

EFFECTS OF VITAMIN E DEPLETION AND REPLETION ON RENIN RELEASE FROM RENIN GRANULES

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The present study was carried out to investigate the effects of vitamin E-deficiency and supplementation of α -tocopheryl acetate (TOCA) on renin release from renin granules. Male Wistar rats were fed either a control or a vitamin E-deficient diet for 4 weeks. Subsequently, the vitamin E-deficient rats received dietary supplementation of TOCA (40 mg/100 g diet) for 5 d. The renin granule fraction was prepared from the kidney cortex homogenate by a discontinuous sucrose density gradient centrifugation. The intake of vitamin E-deficient diet for 4 weeks resulted in an increased level of endogenous lipid peroxides in the renin granule fraction, accompanied by a marked decrease in α -tocopherol content, and led to a significant increase in the rate of renin release from the granules during incubation at 37°C. These changes in α -tocopherol content, lipid peroxide level and renin release in the renin granule fraction were restored to the control values by dietary TOCA supplementation. Similarly, dietary supplementation of *N,N'*-diphenyl-*p*-phenylenediamine (80 mg/100 g diet), which has an antioxidative ability, suppressed the increases in lipid peroxidation and renin release due to vitamin E-deficiency, although this compound was ineffective in restoring α -tocopherol levels. These results suggest that vitamin E functions in maintenance of membrane integrity of renin granules by inhibiting the lipid peroxidation.

Keywords—rat renin granule; vitamin E-deficiency; dietary supplementation of α -tocopheryl acetate; renin release; α -tocopherol content; lipid peroxidation

INTRODUCTION

Since the membranes of subcellular organelles contain a large amount of unsaturated phospholipids and since the function of a cell would be influenced by the nature of phospholipids, the peroxidation of phospholipids might induce membrane damage and impairment of cellular function.¹⁻⁶⁾ On the other hand, vitamin E reacts rapidly with free radicals, and its presence in the cellular and subcellular membranes is the first line of defence against peroxidation of polyunsaturated fatty acid constituents of vital phospholipids.^{7,8)}

In a recent paper, we demonstrated that renin release from renin granules was markedly stimulated by ascorbic acid or ferrous ions, accompanied by increased formation of lipid peroxides in the renin granule fraction.⁹⁾ Furthermore, we found that the rate of renin release from the gran-

ules was augmented by dietary vitamin E-deficiency.¹⁰⁾ The present study was designed to further investigate the effects of *in vivo* repletion of this vitamin on renin release from the granules of vitamin E-deficient rats.

MATERIALS AND METHODS

Animals and Diets—Male Wistar rats weighing 70–80 g were used. For at least 1 week before the study, the rats were fed a standard laboratory rat chow (Oriental Yeast Co., MF) and received tap water *ad libitum*. The animals were divided into control and experimental groups. The experimental animals were maintained on a vitamin E-deficient basal diet consisted of the components described in Table I, while the control animals were given a control diet prepared by the addition of 2 mg of *dl*- α -tocopheryl acetate (TOCA) to 100 g of the basal diet for 4 weeks. Subsequently,

parts of vitamin E-deficient animals received dietary supplementation of TOCA or *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) for 5 d. The supplemental diet of TOCA or DPPD was prepared by the addition of 40 mg of TOCA or 80 mg of DPPD to 100 g of vitamin E-deficient basal diet.

Processing of Blood Samples and Preparation of Kidney Cortex Homogenate — The peritoneal cavity was opened under pentobarbital anesthesia (30 mg/kg, *i.p.*), and the renal artery and vein were ligated. Both kidneys were removed and immediately cooled. An arterial blood sample was withdrawn through the aorta with a syringe wetted with heparin, immediately cooled, and centrifuged. The kidney cortex was removed from the medulla, sectioned into thin slices, and homogenized with ice-cold physiological saline (1:8, w/v). The homogenate and plasma were analyzed for α -tocopherol and lipid peroxides.

Preparation of Renin Granule Fraction — Renin granule fraction was prepared from the kidney cortex by discontinuous sucrose density gradient centrifugation.^{11,12)} Renal cortical slices were homogenized with ice-cold 0.45 M sucrose (1:8, w/v). After separating the unbroken cells,

cell debris, and nuclei by centrifugation at $500 \times g$ for 10 min, the homogenate was layered on a discontinuous sucrose density gradient solution (1.2 M to 1.7 M with 0.1 M intervals) and centrifuged at $60000 \times g$ for 90 min. Renin granules were mainly equilibrated in the fraction corresponding to 1.5 M sucrose. This fraction was used for measuring α -tocopherol and lipid peroxide contents, and the amount of renin release from the granules.

Incubation System for Renin Granule Fraction — Half ml of renin granule fraction (0.45–0.50 mg protein) was suspended in 0.5 ml of 0.1 M Tris-HCl buffer (pH 7.4) containing 0.15 M KCl, and incubated at 37°C for 60 min or 120 min. The incubation mixture was separated into the supernatant and sediment by centrifugation at $105000 \times g$ for 60 min. The sediment was resuspended in Tris-HCl buffer (pH 7.4). The supernatant and sediment were analyzed for renin activity.

Renin Assay — Renin activity in the sample was measured by radioimmunoassay of angiotensin I¹³⁾ generated after incubation with semi-purified rat renin substrate, which was prepared according to the procedure described previously.¹⁴⁾ The incubation medium consisted of: 1) samples containing renin, 50 μ l; 2) renin substrate dissolved in 0.25 M phosphate buffer (pH 7.0) containing 20 mM EDTA, 0.5 ml; 3) 5% diisopropylphosphorofluoridate, 20 μ l; 4) 6.6% 8-hydroxyquinoline sulfate, 10 μ l; 5) 10% dimer-caprol, 3 μ l. After incubation at 37°C for 15 min, the reaction was stopped by heating for 5 min in a boiling water bath. Total renin was taken to be sum of renin content in the supernatant and sediment, and the amount of renin release during incubation was expressed as a percentage of total renin.

Measurement of α -Tocopherol Content — The plasma α -tocopherol content was determined following the method of Abe and Katsui.¹⁵⁾ A mixture of 0.2 ml of plasma and 1.0 ml of ethanol was vigorously shaken with 4.0 ml of *n*-hexane. The mixture was centrifuged at 3000 rpm for 10 min, and fluorescence of *n*-hexane layer was

TABLE I. Components of Basal Vitamin E-Deficient Diet

Ingredient	Percentage
Corn starch	36
Casein	25
Alpha starch of wheat	10
Powdered filter paper	8
Salt mixture	6
Granulated sugar	5
Vitamin mixture ^{a)}	2
Stripped corn oil	8

a) One gram of vitamin mixture contains 500 IU vitamin A, 1.2 mg vitamin B₁, 4.0 mg vitamin B₂, 0.8 mg vitamin B₆, 0.5 ng vitamin B₁₂, 30 mg vitamin C, 100 IU vitamin D₂, 5.2 mg vitamin K₃, 20 ng biotin, 0.2 mg folic acid, 5 mg calcium pantothenate, 5 mg *p*-aminobenzoic acid, 6 mg niacin, 6 mg inositol, and 200 mg choline chloride.

measured with excitation at 295 nm and emission at 320 nm using a Hitachi model 650-40 fluorescence spectrophotometer. α -Tocopherol solution (2 μ g/ml) dissolved in ethanol was used as an external standard. The plasma α -tocopherol content was expressed as mg per dl. The α -tocopherol contents in the kidney cortex homogenate and renin granule fraction were determined according to the method of Taylor *et al.*¹⁶⁾ After 0.5 ml of 25% ascorbic acid and 1.0 ml of ethanol were added to 1.5 ml each of the homogenate and renin granule fraction, each mixture was incubated with 1.0 ml of 10 N KOH at 37°C for 30 min, and then extracted with 4.0 ml of *n*-hexane. Analytical procedures for fluorescence of *n*-hexane layer and the external standard were similar to those used in the measurement of plasma α -tocopherol. The α -tocopherol contents in the kidney cortex and renin granule fraction were expressed as nmol per mg of protein.

Measurement of Lipid Peroxides — Malondialdehyde (MDA) production, as measured by the thiobarbituric acid (TBA) test, served as an index of lipid peroxidation. Plasma lipid peroxides were determined according to the method of Yagi.¹⁷⁾ Twenty μ l of plasma was mixed with 4.0 ml of 1/12 N sulfuric acid and 0.5 ml of 10% phosphotungstic acid, and the mixture was centrifuged at 3000 rpm for 10 min. The sediment was suspended in 4.0 ml of distilled water, and then heated with 1.0 ml of 0.67% TBA at 95°C for 60 min. The reaction product was extracted with 5.0 ml of *n*-butanol, and measured fluorometrically with excitation at 515 nm and emission at 553 nm using a Hitachi model 650-40 fluorescence spectrophotometer. Tetraethoxypropane was used as the standard, and lipid peroxide formation in plasma was expressed in terms of MDA (nmol per ml of plasma).

Lipid peroxides in the kidney cortex homogenate and renin granule fraction were determined according to the method of Ohkawa *et al.*¹⁸⁾ The reaction mixture consisted of : 1) sample, 0.2 ml; 2) 8.1% sodium dodecyl sulfate, 0.2 ml; 3) 20% acetate buffer (pH 3.5), 1.5 ml; 4) 0.8% TBA, 1.5 ml. The mixture was heated at 95°C for 60 min,

and reaction product was extracted with 5.0 ml of the mixture of *n*-butanol and pyridine (15:1, v/v). Analytical procedures for fluorescence of organic solvent phase were the same as those for plasma lipid peroxides. Lipid peroxide formation in the kidney cortex homogenate and renin granule fraction was expressed in terms of MDA (nmol per mg of protein).

Measurement of Protein Content — The protein content was determined by the method of Lowry *et al.*¹⁹⁾

Statistical Analysis — All values were expressed as the mean \pm S.E. Student's *t*-test was used to determine whether differences between the experimental and control groups were significant. Differences were not considered significant if $p > 0.05$.

RESULTS

Throughout the experimental period, no significant difference could be detected in average body weight or kidney weight per 100 g of body weight between the control and experimental rats. We checked vitamin E status of each animal by the hemolytic action of dialuric acid on erythrocytes.²⁰⁾ The animals maintained on the control and vitamin E-deficient diets for 4 weeks showed $0.5 \pm 0.3\%$ and $93.7 \pm 3.0\%$ erythrocyte hemolysis, respectively. But, dietary supplementation of TOCA or DPPD for further 5 d prevented effectively erythrocyte hemolysis due to vitamin E-deficiency, *i.e.*, erythrocytes of the animals received TOCA and DPPD exhibited $0.4 \pm 0.2\%$ and $0.9 \pm 0.7\%$ hemolysis, respectively.

As shown in Table II, the intake of vitamin E-deficient diet for 4 weeks resulted in the decreases in the mean α -tocopherol contents of plasma and kidney cortex tissues, being 16% and 26% of each control value. The reduced α -tocopherol levels in these preparations were restored to the control levels by dietary supplementation of TOCA, but not at all by dietary supplementation of DPPD.

Vitamin E-deficient rats had almost a threefold increase in the plasma lipid peroxide level. However, this increased level of plasma lipid peroxides was suppressed below the control value

by dietary supplementation of TOCA or DPPD, although no significant differences could be detected between the control and supplemented groups. Similar results were obtained with lipid peroxide formation in the kidney cortex tissues (Table II).

A negative correlation between α -tocopherol content and lipid peroxide formation was also observed in the renin granule fraction of vitamin E-deficient rats. As shown in Table III, the α -tocopherol content in the renin granule fraction was decreased to 27% of control value by the intake of vitamin E-deficient diet for 4 weeks

while lipid peroxide formation in this fraction was increased to 160% of control value. These alterations in α -tocopherol content and lipid peroxide formation were restored to each control level by dietary TOCA supplementation for a further 5 d. On the other hand, dietary DPPD supplementation completely restored the increased level of lipid peroxides to the control value, but did not affect the α -tocopherol content in the renin granule fraction, as observed in plasma of vitamin E-deficient rats.

Fig. 1 shows the effect of vitamin E-deficiency and supplementation of TOCA and DPPD on

TABLE II. *Effects of Vitamin E-Deficiency and Supplementation of TOCA or DPPD on α -Tocopherol Content and Lipid Peroxide Formation in Plasma and Kidney Cortex*

Diet	α -Tocopherol content		Lipid peroxide formation	
	Plasma (mg/dl)	Kidney cortex (ng/mg protein)	Plasma (nmol MDA/ml)	Kidney cortex (nmol MDA/mg protein)
Control	1.17 \pm 0.04	86.7 \pm 5.0	3.30 \pm 0.39	1.14 \pm 0.04
Vitamin E-deficient	0.19 \pm 0.01 ^{b)}	22.3 \pm 4.4 ^{b)}	10.57 \pm 1.25 ^{b)}	1.65 \pm 0.10 ^{a)}
TOCA supplemented	1.46 \pm 0.07 ^{a,d)}	87.9 \pm 6.4 ^{d)}	2.20 \pm 0.47 ^{d)}	1.13 \pm 0.10 ^{c)}
DPPD supplemented	0.21 \pm 0.01 ^{b)}	20.4 \pm 3.3 ^{b)}	2.71 \pm 0.49 ^{d)}	1.02 \pm 0.10 ^{c)}

Each value represents the mean \pm S.E. of six animals. a) $p < 0.01$, b) $p < 0.001$; significantly different from each control group, c) $p < 0.01$, d) $p < 0.001$; significantly different from each vitamin E-deficient group.

TABLE III. *Effects of Vitamin E-Deficiency and Supplementation of TOCA or DPPD on α -Tocopherol Content and Lipid Peroxide Formation in the Renin Granule Fraction*

Diet	α -Tocopherol content (ng/mg protein)	Lipid peroxide formation (nmol MDA/mg protein)
Control	108.6 \pm 18.7	1.30 \pm 0.10
Vitamin E-deficient	28.8 \pm 9.3 ^{a)}	2.08 \pm 0.30 ^{a)}
TOCA supplemented	135.7 \pm 24.1 ^{b)}	1.15 \pm 0.12 ^{b)}
DPPD supplemented	29.6 \pm 8.8 ^{a)}	1.01 \pm 0.16 ^{b)}

Each value represents the mean \pm S.E. of four separate experiments. a) $p < 0.05$; significantly different from each control group, b) $p < 0.05$; significantly different from each vitamin E-deficient group.

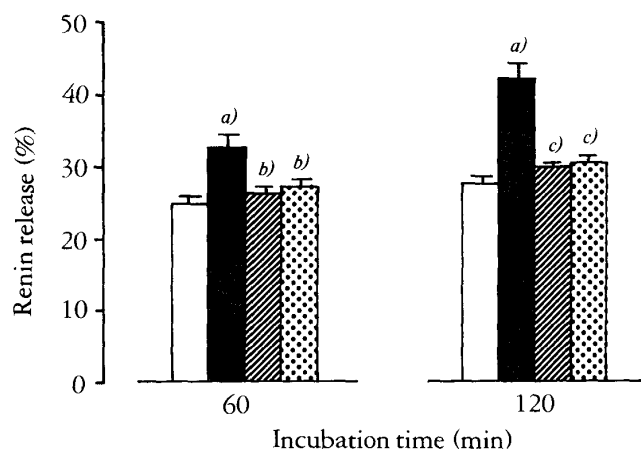


FIG. 1. *Effects of Vitamin E-Deficiency and Supplementation of TOCA or DPPD on Renin Release from Renin Granules*

Each column represents the mean \pm S.E. of four separate experiments. : control; : vitamin E-deficient; : TOCA supplemented; : DPPD supplemented.

a) $p < 0.01$; significantly different from each control group; b) $p < 0.05$, c) $p < 0.01$; significantly different from each vitamin E-deficient group.

renin release from the granules. When the renin granule fraction of control rats was incubated at 37°C for 60 min, $24.8 \pm 0.9\%$ of total renin appeared in the medium. In vitamin E-deficient rats, the higher rate of renin release ($32.5 \pm 1.8\%$ of total renin) was observed with incubation of renin granule fraction under the same conditions. In addition, incubation of this fraction at 37°C for 120 min led to a further increase in renin release ($43.0 \pm 2.3\%$ of total renin) compared with the control value ($27.5 \pm 0.9\%$ of total renin). However, the stimulatory effect of vitamin E-deficiency on renin release was not observed in rats supplemented with TOCA and the rate of renin release was completely restored to the control value. The same results were obtained with DPPD supplementation to the vitamin E-deficient rats.

DISCUSSION

Vitamin E-deficiency has been shown to produce various pathological conditions, such as

myopathy, erythrocyte and leucocyte abnormalities, and testicular degeneration.²¹⁻²⁴ Deficiency of this vitamin has also shown to cause damage of membrane structure and failures of metabolic function in a variety of subcellular organelles.²⁵⁻²⁸ In the present study, we found that a lack of dietary vitamin E increased the amount of renin release from renin granules, accompanied by an increased level of endogenous lipid peroxides in the renin granule fraction. The same increase in endogenous lipid peroxides was observed in the plasma and kidney cortex tissues of vitamin E-deficient rats. Erythrocytes of these animals exhibited approximately 94% hemolysis against dialuric acid.

It is thought that vitamin E has an important role in terminating peroxidative reactions of polyunsaturated fatty acid, because of its antioxidant capacity and lipophilic character.⁸ It has also been suggested that because of the amphiphilic structure, vitamin E has a strong physical interaction with polyunsaturated fatty acids in the membranes.⁷ This vitamin is predominantly present in the membranes of the cells and subcellular organelles and only limited amounts are found in the soluble fraction.²⁹ When the properties of high physical interaction and antioxidant capacity are combined, one expects an important stabilizing action of this vitamin in the biomembranes. In this study, oral supplementation of TOCA to the vitamin E-deficient animals suppressed the elevation of lipid peroxide formation in the renin granule fraction as well as the plasma and kidney cortex tissues, which returned to each control value 5 d after supplementation. Simultaneously, the elevated of renin release from the granules due to vitamin E depletion was completely restored to the control value. In addition, the high degree of erythrocytes hemolysis induced by vitamin E-deficiency returned to the normal value. These findings suggest that there is a close association between lipid peroxide formation and renin release in the renin granule fraction and that vitamin E acts as a lipid antioxidant in the membranes of renin granules.

To further examine the correlation between

lipid peroxidation and renin release in the renin granule fraction, we conducted DPPD supplementation to vitamin E-deficient rats. DPPD is well known to have a strong antioxidative effect on lipid peroxidation in microsomes³⁰⁾ and mitochondria,³¹⁾ although its antihemolytic ability is lower than that of TOCA.³²⁾ Thus, in the present study, DPPD supplemented diet, which contained twice amounts of DPPD (80 mg/100 g diet) compared with TOCA (40 mg/100 g diet) supplemented diet, was given to vitamin E-deficient rats. DPPD supplementation for 5 d suppressed the elevated levels of lipid peroxides in the plasma, kidney cortex tissues and renin granule fraction following vitamin E-deficiency to the control values, although this agent did not affect the reduced levels of α -tocopherol in these preparations. Simultaneously, the high rate of renin release from the granules of vitamin E-deficient rats was returned to the control value. Thus, we confirmed that there is a positive correlation between lipid peroxidation and renin release in the renin granule fraction.

The findings in the present study indicate that dietary TOCA supplementation shows a protective effect against increases in lipid peroxidation and renin release in the renin granule fraction by vitamin E-deficiency. These results strongly suggest that vitamin E functions in maintenance of membrane integrity of renin granules by inhibiting the lipid peroxidation.

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REFERENCES

- 1) B.O.Goldstein, M.G.Rozen, and R.L.Kunis: Role of red cell membrane lipid peroxidation in hemolysis due to phenylhydrazine, *Biochem. Pharmacol.*, **29**, 1355–1359 (1980).
- 2) S.R.Ribarov and L.C.Benov: Relationship between the hemolytic action of heavy metals and lipid peroxidation, *Biochim. Biophys. Acta*, **640**, 721–726 (1981).
- 3) R.C.McKnight, F.E.Hunter, and W.H.Oehlert: Mitochondrial membrane ghosts produced by lipid peroxidation induced by ferrous ion. I. Production and general morphology, *J. Biol. Chem.*, **240**, 3439–3446 (1965).
- 4) O.Shimada and H.Yasuda: Lipid peroxidation and its inhibition by tinoridine. II. Ascorbic acid-induced lipid peroxidation of rat liver mitochondria, *Biochim. Biophys. Acta*, **572**, 531–536 (1979).
- 5) I.D.Desai, P.L.Sawant, and A.L.Tappel: Peroxidation and radiation damage to isolated lysosomes, *Biochim. Biophys. Acta*, **86**, 277–285 (1964).
- 6) E.D.Wills and A.E.Wilkinson: Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release, *Biochem. J.*, **99**, 657–666 (1966).
- 7) J.A.Lucy: Functional and structural aspects of biological membranes: A suggested structural role for vitamin E in the control of membrane permeability and stability, *Ann. N. Y. Acad. Sci.*, **203**, 4–11 (1972).
- 8) A.L.Tappel: Vitamin E and free radical peroxidation of lipids, *Ann. N. Y. Acad. Sci.*, **203**, 12–28 (1972).
- 9) Y.Matsumura, T.Shimizu, Y.Ohno, and S.Morimoto: Renin release and lipid peroxidation in renin granules of the rat, *Chem. Pharm. Bull.*, **30**, 3742–3747 (1982).
- 10) Y.Matsumura, Y.Ohno, N.Miyawaki, T.Shimizu, and S.Morimoto: Effect of vitamin E-deficiency on renin release from renin granules, *J. Pharm. Dyn.*, **5**, 1030–1032 (1982).
- 11) S.Morimoto, K.Yamamoto, and J.Ueda: Isolation of renin granules from the dog kidney cortex, *J. Appl. Physiol.*, **33**, 306–311 (1972).
- 12) S.Morimoto, R.Abe, A.Fukuhara, K.Tanaka, and K.Yamamoto: Effect of sodium restriction on plasma renin activity and renin granules in the rat kidney, *Am. J. Physiol.*, **234**, F367–F371 (1979).
- 13) E.Haber, T.Koerner, L.B.Page, B.Kliman, and A.Purnode: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal subjects, *J. Clin. Endocrinol. Metabol.*, **29**, 1349–1355 (1969).
- 14) S.Morimoto, K.Yamamoto, K.Horiuchi, H.Tanaka, and J.Ueda: A release of renin from dog kidney cortex slices, *Jpn. J. Pharmacol.*, **20**, 536–545 (1970).
- 15) K.Abe and G.Katsui: Fluorometric determination of tocopherol in serum, *Jpn. J. Food Nutr.* (in Japanese), **28**, 277–280 (1975).
- 16) S.L.Taylor, M.P.Lamden, and A.L.Tappel: Sensitive fluorometric method for tissue tocopherol analysis, *Lipids*, **11**, 530–538 (1976).
- 17) K.Yagi: A simple fluorometric assay for lipoperoxide in blood plasma, *Biochem. Med.*, **15**, 212–216 (1976).
- 18) H.Ohkawa, N.Ohishi, and K.Yagi: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal. Biochem.*, **95**, 351–358 (1979).
- 19) O.H.Lowry, N.J.Rosenbrough, A.L.Farr, and R.J.

- Randall: Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, **193**, 265–275 (1951).
- 20) H.Ikehata and Y.Sugiyama: Studies on the hemolysis caused by vitamin E deficiency (I) Hemolysis test with dialuric acid, *Vitamins* (in Japanese), **37**, 31–36 (1968).
- 21) E.Gabriel, L.J.Machlin, R.Filipski, and J.Nelson: Influence of age on vitamin E requirement for resolution of necrotizing myopathy, *J. Nutri.*, **110**, 1372–1379 (1980).
- 22) C.S.Rose and György: Specificity of hemolytic reaction in vitamin E-deficient erythrocytes, *Am. J. Physiol.*, **168**, 414–420 (1952).
- 23) R.H.Harris, L.A.Boxer, and R.L.Baehner: Consequences of vitamin E-deficiency on the phagocytic and oxidative functions of the rat polymorphonuclear leukocyte, *Blood*, **55**, 338–343 (1980).
- 24) K.E.Mason and M.K.Horwitt: The tocopherol, Effect of deficiency in animals, "The vitamins," ed. by W.H.Sebrell and R.S.Harris, Academic Press, New York, 1972, pp. 272–278.
- 25) L.P.Thung, L.Manning, B.Wiseman and M.P.Carpenter: Effect of vitamin E on testis prostaglandin metabolism and liver hydroxylation, *Fed. Proc.*, **37**, 707 (abs.) (1978).
- 26) K.Schwarz: The cellular mechanisms of vitamin E action: Direct and indirect effects of α -tocopherol on mitochondrial respiration, *Ann. N. Y. Acad. Sci.*, **203**, 45–52 (1972).
- 27) C.Vajanamarhutue, P.Wilairat, and P.Komaratat: Effect of vitamin E deficiency on the activities of lipid-requiring enzymes in rabbit liver and muscle, *J. Nutr.*, **109**, 848–855 (1979).
- 28) M.Frigg and H.P.Rohr: Ultrastructural and stereological study on the effect of vitamin E on liver mitochondrial membranes, *Exp. Mol. Pathol.*, **24**, 236–243 (1976).
- 29) D.M.Lussier and R.M.Roy: Distribution of vitamin E among tissue and subcellular fractions in sublethally irradiated mice, *Radiat. Res.*, **70**, 236–240 (1977).
- 30) T.F.Slater, and B.C.Sawer: The stimulatory effects of carbon tetrachloride on peroxidative reactions in rat liver fractions in vitro: Inhibitory effect of free-radical scavengers and other agents, *Biochem. J.*, **123**, 823–828 (1971).
- 31) T.Fujita and Y.Fujimoto: Effect of various diuretics on lipid peroxidation in rat renal cortical mitochondria and in the supernatant, *Jpn. J. Pharmacol.*, **31**, 795–800 (1981).
- 32) J.Bunyan, J.Green, E.E.Edwin, and A.T.Diplock: Studies on vitamin E, 3, The relative activities of tocopherols and some other substances *in vivo* and *in vitro* against dialuric acid-induced hemolysis of erythrocytes, *Biochem. J.*, **75**, 460–467 (1960).