of cysteine oxidation. Thus, Beatty and Reed reported a rate of 30 nmoles of cysteine oxidized per minute. However we show here that in water with physiological amounts of Cu(II) added or in blood plasma, cysteine is oxidized at a rate that is, at least, an order of magnitude higher than that reported by Beatty and Reed [10]. This difference in oxidation rate is important because the very toxic thiyl and hydroxyl radicals are generated only when cysteine is oxidized [13].

The question of finding an optimal treatment against paracetamol intoxication remains open. An interesting new approach is the use of liposomally entrapped glutathione [21, 22]. We found [7] that methionine protects against paracetamol induced GSH depletion. N-acetyl cysteine, given orally may also protect against GSH depletion induced by paracetamol. The fact that cysteine induces a depletion of GSH may be of both theoretical and practical importance.

The relevant facts reported in this paper are. (1) Cysteine administered to rats, i.p. or orally, results in a depletion of hepatic GSH. (2) N-acetyl cysteine, given i.p., also caused a depletion of hepatic GSH. However, when given orally, NAC did not affect liver GSH. (3) Neither cysteine, administered orally or i.p. nor NAC given i.p. prevented the paracetamol induced depletion. However, NAC administered orally prevented paracetamol-induced GSH depletion. (4) The rate of oxidation of cysteine in water or in blood plasma was always higher than that of NAC. Since the rapid thiol oxidation, which generates free radicals, is essential to explain cysteine cytotoxicity, the lower rate of autooxidation of NAC when compared with cysteine explains the different effects of both amino acids on liver GSH and other cytotoxic effects of cysteine.

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Inhibition of oxygen radical metabolism in phorbol ester-activated polymorphonuclear leukocytes by an antitumor promoting copper complex with superoxide dismutase-mimetic activity

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A relationship between chronic inflammation and the induction of malignancy has been proffered following observations that metabolically activated polymorphonuclear leukocytes (PMNs) produce genetic lesions in bacterial and mammalian cells [1–3], presumably as a consequence of their generation of reactive oxygen species. Although oxygen radical production by leukocytes is readily elicited by tumor promoting phorbol esters [4, 5], the role of inflammation in the promotion stage of carcinogenesis is unclear. Most all epidermal promoters are irritants; however, not all inflammatory agents are promoters [6]. That oxygen radicals may mediate a component of the promotion process appears likely though. Free radical generating compounds, such as benzoyl peroxide, are tumor promoters [7]. A previous study has shown that there appears to be a correlation among phorbol esters as to their relative activities as tumor promoters in mouse epidermis and their abilities to stimulate oxygen radical metabolism in PMNs [5]. Moreover, phorbol esters provoke a rapid and sustained decrease in epidermal superoxide dismutase (SOD) and

catalase activities [8], the foremost detoxifying enzymes for oxygen radicals. Finally, antioxidants [9] and a novel copper complex with SOD-like chemical reactivity, Cu(II)(3,5-diisopropylsalicylic acid)$_2$ (CuDIPS) [10], are potent inhibitors of tumor promotion. We report in the present communication that this later inhibitor, CuDIPS, is an effective quencher of oxygen radical generation by phorbol ester-activated PMNs.

CuDIPS was synthesized from cupric chloride and 3,5-diisopropylsalicyclic acid (DIPS) [11]: elemental analysis (C, H) and decomposition range of the copper complex were in accord with literature values. Zn (II)(3,5-diisopropylsalicylic acid)$_2$ (ZnDIPS) was the gift of Prof. John R. J. Sorenson, University of Arkansas for Medical Sciences, Little Rock, AR. All other reagents were of the highest quality obtainable commercially. PMNs were isolated from the blood of healthy normal volunteers [5] and suspended in Dulbecco's phosphate buffered saline plus 0.1% glucose. Chemiluminescence responses of 7×10^6 cells in 3 ml buffer were monitored using a model 3003 liquid scintillation spectrometer (Packard Instruments) operated at ambient temperature and in the out-of-coincidence mode [5]. Results are expressed as counts/unit time minus background (~3800 counts/0.2 min). Data are presented as peak (maximum) responses and temporal curves. Stimulation of superoxide anion ($O_2^{\cdot-}$) production by PMNs was determined by the SOD-inhibitable reduction of cytochrome *c* as previously described [5]. Measurements of the SOD-mimetic activities of CuDIPS in enzymic (xanthine-xanthine oxidase) and chemical (KO_2) systems were performed as described in Younes and Weser [12].

A natural resultant of the increased oxygen radical metabolism of PMNs that accompanies phagocytosis or chemical stimulation is chemiluminescence (CL) and, as such, CL can be used as an index of the generation of and reactions mediated by oxygen radicals [13]. Interaction of the potent tumor promotor 12-*O*-tetradecanoylphorbol-13-acetate (TPA) with PMNs produced a rapid burst of CL. A distinct CL response occurred within 0.2 min following TPA addition and rose rapidly to reach a peak at 5 min that was 25-fold greater than the CL of the resting PMN. As shown in Fig. 1A, addition of either 150 μg bovine erythrocyte SOD (a maximally inhibitory dose) or 10 μM CuDIPS just prior to TPA inhibited the peak CL response by 80 and 95% respectively. The dose-response curve for the inhibition of TPA-stimulated CL by CuDIPS is shown in Fig. 1B. Inhibition was seen over a broad concentration range between 0.01 and 10 μM CuDIPS. Cell viability, as monitored by trypan blue exclusion, was unaffected by the highest dose of CuDIPS, suggesting that inhibition of CL is not simply a manifestation of cytotoxicity.

The oxygen radical scavenging activities of CuDIPS and two analogs are presented in Table 1, utilizing cellular, enzymic and chemical sources of $O_2^{\cdot-}$. Values are expressed as the concentrations producing a 50% inhibition (IC_{50}). The SOD-like chemical reactivity of CuDIPS has been described previously [14], and our observations with the xanthine–xanthine oxidase and KO_2 systems are in agreement with reported values. Inhibitory activity of CuDIPS towards the reduction of cytochrome *c* by ectopically generated $O_2^{\cdot-}$ in TPA-stimulated PMNs was consonant with the non-cellular assay systems. However, inhibition of TPA-stimulated CL in these cells was accomplished at substantially lower concentrations. The chromaphore reduction assays for $O_2^{\cdot-}$ are primarily aqueous in their constituency whereas CuDIPS is extremely lipophilic (soluble in diethyl ether) [15]. It might be anticipated that CuDIPS will partition to and accumulate in cell membranes which also serve as sites of $O_2^{\cdot-}$ generation, accumulation and reactivity, thus rendering the copper chelate a more effective inhibitor of $O_2^{\cdot-}$-dependent CL reactions. Such a view might also explain the lack of total abrogation of the CL response by SOD, which due to its size and lack of lipophilic character does not penetrate well into cells [16]. Analogs of CuDIPS, namely the ligand DIPS alone and the corresponding zinc chelate, ZnDIPS, were essentially without SOD-mimetic activity in any of the assay systems, underscoring the specificity of the action of CuDIPS. Concordantly, ZnDIPS and DIPS, unlike CuDIPS, do not antagonize phorbol ester action in mouse epidermis [10, 17].

Among the different antagonists of tumor promotion that we have examined, CuDIPS was the most potent inhibitor of TPA-stimulated CL (e.g. CuDIPS > phenolic antioxidants > protease inhibitors > retinoids > non-steroidal antiinflammatory drugs [5], (unpublished observations). It should be noted that such a ranking is not observed for these agents as antagonists of TPA-mediated tumor promotion in mouse epidermis. Most notably, the retinoids and protease inhibitors are substantially more potent than the other compounds [9, 18, 19]. The disparity between the CL and *in vivo* observations undoubtedly reflects differ-

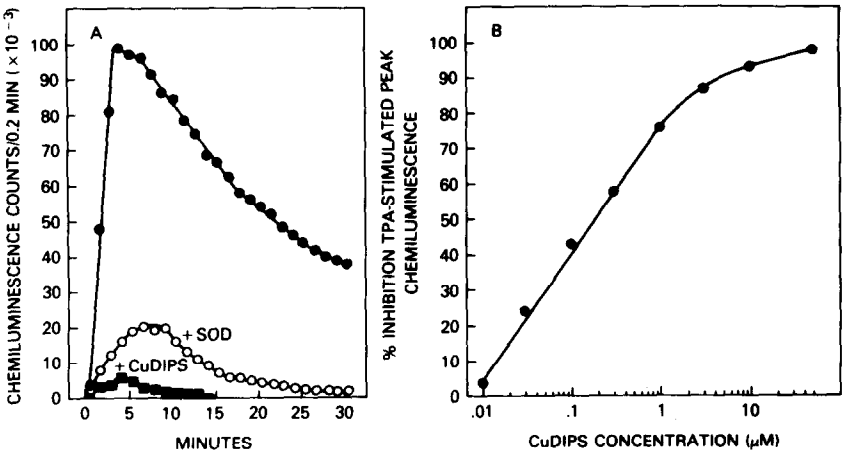

Fig. 1. (A) Effects of CuDIPS and SOD on TPA-stimulated CL. CuDIPS (10 μM) (■), SOD (150 μg) (○), or DMSO (●) was added to vials 1 min prior to TPA (100 ng/ml), and CL was monitored for 30 min. Final DMSO concentration in all vials was 0.6%. (B) Dose–response curve for the inhibition of TPA-stimulated CL by CuDIPS. The percentage of inhibition of CL was calculated by measuring peak CL responses in the presence and absence of the indicated concentrations of CuDIPS.

Table 1. Oxygen radical scavenging activities of CuDIPS and analogs

Compound	IC_{50} (μM)			
	TPA-stimulated CL in PMNs	TPA-stimulated reduction of cytochrome *c* by PMNs	Xanthine-xanthine oxidase reduction of nitroblue tetrazolium	KO_2 reduction of nitroblue tetrazolium
CuDIPS	0.2	1.2	1.8	3.2
ZnDIPS	>10*	Inactive†	Inactive	Inactive
DIPS	Inactive	Inactive	Inactive	Inactive

* Twenty-eight percent inhibition at 10 μM.
† No activity at 10 μM.

ences in mechanism and sites of action as well as pharmacokinetic properties of these agents.

The finding that a biomimetic SOD with antipromoting activity is a potent inhibitor of TPA-activated PMN oxygen radical metabolism amplifies the possible role of phagocytes in the promotion process. However, the possibility remains that CuDIPS may act independently of an inflammatory response by quenching radicals generated in other cell populations.

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