENZYMES MYELOPEROXIDASE AND EOSINOPHIL PEROXIDASE BY DAPSONE

PAULA M. BOZEMAN,*† DOUGLAS B. LEARN‡ and EDWIN L. THOMAS§

* Division of Cardiopulmonary-Critical Care Medicine, St. Jude Children's Research Hospital, Memphis, TN 38105; ‡ Skin Biology Research, Schering–Plough HealthCare Products, Memphis, TN 38151; and § Dental Research Center and Department of Biochemistry, University of Tennessee, Memphis, TN 38163, U.S.A.

(*Received* 4 *February* 1991; *accepted* 8 *April* 1992)

**Abstract**—Dapsone (4,4′-diaminodiphenylsulfone) is an antimicrobial substance that also has anti-inflammatory activity, which has been attributed to inhibition of the leukocyte enzyme myeloperoxidase (MPO). We observed that dapsone was a much better inhibitor of the eosinophil peroxidase (EPO) in an assay that measured peroxidase-catalyzed oxidation of tetramethylbenzidine at pH 5.4. To clarify the specificity and pH-dependence of dapsone inhibition of the purified enzymes under more physiologic conditions, we studied peroxidase-catalyzed oxidation of chloride to the antimicrobial and cytotoxic agent hypochlorous acid. Taurine was added as a trap for hypochlorous acid, to prevent inactivation of the enzymes or chlorination of dapsone by hypochlorous acid. Dapsone was much more effective as an inhibitor of both MPO and EPO when chloride rather than tetramethylbenzidine was the substrate. Inhibition of both enzymes was greater at neutral pH than at acid pH (pH 7 vs pH 5), but EPO was more sensitive to inhibition than MPO regardless of pH. Inhibition was increased by lowering chloride, raising hydrogen peroxide, or lowering the enzyme concentration. Inhibition was accompanied by irreversible loss of enzyme activity, which was correlated with loss of the heme absorption spectrum, indicating chemical modification of the enzyme active site. EPO, but not MPO, was partially protected against inactivation by adding physiologic levels of bromide along with chloride. The results suggest that dapsone could prevent MPO- and EPO-mediated tissue injury at sites where the peroxidase enzymes are secreted and diluted into the neutral pH environment of the tissue interstitial space. Dapsone might not inhibit peroxidase-mediated antimicrobial activity, which occurs at high enzyme concentrations in the acid environment of phagolysosomes.

Dapsone (4,4′-diaminodiphenylsulfone) is an antimicrobial substance that has been used for many years to treat leprosy [1] and in recent years to treat *Pneumocystis carinii* pneumonia in AIDS patients [2]. Dapsone is a member of the class of compounds that are analogues of *p*-aminobenzoic acid and interfere with microbial folic acid synthesis [3].

Dapsone has also found limited use in the treatment of inflammatory diseases [4–10], primarily skin disorders that are not known to be initiated by infectious agents [4, 9, 11–14]. A possible basis for anti-inflammatory activity emerged from studies indicating that dapsone inhibits myeloperoxidase (MPO‖) [15], an enzyme found in two classes of inflammatory cells, the monocytes and neutrophils (polymorphonuclear leukocytes). Neutrophils combat infection by engulfing and killing microorganisms in intracellular compartments known as phagolysosomes [16]. MPO is one of a number of antimicrobial agents that are secreted into phagolysosomes. MPO and other lysosomal components are also secreted into the extracellular medium at sites of infection and inflammation and contribute to inflammatory tissue injury [17–19].

Stimulated leukocytes produce hydrogen peroxide ($H_2O_2$), and MPO catalyzes the oxidation of halide ions by $H_2O_2$ to produce oxidizing and halogenating agents [20]. The physiologic substrate for MPO is thought to be chloride ion ($Cl^-$), which is oxidized to the potent oxidizing and chlorinating agent hypochlorous acid (HOCl). The toxicity of the MPO/$H_2O_2$/$Cl^-$ system to microorganisms and host tissues may be due to the direct reaction of HOCl with cell components, or may be mediated by other oxidizing and chlorinating agents such as chloramines, which are formed in the reaction of HOCl with ammonia and amines.

Like other hemoprotein peroxidases, MPO also catalyzes the oxidation of a wide variety of organic compounds including phenols and aromatic amines. Dapsone has two primary aromatic amine moieties, suggesting that dapsone is a substrate for MPO and inhibits by competing with other substrates for binding and oxidation at the enzyme active site. Figure 1 compares the structure of dapsone with that of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB).

† Corresponding author: Dr. Paula M. Bozeman, Division of Cardiopulmonary-Critical Care Medicine, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105. Tel. (901) 531-2351; FAX (901) 527-2270.

‖ Abbreviations: DETAPAC, diethylenetriaminepentaacetate; EPO, eosinophil peroxidase; MPO, myeloperoxidase; Nbs, 5-thio-2-nitrobenzoic acid or "TNB"; $Nbs_2$, 5,5′-dithiobis(2-nitrobenzoic acid) or "DTNB"; PBS, phosphate-buffered saline (0.14 M NaCl and 15 mM potassium phosphate buffer, pH 7.2); PMA, phorbol myristate acetate; TauNHCl, taurine-monochloramine; and TMB, 3,3′,5,5′-tetramethylbenzidine.

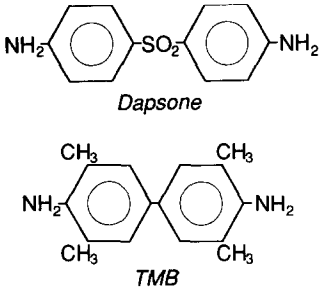


Fig. 1. Structures of the inhibitor dapsone and substrate TMB.

Dapsone has proved useful in our studies of MPO-catalyzed $Cl^-$ oxidation by isolated neutrophils [21, 22]. Unlike other peroxidase inhibitors such as azide and cyanide, dapsone does not inhibit catalase in neutrophils or target cells [23]. Relatively low dapsone concentrations (<0.1 mM or 30 μg/mL) were sufficient to block $Cl^-$ oxidation when neutrophil $H_2O_2$ production and MPO secretion were stimulated by phorbol myristate acetate (PMA) or high levels of opsonized zymosan. Under these conditions, $Cl^-$ oxidation occurs primarily in the extracellular medium [21, 24] at the buffer pH of 7.0 to 7.4.

On the other hand, we observed that dapsone was a poor inhibitor of MPO in a non-physiologic assay of peroxidase activity that measures oxidation of TMB at pH 5.4 [25]. Dapsone did inhibit the eosinophil peroxidase (EPO), an enzyme found in another class of inflammatory cells, the eosinophilic leukocytes. The biological role of EPO is similar to that of MPO, but EPO is less active as a catalyst for $Cl^-$ oxidation [26]. At present, it is not known whether $Cl^-$ is the physiologic substrate, or whether EPO catalyzes the oxidation of bromide ($Br^-$), iodide ($I^-$), or thiocyanate ($SCN^-$) *in vivo* [26–28].

One aim of this study was to determine whether dapsone inhibition of MPO is pH dependent, which could account for differences in results obtained with isolated neutrophils and the TMB-oxidation assay. The pH-dependence of inhibition could be important *in vivo*. The inflammatory activity of MPO is expressed at neutral pH in the tissue interstitial space, whereas antimicrobial activity is expressed in the acidified phagolysosome compartment. If dapsone is more effective at neutral pH, then dapsone might block MPO-mediated tissue injury but not interfere with the role of MPO in host-defense against infection.

Another aim was to determine whether dapsone is a better inhibitor of EPO than MPO in the more physiologic assay that measures $Cl^-$ oxidation. If EPO is especially sensitive to inhibition, then dapsone may be useful in treating inflammatory disease processes involving eosinophils. These leukocytes have a role in allergic phenomena and are found in high numbers in certain mucosal tissues, especially the intestinal mucosa, and inflamed skin [29].

## MATERIALS AND METHODS

MPO and EPO were purified from human leukemic leukocytes [25]. Concentrations were calculated assuming extinction coefficients of 89,000 $M^{-1}$ $cm^{-1}$ for MPO at 430 nm [30] and 112,000 $M^{-1}$ $cm^{-1}$ for EPO at 415 nm [31]. Carboxymethylcellulose (CM-52) preswollen microcrystalline resin was from Whatman Biosystems Ltd., Maidstone, Kent, England. Dapsone, diethylenetriaminepentaacetate (DETAPAC), hypotaurine, 5,5′dithiobis(2-nitrobenzoic acid) ($Nbs_2$), taurine, TMB, thiodipropionic acid, and Triton X-100 were from the Sigma Chemical Co., St. Louis, MO. Dapsone (0.1 M) was dissolved in 0.46 M HCl and diluted to 1 mM in water, phosphate-buffered saline (PBS), pH 7.2, or 0.3 M sucrose with 50 mM sodium acetate buffer, pH 5.4 or 7, and the pH was readjusted with potassium hydroxide. DETAPAC (0.1 M) was adjusted to pH 7 with potassium hydroxide. 5-Thio-2-nitrobenzoic acid (Nbs) (0.6 mM) was prepared by adding 4 μL of 2-mercaptoethanol to 100 mL of 1 mM $Nbs_2$ in PBS with 0.1 mM DETAPAC. Dilutions of $H_2O_2$ (Fisher Chemical Co., Pittsburgh, PA) were prepared in sterile water, 0.1 M sodium sulfate, or the sucrose/acetate buffer solutions. Catalase crystals (Boehringer Mannheim, Indianapolis, IN) were washed twice in water and dissolved in PBS.

*$Cl^-$ oxidation.* Incubations of MPO or EPO at 37° with $Cl^-$, $H_2O_2$, and taurine (10 mM) were stopped by adding catalase (15 μg), and then the taurine-monochloramine (TauNHCl) concentration was measured by a modification of the method based on oxidation of Nbs [32]. DETAPAC (0.1 mM) was included in the Nbs and diluent solutions to inhibit metal ion-catalyzed oxidation of Nbs [33]. In addition, absorbance was measured at 409 nm rather than 412 nm, and an extinction coefficient of 14,050 $M^{-1}$ $cm^{-1}$ was determined for Nbs at 409 nm. Oxidant concentration (μM TauNHCl) was calculated as previously described [32].

*Peroxidase activity.* Activity was calculated from the rate of $H_2O_2$-dependent oxidation of TMB [25]. Assay mixtures were warmed to 37°, and 0.05 mL of 0.1 M TMB in *N,N*-dimethylformamide was added. Incubations were started by adding 0.2 mL of $H_2O_2$ to give 0.3 mM $H_2O_2$ in 3.5 mL total volume containing 1.4 mM TMB, 30 μg Triton X-100, 0.3 M sucrose, and 50 mM sodium acetate buffer, pH 5.4, with 2-3 nM MPO or EPO. After 3 min at 37°, incubations were stopped by adding 0.1 mL of 0.3 mg/mL catalase and 3.4 mL of cold 0.2 M acetic acid. Portions (0.6 mL) were diluted with 0.75 mL of 0.2 M acetic acid to give a final 4.5-fold dilution. The diluted mixtures were clarified by centrifugation and absorbance was measured at 655 nm. Absorbance values reported below are 4.5 times the value measured after dilution.

*Spectrophotometric experiments.* Incubation mixtures (30 mL) contained 30 nM MPO, 10 or 100 mM $Cl^-$, $H_2O_2$, 10 mM taurine, and dapsone in 15 mM phosphate buffer, pH 7 or 5. Incubations at 37° were stopped by adding 1 μL of 0.3 mg/mL catalase, 0.3 mL of 10 mM hypotaurine, and 10 mL of either 50 mM acetic acid or 50 mM sodium acetate buffer,

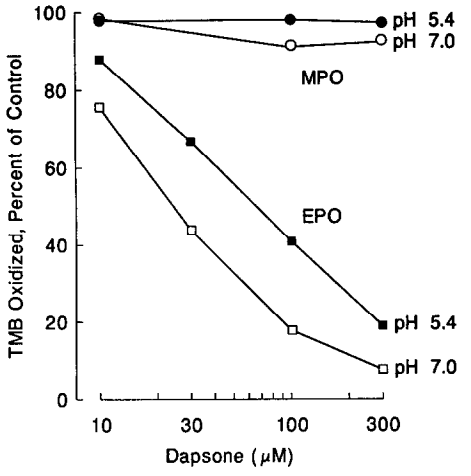


Fig. 2. Inhibition of TMB oxidation. Assays with 2.5 nM MPO (●, ○) or EPO (■, □) were at pH 5.4 (●, ■) as described in Materials and Methods, or with all solutions adjusted to pH 7 (○, □). Control values were 4.5 and 2.3 absorbance units per 3 min at pH 5.4 and 7. (Number of experiments, N, was 10 with MPO and 3 with EPO.)

pH 4.7, to adjust the pH to 4.7. All subsequent steps were at pH 4.7. The mixtures were applied to columns each prepared from 0.25 g of CM-52 resin equilibrated in 25 mM acetate buffer. After washing with 50 mL of 0.2 M NaCl in 25 mM acetate buffer, MPO was eluted by raising NaCl to 0.5 M, and the eluate was adjusted to 1 mL total volume. After absorbance was measured, peroxidase activity and protein concentration [34] were determined. In similar experiments with 0.1 μM EPO, the enzyme was concentrated 10-fold by centrifugal filtration rather than chromatography.

## RESULTS

*Inhibition of TMB oxidation.* To determine whether inhibition by dapsone is pH dependent, the effect of pH was first examined in the TMB oxidation assay. Figure 2 shows that dapsone up to 0. 3 mM did not inhibit MPO at pH 5.4, and caused only slight inhibition at pH 7. Dapsone did inhibit EPO, and was about 3 times more effective at pH 7 than at pH 5.4. The results indicate that dapsone was more effective as an inhibitor of EPO than of MPO, and more effective against both enzymes at neutral pH.

MPO and EPO have identical activity per mole of enzyme at pH 5.4 in the TMB assay [25]. The enzymes were also found to have identical activity at pH 7, equal to about half the activity at pH 5.4. Therefore, the greater sensitivity of EPO to dapsone inhibition was not due to a difference in the ability of MPO and EPO to catalyze the oxidation of TMB.

*Inhibition of* $Cl^-$ *oxidation.* Dapsone inhibition of MPO-catalyzed $Cl^-$ oxidation was studied under conditions similar to those in experiments with isolated neutrophils. The MPO concentration (50 nM) was the same as would be obtained if half the MPO content of a suspension of $4 \times 10^6$ neutrophils/mL was secreted into the medium [25, 27, 35]. The $Cl^-$ concentration was 0.1 M, and 0.1 mM $H_2O_2$ was added at the start of the incubation. This amount of $H_2O_2$ is the same as utilized in $Cl^-$ oxidation during 1 hr by PMA-stimulated neutrophils at 2–4 $\times$ $10^6$/mL [21, 24]. The amine compound taurine (10 mM) was added as a trap for HOCl, to prevent inactivation of MPO by HOCl [36], reduction of HOCl by $H_2O_2$ [37], or chlorination of the amine moieties of dapsone [38]. After 1 hr at 37°, the amount of $Cl^-$ oxidation was determined by measuring the concentration of TauNHCl.

Figure 3A shows that in the absence of dapsone, the yield of TauNHCl was about 0.1 mM, indicating that the amount of $Cl^-$ oxidized was about equal to the amount of $H_2O_2$ added. Dapsone at 2–3 μM inhibited $Cl^-$ oxidation by 50% at pH 7. Similar results were obtained either by adding 0.1 mM $H_2O_2$ at the start of the incubation or, in other experiments, by using the glucose oxidase $H_2O_2$-generating system to produce the same amount of $H_2O_2$ over the course of an hour. The results are consistent with experiments indicating that dapsone inhibits neutrophil $Cl^-$ oxidation at neutral pH.

Figure 3A also shows that dapsone inhibited MPO activity rather than simply competing with $Cl^-$ as a substrate and thus lowering the amount of $H_2O_2$ available for $Cl^-$oxidation. That is, the amount of $H_2O_2$ that could be consumed in oxidizing 2–3 μM dapsone would be too small relative to the 0.1 mM $H_2O_2$ to lower $Cl^-$ oxidation by 50%. Similarly, scavenging of HOCl by dapsone could not account for inhibition, because the decrease in the yield of TauNHCl was much greater than the amount of dapsone. Scavenging could account for this result only if 2–3 μM dapsone competed very effectively with 10 mM taurine for reaction with HOCl and consumed 50 μM HOCl, or if 2–3 μM dapsone consumed 50 μM TauNHCl. To test for scavenging, 0.1 mM HOCl was added to 10 mM taurine in the presence and absence of 0.1 mM dapsone, and the yield of TauNHCl was measured. In addition, 0.1 mM dapsone was incubated for 1 hr at 37° with 0.1 mM TauNHCl, and then TauNHCl was measured. Dapsone did not interfere with TauNHCl formation by consuming HOCl, and did not react with TauNHCl, indicating that dapsone could not act as a scavenger under these conditions.

Figure 3B shows that MPO was not inhibited at pH 5, but 2–3 μM dapsone inhibited EPO by 50%. These results were consistent with the TMB oxidation assay, which indicated that EPO was more sensitive to inhibition. Dapsone was much more effective as an inhibitor of both enzymes when $Cl^-$ rather than TMB was the substrate.

A direct comparison of dapsone inhibition of EPO at pH 5 and 7 could not be made with this enzyme concentration (50 nM), because EPO-catalyzed $Cl^-$ oxidation was very slow at pH 7. However, Fig. 3C shows that when $Cl^-$ was increased to 0.3 M, EPO-catalyzed $Cl^-$ oxidation was nearly complete within 1 hr and was 50% inhibited by 1 μM dapsone. For comparison, results are also shown for MPO at 0.3 M $Cl^-$. Raising $Cl^-$ from 0.1 to 0.3 M made dapsone

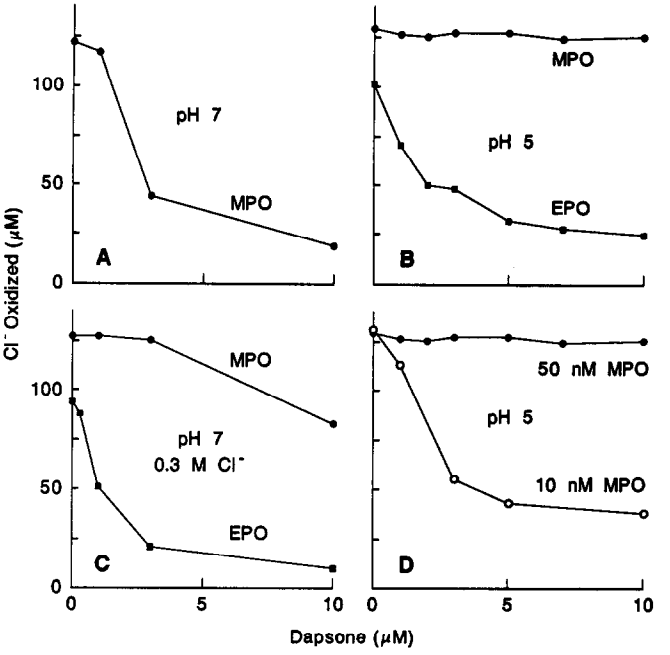


Fig. 3. Inhibition of $Cl^-$ oxidation. The amount of oxidized $Cl^-$ was measured at the end of 1-hr incubations of MPO or EPO with $Cl^-$, 0.1 mM $H_2O_2$, 10 mM taurine, and the indicated concentrations of dapsone. (A) 50 nM MPO (●), 0.1 M $Cl^-$, pH 7. (B) 50 nM MPO (●) or EPO (■), 0.1 M $Cl^-$, pH 5. (C) 50 nM MPO (●) or EPO (■), 0.3 M $Cl^-$, pH 7. (D) 50 nM (●) or 10 nM (○) MPO, 0.1 M $Cl^-$, pH 5. [(A) N = 5; (B–D) N = 3.]

less effective as an inhibitor of MPO. Therefore, this comparison of dapsone inhibition of EPO at pH 5 and 7 underestimates the pH-dependence. The results indicate that dapsone was much more effective as an inhibitor of both enzymes at pH 7 than at pH 5, and that dapsone was more effective against EPO than MPO regardless of pH.

*Inactivation by dapsone.* If dapsone is a competitive inhibitor of $Cl^-$ oxidation, then inhibition would depend on the $Cl^-$ concentration but would be independent of enzyme concentration. However, Fig. 3D shows that inhibition depended on the enzyme concentration. When MPO was lowered from 50 to 10 nM, dapsone was able to inhibit MPO-catalyzed $Cl^-$ oxidation at pH 5. A similar effect of enzyme concentration was observed with EPO (not shown).

To examine the effect of dapsone on enzyme activity, the time-course of $Cl^-$ oxidation was measured (Fig. 4). In the absence of dapsone, $Cl^-$ oxidation was nearly linear with time during the first 5 min. Within 30 min, the TauNHCl concentration approached a stable plateau level equal to the amount of $H_2O_2$ added at the start of the incubation, indicating that the reaction went to completion and all the $H_2O_2$ was consumed. With 1 μM dapsone, the initial rate of $Cl^-$ oxidation was inhibited, and in addition, a plateau level of TauNHCl was obtained that was much lower than in the absence of dapsone. Competitive inhibition would slow the initial rate, but the same plateau level would eventually be reached with or without the inhibitor. Therefore, the results suggested that dapsone caused progressive inactivation of MPO during the incubation.

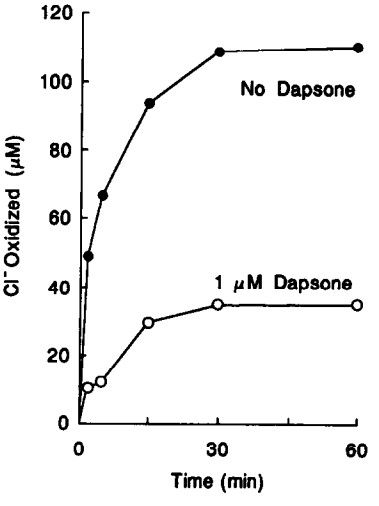


Fig. 4. Time-course of $Cl^-$ oxidation. Incubation mixtures at pH 7 contained 30 nM MPO, 0.1 M $Cl^-$, 0.1 mM $H_2O_2$, 10 mM taurine, and no dapsone (●) or 1 μM dapsone (○). (N = 3.)

Figure 5 shows results of an experiment that confirmed inactivation. In the absence of dapsone (Fig. 5A), adding more $H_2O_2$ after the plateau level

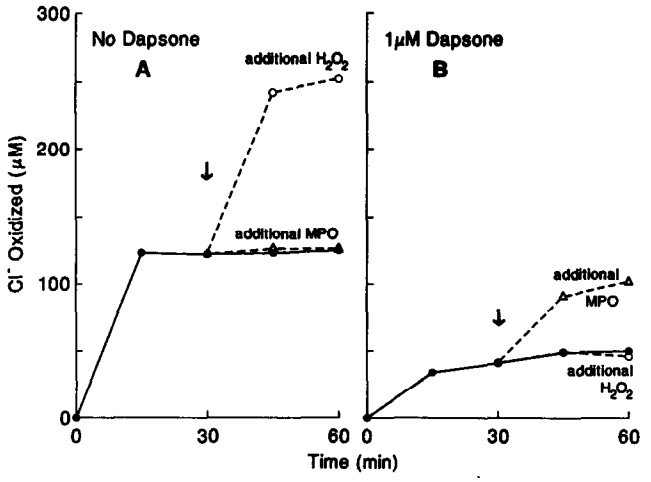


Fig. 5. Effect of adding more $H_2O_2$ or MPO after $Cl^-$ oxidation had stopped. Incubation mixtures at pH 7 contained 30 nM MPO, 0.1 M $Cl^-$, 0.1 mM $H_2O_2$, 10 mM taurine, and no dapsone (A, ●) or 1 μM dapsone (B, ●). After 30 min, 0.1 mM $H_2O_2$ (--○--) or 30 nM MPO (-- △ --) was added. (N = 3).

of TauNHCl was achieved caused a second burst of $Cl^-$ oxidation, indicating that MPO was active. Adding more MPO had no effect, indicating that all the $H_2O_2$ had been consumed. The opposite results were obtained when 1 μM dapsone was present (Fig. 5B). Adding more $H_2O_2$ had no effect because all the MPO was inactivated. Adding more MPO caused a second burst of $Cl^-$ oxidation, indicating that MPO was inactivated before all of the $H_2O_2$ was consumed.

*Mechanism of inactivation.* Like other hemoprotein peroxidases, the form of MPO that oxidizes halide ions is the Compound I oxidation state, which is produced in the reaction of $H_2O_2$ with the ferric form of the enzyme [39–41]. The Compound I form of peroxidases also carries out one- or two-electron oxidation of other substrates such as phenols and aromatic amines [42]. One-electron reduction of Compound I by these substrates yields Compound II. The enzymes are inactive toward halide ions while in the Compound II form [39, 43], but Compound II oxidizes other substrates and is reduced back to the ferric form [44, 45]. Moreover, if excess $H_2O_2$ is removed, the enzyme reverts to the active ferric form, due to reduction of Compound II by endogenous electron donors [45]. Therefore, conversion of MPO to Compound II is a reversible form of inactivation.

To determine whether dapsone caused reversible inactivation of MPO in the $Cl^-$ oxidation assay, MPO was incubated with $Cl^-$ $H_2O_2$, taurine, and dapsone under conditions that caused inactivation, the mixture was diluted, and MPO was assayed at pH 5.4 with TMB as the substrate. Aromatic amines such as TMB have been shown to be good substrates for the Compound II forms of other peroxidases [42]. Figure 6A shows that MPO lost activity toward TMB, suggesting that inactivation was irreversible. Dapsone at 1 μM almost completely inactivated 30 nM MPO at pH 7, consistent with results of the $Cl^-$ oxidation assay. Also shown are controls in which $H_2O_2$ was omitted from the first incubation. No inhibition was obtained, indicating that simultaneous incubation with dapsone and $H_2O_2$ at pH 7 was required for inactivation.

In experiments described in a later section, MPO was freed of $H_2O_2$ and dapsone by a chromatographic procedure and then assayed with TMB as the substrate. This procedure should permit any MPO Compound II to revert to the ferric form. Loss of activity was also observed in these experiments, confirming that inactivation was irreversible, and that inactivation did not result from accumulation of MPO in the Compound II form.

The loss of activity toward TMB also made it possible to quantify the extent of inactivation that occurred during incubations of MPO or EPO with $Cl^-$, $H_2O_2$, taurine, and dapsone at pH 5 and 7. Because activity of the enzymes is high in the TMB assay, activity could be measured after extensive dilution of the incubation mixtures. Dilution lowered dapsone concentrations far below the levels required to inhibit in the TMB assay. Dilution also lowered $Cl^-$ to levels that do not interfere [25], and any unreacted $H_2O_2$ was diluted to levels that would not contribute significantly to color development with TMB. Hypotaurine was added to reduce any TauNHCl [32] and thus prevent the oxidation of TMB by TauNHCl.

Figure 6B shows results of an experiment in which MPO or EPO was incubated with $Cl^-$, $H_2O_2$, taurine, and dapsone at pH 5, and was then diluted and assayed with TMB at pH 5.4. Consistent with results above, there was little inactivation of MPO at pH 5, but EPO was inactivated by low (1–10 μM) dapsone concentrations. The amount of dapsone required to inactivate EPO during the incubation with $H_2O_2$ and $Cl^-$ was far below the levels required

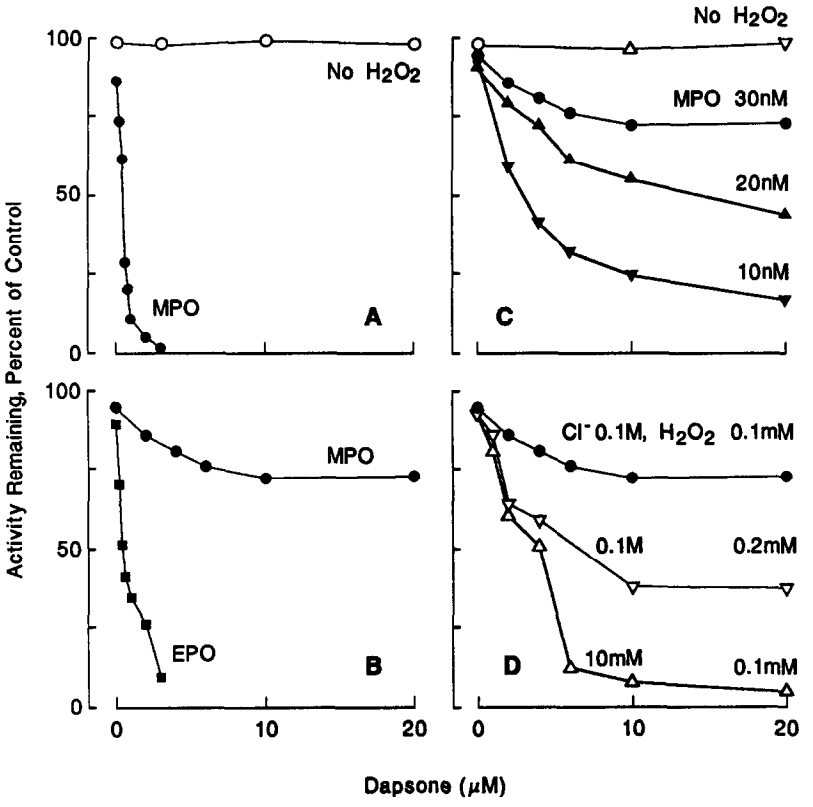


Fig. 6. Loss of enzyme activity. Incubation mixtures at pH 7 (A) or pH 5 (B–D) contained MPO or EPO, $H_2O_2$, $Cl^-$, 10 mM taurine, and the indicated concentrations of dapsone. (A) 30 nM MPO, 0.1 M $Cl^-$, and no $H_2O_2$ (○) or 0.1 mM $H_2O_2$ (●). (B) 30 nM MPO (●) or EPO (■), 0.1 M $Cl^-$, 0.1 mM $H_2O_2$. (C) 30 nM (○, ●), 20 nM (△, ▲), or 10 nM (▽, ▼) MPO, 0.1 M $Cl^-$, and no $H_2O_2$ (○, △, ▽) or 0.1 mM $H_2O_2$ (●, ▲, ▼). (D) 30 nM MPO, with 0.1 M $Cl^-$ and 0.1 mM $H_2O_2$ (●), 0.1 M $Cl^-$ and 0.2 mM $H_2O_2$ (▽), or 10 mM $Cl^-$ and 0.1 mM $H_2O_2$ (△). After 1 hr, 0.1 mM hypotaurine was added, the mixtures were diluted 14-fold, and peroxidase activity was assayed with TMB at pH 5.4. Control values were 54 absorbance units per 3 min for 30 nM enzyme. [(A) N = 5; (B–D) N = 3.]

to inhibit when dapsone was added at the start of a TMB oxidation assay (see Fig. 2).

Panels C and D of Fig. 6 show that inactivation of MPO by dapsone could be obtained at pH 5 by lowering the MPO concentration, raising $H_2O_2$, or lowering $Cl^-$. The results suggest that dapsone and $Cl^-$ competed for oxidation, and that an oxidized form of dapsone reacted with and inactivated MPO. Raising $H_2O_2$ or lowering $Cl^-$ allowed more dapsone to be oxidized and thus more of the inactivator to be produced. When MPO was lowered, the number of inactivated enzyme molecules was a larger fraction of the total enzyme molecules, and a higher percent inhibition was observed.

Figure 7 shows the time-course of inactivation of 30 nM MPO at pH 7 and 10 nM MPO at pH 5. Inactivation was progressive during the 1 hr incubation, but significant inactivation was observed within the first 5 min. Therefore, inactivation contributed to inhibition of $Cl^-$ oxidation during the initial linear phase of $Cl^-$ oxidation as well as to the later phase when $Cl^-$ oxidation came to a stop.

Diluting and assaying activity with TMB also made it possible to quantify inactivation of the enzymes under conditions that did not permit measurements of $Cl^-$ oxidation. For example, inactivation of MPO and EPO was not altered by substituting hypotaurine for taurine. Hypotaurine is oxidized to taurine by HOCl or chloramines [32], and no oxidant accumulates in the presence of excess hypotaurine. As shown below, inactivation was also obtained in the presence of a 10-fold excess of the scavenger thiodipropionate. Therefore, accumulation of an oxidant such as TauNHCl was not required for inactivation.

*Effect of bromide.* Results shown above indicated that when the $Cl^-$ concentration was increased, higher levels of dapsone were required for inactivation. Because physiologic fluids contain $Br^-$ as well as $Cl^-$ and $Br^-$ is a better substrate than $Cl^-$ for EPO, the effect of $Br^-$ on inactivation was also examined. The thioether compound thiodipropionate, which is readily oxidized by chlorine- or bromine-containing oxidants [32], was added to prevent inactivation of the enzymes by hypobromous acid (HOBr), reduction of HOBr by $H_2O_2$, or bromination of dapsone.

Table 1 compares inactivation of MPO and EPO

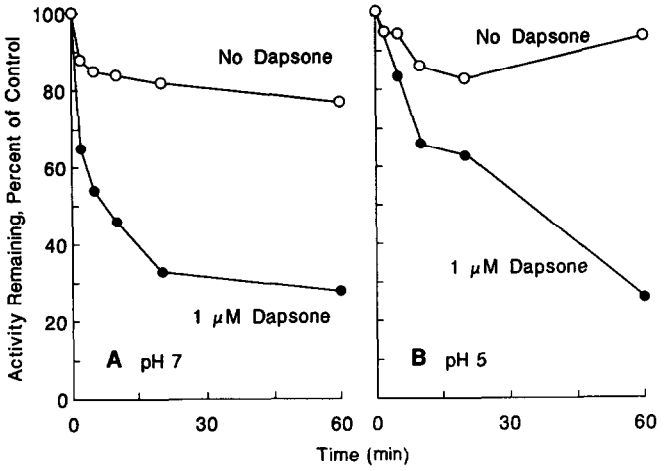


Fig. 7. Time-course of inactivation. Incubation mixtures contained MPO, 0.1 M $Cl^-$, 0.1 mM $H_2O_2$, 10 mM taurine, and no dapsone (○) or 1 μM dapsone (●). (A) 30 nM MPO, pH 7. (B) 10 nM MPO, pH 5. At the indicated times, 0.1 mM hypotaurine was added, the mixtures were diluted 14-fold, and peroxidase activity was assayed with TMB at pH 5.4. Control values were 54 absorbance units per 3 min for 30 nM enzyme. (N = 5.)

Table 1. Effect of bromide on inactivation

| Dapsone (μM) | Activity remaining (%) | | | |
|---|---|---|---|---|
| | MPO | | EPO | |
| | Without $Br^-$ | With $Br^-$ | Without $Br^-$ | With $Br^-$ |
| 0 | 87 | 84 | 97 | 99 |
| 0.1 | 65 | 66 | 34 | 96 |
| 0.3 | 51 | 55 | 21 | 91 |
| 1.0 | 11 | 13 | 5 | 82 |
| 3.0 | 4 | 6 | 0 | 75 |

Incubation mixtures at pH 7 contained 0.1 μM MPO or EPO, 0.1 M $Cl^-$, 0.1 mM $H_2O_2$, 10 mM taurine, 1 mM thiodipropionate, the indicated concentrations of dapsone, and either no $Br^-$ or 0.1 mM $Br^-$. After 1 hr, portions were diluted 50-fold, and activity was measured with TMB. The control was MPO or EPO incubated for 1 hr without $H_2O_2$, which had an activity of 180 absorbance units per 3 min. (N = 11 for MPO and 3 for EPO.)

at pH 7 with 0.1 M $Cl^-$ and with both 0.1 M $Cl^-$ and 0.1 mM $Br^-$. This level of $Br^-$, which is at the high end of the physiologic range of plasma $Br^-$ concentrations [26, 28], protected EPO but not MPO against inactivation. In a similar experiment with dapsone held constant at 1 μM and $Br^-$ at 0.01, 0.05, and 0.1 mM, the EPO activity remaining was 34, 54, and 76% of the control. In contrast, protection of MPO against inactivation was observed only at $Br^-$ concentrations of 1 mM or higher. Therefore, MPO rather than EPO was more readily inactivated when equal concentrations of the enzymes were compared at the same level of $H_2O_2$ and the presence of both 0.1 M $Cl^-$ and 0.1 mM $Br^-$.

*Correlation of inactivation with spectrophotometric changes.* MPO was incubated at pH 7 with $Cl^-$, $H_2O_2$, taurine, and dapsone at a low MPO concentration (30 nM) that favored a high percent inactivation. Catalase and hypotaurine were then added to eliminate any $H_2O_2$ and TauNHCl. The incubation mixtures were acidified and passed over ion-exchange columns to absorb MPO and remove dapsone. MPO was then eluted from each column with a small volume of high ionic strength buffer at pH 4.7 to obtain the enzyme at a higher concentration (1 μM) that permitted examination of the absorption spectrum. Activity of the recovered enzyme was measured with TMB at pH 5.4, and protein was measured to correct absorbance and activity measurements for variations in recovery.

Figure 8 shows that incubation with dapsone and $H_2O_2$ at pH 7 resulted in loss of both enzyme activity and the principal heme (Soret) absorbance at 430 nm. Complete inactivation was correlated with loss of

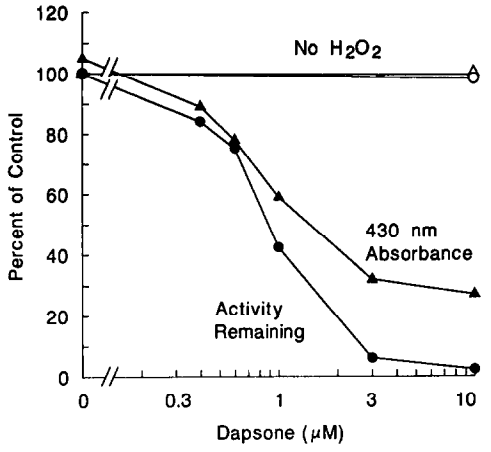


Fig. 8. Correlation of inactivation and loss of MPO heme absorbance. Incubation mixtures at pH 7 contained 30 nM MPO, 0.1 M $Cl^-$, 10 mM taurine, the indicated concentrations of dapsone, and either no $H_2O_2$ (△, ○) or 50 μM $H_2O_2$ (▲, ●). After 1 hr, MPO was freed of dapsone and concentrated to 1 μM. Absorbance at 430 nm was measured (△, ▲). Portions were diluted 350-fold, and peroxidase activity was measured with TMB at pH 5.4 (○, ●). The control, which was not incubated or chromatographed, was 1 μM MPO in 0.5 M NaCl with 25 mM acetate buffer, pH 4.7, which had an absorbance of 0.089 at 430 nm and peroxidase activity of 1800 absorbance units per 3 min. (N = 7.)

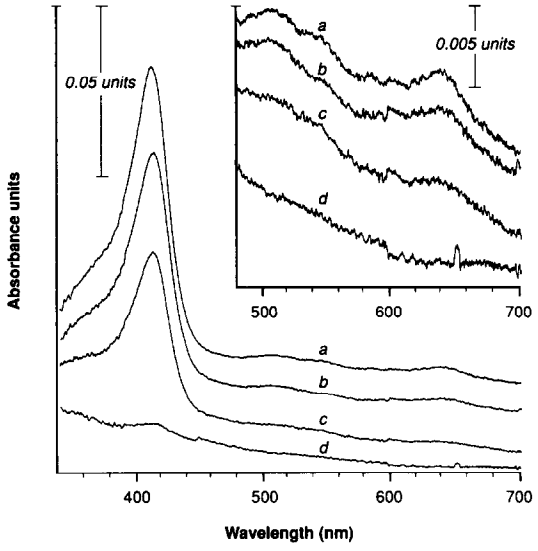


Fig. 9. Absorption spectra of active and inactivated EPO. Incubation mixtures at pH 7 contained 0.1 μM EPO, 0.1 M $Cl^-$, and 10 mM taurine, with no $H_2O_2$ or dapsone (*a*), 100 nmol/ml $H_2O_2$ added over the course of 1 hr (*b*), $H_2O_2$ and 3 μM dapsone (*c*), or $H_2O_2$ and 10 μM dapsone (*d*). After 1 hr, EPO in the mixtures was concentrated 10-fold, and the spectra were obtained. The inset figure shows the spectra at 5-fold higher sensitivity. Portions were diluted 350-fold, and the activity was measured with TMB at pH 5.4. Activity of the enzyme in *b*, *c* and *d* was 78, 54, and 2% of that in *a*, which was 1800 absorbance units per 3 min. (N = 3.)

about two-thirds of the 430 nm absorbance. No activity or absorbance was lost when $H_2O_2$ or dapsone was omitted. Loss of 430 nm absorbance was due to bleaching of the heme absorbance, rather than a shift to higher wavelengths, which would occur if MPO was converted to a reversibly inactivated form such as Compound II or III [46, 47] or to "sulfheme" or "sulfperoxidase" forms, which are produced in the reaction of hemoproteins with sulfhydryl and other sulfur-containing inhibitors [48, 49].

When the same experiments were carried out at pH 5, dapsone had no effect under conditions that resulted in complete inactivation at pH 7. However, inactivation and loss of 430 nm absorbance were obtained by raising $H_2O_2$, lowering $Cl^-$, or both. The activity remaining after incubation with 10 μM dapsone was 65, 35, or 1% of the control when $H_2O_2$ was raised from 50 to 100 μM, $Cl^-$ was lowered from 0.1 to 0.01 M, or both. The remaining 430 nm absorbance was 75, 58, or 44% of the control. Therefore, the inactivation process appeared to be the same at pH 5 and 7.

In similar experiments with EPO, complete inactivation was correlated with loss of 80–90% of the heme absorbance. Figure 9 shows that inactivation was accompanied by bleaching of the heme absorbance at 415 nm and higher wavelengths, rather than a shift in the absorbance. The results indicate that inactivation of MPO and EPO was the result of a chemical attack on the enzymes that chemically modified the heme groups or which removed iron or heme from the active sites.

## DISCUSSION

*Mechanism of inhibition.* In a study that measured the initial rate of MPO-catalyzed oxidation of $I^-$, it was reported that dapsone was a competitive inhibitor, that inhibition was independent of MPO concentration, and that dapsone did not cause loss of MPO activity [15]. In our study of MPO- and EPO-catalyzed $Cl^-$ oxidation, inhibition was competitive in the sense that inhibition decreased as the $Cl^-$ concentration increased. However, the results suggest that $Cl^-$ and dapsone competed for oxidation, and that an oxidized form of dapsone reacted with and inactivated the enzymes. Inactivation rather than competition for oxidation was the principal mechanism of inhibition.

In two recent studies, dapsone-dependent conversion of MPO to the Compound II state was proposed to account for inhibition of $Cl^-$ oxidation [50, 51]. Spectrophotometric experiments were carried out at high enzyme concentrations and short times of incubation, conditions that are convenient for obtaining absorption spectra. Under these conditions, conversion of MPO to Compound II was observed, and no irreversible inactivation or loss of the heme spectrum was reported. In studies reported here, conditions were selected to resemble those in experiments with isolated leukocytes. Irreversible inactivation with loss of the heme spectra of both MPO and EPO was the major form of inhibition by

dapsone, and enzyme concentration and time were found to be important variables.

*Oxidized forms of dapsone.* There are a number of reactive products that might be formed during dapsone oxidation that could chemically modify and inactivate the enzymes. If dapsone, like other aromatic amines ($ArNH_2$), undergoes one-electron oxidation by the Compound I and/or Compound II forms of the enzymes, then free-radicals such as $ArNH_2^+\cdot$ would be produced that might attack heme or adjacent sites required for activity. Two-electron oxidation of dapsone catalyzed by MPO was reported to yield the hydroxylamine derivative, $ArNH_2OH$ [38], which might also react with the enzymes. Because dapsone appears non-toxic to isolated cells, oxidation of dapsone to the hydroxylamine derivative *in vivo* was proposed to account for the toxicity of high-dose dapsone [38]. Recent studies indicate that oxidation of dapsone by liver microsomes rather than by MPO or EPO may account for formation of dapsone-hydroxylamine *in vivo* [52].

Additional reactive derivatives could be formed in the chlorination of dapsone by HOCl to yield the chloramines, ArNHCl and $ArNCl_2$. In particular, the dichloramine $ArNCl_2$ might chlorinate or insert into nucleophilic groups at the enzyme active site [53]. However, this study was carried out with a high concentration of taurine to compete with dapsone for chlorination. Moreover, oxidation of $Cl^-$ to HOCl and chlorination of dapsone would be favored at high $Cl^-$ concentrations rather than the low $Cl^-$ concentrations that favored inactivation. Therefore, attack by a dapsone radical or the hydroxylamine derivative appears more likely, and dapsone might qualify as a suicide-substrate.

*Competition between dapsone and halides or TMB as substrates.* Dapsone was more effective as an inhibitor of both MPO and EPO when $Cl^-$ rather than TMB was the substrate, and more effective against both enzymes at neutral rather than acid pH. These results are consistent with competition between dapsone and either $Cl^-$ or TMB as substrates. Dapsone should compete more effectively for oxidation when $Cl^-$ is the substrate because TMB is a better substrate than $Cl^-$ for both MPO and EPO. The enzymes were saturated with respect to TMB in the peroxidase activity assay; raising TMB above 1.4 mM did not increase the rate of oxidation. In comparison, 0.1 to 0.3 M $Cl^-$ was required to obtain maximum rates of $Cl^-$ oxidation, particularly at pH 7 or when EPO was the catalyst. Therefore, dapsone competed with a large excess of substrate in the TMB oxidation assay, but only a small excess or a sub-saturating level of substrate in the $Cl^-$ oxidation assay.

Competition between dapsone and $Cl^-$ could also account for the greater inhibition of EPO, because $Cl^-$ is a better substrate for MPO than EPO. Adding $Br^-$, which is a better substrate than $Cl^-$ for EPO, partially protected EPO against inactivation. Nevertheless, dapsone was also more effective against EPO when TMB was the substrate, although the enzymes oxidized TMB at the same rate. This observation suggests that EPO has a greater affinity for dapsone than does MPO. Structural differences between EPO and MPO might also make EPO more susceptible to chemical modification and inactivation by an oxidized form of dapsone. Deprotonation of residues at the active sites might make both enzymes more susceptible to chemical modification at neutral pH.

*Dapsone inhibition* in vitro *and* in vivo. The characteristics of dapsone inhibition of MPO suggest that dapsone may have as yet unexploited potential as an anti-inflammatory agent. Inhibition was favored by low enzyme concentration and neutral rather than acid pH. These conditions may be found at sites of inflammation where MPO is secreted and diluted into the tissue interstitial fluid. In contrast, the amount of MPO inside monocytes and neutrophils is very high [35], and killing of microorganisms takes place in phagolysosomes, which undergo progressive acidification [54].

Dapsone blocks the oxidative toxicity of PMA-stimulated neutrophils to isolated pulmonary artery endothelial cells [55] and cultured tumor cells [22] in $Cl^-$ containing media, and protects cultured tumor cells in an $I^-$-supplemented medium [56]. These results suggest that dapsone could block toxicity to normal host tissues *in vivo* by inhibiting that portion of the MPO which is secreted and exposed to the extracellular environment.

Several studies attempted to determine whether dapsone interferes with antimicrobial activity in neutrophil phagolysosomes, although possible effects of pH and enzyme concentration were not considered. Dapsone at 0.1 mM does not interfere with phagocytosis or killing of the gram-negative bacterium *Escherichia coli*, but inhibits killing of yeast-phase *Candida albicans* by up to 50% [15]. Because some of the yeast may have been killed in the extracellular medium rather than in phagolysosomes, the results do not necessarily indicate inhibition of MPO in phagolysosomes. Similarly, dapsone causes up to a 70% inhibition of MPO-catalyzed oxidation of $I^-$ by stimulated neutrophils, as measured by incorporation of radioactive $I^-$ into proteins [15]. In this and another study [56], iodination was assumed to be intracellular, and the results were taken to indicate inhibition of MPO in phagolysosomes. However, other studies have indicated that iodination occurs primarily in the extracellular medium [57] and that the amount of iodination is poorly correlated with intracellular antimicrobial activity [58]. Therefore, further studies are required to determine whether antimicrobial activity in phagolysosomes is inhibited.

Although high-dose dapsone has toxic side-effects [4, 59, 60], increased susceptibility to infection has apparently not been noted. Results reported here suggest that dapsone would not inhibit MPO in phagolysosomes. In addition, the inhibition of microbial folic acid synthesis by dapsone might counterbalance any defect in host-defense.

In both the TMB and $Cl^-$ oxidation assays, dapsone was a more effective inhibitor of EPO than of MPO. Therefore, dapsone might be especially effective in treating inflammatory disease processes in which EPO mediates tissue injury by stimulated eosinophils. The extraordinary efficacy of dapsone in treating certain conditions such as dermatitis herpetiformis [4] might indicate that eosinophils

have a role in pathogenesis. Nevertheless, the relative susceptibility of MPO and EPO to inhibition *in vivo* would depend on a number of factors including enzyme concentrations, pH, $H_2O_2$ levels, the mix of substrates including $Cl^-$ and $Br^-$, and local dapsone concentrations.

*Acknowledgements*—We thank William Weatherred and M. Margaret Jefferson for technical assistance. This work was supported by an American Lung Association Training Fellowship, NIH Grants HL 02373, AI 16795, DE 04235, and CA 21765, and by the American Lebanese Syrian Associated Charities (ALSAC).

## REFERENCES

1. Chang YT, Wolcott RR and Doull JA, Sulfone therapy of leprosy. *Med Clin North Am* **38**: 599–610, 1954.
2. Medina I, Mills J, Leoung G, Hopewell PC, Lee B, Modin G, Benowitz N and Wofsy CB, Oral therapy for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. A controlled trial of trimethoprim-sulfamethoxazole versus trimethoprim-dapsone. *N Engl J Med* **323**: 776–782, 1990.
3. Mandell GL and Sande MA, Antimicrobial agents —drugs used in the chemotherapy of tuberculosis and leprosy. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Eds. Gilman AG, Goodman LS, Rall TW and Murad F), pp. 1199–1218. Macmillan, New York, 1985.
4. Lang PG, Sulfones and sulfonamides in dermatology today. *J Am Acad Dermatol* **1**: 479–492, 1979.
5. Ruzicka T, Bauer A, Glück S and Born M, Effects of dapsone on passive arthus reaction and chemotaxis and phagocytosis of polymorphonuclear leukocytes. *Arch Dermatol Res* **270**: 347–351, 1981.
6. Stendahl O, Molin L and Lindroth M, Granulocyte-mediated release of histamine from mast cells. Effect of myeloperoxidase and its inhibition by antiinflammatory sulfone compounds. *Int Arch Allerg Appl Immunol* **70**: 277–284, 1983.
7. Grindulis KA and McConkey B, Outcome of attempts to treat rheumatoid arthritis with gold, penicillamine, sulphasalazine, or dapsone. *Ann Rheum Dis* **43**: 398–401, 1984.
8. von Ritter C, Grisham MB and Granger DN, Sulfasalazine metabolites and dapsone attenuate formyl-methionyl-leucyl-phenylalanine-induced mucosal injury in rat ileum. *Gastroenterology* **96**: 811–816, 1989.
9. Roger H, Thevenet JP, Souteyrand P and Sauvezie B, Subcorneal pustular dermatosis associated with rheumatoid arthritis and raised IgA: simultaneous remission of skin and joint involvements with dapsone treatment. *Ann Rheum Dis* **49**: 190–191, 1990.
10. Berlow BA, Liebhaber MI, Dyer Z and Spiegel TM, The effect of dapsone in steroid-dependent asthma. *J Allergy Clin Immunol* **87**: 710–715, 1991.
11. Hall RP, Lawley TJ, Smith HR and Katz SI, Bullous eruption of systemic lupus erythematosus. Dramatic response to dapsone therapy. *Ann Intern Med* **97**: 165–170, 1982.
12. Convit J, Goihman-Yahr M and Rondon-Lugo AJ, Effectiveness of dapsone in Behcet's disease. *Br J Dermatol* **111**: 629–630, 1984.
13. Falk DK, Latour DL and King LE Jr, Dapsone in the treatment of erosive lichen planus. *J Am Acad Dermatol* **12**: 567–570, 1985.
14. Steffen C, Eosinophilic pustular folliculitis (Ofuji's disease) with response to dapsone therapy. *Arch Dermatol* **121**: 921–923, 1985.
15. Stendahl O, Molin L and Dahlgren C, The inhibition of polymorphonuclear leukocyte cytotoxicity by dapsone. *J Clin Invest* **62**: 214–220, 1978.
16. Thomas EL, Lehrer RI and Rest RF, Human neutrophil antimicrobial activity. *Rev Infect Dis* **10**: S450–S456, 1988.
17. Clark RA, Extracellular effects of the myeloperoxidase–hydrogen peroxide–halide system. *Adv Inflamm Res* **5**: 107–146, 1983.
18. Klebanoff S, Phagocytic cells: products of oxygen metabolism. In: *Inflammation: Basic Principles and Clinical Correlates* (Eds. Gallin JI, Goldstein IM and Snyderman R), pp. 391–444. Raven Press, New York, 1988.
19. Weiss SJ, Tissue destruction by neutrophils. *N Engl J Med* **320**: 365–376, 1989.
20. Thomas EL and Leam DB, Myeloperoxidase-catalyzed oxidation of chloride and other halides: The role of chloramines. In: *Peroxidases: Chemistry and Biology* (Eds. Grisham MB and Everse J), Vol. 1, pp. 83–104. CRC Press, Boca Raton, FL, 1990.
21. Thomas EL, Grisham MB and Jefferson MM, Myeloperoxidase-dependent effect of amines on functions of isolated neutrophils. *J Clin Invest* **72**: 441–454, 1983.
22. Learn DB and Thomas EL, Inhibition of tumor cell glutamine uptake by isolated neutrophils. *J Clin Invest* **82**: 789–796, 1988.
23. Thomas EL, Bozeman PM and Learn DB, Lactoperoxidase: structure and catalytic properties. In: *Peroxidases: Chemistry and Biology* (Eds. Grisham MB and Everse J), Vol. 1, pp. 123–142. CRC Press, Boca Raton, FL, 1990.
24. Weiss SJ, Klein R, Slivka A and Wei M, Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. *J Clin Invest* **70**: 598–607, 1982.
25. Bozeman PM, Learn DB and Thomas EL, Assay of the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. *J Immunol Methods* **126**: 125–133, 1990.
26. Jong EC, Henderson WR and Klebanoff SJ, Bactericidal activity of eosinophil peroxidase. *J Immunol* **124**: 1378–1382, 1980.
27. Thomas EL and Fishman M, Oxidation of chloride and thiocyanate by isolated leukocytes. *J Biol Chem* **261**: 9694–9702, 1986.
28. Weiss SJ, Test ST, Eckmann CM, Roos D and Regiani S, Brominating oxidants generated by human eosinophils. *Science* **234**: 200–203, 1986.
29. Spry CJF, *Eosinophils. A Comprehensive Review and Guide to the Scientific and Medical Literature.* Oxford University Press, New York, 1988.
30. Agner K, Studies on myeloperoxidase activity. I. Spectrophotometry of the MPO-$H_2O_2$ compound. *Acta Chem Scand* **17**: S332–S338, 1963.
31. Bolscher BGJM, Plat H and Wever R, Some properties of human eosinophil peroxidase, a comparison with other peroxidases. *Biochim Biophys Acta* **784**: 177–186, 1984.
32. Thomas EL, Grisham MB and Jefferson MM, Preparation and characterization of chloramines. *Methods Enzymol* **132**: 569–585, 1986.
33. Thomas EL, Learn DB, Jefferson MM and Weatherred W, Superoxide-dependent oxidation of extracellular reducing agents by isolated neutrophils. *J Biol Chem* **263**: 2178–2186, 1988.
34. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
35. Bos A, Wever R and Roos D, Characterization and quantification of the peroxidase in human monocytes. *Biochim Biophys Acta* **525**: 37–44, 1978.
36. Stelmaszynska T and Zgliczynski JM, Myeloperoxidase

of human neutrophilic granulocytes as chlorinating enzyme. *Eur J Biochem* **45**: 305–312, 1974.
37. Thomas EL, Myeloperoxidase, hydrogen peroxide, chloride antimicrobial system: Nitrogen-chlorine derivatives of bacterial components in bactericidal action against *Escherichia coli*. *Infect Immun* **23**: 522–531, 1979.
38. Uetrecht J, Zahid N, Shear NH and Biggar WD, Metabolism of dapsone to a hydroxylamine by human neutrophils and mononuclear cells. *J Pharmacol Exp Ther* **245**: 274–279, 1988.
39. Harrison JE, The functional mechanism of myeloperoxidase. In: *Cancer Enzymology*, (Eds. Schultz J and Ahmad F), Vol. 2, pp. 305–316. Academic Press, New York, 1976.
40. Schultz J, Myeloperoxidase. In: *The Reticuloendothelial System* (Eds. Sbarra AJ and Strauss RR), Vol. 2, pp. 231–254. Plenum Press, New York, 1980.
41. Zgliczynski JM, Characteristics of myeloperoxidase from neutrophils and other peroxidases from different cell types. In: *The Reticuloendothelial System* (Eds. Sbarra AJ and Strauss RR), Vol. 2, pp. 255–277. Plenum Press, New York, 1980.
42. Chance B, The iron-containing enzymes. C. The enzyme–substrate compounds and mechanism of action of the hydroperoxidases. In: *The Proteins* (Eds. Summer JB and Myrback K), Vol. 2, Part 1, pp. 428–453. Academic Press, New York, 1951.
43. Morrison M and Schonbaum GR, Peroxidase-catalyzed halogenation. *Annu Rev Biochem* **45**: 861–888, 1976.
44. Theorell H, Ehrenberg A and Chance B, Electronic structure of the peroxidase–peroxide complexes. *Arch Biochem Biophys* **37**: 237–239, 1952.
45. Odajima T and Yamazaki I, Myeloperoxidase of the leukocyte of normal blood. I. Reaction of myeloperoxidase with hydrogen peroxide. *Biochim Biophys Acta* **206**: 71–77, 1970.
46. Winterbourn CC, Garcia RC and Segal AW, Production of the superoxide adduct of myeloperoxidase (compound III) by stimulated human neutrophils and its reactivity with hydrogen peroxide and chloride. *Biochem J* **228**: 583–592, 1985.
47. Cuperus RA, Muijsers AO and Wever R, The superoxide dismutase activity of myeloperoxidase; Formation of Compound III. *Biochim Biophys Acta* **871**: 78–84, 1986.
48. Nakamura S, Nakamura M, Yamazaki I and Morrison M, Reactions of ferryl lactoperoxidase (compound II) with sulfide and sulfhydryl compounds. *J Biol Chem* **259**: 7080–7085, 1984.
49. Doerge DR, Mechanism-based inhibition of lactoperoxidase by thiocarbamide goitrogens. *Biochemistry* **25**: 4724–4728, 1986.
50. Kettle AJ and Winterbourn CC, Mechanism of inhibition of myeloperoxidase by anti-inflammatory drugs. *Biochem Pharmacol* **41**: 1485–1492, 1991.
51. van Zyl JM, Basson K, Kriegler A and van der Walt BJ, Mechanisms by which clofazimine and dapsone inhibit the myeloperoxidase system. A possible correlation with their anti-inflammatory properties. *Biochem Pharmacol* **42**: 599–608, 1991.
52. Riley RJ, Roberts P, Coleman MD, Kitteringham NR and Park BK, Bioactivation of dapsone to a cytotoxic metabolite: *In vitro* use of a novel two compartment system which contains human tissues. *Br J Clin Pharmacol* **30**: 417–426, 1990.
53. Thomas EL, Jefferson MM and Grisham MB, Myeloperoxidase-catalyzed incorporation of amines into proteins: Role of hypochlorous acid and dichloramines. *Biochemistry* **21**: 6299–6308, 1982.
54. Mandel GL, Intraphagosomal pH of human polymorphonuclear neutrophils. *Proc Soc Exp Biol Med* **134**: 447–449, 1970.
55. Martin WJ and Kachel DL, Reduction of neutrophil-mediated injury to pulmonary endothelial cells by dapsone. *Am Rev Respir Dis* **131**: 544–547, 1985.
56. Kazmierowski JA, Ross JE, Peizner DS and Wuepper KD, Dermatitis herpetiformis: Effects of sulfones and sulfonamides on neutrophil myeloperoxidase-mediated iodination and cytotoxicity. *J Clin Immunol* **4**: 55–64, 1984.
57. Odeberg H, Olofsson T and Olsson I, Myeloperoxidase-mediated extracellular iodination during phagocytosis in granulocytes. *Scand J Haematol* **12**: 155–160, 1974.
58. Klebanoff SJ and Clark RA, Iodination by human polymorphonuclear leukocytes: A re-evaluation. *J Lab Clin Med* **89**: 675–686, 1977.
59. Snavely SR and Hodges GR, The neurotoxicity of antibacterial agents. *Ann Intern Med* **101**: 92–104, 1984.
60. Kelly JW, Scott J, Sandlund M, Van der Weyden MB and Marks R, Vitamin E and dapsone-induced hemolysis. *Arch Dermatol* **120**: 1582–1584, 1984.