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The Role of Vitamin E in Blood and Cellular Aging

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HEMATOLOGICAL ASPECTS OF VITAMIN E

It has been recognized that vitamin E is an essential component of blood and may function as an antioxidant and a membrane stabilizer for protecting blood components, such as erythrocytes, leukocytes, and platelets, from oxidative damage. From many observations made through in vivo and in vitro studies of animal and human blood, the role of vitamin E has become clearer. Almost all these findings have been made by taking into account phenomena of vitamin E deficiency and the effects of supplementation of the vitamin.

A variety of vitamin E deficiency syndromes are readily produced in animals, but humans have not been shown to develop symptoms related to vitamin E deprivation. Because of the widespread occurrence of tocopherol in food, nutritional vitamin E deficiency in humans is extremely uncommon. Hematological manifestations of vitamin E deficiency in humans are generally limited to prematurely delivered neonates and pathological states associated with chronic fat malabsorption (1–7). Among several disorders characterized by steatorrhea, cystic fibrosis with pancreatic achylia represents one of the most common causes of fat malabsorption syndrome (1,8). All patients with pancreatogenic steatorrhea are deficient in vitamin E, and the plasma α-tocopherol levels correlate with indices of intestinal malabsorption (9). The survival of erythrocytes in vitamin E-deficient patients is moderately but significantly decreased. The life span of red blood cells have been found to be shortened in such patients to an average half-life of 19.0 $\pm$ 1.3 days; following vitamin E supplementation, the half-life increases to 27.5 $\pm$ 0.9 days. In vivo studies of peroxide-induced hemolysis revealed that normal erythrocytes resist hemolysis during incubation, but vitamin E-deficient erythrocytes show abnormal oxidant susceptibility, evidenced by a greater than 5% hemoglobin release (9).

Another manifestation of human vitamin E deficiency has been found in patients with abetalipoproteinemia caused by fat malabsorption, attributable to the blockage of chylomicron formation and resulting in the absence of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in blood (10–12). Since vitamin E is normally absorbed via the chylomicrons and transported mostly by LDL and VLDL, in the absence of these lipoproteins vitamin E is undetectable in the serum of patients (13). The most characteristic abnormal feature of erythrocytes in abetalipoproteinemia is the presence of acanthocytes (spiky red cells) with excess sphingomyelin (10,11,13,14). An increase in this phospholipid, which is located in the

outer membrane layer, causes a decrease in erythrocyte membrane fluidity, producing acanthocytes (10,15). Although malabsorption of vitamin E has been reported to make acanthocyte lipids more sensitive than normal erythrocyte lipids to oxidative damage that can be prevented by treatment with water-soluble vitamin E (α-tocopheryl polyethylene glycol succinate), neither the extent of peroxidation in serum and erythrocytes nor the improvement of the morphological abnormality of erythrocytes by vitamin E has been investigated (16). Long-term treatment of patients with vitamin E increases serum vitamin E concentrations and reduces in vitro hemolysis (11). Since a recent report suggested that even in very severe vitamin E deficiency, the requirement for vitamin E, a chain-breaking (peroxyl radical-trapping) antioxidant, cannot be met by other exogenous or endogenous antioxidants (17), it seems likely, although not proved, that the role of vitamin E in the blood of patients with abetalipoproteinemia is not only to protect against peroxidation but also to function in membrane stabilization independently of a redox system (18,19). Morphological alterations of erythrocytes also appear in patients with sickle cell anemia (20). Although sickle cells regain their normal shape upon oxygenation, a portion of the circulating red cells retain their abnormal shape even when the blood is fully oxygenated (20,21). Sickle cells are deficient in vitamin E and spontaneously generate approximately twice the normal amount of activated oxygen; consequently, the production of hydrogen peroxide (H_2O_2) is increased (22). These cells are therefore more susceptible to oxidative stress than normal cells, resulting in the accumulation of two to three times the normal amount of malondialdehyde (MDA), an end product of lipid peroxidation (23). Treatment of sickle cell anemia patients with large amounts of vitamin E causes their plasma tocopherol levels to increase from 0.7 to 2.3 mg/g lipid and the proportion of irreversibly sickled red cells to decrease from 25 to 11% (21). It is unclear, however, whether sickle cells regain their normal shape due to the antioxidant properties of vitamin E or whether the vitamin enhances erythropagocytosis directly.

VITAMIN E IN CELLULAR AGING

One of the most neglected topics in vitamin E research concerns the role of vitamin E in aging. Although vitamin E expands the life span of a number of species, including rotifer, paramecium, turbatrix, drosophila, and rat, and actual mechanism of this life span elongation effect by vitamin E is still poorly understood (24–30). In research on cellular aging, the red blood cell provides a good model for studying the aging process because it is simple to separate aged red cells from younger cells (31). It has been found that aged red cells are more dense than younger cells and are osmotically fragile (32); also, the activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase have been found to be decreased in aged red cells (33,34). In addition, membrane lipid concentrations of aged cells, especially of arachidonate in the phospholipids, decreases significantly during the aging process (35). From these observations and on the basis of the free-radical aging theory (36), it is reasonable to assume that lipid peroxidation in vivo may induce red blood cell senescence and that antioxidants, including vitamin E, may delay the aging process. Nevertheless, a recent report by Burton et al. (37) suggested that young and old human red blood cells contain about the same amount of α-tocopherol. Furthermore, young cells have a lower ratio of α-tocopherol to arachidonic acid than old cells. These results are incompatible with the preceding argument, and hence the explanation for the mechanism of red blood cell aging has become more complicated.

It has been suggested that to remove senescent red blood cells from the circulation, macrophages distinguish them from mature red cells. Kay (38) proposed that a senescent cell antigen, which develops on the surface of aged red cells, is recognized and bound by the Fab region of

an IgG autoantibody in the serum; subsequently the Fc region of the IgG is recognized and bound by macrophages, resulting in phagocytosis of the red cell. This senescent cell antigen arises as a breakdown product (M_r = 62,000) of a membrane protein designated band 3, which is actually an anion channel in erythrocyte membranes. In addition to senescent red cells, this antigen is present in lymphocytes, platelets, and neutrophils, but not in young red cells. Erythrocytes from vitamin E-deficient rats, regardless of their age, behave like old erythrocytes from normal rats: that is, they show an increase in susceptibility to phagocytosis and IgG binding, an impairment in anion transport ability, and a decline in the activity of glyceraldehyde-3-phosphate dehydrogenase, a main segment of band 3 (39). Regrettably, these abnormalities are not reversed by the addition of exogenous vitamin E to the assay medium. Kay et al. (39) proposed that the oxidative degradation of band 3 and the generation of senescent cell antigen in vivo may provide a possible mechanism for erythrocyte aging. However, neither the effect of vitamin E supplementation on the aging process in vivo nor the content of vitamin E and lipid peroxides in the membranes of old and young red cells have been investigated. Since sickle cells generate more malondialdehyde than normal cells, these cells bind considerably more IgG and are ingested by macrophages (22). Thus, simple proteolysis of a band 3 membrane component is not enough to explain red cell aging. Consequently, it seems likely that oxidation of either erythrocyte lipids or proteins may contribute to cellular aging.

Although the mean life span of normal human platelets is thought to average 6.9–9.9 days (40), in vitamin E-deficient neonates and children platelet life span is significantly decreased to a half-life of 2.0–2.4 days. Plasma vitamin E levels during the period of E deficiency were found to be markedly decreased and MDA formation to be increased, leading to hyperaggregability. Long-term vitamin E repletion to these patients caused platelet survival times to become completely normal at 4.2–4.7 days (41). The mechanism for this effect of vitamin E, however, is not known.

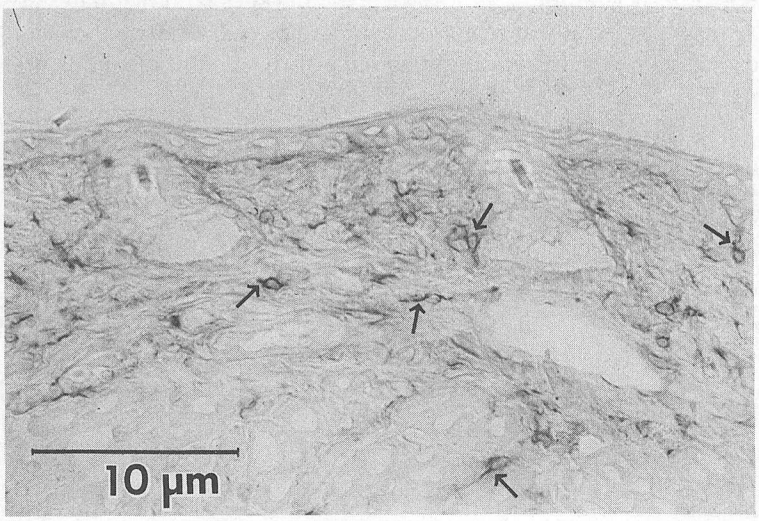

FIGURE 1 Arrows show $CD4^+$ T lymphocytes with dendrites in aged rat skin. The frozen section (8 μm thick) was stained by an avidine-biotin method with a monoclonal antibody to W3/25 (T helper cells). The antibody stained only round T lymphocytes without dendrites in young rat skin (data not shown). (Courtesy of Drs. T. Toda and J. Ohno, with permission.)

The role of vitamin E in the aging process of other cells in the blood is still unknown. Recently, however, it was shown that lymphocyte T cells are morphologically transformed to dendrite-shaped cells during aging (Fig. 1) (42). Consequently, it is interesting to consider, although firm evidence is lacking, that the mechanism for the appearance of these abnormal T cells may be related to that for the appearance of spiky red cells and sickle cells and that vitamin E may play a biologically active role in T cell aging. Since a great deal of vitamin E research is currently in progress, it can be expected that the actual link between vitamin E and cellular aging will be clarified and that the mechanism of vitamin E activity in the aging process will be made clear.

BIOCHEMICAL ASPECTS OF VITAMIN E

Vitamin E and the Susceptibility of Erythrocytes to Oxidative Stress

Erythrocyte membranes are presumed to be highly susceptible to peroxidation because of the presence of hemoglobin, which catalyzes oxidation under circumstances of high oxygen concentration, and erythrocyte membranes are rich in polyunsaturated lipids. Erythrocytes must, therefore, be protected by antioxidants and antioxidative enzymes. When these antioxidative substances are deficient in blood, the susceptibility of erythrocytes to oxidative stress is enhanced and hence the free radicals generated may attack the membranes, leading to their oxidative destruction; finally, the cells may be hemolyzed. It is well documented that even a low concentration of vitamin E can protect erythrocytes from oxidative damage and that hemolysis takes place in vitro after vitamin E depletion through oxidation (Fig. 2) (43, 44). Thus, vitamin E in erythrocyte membranes plays an important role in protection of the membrane against free radicals.

Mino et al. (44) demonstrated that when an azo compound, AAPH [2,2′-azobis-(2-amidino propane) dihydrochloride] was used as a radical initiator, the peroxidizability in vitamin E-deficient red cell membranes from neonates increased more than the peroxidizability of adult red cell membranes after depletion of vitamin E. Erythrocyte membranes from neonates are rich in polyunsaturated fatty acids, especially arachidonic acid and eicosapentatrienoic acid, compared with adult erythrocytes. To verify the peroxidizability of the membrane lipids, the

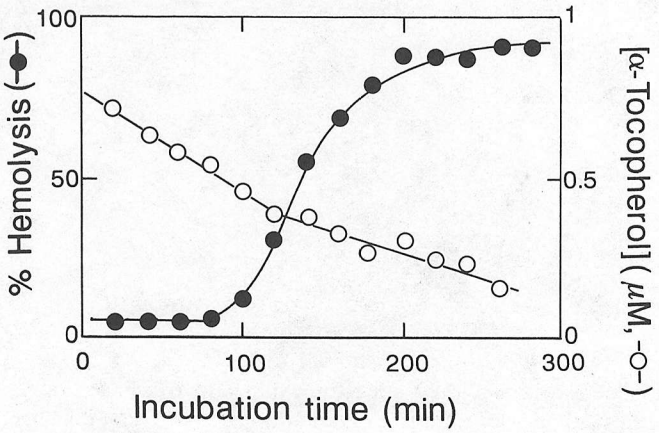

FIGURE 2 Hemolysis and consumption of α-tocopherol in rabbit erythrocytes induced by AAPH at 37°C in air. [Reproduced from E. Niki (43), with permission.]

authors calculated the amount of active hydrogen numbers (biallylic hydrogen numbers in fatty acid), which is a reliable index for assessing the extent of hydrogen abstraction by free radicals, and showed that the number is significantly higher in neonates relative to adults, resulting in high peroxidizability. Interestingly, when erythrocytes from vitamin E-deficient rats were hemolyzed using the xanthine-xanthine oxidase system, the morphological features of the damaged cells were quite different from those of the AAPH-damaged cells. As shown in Figure 3, xanthine-xanthine oxidase reaction produces spiky cells (by echinocytosis) but AAPH produces cup cells (by stomatocytosis) (45). Generally, it is considered that echinocytes are formed through the evagination of the outer leaflet of the membrane caused by changes in outer leaflet ordering; on the contrary, changes in the inner half of the bilayer induce cup formation (46). On this basis, it can be concluded that the free radicals induced by these radical initiators attack at different parts of the membrane by different mechanisms. Chain-breaking antioxidants, such as α-tocopherol, scavenge radicals arising from either initiator within the lipid core of erythrocyte membranes; consequently, the oxidative hemolysis of erythrocytes is efficiently inhibited. Similar susceptibility of erythrocytes to oxidative stress and the predominant inhibition of oxidative damage by α-tocopherol have been found in such pathological states as abetalipoproteinemia, cystic fibrosis, and aged diabetes, states in which the polyunsaturated fatty acids in the erythrocyte membranes are abnormally increased (9,16,47).

On the other hand, the attack of free radicals on erythrocytes can also lead to the oxidative destruction of membrane proteins, resulting in fragmentation, amino acid modification, cross-linking, and fluorescent pigmentation (45,48). The oxidation of rat red blood cells by either xanthine-xanthine oxidase reaction or AAPH leads not only to an increase in thiobarbituric acid-reactive substances (TBARS) and chemiluminescence in erythrocytes but also to a depletion of spectrin and the sulfhydryl groups (−SH) of membrane proteins (45,49–51). Although membrane tocopherol, even below a critically low level, suppresses lipid peroxidation, protein damage and the loss of SH groups are not inhibited. This difference in the eficacy of α-tocopherol can be explained by the fact that when radicals are generated outside the membranes, membrane proteins are damaged concurrently with the consumption of membrane tocopherol; on the contrary, when radicals are initiated within the lipid core of membranes, the depletion of SH groups and the formation of TBARS are predominantly suppressed

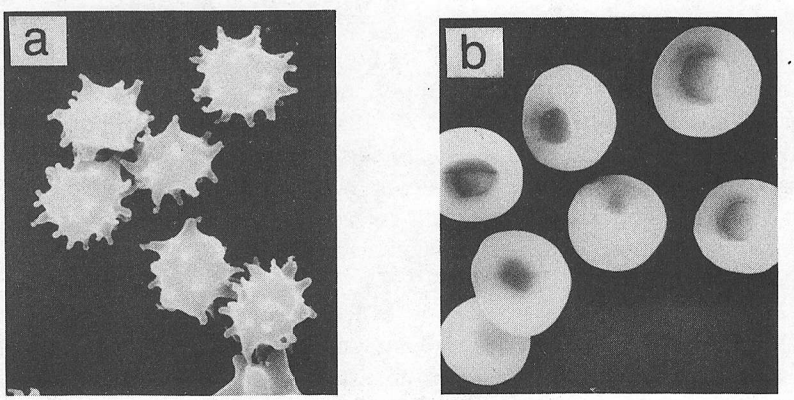

FIGURE 3 Scanning electron micrographs of erythrocytes subjected to the xanthine-xanthine oxidase reaction (a) and the AAPH reaction (b) (each ×6000). (Courtesy of Dr. M. Mino, with permission.)

by membrane tocopherol (51). The inhibition of membrane protein damage by α-tocopherol differs depending on the site of initial radical generation.

Vitamin E in Leukocyte and Platelet Function

Polymorphonuclear leukocytes (PMNs), similarly to platelets and lymphocytes, are capable of achieving a heightened state of functional and metabolic activation in response to immunochemical signals. This response is often altered by oxidation or vitamin E deficiency. Vitamin E-deficient rat PMNs have almost a twofold increase in peroxidized membrane lipids (MDA) due to enhanced oxygen consumption and hydrogen peroxide release; therefore, the susceptibility of these cells to oxidative stress is enhanced relative to that of normal PMNs. Furthermore, vitamin E-deficient PMNs are unable to respond normally to chemotactic and phagocytic stimuli. This oxidant-inflicted damage to vitamin E-deficient PMNs was suggested to occur before phagocytic events, and hence chemotactic and phagocytic functions are impaired; in contrast, autooxidative damage to vitamin E-replete PMN occurs in tempo with the phagocytic event, resulting in an enhancement of these functions (52). These defects are rapidly corrected by in vivo repletion of vitamin E-deficient rats with parenteral vitamin E. In the PMNs of vitamin E-replete rats, hydrogen peroxide (H_2O_2) released from the PMNs is markedly decreased, but superoxide anion (O_2^-) is slightly increased. Therefore it is concluded that H_2O_2 initiates the peroxidative damage to PMNs (53). Recent findings, however, show that a decrease of 20% or more in O_2^- occurs in vitamin E-replete rat PMNs (54). Although this discrepancy complicates the explanation for the antioxidant activity of vitamin E in PMN function, that the bactericidal functioning of vitamin E-replete PMNs is reduced (53) leads to the conclusion that vitamin E may attenuate the original oxidative activity in PMNs.

On the other hand, in infants with a congenital deficiency in glutathione synthetase activity, PMNs are unable to synthesize glutathione and hence the cellular membranes are susceptible to oxidative damage, resulting in impairment of the phagocytic function. Vitamin E repletion enhances the phagocytic functioning of normal PMNs by its ability to scavenge oxygen by-products (55).

Platelet-activating factor (PAF) is known to be metabolized in PMNs through certain stimuli and to be a potent phospholipid mediator that causes activation of leukocytes and platelets, thus contributing to a hemostatic eent. Recent findings by Fukuzawa et al. (56) show that the synthesis of PAF is significantly enhanced in vitamin E-deficient rat PMNs (Fig. 4). Although the activity of the acetyltransferase, which transfers an acetyl moiety to 2-lyso-PAF to form PAF, is higher in vitamin E-deficient than in vitamin E-supplemented PMNs, in vitro addition of vitamin E does not inhibit the increased activity of the enzyme. From these results it can be concluded that vitamin E does not directly inhibit the activity of this enzyme but rather may inhibit the activity of phospholipase A_2, which produces 2-lyso-PAF in PMN. In any case, the mechanism of this effect of vitamin E in PMN is still unclear.

One of the most important functions of platelets is in aggregation, which under normal condition exert a hemostatic effect and may be crucial for the development of thrombosis (57). In response to various stimuli, arachidonic acid is released from platelet membranes through the activation of phospholipase A_2. Arachidonic acid is then converted enzymatically to thromboxane A_2 (TXA_2) and prostaglandins. TXA_2 synthesized in platelets is the most potent proaggregatory and thrombotic metabolite, whereas prostaglandin I_2 (PGI_2), produced in the endothelium of the vessel wall, has significant antiaggregatory and antithrombotic effects (41,57). By a balance in the biosynthesis of these two compounds, platelet aggregation and its inhibition are regulated under normal conditions (Fig. 5).

In vitamin E-deficient rats, platelet aggregation induced by collagen and the contents of prostaglandin E_2 and $F_{2\alpha}$ in serum were found to be increased compared to those in normal

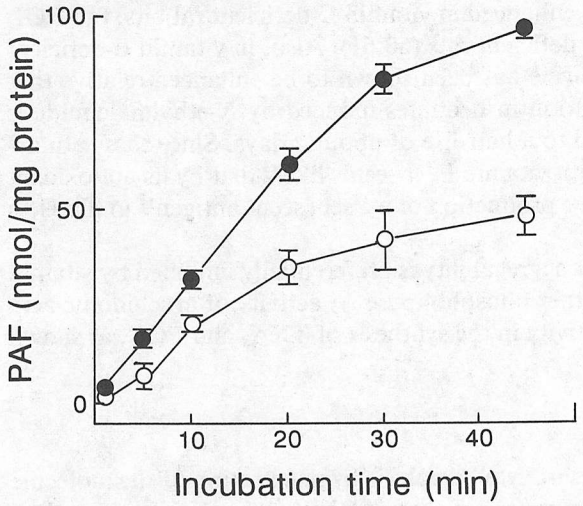

FIGURE 4 Time course for the incorporation of [^{3}H]acetyl-CoA into lyso-PAF by PMN homogenates prepared from vitamin E-supplemented (open circles) and vitamin E-deficient (solid circles) rats. The values for the two groups were significantly different ($P < 0.001$) at all time points. [Reproduced from K. Fukuzawa (56), with permission.]

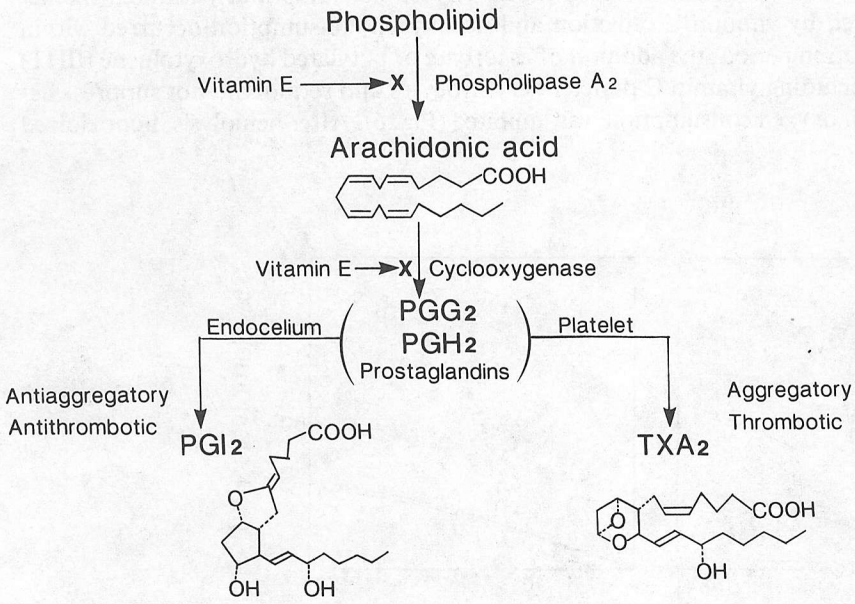

FIGURE 5 Biosynthesis of prostaglandin I_2 (PGI_2) and thromboxane A_2 (TXA_2) and its inhibition by vitamin E.

rats (58,59). Not only TXA_2 production is enhanced in vitamin E-deficient rabbits, but PGI_2 in vessel walls is significantly decreased in deficient rats (60,61). Also, in vitamin E-deficient neonates, platelet aggregability to epinephrine has been shown to be enhanced relative that in normal neonates. Platelet MDA formation in neonates induced by *N*-ethylmaleimide is increased and platelet survival is decreased to a half-life of about 2 days. Since these abnormalities are improved following therapy with vitamin E, it seems likely that by its antioxidant activity, vitamin E may inhibit the oxidative production of a "senescent antigen" in platelets (41,62,63).

Thus, the mechanism by which platelet aggregability is preferentially inhibited by vitamin E is thought to involve the inhibition of either phospholipase A_2 activity of arachidonic acid release from platelets or cyclooxygenase activity in the synthesis of TXA_2 and PGI_2, as shown in Figure 5.

Vitamin E in Nonoxidative Hemolysis

Retinol (vitamin A) is known to induce erythrocyte hemolysis by penetration of the molecule into membranes (64). Although it has been proposed that this hemolysis may be caused by the physical disruption of micelles in the membrane rather than by oxidative damage (18), this idea has been generally criticized because retinol, which is very unstable to oxidation because of its polyunsaturation, may undergo facile, self-initiated free-radical chain oxidation and may be capable of initiating the oxidative destruction of erythrocyte membrane lipids, resulting in hemolysis. Despite the uncertainty, there are no reports to demonstrate whether retinol-induced hemolysis arises from oxidative damage. Recently, Urano (19) proposed that erythrocyte hemolysis is actually caused by physical damage to the erythrocyte membrane. When a suspension of vitamin E-deficient rabbit erythrocytes was incubated with retinol, the erythrocytes were hemolyzed concurrently with oxygen consumption. Although the hemolysis was inhibited by vitamin E repletion and no oxygen consumption occurred within the 30 minute incubation period, the addition of ascorbate or butylated hydroxytoluene (BHT) into a suspension including vitamin E-deficient erythrocytes and retinol did not suppress hemolysis, even though oxygen consumption was inhibited (Fig. 6). After hemolysis, nonoxidized

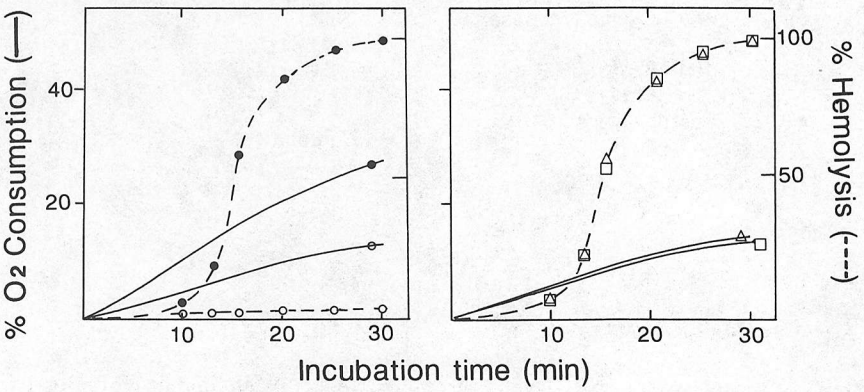

FIGURE 6 Rate of oxygen consumption and hemolysis in rabbit erythrocytes initiated by retinol and its inhibition by antioxidants. The erythrocyte suspensions were incubated with 1.7×10^{-3} μmol retinol (solid circles), retinol + 0.2×10^{-2} μmol α-tocopherol (open circles), retinol + 0.2×10^{-2} μmol ascorbic acid (open triangles), or retinol + 0.2×10^{-2} μmol BHT (open squares).

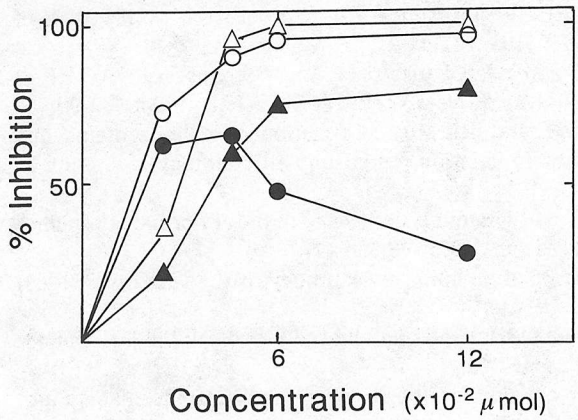

FIGURE 7 Inhibitory effect of α-tocopherol and its derivatives on retinol-induced rabbit erythrocyte hemolysis. The suspensions were incubated with α-tocopherol (solid circles), α-tocopheryl acetate (open circles), α-tocopheryl nicotinate (open triangles), and 6-deoxy-α-tocopherol (solid triangles) at 37°C for 20 minutes.

retinol and α-tocopherol were found in the broken membranes but the oxidized forms were not generally found. These results suggest that the added retinol is not oxidized in the suspension but rather penetrates into the erythrocyte membranes to cause physical damage to the erythrocytes.

Not only α-tocopherol but also its acetate and nicotinate inhibit hemolysis; in fact, the inhibitory effect of the acetate and nicotinate is higher than that of α-tocopherol by 100% (Fig. 7) (19,65). This result also shows the physical membrane-stabilizing effect of α-tocopherol. Although the mechanism of this effect is unclear as to detail, it has been reported that the chroman ring, rather than isoprenoid side chain of α-tocopherol, is important for the membrane stabilization of erythrocytes (66–69).

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