Proceedings of the Nutrition Society (1986), **45**, 333–344

Regulation of phagocyte function by α-tocopherol

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Before phagocytic stimulation, the human polymorphonuclear leucocyte (PMN) consumes only small amounts of oxygen and relies primarily on an aerobic glycolysis for energy, a finding consistent with the paucity of mitochondria in the cells (Cheson *et al.* 1979). Within seconds after stimulation, however, the PMN activates a specialized pathway that results in a 100-fold increase in O_2 consumption (Root & Metcalf, 1977; Badwey *et al.* 1980). This pathway culminates in a reaction in which molecular O_2 is reduced to superoxide ($O_2^{\cdot -}$). The superoxide, in turn, undergoes further reactions to form toxic derivatives such as hydrogen peroxide, hydroxyl radical ($OH^{\cdot -}$), organic O_2 radicals and hypochlorite ion, which are then used by the cell to destroy ingested micro-organisms (Babior & Crowley, 1973; Badwey & Karnovsky, 1980; Tauber *et al.* 1983). The massive increases in O_2 consumption and O_2 radical production are referred to as the respiratory burst.

At the heart of the respiratory burst is a flavoprotein enzyme, NADPH oxidase, which catalyses the one electron reduction of O_2 to superoxide according to the following reaction:

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2^{\cdot -}.$$

Because of the high rates of superoxide production by the stimulated PMN, it is necessary for the cell to regenerate NADPH. This is accomplished by the first two reactions of the hexose monophosphate shunt, glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PD) and 6-phospholgluconate dehydrogenase (*EC* 1.1.1.43) which reduce $NADP^+$ back to NADPH. Measurements of hexose monophosphate shunt activity in PMN have revealed twenty-fold increases in metabolic activity following phagocytic stimulation (Curnutte & Boxer, 1985). As previously indicated, the immediate product of the NADPH oxidase reaction is superoxide ion. Although superoxide itself is only weakly bactericidal, this reactive O_2 radical is capable of undergoing further reactions to generate other toxic O_2 derivatives such as H_2O_2. Superoxide generated either on the surface of the cell, or within the confines of the phagolysome, can leak into the cytoplasm and cause cellular damage as on the surface of the plasma membrane. The cytoplasm of the leucocyte contains an enzyme, superoxide dismutase (*EC* 1.15.1.1; SOD) that rapidly destroys superoxide according to the following reaction:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2^{\cdot -}.$$

The H_2O_2 generated by this reaction is detoxified in the leucocyte cytoplasm by the glutathione cycle. The NADPH consumed by the glutathione cycle is also regenerated by the reactions of the hexose monophosphate shunt.

The central role of NADPH oxidase in the respiratory burst is highlighted by studies with PMN from patients with chronic granulomatous disease (Gallin *et al.* 1983; Tauber *et al.* 1983). PMN from patients with both X-linked and autosomal recessive chronic granulomatous disease show no measurable respiratory burst. PMN from patients with chronic granulomatous disease undergo normal to rapid rates of chemotaxis, ingestion and degranulation. These normal functions coupled with the defective respiratory burst explain why chronic granulomatous disease patients are plagued with organisms which are catalase (*EC* 1.11.1.6)-positive but not those which are catalase-negative. Catalase-negative microbes are unable to destroy their endogenously produced H_2O_2 so that when they are ingested by chronic granulomatous disease cells they release small but significant quantities of H_2O_2 within the confines of the phagolysome. This microbially derived H_2O_2 is then used by the myeloperoxidase system to generate toxic O_2 derivatives which are then capable of killing the micro-organism. Since catalase-positive organisms destroy all their H_2O_2, there is no supplemental source available to the chronic granulomatous disease leucocyte.

The importance of oxidizing-radicals to the microbial system of the PMN is shown by experiments in which they are intercepted before they can interact with their targets. Microbicidal activity is inhibited by catalase, by SOD, or by O_2-radical scavengers such as mannitol. Since the O_2 radicals produced by the PMN are capable of damaging not only bacteria but also normal tissues in sites of inflammation, these radical scavengers along with α-tocopherol are being studied as anti-inflammatory agents.

There is growing evidence that PMN are activated in vivo by complement or immune complexes in disorders such as adult respiratory distress syndrome (ARDS) and thermal injury (Rinaldo & Rogers, 1982; Wolach *et al.* 1984). In animal models of ARDS, lung damage can be minimized when O_2-radical scavengers are administered during the course of the disease (Till *et al.* 1985; Tvedten *et al.* 1985). It would appear then that anti-oxidants can be employed to manipulate chemotaxis and phagocytosis of PMN through their ability to scavenge released oxidant products as well as attenuate tissue injury affected by their products.

Consequence of α-tocopherol deficiency on PMN function

In neonates, both random motility of PMN and chemotaxis are diminished (Miller, 1971). Other abnormalities of PMN phagocytes have been noted in human newborn infants. These include the decreased intracellular killing in the presence of a stress or a secondary illness. The aetiology of these abnormalities of phagocyte function remain unknown but possibly could relate to differences in membrane

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properties between newborn and adult PMN (Wright *et al.* 1975). Others have found by treating PMN with 2,3-dihydroxybenzoic acid (2,3-DHB) that chemotactic activity could be improved (Shigeoka *et al.* 1981). Potentially, the active metabolic state of newborn PMN in concert with the relative α-tocopherol deficiency at birth could contribute to alterations in cellular function through membrane damage by predisposing the PMN to auto-oxidant-induced injury (Baehner *et al.* 1977; Oski, 1977; Park *et al.* 1970). We previously assessed whether α-tocopherol deficiency could affect PMN functions (Harris *et al.* 1980). Thus, we rendered weanling rats α-tocopherol deficient and evaluated PMN behaviour. Following 8 weeks of an α-tocopherol-deficient diet, serum α-tocopherol levels were 0·6 (SD 0·1) mg/l compared with levels in control rats of 11·7 (SD 0·6) mg/l. PMN obtained from the peritoneal cavity of rats revealed that directed cell movement but not random motility was depressed in the α-tocopherol-deficient animals. Similarly, the ingestion of both C3b opsonized or IgG opsonized paraffin-oil droplets was significantly compromised in PMN obtained from the α-tocopherol-depleted animals (Table 1). We further found an increase of 1·5-fold in the amount of peroxidized lipid membrane as assayed by the formation of malonyl dialdehyde in the α-tocopherol-depleted compared with control PMN. Further studies revealed that α-tocopherol-deficient PMN were metabolically more active than controls as evidenced by an increase in O_2 consumption and release of H_2O_2 into the extracellular medium. We developed the hypothesis that the membrane-associated NADPH oxidase responsible for the consumption of O_2 and generation of O_2 by-products in the α-tocopherol-deficient PMN was situated in an abnormal lipid milieu which in turn facilitated greater activation of the enzyme and generation of auto-toxic concentrations of H_2O_2.

To appreciate the consequence of α-tocopherol depletion, it is necessary to outline the role of the vitamin in inhibiting the oxidation of unsaturated fatty acids. The oxidation of fatty acids is dependent on the presence of double bonds which are present in the unsaturated fatty acids but not in the saturated fats

Table 1. *C3b and Fc receptor-mediated ingestion of opsonized paraffin-oil droplets by α-tocopherol-deficient rat polymorphonuclear leucocytes (PMN) (kg paraffin oil/10^7 PMN per min)*

(Values are means and standard deviations of at least eight samples)

	Control		α-Tocopherol-deficient	
	Mean	SD	Mean	SD
C3b receptor-mediated ingestion	0·093	0·091	0·051**	0·071
Fc receptor-mediated ingestion	0·0976	0·081	0·052**	0·029

**$P<0·005$.

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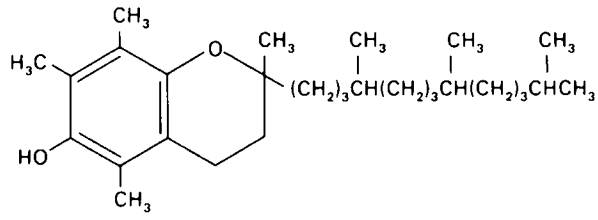

Fig. 1. Structure of α-tocopherol. The phenolic group on the chroman ring (left) permits reactivity with free radicals, whereas the fatty acid chain allows for lipid solubility.

(Mead, 1976). Such auto-oxidation of an unsaturated fatty acid, like linoleic acid, has been postulated to occur as follows: (1) loss of a hydrogen ion atom from the carbon in the α position of the double bond; (2) non-enzymic oxidation of the C atom to a free peroxidic radical; (3) reaction of the unsaturable lipid-free radicals with other unsaturated fatty acid molecules which generate lipid hydroperoxides, and more lipid-free radicals.

Because of its capacity to scavenge lipid-free radicals through the phenolic group found on the chroman ring of the molecule (Fig. 1), α-tocopherol prevents the oxidation reactions described previously. In turn the fatty acid and side chain of the α-tocopherol molecule account for its solubility in lipids thereby rendering the vitamin useful in preventing oxidation within the plasma membrane.

Effects of antioxidants including α-tocopherol on normal PMN function

Clearly, α-tocopherol deficiency leads to sufficient damage which compromises PMN function. On the other hand, what are the consequences of supplementing the diet with α-tocopherol on PMN function? To answer this question, we administered 1·07 (1600 units) α-tocopherol/d to adult volunteers for 2 weeks (Baehner *et al.* 1977). Serum α-tocopherol levels rose from a range of 11·2 (SD 1·6) to 18·2 (SD 0·4) mg/l. As noted in Table 2, rate of uptake of C3-opsonized lipopolysaccharide-coated paraffin-oil droplets increased from 0·060 (SD 0·015) mg/10^7 PMN per min before α-tocopherol supplementation to values of 0·76 (SD 0·009) mg/10^7 PMN per min during α-tocopherol ingestion. On the other hand, despite the greater ability to phagocytose, α-tocopherol-replete PMN showed a mildly reduced ability to kill bacteria compared with control at 100 min. The extent of this acquired abnormality was far less than that observed for PMN in patients with chronic granulomatous disease. These results suggest that the decreased release of H_2O_2 by α-tocopherol-replete PMN may result in less oxidative damage to their membranes so that they are more efficient in ingestion of C3-opsonized lipopolysaccharide-coated paraffin-oil droplets but that the failure to produce this reactive species also serves to protect ingested micro-organisms from the peroxide-mediated attack which results in their demise.

We found that α-tocopherol-replete PMN released superoxide normally to the extracellular medium but released only 45% of the amount of H_2O_2 as control

Table 2. *Rate of uptake of C3-coated lipopolysaccharide paraffin-oil droplets in peripheral blood polymorphonuclear leucocytes (PMN) from volunteers studied before (control) and during ingestion of α-tocopherol (1·07 g (1600 units)/d) for 14 d*

(Results are expressed as means and standard deviations. Statistical analysis was based on Student's *t* test with paired values, with means compared with control values. Benzoic acid and 2,3-dihydroxybenzoic acid (2,3-DHB) were added to normal PMN in vitro)

Treatment	Ingestion rate (mg paraffin oil/10^7 PMN per min) Mean	SD	Statistical significance of difference: $P<$
Control	0·060	0·015	0·025
α-Tocopherol replete	0·076	0·009	NS
Benzoic acid (10^{-3} M)	0·066	0·018	0·002
2,3-DHB: 10^{-3} M	0·092	0·014	0·002
10^{-4} M	0·087	0·050	0·025
10^{-5} M	0·070	0·011	NS

NS, not significant.

PMN. α-Tocopherol also serves to scavenge endogenously generated H_2O_2 within the cell. To assess the availability of H_2O_2 within the cell, [^{14}1-C]glucose oxidation through the hexose monophosphate shunt, which depends on generation of intracellular H_2O_2, was determined. In the control, PMN H_2O_2-dependent [1-^{14}C]glucose oxidation increased by 4·4-fold during phagocytosis of latex particles over 15 min but only increased by 2·9-fold in the α-tocopherol-replete PMN. Although H_2O_2 released both extracellularly and intracellularly were reduced in the α-tocopherol-replete PMN, the cells were able to utilize O_2 normally.

The site of H_2O_2 produced by NADPH oxidase was determined in PMN obtained from the blood of volunteers receiving 1·07 g (1600 units) α-tocopherol/d (Table 3). For these studies, PMN were incubated in the presence of NADPH and cerium chloride and allowed to generate H_2O_2 by phagocytosing opsonized zymosan particles (Butterick *et al.* 1983). The H_2O_2 formed from NADPH reacts with the cerium to form an electron-dense reaction product, cerium perhydroxide, detected either visually or by X-ray microanalysis.

$$\text{NADPH} + O_2 + H^+ \xrightarrow{\text{NADPH oxidase}} \text{NADP}^+ + H_2O_2.$$

$$2H_2O_2 + CeCl_3 \rightarrow Ce(OH)_2OOH + H^+ + 3Cl^-.$$

The reaction product was identified on the plasma membrane as well as within phagocytic vesicle membranes surrounding ingested zymosan when NADPH was employed as the substrate with normal PMN. When PMN obtained from the

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Table 3. *X-ray microanalysis for the presence of cerium precipitates in phagocytic vesicles of intact human polymorphonuclear leucocytes (PMN) after zymosan ingestion*

(Twenty vesicles were analysed per variable. Statistical significance was based on comparisons between values observed in the untreated control and α-tocopherol-replete subjects and the untreated with and without catalase)

	No. of positive vesicles	
	NADPH (0·71 mM)	No additive
Control	18	9
α-Tocopherol-replete	0••	5•
Catalase (*EC* 1.11.1.6; 1500 units)	2••	2•

•$P<0.05$, ••$P<0.005$.

volunteers receiving α-tocopherol were incubated with NADPH, no reaction product developed either within the phagocytic vesicles or on the plasma membrane, suggesting that α-tocopherol is capable of scavenging H_2O_2 needed for the formation of this reaction product.

Because we observed that α-tocopherol-replete cells destroy bacteria less efficiently than control cells, we turned our attention to another scavenger of H_2O_2, 2,3-DHB, in an effort to manipulate better the auto-oxidative reactions engendered by activated PMN (Graziano *et al.* 1976). This drug has been identified as an orally-effective iron chelator (Graziano *et al.* 1974). It is an aromatic compound having *para*-situated hydroxy groups which have the capability of reacting with free radicals to form longer-lived semiquinones. The semiquinones subsequently react with another free radical to form quinones. A scheme whereby 2,3-DHB might react is shown in Fig. 2.

We found that PMN phagocytosed at a faster rate and augmented their directed cell movement in the presence of 2,3-DHB in vitro (Table 2). The concentrations

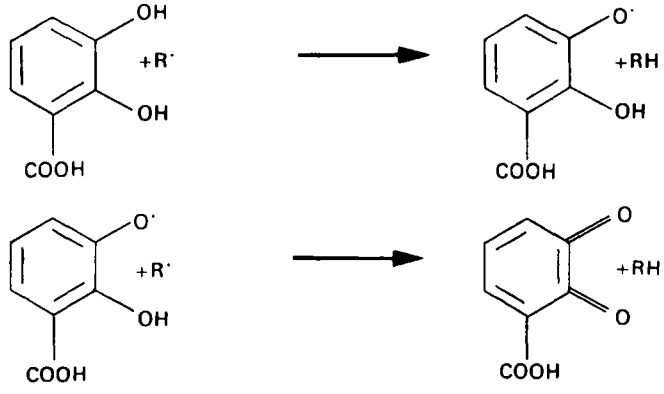

Fig. 2. Scheme whereby 2,3-dihydroxybenzoic acid might react.

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of 2,3-DHB, needed to alter PMN functions in vitro were readily attainable in vivo. The administration of 25 mg 2,3-DHB/kg for use in Fe-chelation chemotherapy has resulted in a plasma concentration of 0·5–0·6 mM. In PMN 2,3-DHB failed to inhibit the four-fold enhancement of cytosol enzymes responsible for hexose monophosphate shunt activity during phagocytosis (Boxer *et al.* 1978). Analogues of 2,3-DHB, which in some instances scavenge hydroxyl radicals, were ineffective in enhancing PMN motile functions. Unlike α-tocopherol, the effect of 2,3-DHB, once removed from the extracellular media, was abrogated. α-Tocopherol is incorporated into the membrane lipid bilayer and can reduce intracellular levels of H_2O_2 sufficiently to impair bacterial killing, whereas 2,3-DHB failed to alter the bactericidal capacity of the PMN. Hence, 2,3-DHB may be a more useful drug in some situations in enhancing PMN motile responses without compromising bactericidal potency.

Effect of α-tocopherol on PMN function in a patient with glutathione synthase (EC 6.3.2.3) *deficiency*

A number of patients with glutathione synthase deficiency have been described in which reduced glutathione in the erythrocytes was markedly depressed and associated which chronic nonsperocytic haemolytic anaemia and haemolytic crises following drug ingestion (Boivin & Galand, 1965; Meister, 1974). The inheritance follows an autosomal recessive pattern. In the oxoprolinuric variant of glutathione synthase deficiency, nucleated cells such as leucocytes and fibroblasts have been found to have depressed glutathione levels (Spielberg *et al.* 1978). PMN functions in one child suffering from neutropenia, following episodes of recurrent otitis media, revealed levels of glutathione synthase 5% of normal and levels of glutathione at 10–20% of normal (Spielberg *et al.* 1979). While the chemotaxis of phatocytosis was normal, killing of ingested staphylococci was moderately impaired. H_2O_2 production by phagocytosing PMN was increased while iodination and microtubule assembly were markedly impaired. All these defects were corrected after 3 months of oral α-tocopherol (20 mg (30 units)/kg per d) (Boxer *et al.* 1979). Cellular levels of glutathione, however, did not rise. These findings suggest that the patient's PMN could not correctly detoxify H_2O_2 which then led to microtubule dysfunction and impaired degranulation of lysosomes into phagosomes thus compromising iodination and bacterial killing. It would appear that α-tocopherol protected the glutathione synthase-deficient cells against oxidative damage.

Attenuation of lung damage in animals by antioxidants

The pathophysiological mechanisms underlying ARDS are an area of intense research interest. Indirect evidence exists regarding the ability of the PMN to produce acute lung injury by elaboration of toxic O_2 metabolites (Till *et al.* 1982; Schraufstaller *et al.* 1984). However, little has changed regarding pharmacological management of the disease. While compounds such as corticosteroids and inhibitors of arachidonic acid metabolism have shown promise in animal studies,

confirmation of these findings in human diseases proves difficult and controversial (Brigham *et al.* 1981; Perkowski *et al.* 1983). In an effort to discover alternative methods, we evaluated the ability of 2,3-DHB to prevent acute lung injury (Baldwin *et al.* 1986).

Various models have been utilized to study acute lung injury. Many have implicated PMN-derived O_2 metabolites as a cause of the damage (Till *et al.* 1982, 1985). One such model involves infusing cobra (*Naja naja*) venom factor (CVF) into the rat (Till *et al.* 1982). The lung injury in this manner appears to be mediated by PMN-generated O_2 metabolites since both PMN depletion or previous administration of O_2-metabolite scavengers have a protective effect. Changes observed after treatment of the rat with CVF resemble those seen in adult ARDS; they include: (1) complement activation with formation of chemotactic fragments; (2) histological evidence of PMN accumulation in lung capillaries and alveolar spaces; (3) high permeability pulmonary oedema assessed by the leakage of radio-labelled albumin from the vascular space to the alveolar interstitium; (4) histological evidence of pulmonary oedema, alveolar haemorrhage and fibrin deposition. Using the CVF model, we tested the hypothesis that 2,3-DHB could protect the lung from such injury.

Using a permeability index that measures the amount of intravenously administered ^{125}I-labelled albumin that accumulates in lung tissue, we found that pretreatment with 2,3-DHB reduced ($P<0{\cdot}05$) lung injury in CVF-treated rats in a dose-dependent manner (Table 4). Morphometric analysis of lung tissue indicated that the protection by 2,3-DHB was not caused by inhibition of CVF-induced PMN sequestration within lung vasculature. Because Fe-saturated 2,3-DHB did not attenuate lung injury, and because in vitro experiments demonstrated that 2,3-DHB inhibited Fe–H_2O_2-induced peroxidation of phospholipid lyposomes, we suggested that 2,3-DHB may be protecting the lung via chelation of Fe as well as serving to protect the lung from H_2O_2-mediated damage.

Experimental thermal injury of rat skin (70°; 30 s) results also in activation of complement which leads to the appearance of C_5-related chemotactic activity in the serum, transient neutropenia and accumulation of PMN in pulmonary capillaries (Till *et al.* 1985). These events ultimately lead to acute lung injury similar to that observed following the infusion of CVF into rats. Protection from acute lung injury following remote thermal injury (involving skin) can be achieved by depleting animals of complement or blood PMN or by systemic treatment of animals with combinations of catalase and SOD. The protective effects of catalase and SOD provide strong evidence that O_2-derived free radicals released from complement-activated PMN are important mediators of lung injury secondary to skin burns. The nature of the O_2 species appears to involve H_2O_2 and perhaps hydroxyl radical. In recent studies it was found that acute lung injury could be prevented by treatment of thermally-injured rats with the antioxidant α-tocopherol or with scavengers of hydroxyl radicals such as 2,3-DHB (Table 4). The role of Fe is not completely understood in the development of tissue injury, secondary to skin burns, but could be explained by its essential role in the classic Fe-dependent

Table 4. *Effect of 2,3-dihydroxybenzoic acid (2,3-DHB) and α-tocopherol on lung vascular permeability*

(Mean values with their standard errors)

		Lung vascular permeability†		
		Index		
Stimulus	Treatment	Mean	SD	Statistical significance of treatment effect: $P<$
Saline*	None	0·018	0·02	—
CVF:	None	0·59	0·06	—
	2,3-DHB (100 mg/kg)	0·20	0·05	0·001
Skin burn:	None	0·68	0·06	—
	2,3-DHB (100 mg/kg)	0·32	0·01	0·001
	α-Tocopherol	0·34	0·04	0·01

CVF, cobra venom factor.

*9 g sodium chloride/l.

†Lung vascular permeability index values were determined at 30 min following infusion of CVF or 3 h following thermal injury.

Fenton reaction in which hydroxyl radical is formed from H_2O_2.

Hydroxyl radical is highly reactive and can lead to generation of lipid peroxides. In the studies involving thermal injury, evidence was provided that the generation of lipid-peroxidation products which are detectable in burned skin, lung tissue and plasma may be dependent on hydroxyl release from complement-activated PMN. We found that both PMN depletion and pretreatment with hydroxyl-radical scavengers almost completely prevented the appearance of conjugated dienes in plasma of thermally injured animals. They also found that hydroxyl-radical scavengers and α-tocopherol decreased the plasma levels of conjugated dienes suggesting that lipid-peroxidation products are in some manner correlated with both the skin injury and lung injury. These findings provide some suggestions for treatments in clinical situations where there is reason to believe that O_2-derived free radicals may be responsible for tissue injury. One such clinical situation is ARDS where increased amounts of oxidants are found in the breath of patients with the disorder (Baldwin *et al.* 1986).

Potential toxicity of large supplements of α-tocopherol

Among the events accompanying phagocytosis by PMN are: (1) recognition of the target by cell-surface receptors; (2) triggering of the respiratory burst and granule release; (3) endocytic engulfment of the target (Curnutte & Boxer, 1985). Recently the structural entities participating in Fc and C3b receptor-mediated recognition have been identified (Fearon, 1980; Mellman & Unkeless, 1980). However, the physiological transduction mechanisms mediating the broad spectrum of functional changes are not known. Trans-membrane ion fluxes may constitute one early step in metabolic signalling during antibody phagocytosis

Table 5. *Effect of α-tocopherol treatment on Fc receptor-mediated ingestion of opsonized paraffin-oil droplets by human polymorphonuclear leucocytes (PMN) (mg paraffin oil/10^7 PMN per min)*

(Volunteers were studied before and during ingestion of α-tocopherol (1·07 g (1600 units)/d) for 28 d)

	Control	Control (1500 units catalase; *EC* 1.11.1.6)	α-Tocopherol treatment
Mean	0·101	0·012	0·028
SD	0·029	0·006	0·007

All values for controls when compared with treated cells were significantly different ($P<0·005$).

(Young *et al.* 1983). Arachidonic acid metabolism may be triggered by a phospholipase activity of the Fc receptors. Additionally, it has been suggested that a cell surface sulphydryl group participating in a sulphydryl-oxidation reaction may play a large role in IgG-dependent endocytic triggering (Petty, 1985). We have hypothesized that release of oxidative factors by phagocytes may constitute a driving force in the IgG-mediated endocytosis of targets. To test this hypothesis, we challenged PMN obtained from patients with chronic granulomatous disease (CGD) with immune complexes or C_3-coated opsonized paraffin-oil droplets. We found that the PMN obtained from the patients with CGD were unable to ingest immune complexes unless they were challenged with complexes capable of generating H_2O_2 (Petty *et al.* 1985). In contrast, normal PMN were able to ingest immune complexes without difficulty. Similarly, the PMN from CGD patients were able to ingest C_3-coated, but not antibody-coated, paraffin-oil droplets. These observations indicate that defective antibody-dependent endocytosis can be associated with PMN obtained from patients with CGD. As we previously indicated, the primary defect in CGD patients has been attributed to the absence of an NADPH oxidase. We suggest that a deficiency in antibody-dependent endocytosis is secondary to the defective respiratory burst of PMN. The PMN from the patients with CGD are then unable to form mixed disulphides between Fc receptor, a necessary prerequisite for Fc-receptor endocytosis.

Based on the observation that PMN from CGD patients fail to internalize IgG immune complexes or IgG-coated particles, we next challenged normal PMN from subjects on 1·07 g (1600 units) α-tocopherol/d for 1 month. As indicated in Table 5, their PMN showed markedly diminished ability to internalize the IgG-coated particles whereas their ability to internalize complement-coated particles was enhanced as previously noted. Additionally, normal PMN were found to have markedly impaired ability to ingest IgG-coated particles in the presence of catalase, a scavenger of H_2O_2. These observations suggest that prolonged ingestion of high dose α-tocopherol potentially can lead to selective inhibition of PMN

phagocytosis. Clinically, one study of premature infants treated prophylactically with high doses of α-tocopherol for prevention of retinopathy revealed that the infants were at risk of developing sepsis and necrotizing enterocolitis (Johnson *et al.* 1985). These particular clinical observations coupled with the in vitro observations relating potential toxicity to high-dose α-tocopherol clearly indicate that the vitamin needs to be administered in moderation.

The author gratefully acknowledges the technical assistance of Laura Mayo and Larry Devall. This work was supported with grants from the NIH A120065 and HL31963.

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