

# The Inhibition of Polymorphonuclear Leukocyte Cytotoxicity by Dapsone

## A POSSIBLE MECHANISM IN THE TREATMENT OF DERMATITIS HERPETIFORMIS

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**ABSTRACT** The effect of the sulfone compound 4,4'-diaminodiphenyl sulfone (dapsone) on normal human polymorphonuclear leukocytes (PMNL) has been investigated in vitro. The drug has a dramatically beneficial effect in dermatitis herpetiformis in which the PMNL and immune complexes has been stressed to be of importance for the development of the skin lesions. Pruritus disappears and the inflammatory eruptions clear within a few days of starting therapy. The effect of dapsone has been evaluated on the different stages of phagocytosis. Using dapsone concentrations (1–30  $\mu\text{g/ml}$ ) comparable with those found after therapeutic doses, we have found that the drug interferes primarily with the myeloperoxidase (MPO)- $\text{H}_2\text{O}_2$ -halide-mediated cytotoxic system in the PMNL. No effect was observed on random locomotion, chemotaxis, phagocytic ingestion, oxidative metabolism, or the release of lysosomal enzymes. Kinetic studies in a cell-free system with purified MPO revealed a competitive type of inhibition using varying concentrations of NaI. Furthermore, the inhibition resulted in reduced candidicidal activity during phagocytosis of *Candida albicans*, and reduced cytotoxicity to adjacent mammalian cells measured as the  $^{51}\text{Cr}$  release from virus-induced lymphoma cells. Because the MPO- $\text{H}_2\text{O}_2$ -halide system not only fulfills the antimicrobial activity but is suggested to be a modulator of the inflammatory reaction as well, the action of dapsone in dermatitis herpetiformis may in part be explained by its effect on this system.

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## INTRODUCTION

Dermatitis herpetiformis (DH)<sup>1</sup> is a chronic skin disease with pleomorphic and symmetrical eruptions consisting of erythematous, papular, vesicular, and bullous lesions (1). Severe pruritus is often an early and predominant symptom. Histopathologically, neutrophil and eosinophil leukocytes accumulate within the dermal papillae forming microabscesses at the tip of the papillae. The collagen undergoes necrosis and subepidermal vesicles are formed. The cause of the disease is unknown but immunoglobulins, in particular IgA, have been demonstrated as fixed beneath unaffected epidermis and, as in other bullous eruptions, a possible immune reaction has been suggested (1). Changes of the intestinal villi like those found in coeliac disease are found in most of patients suffering from DH. Recently, a gluten-free diet has been shown to improve the enteropathy of DH and a diminishing, sometimes also the disappearance, of skin symptoms is seen after 4-mo periods or more of a gluten-free diet (2).

It is well known that dapsone (4,4'-diaminodiphenyl sulfone) has a dramatically beneficial effect on DH (3). Pruritus disappears and the eruptions usually clear within a few days of starting therapy, and recurs after the cessation of treatment within a

<sup>1</sup>Abbreviations used in this paper: Cyt c, horse heart ferricytochrome c; DH, dermatitis herpetiformis; KRG, Krebs-Ringer phosphate buffer with 5 mM glucose, pH 7.2; MPO, myeloperoxidase; PBS, phosphate-buffered saline; PMNL, polymorphonuclear leukocytes; STZ, serum-treated zymosan; YCAB, ascitic Moloney virus-induced lymphoma.

day or two. Good therapeutic results have also been reported in bullous diseases of mixed nature (4, 5), subcorneal pustular dermatosis (6), generalized pustular psoriasis (7), leukocytoclastic vasculitis (8), and recently, in some cases of rheumatoid arthritis (9) and Crohn's disease (10). Immune complexes have been implicated in the pathogenesis of most of the disorders mentioned.

The mechanisms involved in the effect of dapsone are still being disputed. In high doses over a prolonged period of time, dapsone appears to suppress the Arthus reaction in guinea pigs (11). Dapsone also shows anti-inflammatory activity comparable with established nonsteroidal anti-inflammatory drugs such as indomethacin (9). Furthermore, an inhibition of lysosomal enzyme release comparable with that of corticosteroids has been shown (12). The concentrations of dapsone required for the inhibition were, however, much higher than the blood levels after a normal therapeutic dose of dapsone (13). Alternative mechanisms must thus be sought for explaining the therapeutical effect of dapsone (14).

In the present investigation we have analyzed in vitro the influence of dapsone on various aspects of phagocytosis with polymorphonuclear leukocytes (PMNL) from normal healthy individuals. Using dapsone concentrations comparable with those concentrations found after therapeutic doses in DH and leprosy, we have found that the drug interferes primarily with the myeloperoxidase-mediated cytotoxic reaction in the PMNL. Because this cytotoxic system not only fulfills the antimicrobial activity (15) but is suggested to be a modulator of the inflammatory reaction as well (16, 17), the action of dapsone in DH may in part be explained by this mechanism.

## METHODS

**Leukocyte preparation.** Blood was obtained from apparently healthy adult volunteers (age 18–35 yr), and the leukocytes were isolated according to Bøyum (18) using Isopaque (Nyegaard Ltd., Oslo, Norway)-dextran sedimentation for the separation of cells from EDTA blood. After separation, washing, and hypotonic lysis of the contaminating erythrocytes, the leukocytes were suspended to  $1 \times 10^7$  cells per milliliter in Krebs-Ringer phosphate buffer containing 5 mM glucose, pH 7.2 (KRG). A differential count was performed to determine the number of PMNL. Trypan blue exclusion was used to assay viability.

**Motility measurement system.** Leukocyte motility was studied by a modification of the method described by Nelson et al. (19). Briefly, 1.5% agarose was dissolved in sterile water by heating. After cooling to 50°C, the agarose was mixed with an equal volume of prewarmed Gey's solution (20) in double its usual concentration. 8 ml of the agarose medium was poured into 60 × 15 mm tissue culture dishes (60 × 15; Flow Laboratories, Scotland). Six sets of three wells were cut in the agarose. The wells had a diameter of 2.4 mm and in each set they were placed 2.4 mm apart.

In each set of wells, 10  $\mu$ l of the cell suspension ( $2 \times 10^7$

PMNL/ml) was placed in the middle well, 10  $\mu$ l of Gey's solution was placed in the inner well, and as an attractant, 10  $\mu$ l of human normal serum was placed in the outer well. To determine the random locomotion of the PMNL population, the inner and outer wells were filled with Gey's solution.

The dishes were incubated for 2 h at 37°C. After fixation in methanol for 30 min, the agarose was removed, and the cells stained with Giemsa for 15 min. The distances of migration were measured with an ocular micrometer (21).

**Phagocytic uptake.** The ability of isolated leukocytes to ingest heat-killed yeast cells (*Saccharomyces cerevisiae*) was assayed as described previously (22). 200 phagocytic cells were counted and the phagocytic uptake was calculated as the mean number of yeast particles per phagocytic cell.

**Measurement of leukocyte iodination** was carried out essentially as described by Olsson et al. (23). The reaction mixture contained  $1 \times 10^6$  leukocytes, 10% pooled human serum, 30 nmol of sodium iodide (0.5  $\mu$ Ci of  $^{125}$ I),  $5 \times 10^7$  yeast particles, and KRG to a final volume of 0.5 ml. The tubes were incubated at 37°C and the reaction was terminated after 30 min with 0.1 ml of a 0.1-M sodium thiosulphate solution. 5 ml of cold 10% trichloroacetic acid (TCA) was then added. After centrifugation, the precipitates were washed three times with 5 ml of TCA. The iodination was expressed as nanomoles of  $I^-$  precipitated per  $1 \times 10^6$  PMNL per 30 min.

**Microbicidal activity** of leukocytes against *Escherichia coli* O(somatic)55:K(capsular)59 and *Candida albicans* was measured as described before (24). The microbicidal activity was calculated as the percentage of surviving bacteria or fungi in the presence of leukocytes compared to controls, where the leukocytes had been omitted.

**Superoxide generation** was assayed essentially as described by Curnutte and Babior (25). The reaction mixtures contained  $5 \times 10^6$  PMNL, 75  $\mu$ M horse heart ferricytochrome c (Cyt c, type III, Sigma Chemical Co., St. Louis, Mo.), 5 mg serum-treated zymosan (STZ) and KRG to a final volume of 3 ml. The reaction was initiated by incubating 1.5 ml of the reaction mixture at 37°C in a water bath whereas the other 1.5 ml was kept at 0°C in melting ice and used as a blank. After 30 min, the test tubes were placed in melting ice and then centrifuged at 4°C (750 g, 10 min). The supernate was collected and assayed for the amount of reduced Cyt c with a Beckman DU-2 spectrophotometer (Beckman Instruments, Inc., Electronic Instruments Div., Schiller Park, Ill.) at 550 nm. The amount of reduced Cyt c was calculated using an absorbance coefficient of 15.5/mM at 550 nm (26). The superoxide-dependent Cyt c reduction was expressed as the difference in Cyt c reduction between reaction mixtures containing no superoxide dismutase (Sigma Chemical Co.) and those containing 200 U/ml of superoxide dismutase. These controls were run with each set of experiments.

**Lysosomal enzyme release.** The extracellular release of the PMNL granule-associated enzyme  $\beta$ -N-acetylglucosaminidase (EC 3.21.30) was assayed from reaction mixtures identical to those employed for the determination of superoxide generation, but in the absence of Cyt c, using the fluorochrome substrate 4-methylumbelliferyl 2-deoxy-2-acetamido- $\beta$ -D-glucopyranoside (27). Maximum enzyme release was determined as the amount released by 0.2% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.).

**Cell-free myeloperoxidase-mediated iodination.** Myeloperoxidase (MPO) isolated from human PMNL (23) was kindly supplied by Dr. Inge Olsson, Lund, Sweden. The reaction mixtures contained varying concentrations (0.2–10  $\mu$ g/ml) of MPO,  $1 \times 10^7$  yeast cells, 120 nmol of NaI (1.0  $\mu$ Ci of  $^{125}$ I), and 0.05 M Tris-HCl buffer, pH 7.0, to a final volume of 2.0 ml. The reaction was initiated by the addition

of 0.5 ml of glucose (20 mg/ml in 20 mM  $\text{NH}_4\text{Cl}$  and  $\text{CaCl}_2$ ) and glucose oxidase (100  $\mu\text{g/ml}$ ; EC 1.1.3.4, Sigma Chemical Co.) (23). The ammonium and  $\text{Ca}^{++}$  ions are reported to enhance the peroxidase reaction (28). However, similar results were obtained using the Tris-HCl buffer. After 30 min, the reaction was terminated by the addition of 0.1 ml of a 0.1-M sodium thiosulphate solution, and 5 ml of 10% TCA was added. The precipitate was washed three times with 5 ml of TCA and assayed for radioactivity.

To determine the type of inhibition of dapsone on MPO-mediated iodination, the kinetics of the reaction was studied using varying concentration of iodide (0.05–1.0 mM). The incubation mixture contained 2.5  $\mu\text{g}$  MPO, iodide (0.5  $\mu\text{Ci}$   $^{125}\text{I}$ ),  $1 \times 10^7$  yeast cells, and Tris-HCl to a final volume of 2.0 ml. The reaction was initiated by the addition of 0.5 ml glucose and 0.2 ml glucose oxidase, and incubated for 15 min. The interaction between dapsone and MPO was further analyzed by evaluating the absorption spectrum using a Beckman DU-2 spectrophotometer.

**MPO- $\text{H}_2\text{O}_2$ -halide-mediated cytotoxicity.** The cytotoxic effect of the MPO- $\text{H}_2\text{O}_2$ -halide system was performed essentially as described by Clark et al. (16) assayed the  $^{51}\text{Cr}$  release from prelabeled mammalian target cells. An ascitic Moloney virus-induced lymphoma (an in vitro subline, YCAB) (29) was used as the target source. The tumor cells were grown in vitro in RPMI 1640 medium, supplemented with 10% fetal calf serum and antibiotics, harvested by centrifugation, washed twice in phosphate-buffered saline (PBS) and suspended in PBS to  $2 \times 10^7$  cells/ml (29). The cells were labeled by incubating  $2 \times 10^7$  cells with 200  $\mu\text{Ci}$  of  $\text{Na}_2^{51}\text{CrO}_4$  at  $37^\circ\text{C}$  for 1 h. After washing the cells four times in PBS, the cells were suspended to  $1 \times 10^7$  cells in 0.03 M sodium phosphate buffer, pH 7.0, supplemented with 1.5 mM  $\text{KH}_2\text{PO}_4$ , 1.5 mM  $\text{MgSO}_4$ , and 0.1 M NaCl.

The cytotoxicity was assayed from reaction mixtures containing  $10^6$  YCAB cells, 2.5  $\mu\text{g}$  MPO, 0.1  $\mu\text{mol}$   $\text{H}_2\text{O}_2$ , and the sodium phosphate buffer described above to a final volume of 1.0 ml. After a 60-min incubation at  $37^\circ\text{C}$ , the test tubes were transferred to melting ice and centrifuged at  $4^\circ\text{C}$  (250 g, 5 min). The supernates were collected and assayed for radioactivity. Maximum  $^{51}\text{Cr}$  release was determined by counting the supernates from tubes where 0.2% Triton X-100 had been added. The cytotoxicity was expressed as percent of maximum releasable activity in duplicate samples.

**Dapsone.** The drug (4,4'-diaminodiphenyl sulfone), a generous gift from ICI (Pharmaceutical Division, Macclesfield, England) was solubilized in physiological saline to 300  $\mu\text{g/ml}$  and then diluted in the appropriate medium. The same preparation of the drug was used throughout the investigation.

## RESULTS

**Effect of dapsone on random locomotion and chemotaxis of PMNL.** Table I shows that 3, 10, or 30  $\mu\text{g/ml}$  of dapsone had no effect on random mobility or chemotaxis. There was an 80–90% increase in locomotion when the cells were chemotactically stimulated with normal human serum, which is activated in the agar (21).

**Effect on the phagocytic uptake and metabolic activity.** When PMNL were incubated with concentrations of dapsone (3 and 30  $\mu\text{g/ml}$ , respectively) the phagocytic uptake of yeast particles was not changed (Table II). A marked dose-dependent inhibi-

TABLE I  
Effect of Dapsone on Random Locomotion and Chemotaxis of PMNL

Supplements	Random locomotion*	Chemotaxis†
PMNL alone	68±9.5 (3)	63±7.0/114±11 (3)
+ 10 $\mu\text{g/ml}$ dapsone	76±8.8 (3)	72±7.7/117±11 (3)
+ 30 $\mu\text{g/ml}$ dapsone	66±7.5 (3)	61±6.3/111±8 (3)

\* The numbers express in arbitrary units the distance of migration towards the wells containing buffer solution (Gey's) alone.

† The numbers express in arbitrary units the distance of migration of PMNL towards the wells containing only buffer solution and serum, respectively. The increase in migration expresses the chemotactic response. Mean±SEM. The number of experiments within parentheses.

tion of the MPO-mediated iodination of the ingested yeast particles was, however, observed (Table II). In the presence of 3  $\mu\text{g/ml}$  of the drug, the iodination was reduced by 28%, whereas 30  $\mu\text{g/ml}$  caused a more pronounced inhibition of  $\approx 70\%$ . Higher concentrations of the drug inhibited the reaction even further, but because they were too high to be comparable to pharmacological levels in blood, they were not used further in the study. Preincubating the PMNL with the drug for 30 min before adding the yeast cells did not enhance the inhibition. Furthermore, the inhibition was reversed by washing the dapsone-treated cells twice in KRG (Table III).

During phagocytosis, the oxidative metabolism of the PMNL is greatly stimulated as indicated by increased  $\text{O}_2$  consumption (30), superoxide (25) and peroxide production (31). These metabolic changes linked to the MPO-mediated reactions are essential for the intracellular microbicidal activity of the cell (15). Table IV shows that dapsone has no effect on superoxide pro-

TABLE II  
Effect of Dapsone on the Phagocytic Uptake and MPO-Mediated Iodination of Yeast Cells

Supplements	Phagocytic uptake*	Iodination†
		%
PMNL + yeast cells	2.55±0.12 (5)	100
+ yeast cells + 3 $\mu\text{g/ml}$ dapsone	2.73±0.16 (3)	72
+ yeast cells + 30 $\mu\text{g/ml}$ dapsone	2.77±0.18 (5)	32

\* Mean number of yeast cells ingested per PMNL±SEM. The number of experiments within parentheses.

† Percent iodination of the control. 100% =  $0.95 \pm 0.15$  nmol  $\text{I}^-/10^6$  PMNL, 30 min.

**TABLE III**  
*The Reversible Inhibitory Effect of Dapsone on the MPO-H<sub>2</sub>O<sub>2</sub>-Halide Dependent Iodination of Yeast Cells*

Supplements*	Iodination†
PMNL + yeast cells	0.54±0.07 (4)
+ 10 µg/ml dapsone + yeast cells	0.27±0.05 (4)
+ 10 µg/ml washed dapsone + yeast cells	0.55±0.06 (4)

\* PMNL were incubated in KRG with or without 10 µg/ml dapsone for 30 min at 37°C. The cells were then centrifuged, washed twice in KRG, resuspended in KRG, and incubated with yeast cells.

† Expressed as nanomoles of I<sup>-</sup> precipitated per 1 × 10<sup>6</sup> PMNL per 30 min. Mean±SEM. The number of experiments within parentheses.

duction during phagocytosis of STZ. Because superoxide is an intermediate in the reduction of oxygen to hydrogen peroxide (32), the deficient iodination is not due to lack of hydrogen peroxide.

*Effect on lysosomal enzyme release.* Table V shows that 30 and 100 µ/ml of dapsone has no effect on the release of the lysosomal enzyme β-N-acetylglucosaminidase during phagocytosis of STZ.

*Effect on the microbicidal activity.* PMNL treated with dapsone showed normal bactericidal activity against *E. coli* 055:K59, whereas the candidacidal activity was impaired ≈50% (Fig. 1). This is consistent with the requirement for the MPO system in granulocytes, for obtaining maximum microbicidal activity against certain fungi (33). The residual MPO activity (32%) and other systems may, however, fulfill adequate bactericidal activity even during the early postphagocytic period, in contrast to MPO-deficient PMNL, which are showing a reduced bactericidal activity in the early phase of phagocytosis (24).

*Effect on the cell-free MPO-H<sub>2</sub>O<sub>2</sub>-halide system.* To further elaborate on the mechanism of inhibition,

**TABLE IV**  
*Effect of Dapsone on the O<sub>2</sub><sup>-</sup>-Dependent Cytochrome c Reduction in PMNL Exposed to STZ\**

Supplements	Superoxide generation†
PMNL STZ	49±5.6 (3)
PMNL STZ plus 30 µg/ml dapsone	55±2.8 (3)

\* Zymosan was boiled for 30 min, washed twice, and suspended to 20 mg/ml in PBS. Equal volumes of the zymosan suspension and normal human serum were mixed, incubated for 30 min at 37°C, then washed twice in PBS, and suspended to 20 mg/ml.

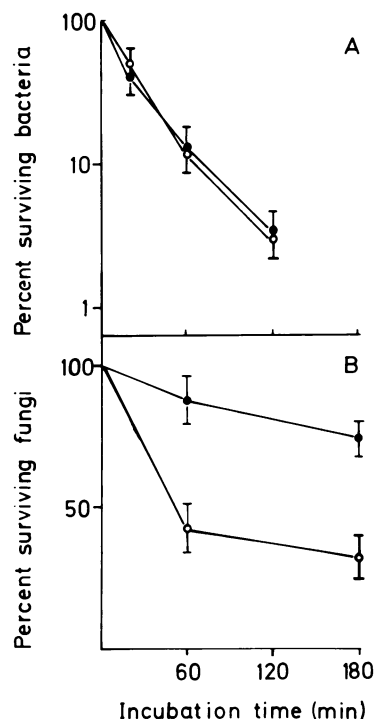
† Expressed as superoxide dismutase-inhibitable Cyt c reduction (nanomoles of reduced Cyt c/5 × 10<sup>6</sup> PMNL, 30 min). Mean±SEM; the number of experiments within parentheses.

**TABLE V**  
*Effect of Dapsone on the Lysosomal Enzyme Release from PMNL during Phagocytosis of STZ*

Supplements	Enzyme release*
PMNL alone	8.9±1.5 (4)
+ STZ	28±5.5 (4)
+ STZ + 30 µg/ml dapsone	29±6.0 (4)
+ STZ + 100 µg/ml dapsone	28±6.2 (4)

\* Expressed as percent of total enzyme (β-N-acetylglucosaminidase) released from 10<sup>7</sup> PMNL by 0.2% Triton X-100 (2.52±0.63 µmol substrate/min). Mean±SEM. The number of experiments are given in parentheses.

we have studied the effect of the drug in a cell-free system using isolated, partly purified human MPO. Dapsone gave a dose-dependent inhibition (Fig. 2), similar to that found during PMNL iodination. Increasing the concentration of MPO did not reverse the inhibition. To determine the type of inhibition caused by the drug, the kinetics of the reaction was studied using varying concentrations of iodide (0.050–1.0 mM) and a constant MPO concentration. A Lineweaver-Burke plot of the reactions in the presence of 10 µg/ml of dapsone indicated that the drug caused a



**FIGURE 1** The microbicidal activity of PMNL against (A) *E. coli* 055:K59 and (B) *C. albicans*. PMNL, (○), PMNL plus 30 µg/ml dapsone, (●). The vertical bars represent SEM for three experiments.

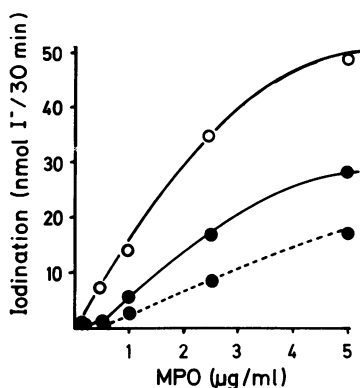


FIGURE 2 The MPO-H<sub>2</sub>O<sub>2</sub>-<sup>125</sup>I-dependent iodination of yeast cells in a cell-free system containing varying concentrations of MPO. MPO-system without dapsone, (○); MPO-system plus 10 µg/ml dapsone, (●); MPO-system plus 30 µg/ml dapsone, (●---●).

competitive type of inhibition (Fig. 3), which ceased with increasing concentrations of iodide.

Studying the absorption spectrum of purified MPO in the presence of dapsone reveals no alteration in spectrum, which would indicate no complex formation between drug and enzyme. However, the drug inhibits the formation of free I<sub>2</sub> from NaI in the MPO-H<sub>2</sub>O<sub>2</sub>-NaI reaction, visualized by observing the lack of blue color formed in the presence of starch and I<sub>2</sub>.

**Effect on the MPO-mediated cytotoxicity.** The results obtained so far indicate that dapsone inhibits the MPO-mediated iodination reaction. The prompt anti-inflammatory effect of the drug in DH can not be fully understood from the inhibition of the intracellular MPO-dependent activity. However, because both

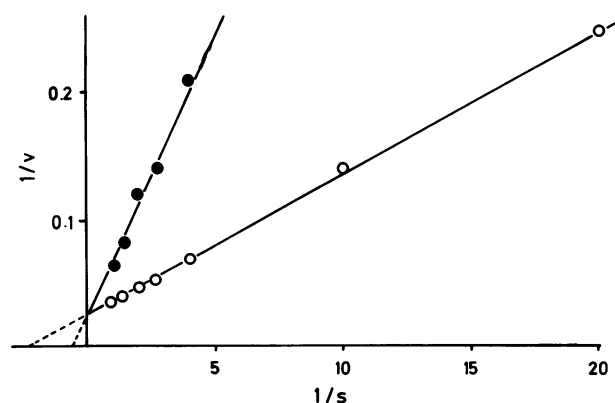


FIGURE 3 Effect of dapsone on iodination in the MPO-H<sub>2</sub>O<sub>2</sub> cell-free system at different iodide concentrations given in a double reciprocal plot. V, reaction velocity (nanomoles iodide oxidized/30 min; S, iodide concentration millimolar); (○), incubated without dapsone; (●), incubated with 10 µg/ml dapsone.

MPO and H<sub>2</sub>O<sub>2</sub> are released into the extracellular fluid during phagocytosis, and the MPO-H<sub>2</sub>O<sub>2</sub>-halide system has been reported to be toxic to certain mammalian cells (16), the effect of dapsone was tested in such an MPO-dependent cytotoxic system. Table VI shows that the MPO-mediated cytotoxicity on YCAB cells was completely inhibited in the presence of 30 µg/ml of dapsone. In the absence of the drug, the complete system caused a 58% release of the releasable radioactivity, whereas control samples, where MPO, H<sub>2</sub>O<sub>2</sub>, or Cl<sup>-</sup> halide were deleted, exhibited a 38% release. In the presence of dapsone, no significant difference was observed between the release from control samples and those with the complete MPO system (34.6 and 33.8%, respectively).

## DISCUSSION

The evidence reported here indicates that dapsone interferes with the MPO-mediated cytotoxic system in normal human PMNL. There was no effect of dapsone on random motility, chemotaxis, phagocytic uptake of yeast cells, or the oxidative metabolism linked to these processes. In fact, the drug appears to interfere relatively specifically with the MPO-H<sub>2</sub>O<sub>2</sub>-halide system, whether measured as the iodination of yeast cells during phagocytosis or as the iodination in a cell-free system using isolated human MPO. Similar results were observed by Lehrer (34) using other sulfonamide compounds. The mechanism whereby the drug interferes with the MPO system is, however, not elucidated. As Lehrer (34) discussed, sulfonamides may act as competitive substrates, thus interfering with the peroxidase reaction. Our kinetic studies, using different concentrations of I<sup>-</sup>, would support this hypothesis. In the intact phagocytic cell, the oxidant properties of the drug may raise the level of H<sub>2</sub>O<sub>2</sub> to toxic levels by reducing the amount of reduced glutathione (35) as in patients lacking glutathione reductase activity (36). However, because the inhibition is reversible and observed also in a cell-free system, a direct effect on the MPO-catalyzed system is more

TABLE VI  
Effect of Dapsone on the MPO-Hydrogen Peroxide-Halide Cytotoxicity System for YCAB Cells

Supplements	Cytotoxicity*
MPO + H <sub>2</sub> O <sub>2</sub> + Cl <sup>-</sup> halide	58.0±1.3 (3)
— H <sub>2</sub> O <sub>2</sub> + halide	38.0±0.6 (3)
MPO + H <sub>2</sub> O <sub>2</sub> + halide + 30 µg/ml dapsone	34.6±2.3 (3)
— H <sub>2</sub> O <sub>2</sub> + halide + 30 µg/ml dapsone	33.8±2.3 (3)

\* Expressed as percent <sup>51</sup>Cr radioactivity released of maximum release from 0.2% Triton X-100-treated YCAB cells. Mean±SEM. The number of experiments within parentheses.

likely. The drug appears to interfere with the formation of  $I_2$  from NaI catalyzed by MPO- $H_2O_2$ , without any direct effect on the enzyme activity or its release.

The clinical course of dapsone treatment of DH correlates with the reversible and dose-dependent inhibition of the drug. The competitive inhibition of formation of free  $I_2$  by the MPO- $H_2O_2$ -halide system is logically in agreement with the increase of itching and skin lesions of DH after the patients' intake of iodine, often used as a diagnostic sign of the disease (1). The present findings might thus give us some ideas concerning the mechanism involved in the therapeutic effect of dapsone in DH. Although the inhibition of the MPO-dependent microbicidal activity results in the decreased fungicidal activity of the PMNL, no clinical signs support the relevance of the enhanced susceptibility to infection in DH. It has, however, recently been demonstrated that phagocytizing PMNL also can act as cytotoxic effector cells against other mammalian cells (16). Since this cytotoxic effect appears to be directed towards any adjacent cell, a general potentiation of the inflammation reaction could occur when these damaged cells released biologically active substances. This cytotoxic effect, mediated by the MPO- $H_2O_2$ -halide system (16) functions outside the phagocytic cell, and is accomplished during phagocytosis of immune complexes (present in DH) (1) during which leakage of lysosomal enzymes is enhanced (37). Because the release of lysosomal enzymes per se is not inhibited by dapsone, but the cytotoxic effect of MPO- $H_2O_2$ -halide is inhibited, this might be a possible mechanism whereby the drug exhibits its rapid anti-inflammatory activity.

It has been shown that the cutaneous and intestinal lesions in DH can be improved by a gluten-free diet (2). Dapsone as well as sulfapyridine (1) improves the skin lesions but not the intestinal lesions. In clinically normal skin in DH, membrane-bound vacuoles were found in the dermis below the epidermal basal lamina in untreated patients as well as in those taking dapsone. In patients on gluten-free diet, however, these vacuoles could not be seen (38). It was suggested that the vacuoles may be involved in an immunological reaction induced by the presence of gluten. It was also suggested that dapsone merely suppresses the pathological process so that it does not become clinically manifest. After dapsone withdrawal there was an apparent increase of vacuoles (39). Possibly, the leukocyte-mediated MPO-dependent cytotoxicity is involved in the inflammatory reaction leading to tissue injury and blister formation in DH. IgA immunoglobulins have been demonstrated in the unaffected skin (40), and would be capable of inducing and establishing target specificity of the MPO-mediated cytotoxicity against the tissue in dermal papillae and epidermal-dermal junction, the area

in which the initial pathological changes can be demonstrated.

Dapsone does not influence the presence of immunoglobulins in the skin, but inhibits the MPO-hydrogen peroxide-halide system and thus, the cytotoxicity. The competitive and reciprocal nature of the inhibition shown in the present investigation may explain the time relations mentioned earlier between clinical symptoms and administration or withdrawal of dapsone and the provocative effect of iodide. The influence by dapsone on the MPO-dependent cytotoxicity might also to some extent explain the effect of dapsone on other inflammatory diseases.

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