TOLERANCE AND SAFETY OF VITAMIN E:
A TOXICOLOGICAL POSITION REPORT

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Abstract—From numerous publications on the “prophylactic” and “therapeutic” use of vitamin E, it may be concluded that the toxicity of vitamin E is very low. It has been demonstrated in animal experiments that vitamin E has neither mutagenic, teratogenic nor carcinogenic properties. Based on studies in humans, a daily dosage of 100-300 mg vitamin E can be considered harmless from a toxicological point of view. Using double-blind studies involving a large number of subjects, it has been demonstrated that large oral doses of up to 3,200 USP-Units/day led to no consistent adverse effects. From a large body of published data, dosage ranges have been deduced which can be characterized as safe for human subjects even where their use extends over a long period of time. It should, however, be noted that oral intake of high levels of vitamin E can exacerbate the blood coagulation defect of vitamin K deficiency caused by malabsorption or anticoagulant therapy. High levels of vitamin E intake are, therefore, contraindicated in these subjects.

Note: The difficulty encountered in the comparative evaluation of data reported in the literature is that the statements about the material used in the studies are sometimes ambivalent since often no distinction is made between the different forms of tocopherols tested or only the generic name vitamin E has been used. Although it would have been preferable to state the dosages in terms of comparable expressions, in this paper the quantity and quality of vitamin E used will be reported as described in the studies cited, i.e. either in mg substance or in International Units (I.U.) or both. For further explanation see footnote (a) in Table 1.

Because of the importance of this topic, and to make it more available to our readership as well as to abstracting services, the Editors are pleased to reprint the following article that originally was prepared as a pamphlet distributed by the Vitamin E Research and Information Service (VERIS). We thank VERIS, 5325 South Ninth Avenue, LaGrange, IL 60525, for their permission to reprint the article “Tolerance and Safety of Vitamin E” by Hermann Kappus and Anthony T. Diplock.

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PREFACE

There is at present major international interest in the possibility that free radical-mediated events in biochemical processes may lie at the heart of the aetiology and natural history of a number of disorders that are of major concern worldwide, in particular cardiovascular disease and cancer, as well as a number of other disease states. Free radicals are atoms or molecules that have one or more unpaired electrons in their atomic structure and are thus generally highly reactive. It follows, therefore, that the mechanisms that exist for the control of free-radical generation, and of their proliferation, are of central importance in considering the prevention of these disease states. Nutritional factors play an important role in this control and among the nutrients to be considered, vitamin E occupies center stage. In the course of normal respiration, a process which is commonly regarded as benign and life-giving, intermediates may be formed randomly which, because of their high chemical reactivity, may cause damage to biological membranes that provide the necessary order to intracellular morphology. The particular function of vitamin E is to protect membranes from oxidative damage. In the absence of adequate amounts of vitamin E in the diet, membrane turnover may accelerate so that the normal order and compartmentalization of cells is destroyed.
Such primary events are thought to be fundamental to the initiation and development of a number of disease processes.

Direct evidence at the biochemical level implies that vitamin E, and other antioxidant nutrients such as selenium, vitamin C and beta-carotene, act as modulators and moderators of biochemical changes that may be expected to lead to pathological change and disease. Direct evidence of the intervention of vitamin E in disease prevention is at present lacking. However, a large and steadily growing body of indirect evidence exists that implies the importance of vitamin E in preventing a number of diseases, in particular cardiovascular disease and certain forms of cancer. This evidence, derived from a number of prospective epidemiological studies in several sites around the world which were begun in the early 1970s, is showing highly significant correlations between low nutritional status at the time of entry into the study with respect to vitamin E, and subsequent higher risk of cardiovascular disease and cancer. These studies are ongoing and at present they serve to give pointers to the need for enhancing vitamin E intakes which may prove to be reinforced as further information accumulates. There is thus a growing body of reliable scientific evidence that links poor dietary intake of the antioxidant nutrients with a higher risk of disease.

It is clear that, as nutritional advice to be given to the public develops in favor of encouraging an enhanced intake of vitamin E, the question of the safety of oral administration of the vitamin must be addressed so that reliable information and advice can be given on this topic. This question becomes particularly important when vitamin E is used in pharmacological amounts for "therapeutic" purposes.

1. INTRODUCTION

Vitamin E is the overall collective term for all biologically active tocopherols and tocotrienols and their derivatives which exhibit "qualitatively, the biological activity of RRR-alpha-tocopherol, the naturally occurring stereoisomer." The nomenclature of vitamin E is briefly summarized in Table 1. In addition to the free phenolic form, oxidation-protected forms such as alpha-tocopheryl acetate and alpha-tocopheryl succinate are employed as well. Their structural formulas are shown in Figure 1.

Whereas vitamin C is known as the most important water-soluble antioxidant, vitamin E can be considered the major lipid-soluble antioxidant which functions to break free radical initiated chain reactions among the polyunsaturated fatty acids of biological membranes. Vitamin E is transported in the lymph and in blood as free tocopherol bound to beta-lipoproteins. Thus, all body tissues, in particular all cell membranes and subcellular organelles (mitochondria, cell nuclei, lysosomes, endoplasmic reticulum), are supplied with vitamin E and are thereby stabilized. There is also a close functional connection between tocopherol and some membrane-associated enzymes. The overall nutritional function of the tocopherols includes antioxidative protective effects towards fats and some vitamins as well as the prevention of the formation of toxic cellular hydroperoxides. Vitamin E is responsible for a protective effect against oxygen attack on the membranes of the erythrocytes.

Vitamin E is, by definition, necessary to life and this is true for human beings as well as for animals. Numerous clinical symptoms in animals are associated with vitamin E deficiency. The symptoms are attributed to the fact that in vitamin E deficiency, protection of the polyunsaturated fatty acids (PUFA) in the membranes no longer occurs or is not present to a sufficient degree, which leads to loss of membrane fluidity and function. Insufficient stabilization of polyunsaturated fatty acids in the cell membranes can lead to various clinical symptoms or can accelerate the development of certain diseases.

Vitamin E was initially considered to be concerned with reproduction since it is responsible for the maintenance of pregnancy and fertility in rats. Currently, its role as a lipid-soluble antioxidant and as a protective component of the membranes of cells and organelles as well as its role as a protective factor against the oxidation of lipids occupies the center stage. Vitamin E inhibits lipid peroxidation; its deficiency leads to premature cell aging and to the binding of immunoglobulin G to erythrocytes and, in addition, it has been reported to play a special role in porphyrin metabolism. Vitamin E affects the metabolism of arachidonic acid; inhibition of thromboxane and leukotriene biosynthesis, and increased prostacyclin formation have been reported. These characteristics may be involved in the biological inhibition of inflammation and in thrombotic diseases. However, other reports suggest a biphasic effect of vitamin E on the arachidonic acid cascade in which small amounts of the vitamin stimulate the biosynthesis of prostaglandin, whereas larger amounts inhibit it. Several other deficiency symptoms have been observed in animals receiving a diet without vitamin E or low in vitamin E; in man, on the other hand, few symptoms exist which can be unequivocally attributed to vitamin E deficiency.

The minimum amount of vitamin E which is required in man to achieve a state of optimal body functioning is subject to discussion. While a fairly low dose is sufficient to prevent deficiency symptoms, no
Tolerance and safety of vitamin E

Table 1. Nomenclature of Tocopherols

<table>
<thead>
<tr>
<th>Description of Product</th>
<th>Configuration</th>
<th>Recommended Trivial Names</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>The only isomer of alpha-tocopherol as yet found in nature</td>
<td>2R,4'R,8'R (1 isomer)</td>
<td>RRR-alpha-tocopherol</td>
<td>d-alpha-tocopherol(^a)</td>
</tr>
<tr>
<td>Totally synthetic alpha-tocopherol such as can be produced from synthetic phytol or isophytol</td>
<td>A mixture (normally equimolar) of all 4 possible racemates (i.e. 8 diastereoisomers in 4 pairs of enantiomers)</td>
<td>all-rac-alpha-tocopherol</td>
<td>dl-alpha-tocopherol(^a)</td>
</tr>
<tr>
<td>Semisynthetic alpha-tocopherol as can be produced from natural phytol</td>
<td>2R,4'R,8'R and 25,4'R,8'R (2 isomers)</td>
<td>2-ambo-alpha-tocopherol</td>
<td>(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Until 1956, the International Unit was defined as the vitamin E activity of 1 mg 2-ambo-alpha-tocopheryl acetate, which contained 2R,4'R,8'R-alpha-tocopheryl acetate and an unspecified amount of 2-epi-alpha-tocopheryl acetate (i.e. 2S,4'R,8'R). After the supply of this compound was exhausted, the International Standard for vitamin E ceased to exist. For labelling purposes, the concept of a unit of biological activity is still used but it has been redefined as 1 mg all-rac-alpha-tocopheryl acetate, the current USP Reference Standard, and renamed “USP Vitamin E-Unit.” It is numerically equal to the discontinued International Unit. The weight/unit relationship is:

1 mg all-rac-alpha-tocopherol = 1 USP-Unit
1 mg RR-alpha-tocopherol = 1.1 USP-Units
1 mg RR-alpha-tocopherol acetate = 1.36 USP-Units
1 mg RR-alpha-tocopherol succinate = 1.49 USP-Units

For further discussion see ref. 3 and refer to United States Pharmacopoeia (USP XXI), page 1118-1119. In this paper, the IUPAC-recommended nomenclature will be used to name the substances and the term “USP-Unit” will be used when the biological activity of vitamin E is discussed.

\(^b\) For dietary purposes, vitamin E activity is expressed as mg RR-alpha-tocopherol equivalents (alpha-TE). To estimate the total alpha-TEs in a diet containing natural or synthetic forms of vitamin E, the number of mg tocopherols present must be multiplied by the following factors:

1 mg alpha-TE = mg RR-alpha-tocopherol \times 1.0
mg RR-beta-tocopherol \times 0.5
mg RR-gamma-tocopherol \times 0.3
mg RR-alpha-tocopherol acetate \times 0.91
mg RR-alpha-tocopherol succinate \times 0.81
mg all-rac-alpha-tocopherol \times 0.74
mg all-rac-alpha-tocopherol acetate \times 0.67

(Ref. 109 and other factors determined by calculation.)

optimal amount has been established with certainty. This is also, in part, due to the fact that the pharmacokinetics and the metabolism of vitamin E have not entirely been elucidated; neither is there a consensus on the level of the blood concentrations in normal and in deficient states. Hemolysis and a shortened lifetime of the erythrocytes have been reported at vitamin E plasma values of 0.5 mg/dl and concentrations below this value indicate the possibility of an existing vitamin E deficiency. The administration of alpha-tocopherol in persons with low vitamin E plasma levels (0.15-0.4 mg/dl) had a favorable effect in increasing the survival time of red blood cells. In contrast, the survival time of erythrocytes was shortened in persons who had received a diet low in vitamin E for 72 to 76 months. According to Hanck, the optimal human plasma concentration of vitamin E is between 1.0 and 1.5 mg/dl.

Although the U.S. National Research Council considers it sufficient if the daily total intake of vitamin E is 10 mg alpha-tocopherol equivalents (RDA-value), some physicians and scientists recommend significantly larger doses. Many persons take daily amounts of 10- to 100-fold of the RDA without the appearance of side effects. This may in part be related to incomplete resorption of the vitamin from the gastrointestinal tract as well as to decrease of the absorption rate that may occur with a high dietary intake of vitamin E.

The present review describes and evaluates—from a toxicological point of view—the results of animal experiments, and of individual human findings, which were reported by physicians and the results of scientifically planned and controlled studies on human subjects. Based on the doses, which were given in the individual trials, and on the resulting effects, reactions, and side effects, consideration is also given to the question of daily intake which will provide an optimal level of vitamin E without risking the development of undesired side effects.

2. ANIMAL EXPERIMENTS

Various derivatives of alpha-tocopherol, e.g. alpha-tocopheryl acetate were studied in animal experi-
ments for acute, subacute, subchronic and chronic toxicity, mutagenicity, carcinogenicity, teratogenicity, and reproductive toxicity. In particular, the following tests were carried out:

2.1 Examination for acute, subacute, subchronic and chronic toxicity

Frogs, rabbits, cats, dogs and monkeys showed no toxic effects whatsoever after oral application of 200 mg/kg vitamin E. According to Hanck, no toxic reactions were observed after oral application of high doses of vitamin E: 2,000 mg/kg in rabbits, 5,000 mg/kg in rats and 50,000 mg/kg in mice (see Table 2).

Studies for determining the toxicity after repeated oral application of vitamin E were also carried out; the duration of application was between 10 and 60 days (see Table 3). In addition to the studies listed in Table 3 with repeated applications over a short period of time, long-term toxicity tests have also been performed; these are summarized in Table 4.

In the examination of subchronic toxicity, free alpha-tocopherol and alpha-tocopheryl acetate were used. The vitamin E was applied orally or mixed with the feed; very different doses were tested. In the oldest studies, 4,000 mg/kg were given to rats over a period of 2 months without any toxic symptoms being noticed. Weissberger and Harris gave 10 or 100 mg vitamin E daily, mixed with the feed, from weaning until 19 weeks to rats and studied the metabolism of phosphorus in bone and several other tissues and compared this with rats which were fed a diet deficient in vitamin E. Vitamin E deficiency, as well as high dosage (100 mg/day/rat), was reported to cause a stimulation of phosphorus metabolism, whereas the intake of 10 mg/day vitamin E had no effect on phosphorus metabolism.

High doses of vitamin E in the form of all-rac-alpha-tocopheryl acetate (35 mg/kg diet and 25-, 50-, 100-, and 1,000-fold of this concentration) were given to rats for 13 weeks. Rats fed with the highest doses showed a lowered feed and protein utilization after 8 weeks. Thirteen weeks after the start of feeding, no changes were found in hemoglobin, serum cholesterol, urine creatine and creatinine, while the serum glutamate pyruvate transaminase (SGPT) was increased in the highest dosage group. The administration of the 25-fold and 50-fold of the normal vitamin E concentration in the diet caused no undesired effects whatsoever. These findings are confirmed by the animal experiments of Krasavage and Terhaar, who observed no undesired effects in the administration of 20, 2000 and 20,000 ppm RRR-alpha-tocopheryl-polyethylene glycol 1000-succinate (TPGS) in the feed of male and female rats for 90 days, on body weight, feed consumption, hematologic parameters and organ weights, serum constituents and micro-morphology.

Table 2. Studies on Acute Oral Toxicity of Vitamin E

<table>
<thead>
<tr>
<th>Species</th>
<th>Highest Dose* Tested without Toxic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>50,000</td>
</tr>
<tr>
<td>Rat</td>
<td>5,000</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2,000</td>
</tr>
<tr>
<td>Cat</td>
<td>200</td>
</tr>
<tr>
<td>Dog</td>
<td>320</td>
</tr>
<tr>
<td>Dog</td>
<td>200</td>
</tr>
<tr>
<td>Monkey</td>
<td>200</td>
</tr>
</tbody>
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<tr>
<th></th>
<th>Reference</th>
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<td>13</td>
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<td>15</td>
<td>15</td>
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</table>

* mg alpha-tocopherol/kg body weight.
Wheldon et al.\textsuperscript{19} fed all-rac-alpha-tocopheryl acetate to rats in daily doses of 500, 1,000 and 2,000 mg/kg body weight for 104 weeks. Hemorrhages were observed at the highest doses, in male rats only, from week 15 on, which could be made to disappear by administration of vitamin K. The prothrombin time was extended in the male rats of all three groups up to week 13, but not later.

The growth and survival rates of the experimental animals were not different from the control animals; slight effects on liver weights and a minor increase in the level of liver enzymes in serum were however noticed. The tumor rate also showed no difference from control animals, with the exception of a trend towards fewer tumors of the mammary glands (see Table 5).

Yang and Desai\textsuperscript{22} studied the effect of low, normal and high vitamin E concentrations in the diet of rats, where the diet contained 0, 25, 250, 2,500, 10,000 and
Table 5. Studies on Chronic Toxicity and Carcinogenicity of Vitamin E

<table>
<thead>
<tr>
<th>Species</th>
<th>Tocopherol Used</th>
<th>Route of Dosage</th>
<th>Dose</th>
<th>Duration</th>
<th>Study Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>All-rac-alpha-tocopheryl acetate</td>
<td>In feed</td>
<td>500, 1,000, 2,000 mg/kg/day (calculated)</td>
<td>104 weeks</td>
<td>No significant effects on clinical-chemical parameters, slight effects on liver weights and small increase of liver enzymes in serum, hemorrhages (anti-vitamin K effect), decrease of mammary tumors.</td>
</tr>
<tr>
<td>Rat</td>
<td>All-rac-alpha-tocopheryl acetate</td>
<td>In feed</td>
<td>0, 25, 250, 2,500, 10,000 and 25,000 I.U./kg</td>
<td>8–16 months</td>
<td>No abnormalities detected at low doses; at high doses increase of alkaline phosphatase, increase of relative heart and spleen weights, no change in prothrombin time.</td>
</tr>
</tbody>
</table>

25,000 I.U. vitamin E (all-rac-alpha-tocopheryl acetate) per kg. The rats were fed these diets for 8 to 16 months. The high doses (10,000 and 25,000 I.U. vitamin E/kg diet) led to a depression of body weight, to a decrease in the ash content of bone and to an increase in the relative weights of heart and spleen and of alkaline phosphatase. The prothrombin time was reduced after 12 months, while the hematocrit value at the highest dose was increased after 16 months. Erythrocytes of rats fed a vitamin E deficient diet showed spontaneous hemolysis in phosphate buffered saline solution, whereas the erythrocytes of rats fed normal or high amounts of vitamin E did not. The excretion of creatine and creatinine via the urine was normal for all rats except those which received the vitamin E deficient diet. These animals had a higher creatinine and a lower creatinine concentration in the urine. The authors conclude from their results that vitamin E deficiency, as well as excessive intake of vitamin E, may lead to undesirable side effects.

The effects of feeding high doses of vitamin E (220 to 2,200 I.U. all-rac-alpha-tocopheryl acetate/kg diet) were examined with the aid of several metabolic parameters in young chicks. Growth was not affected by 1,000 I.U. vitamin E/kg diet; there was, however, a growth depression with 2,200 I.U. kg/diet. 220 I.U./kg reduced the thyroidal uptake and release of $^{131}$I iodiine and the thyroidal hypertrophy following treatment with thiouracil. There was reduced bone calcification at high doses of vitamin E when calcium or vitamin E intake was also low, and the authors conclude that high doses of vitamin E also require higher doses of vitamin D. Dosage with 2,200 I.U. vitamin E/kg induced reticulocytosis and led to a reduced hematocrit value as well as to an extension of prothrombin time, which could be quickly eliminated by injection of vitamin K.

In a 13-week toxicity study, in which rats received 0, 125, 500 or 2,000 mg/kg alpha-tocopheryl acetate by gavage, no effects on body weights and feed consumption were noticed. The extremely high dose of 2,000 mg/kg led, however, to an increased relative liver weight in female animals. In male animals it led to longer prothrombin and thromboplastin times, to reticulocytosis and to a decrease in the hematocrit value and of the hemoglobin concentration. The high dosage caused hemorrhagic diathesis in both male and female animals. At the highest dosage (2,000 mg/kg body weight), an antagonistic effect between vitamin E and vitamin K was observed. In order to eliminate the hemostatic effects in the test groups, vitamin K was added to either the basal diet or to the drinking water. If supplementary vitamin K in rats receiving high doses of vitamin E was omitted or if the vitamin K doses were insufficient, hemorrhages occurred in the intestinal and urinary tracts, in the orbital cavity of the eye and in the meninges. These symptoms were rapidly reversible by sufficient vitamin K. None of these effects were observed at vitamin E levels of 125 mg/kg body weight but alterations in the lungs (interstitial inflammation and adenomatous hyperplasia) were found in all test groups.
Other individual observations with high doses of vitamin E relate to liver alterations, early sclerosis of the aorta, increased liver cholesterol levels and changed concentrations of fatty acids in the liver. In contrast, the application of 500 mg all-rac-alpha-tocopheryl acetate/kg of feed had only small effects on the liver triglycerides, but synergistic effects on the fatty infiltration of the liver induced by ethanol. The administration of high doses of vitamin E had no effect on the cholesterol level in the blood and in the tissues (liver, adrenal glands) of rats, which received 150 mg vitamin E concentrate orally every seventh day. High doses of vitamin E caused local sclerosis and deposits of cholesterol in the aorta.

An increase in mortality and a change in the prothrombin level in rats fed high doses of alpha-tocopherol and irradiated beef diet were found by Mellette and Leone. Furthermore, various authors reported on an increased demand for vitamins D and K as well as on an interdependence between vitamins E and K.

A more serious aspect of a chronic intake of high doses of vitamin E is the potential depression of prothrombin time and blood coagulation abnormalities. Interdependencies between the intake of high doses of vitamin E and vitamin K deficiency symptoms or an influence on blood coagulation caused by high doses of vitamin E, was observed in rats, dogs, chickens and humans. Blood coagulation alterations caused by vitamin E can be experimentally induced in rats and dogs by simultaneous administration of warfarin. Thus, the single consistent finding from all the studies reviewed here was an interaction between vitamin E and vitamin K, which must be regarded as a potentially serious complication of high oral dosages with vitamin E in combination with vitamin E deficiency or antithrombotic drug therapy.

2.2 Examination for mutagenicity

In order to study whether vitamin E has anticlastogenic effects, it was tested for mutagenicity in human lymphocytes in vitro. No indications for an induction of chromosomal damage or for an increase in the sister chromatid exchange rates were found in this test system.

Using the sex-linked recessive lethal test, Beckmann et al. showed in Drosophila that alpha-tocopheryl acetate in the nutrient medium (500 IU/kg) did not affect the mutation rate in irradiated male experimental animals, but caused—after mating with female animals raised on a vitamin-containing diet—a significant reduction of the sex-linked recessive lethal mutations in subsequent generations.

In bacterial cultures (Salmonella), vitamin E led to a significant decrease in point mutations after treatment with malonaldehyde or beta-propiolactone.

2.3 Examination for carcinogenicity

While older publications reported on a tumor-promoting effect of feeding wheat germ extracts or of the administration of synthetic alpha-tocopherol in rats and mice, other studies presented no evidence that oral intake of vitamin E is either tumor-promoting or carcinogenic. In fact, vitamin E has been shown to reduce skin tumors induced by 7,12-dimethylbenzanthracene or croton oil.

Wheldon et al. studied male and female rats in a long-term experiment (104 weeks); the animals received either a basal diet with 39 mg vitamin E/kg and 10 mg vitamin K/kg or the same diet with higher vitamin E concentrations (500, 1,000 or 2,000 mg/kg body weight/day). At the end of the trial, the type and frequency of the tumors were also compared between the individual groups. No relation between neoplastic effects and vitamin E treatment were found. A reciprocal relation existed in both sexes between the dose and the frequency of mammary fibroadenoma but was statistically significant only in the female animals (see Table 5).

2.4 Examination for teratogenesis and reproductive toxicity

Several experiments have been conducted to determine whether vitamin E has teratogenic properties. ISR-mice received daily doses of 591 I.U. RRR-alpha-tocopheryl acetate/30 g mouse by gavage (equivalent to approximately 1.18 × 10^7 I.U. for a woman of 60 kg weight) from days 7–11 of pregnancy; the control animals were either left untreated or they received the same volume of saline solution also by gavage. Of a total of 91 fetuses from 7 births, 1 fetus in the treated group was found to be malformed; no malformations were found among the 177 fetuses (13 births) of the untreated animals and among the 117 fetuses (8 births) of the animals treated with saline solution.

Martin and Hurley fed vitamin E doses of 22.5 to 2,252 mg all-rac-alpha-tocopheryl acetate/kg body weight/day to rats during pregnancy and lactation. Teratogenic effects were not observed and the survival rate of the young animals and the size and weight of the litter were unaffected. Several of the newborns, whose mothers had been treated with 500 mg vitamin E/day (2,252 mg/kg/day), showed delayed opening of the eyelids and other eye problems; the results were, however, not statistically significant when compared to control animals. The authors conclude...
Table 6. Studies on Teratogenesis and Reproductive Toxicity of Vitamin E

<table>
<thead>
<tr>
<th>Species</th>
<th>Tocopherol Used</th>
<th>Route of Dosage</th>
<th>Dose</th>
<th>Duration</th>
<th>Study Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>RRR-alpha-tocopherol polyethylene-glycol 1000-succinate</td>
<td>In feed</td>
<td>0, 0.002, 0.2 and 2% in diet</td>
<td>Days 6-16 of pregnancy</td>
<td>No differences between treatment and placebo group</td>
<td>18</td>
</tr>
<tr>
<td>Rat</td>
<td>RRR-alpha-tocopherol polyethylene-glycol 1000-succinate</td>
<td>Mating on days 112 and 175 (F₁₀ generation); F₀ generation treated until day 268</td>
<td>No effects with regard to reproductive toxicity; clinical and hematological findings normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>RRR-alpha-tocopherol</td>
<td>Gavage</td>
<td>591 I.U./30 g mouse</td>
<td>Days 7-11 of pregnancy</td>
<td>One malformation among 91 fetuses</td>
<td>47</td>
</tr>
</tbody>
</table>

cluded that vitamin E can be regarded as "relatively harmless" for the fetus.

In another experiment, 50 male rats were paired with 100 female albino rats 90-110 days old. The pregnant rats were divided into 5 groups (negative and positive controls, and three treatment groups which received feed containing 0.002, 0.2 or 2% RRR-alpha-tocopherol-polyethylene glycol 1000-succinate [TPGS], a water-soluble form of vitamin E). The diet was fed from day 6 to day 16 of pregnancy. At other times, all groups received the negative control diet. The dams were killed on day 20 of pregnancy; no substance-related changes were observed in the fetuses. Half the rats from a 90-day study were used for a reproduction study with TPGS; doses of 0, 0.002, 0.2 or 2% of this substance were fed to the experimental animals along with their feed. The rats were mated on day 112 for the F₁₀-generation and on day 175 for the F₁₁₀-generation. The reproduction indices (average time of gestation, litter size, sex ratio, mortality of the parents and offspring, internal soft-tissue and skeletal anomalies) showed no difference between the dietary groups. The F₀-generation was kept on their diet from day 265 to day 268 and then killed and examined histologically; clinical and hematological studies had been performed previously. Neither the examination of the organs nor the clinical and hematological examinations revealed any differences between the experimental animals and the controls (see Table 6).

3. OBSERVATIONS IN HUMAN SUBJECTS

If symptoms reported to be attributable to vitamin E deficiency or vitamin E overdosage are analyzed, one has to distinguish between individual cases and group observations, on the one hand, and those findings that are made based on planned studies which include placebo groups, on the other hand. Whereas clinical symptoms attributed to vitamin E deficiency have only rarely been seen, a number of clinical disorders have been linked to the intake of large doses of vitamin E and were reported primarily in letters to the editor. Since, however, the intake of placebo may also lead to side effects, the elucidation of a causal connection between intake and side effects is only possible with the aid of placebo-controlled double-blind studies.

Despite the fact that many reports on the occurrence of symptoms connected with the intake of vitamin E have not been documented in controlled clinical studies and despite the fact that large-scale studies have demonstrated that the frequency of side effects is no greater with vitamin E than with placebo, it is these unconfirmed reports that are frequently mentioned and further promulgated in the literature. Therefore, the following section (3.1) deals only with individual observations and with observations which do not meet proper scientific criteria for human studies; the results of controlled human studies with vitamin E intake are discussed in the subsequent section (3.2).

3.1 Individual cases and group observations

Warnings about the risks of high-dosage vitamin E intake go back to a commentary by H. Roberts, among others. He ascribed nearly 20 partly serious disturbances of health to the intake of large doses of vitamin E, such as thrombophlebitis, hypertension, thyroid dysfunction, hypercholesterolemia, hypertriglyceridemia, alterations in the hormone status, muscle weakness, headaches, vaginal bleeding, stomatitis, urticaria, retarded wound healing, vertigo, diarrhea, intestinal cramps, gynecomastia and breast symptoms. Levy and Boas and Anderson reported on non-specific symptoms such as headaches and intestinal cramps, spells of dizziness, giddiness, and slight constipation in heart patients who received high doses of vitamin E (400-2,400 and up to 3,200 I.U. of vitamin E daily), even though Anderson pointed out that similar symptoms were also reported by patients who had only received placebo. The appearance of fatigue, muscular weakness, exhaustion and creatin-
Tolerance and safety of vitamin E

3.2 Controlled clinical studies (placebo groups, single- and double-blind studies)

The adverse effects reported in numerous uncontrolled studies which have been related to the intake of vitamin E, could, in most cases, not be reproduced in controlled studies and are therefore not considered to be relevant. The experimental design and the results of controlled studies are discussed in the following section. These studies included placebo groups and it is known that "placebo therapy" can also lead to considerable side effects. In order to clarify the warning about risks of high-dosage vitamin E published by various authors, placebo-controlled studies are a prerequisite. A detailed examination of the original reports and the case studies showed that the assumption of a causal relationship between certain symptoms and vitamin E intake was not justified when strict scientific criteria were applied. In no case was there a controlled study in which side effects could be causally related to vitamin E dosage. The controlled double-blind studies discussed below led to entirely different results and conclusions.

In 6 controlled double-blind studies with placebo, in which doses of 600–3,200 mg vitamin E were taken daily for 3 weeks to 6 months, few specific side effects were observed. In order to determine the tolerance of vitamin E, 202 randomized healthy subjects received 600 I.U. all-rac-alpha-tocopheryl acacetate/day or placebo orally for 4 weeks in a double blind trial. No effects with respect to performance, sexuality or general well-being were observed. Muscle weakness and indigestion did not occur. There was also no effect on prothrombin time, total leucocyte count or on the activity of serum creatinine phosphokinase. The serum cholesterol level was slightly increased, but this increase was not statistically significant. The serum triglyceride level was significantly increased in women. Gastrointestinal tolerance was very good and not different from the placebo group. The serum level of thyroid hormones (T/ and T4) was reduced in men, and in women who had not taken oral steroidal contraceptives.

In a 16-week double-blind study, 30 healthy adults received daily doses of 800 I.U. vitamin E or placebo. No differences in the side effects were observed between the vitamin E dosed and the placebo group; in particular, no effect on plasma lipids was found.

Fourteen healthy male college students, 24–25 years of age, took 600 mg (900 I.U.) RRR-alpha-tocopherol daily over a 12-week period. Five students were given a placebo and the single-blind method was used. The levels of vitamin E in plasma, erythrocytes, leucocytes, thrombocytes, and oral mucous membrane were determined. The individuals were asked about their subjective complaints and numerous clinical parameters were measured. The amounts of alpha-tocopherol in blood plasma and in red and white blood cells reached their maximum about four weeks.
after the beginning of the vitamin intake and remained at that level until the end of the treatment, whereas the maximum level in blood platelets and oral mucous membranes was reached 12 weeks after the beginning of the intake. Concentrations were 2.5 to 3 times higher than those measured initially. Values determined in the control group did not differ from the test group. When the intake of tocopherol was stopped, concentrations of alpha-tocopherol in plasma and blood cells dropped almost to their initial values within one week. Alpha-tocopherol levels in buccal mucosal cells remained elevated one month after cessation of vitamin E supplementation.

During the intake of tocopherol there were no changes in the functions of the thyroid gland, liver and kidney, which were assessed by measurement of thyroid hormones (T₃, T₄ and thyroid stimulating hormone [TSH]) and various enzymes such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and creatine phosphokinase (CPK), and blood urea nitrogen (BUN). Furthermore, prothrombin time was not found to be affected. The amount of red and white blood cells, platelets, and the level of hemoglobin did not show any significant changes. The intake of high doses of vitamin E did not lead to a change in the level of cholesterol, phospholipid, triglyceride and total lipid in blood plasma. The test subjects were questioned about their performance or general well-being. No significant differences were found between vitamin E users and those who had not taken vitamin E with respect to clinical, hematological, and biological parameters. Serum glutamate-oxaloacetate transaminase (SGOT) was the only one of the 21 parameters measured which was higher in men who had taken vitamin E, than in controls. The differences in the other parameters were within standard deviation.

Laboratory analysis demonstrated clearly that the intake of high doses of vitamin E leads to an increased concentration of tocopherol in blood, blood cells and tissue cells. The maximum level, which was 2.5 to 3 times the baseline values, was reached in four weeks and did not rise further throughout continued intake. From this it can be concluded that either resorption decreases or excretion increases after the saturation point is reached. The fact that no adverse effects occurred despite continued high intake indicates that RRR-alpha-tocopherol is well tolerated.

No significant side effects were found in a double-blind crossover study involving 52 patients, who received daily amounts of 1,600 I.U. RRR-alpha-tocopheryl succinate over 6 months. Twenty-five clinically healthy persons, 15 diabetics and 15 patients with chronic coronary heart disease received 2,000 I.U. all-rac-alpha-tocopheryl acetate for two weeks. In a second experiment of the same study, 25 diabetics were treated for six weeks with 2,000 I.U. vitamin E or received placebo under a double-blind crossover study design. Neither weakness and fatigue nor thrombophlebitis were observed as a consequence of the vitamin E treatment. Diabetics showed a decrease in serum glucose, serum cholesterol, triglycerides and total lipids in the two-week study. At the end of the six-week study, serum glucose and serum cholesterol of diabetics were also reduced but triglycerides and total lipids showed an increase while HDL remained constant. Thyroid hormone levels were measured and were identical in groups of treated patients as well as in the placebo group.

In a placebo-controlled double-blind study with 77 patients, who received 200 mg/day all-rac-alpha-tocopheryl nicotinate or lactose (placebo), a frequency of side effects of 11.2% was found in the group receiving tocopherol. The placebo group in the study, on the other hand, showed a frequency of side effects of 19%. These were, however, only mild symptoms. This agrees with the results of studies by other authors. Moreover, in other large studies which were not conducted according to the strict principles of double-blind studies, side effects due to administration of vitamin E were rarely observed (see also Tables 7 and 8).

Hale et al. studied the occurrence of clinical abnormalities and examined numerous laboratory parameters of 369 persons, who were vitamin E "users," as compared to 1,861 persons who were "non-users": all were older than 65 years. No significant differences were found between vitamin E users and those who had not taken vitamin E with respect to clinical, hematological, and biological parameters. Serum glutamate-oxaloacetate transaminase (SGOT) was the only one of the 21 parameters measured which was higher in men who had taken vitamin E than in controls. The differences in the other parameters were within standard deviation.

Ten healthy male volunteers, 25–39 years old, received daily doses of 800 mg alpha-tocopherol for 4 weeks. Prior to the start of medication, 2 weeks later and at the end of the 4-week medication period, blood and plasma viscosity, hematocrit, erythrocyte aggregation, colloidal osmotic pressure, the number of leucocytes and erythrocytes as well as the hemoglobin level, total lipids in serum and the concentration of alpha-tocopherol in plasma were determined. Compared to the initial values, the flexibility of erythrocytes and tocopherol levels in serum increased significantly during and after therapy. None of the other parameters showed a significant change over the corresponding initial value.

According to a review of trials on over 9,000 persons who had taken up to 3,000 I.U./day of vitamin E over a period of up to 11 years and 55,000 I.U. for a
few months in a few subjects, no significant adverse effects were observed or no mention of side effects was made. Slight disturbances of well-being were reported by only 80 of about 1,000 additional patients. Therefore, the incidence of undesirable side reactions, mostly mild gastrointestinal complaints, of high-dosage vitamin E therapy was 0.8% of over 10,000 cases reviewed.

Based on the results of numerous controlled clinical studies, in which vitamin E was administered at various dosage levels for extended periods of time, it can be concluded that vitamin E is well tolerated, since undesirable side effects which can be definitely attributed to the intake of vitamin E were induced only in very few cases. Many effects which were reported in several trials could not be reproduced in similarly designed studies, making it impossible to decide if there was indeed a causal relationship. Many symptoms observed in groups receiving vitamin E were also seen in placebo groups. A confirmed side

### Table 7. Studies with Oral Vitamin E in Human Subjects Without Strict Controls

<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>Dose and Substance</th>
<th>Duration</th>
<th>Study Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 adult volunteers</td>
<td>100–800 I.U./day tocopherol</td>
<td>4 months to 21 years (mean 2.9 years)</td>
<td>No evidence of toxicity (clinical-chemical blood parameters and blood coagulation, liver enzymes, thyroid hormones and others were studied)</td>
<td>63</td>
</tr>
<tr>
<td>369 “users” compared to 1,861 “non-users” (over 65 years of age)</td>
<td>Not specified</td>
<td>2 years</td>
<td>21 clinical-chemical parameters were determined and general clinical examinations were carried out, only SGOT was slightly higher in “users” than in “non-users”</td>
<td>75</td>
</tr>
<tr>
<td>10 healthy volunteers</td>
<td>800 mg/day all-rac-alpha-tocopheryl acetate</td>
<td>4 weeks</td>
<td>No adverse effects (clinical-chemical blood parameters determined)</td>
<td>78</td>
</tr>
<tr>
<td>12 warfarin-treated cardiology patients</td>
<td>100–400 I.U. all-rac-alpha-tocopherol</td>
<td>4 weeks</td>
<td>Warfarin effect aggravated (vitamin K-antagonism)</td>
<td>30</td>
</tr>
</tbody>
</table>

### Table 8. Double-Blind Controlled Studies with Oral Vitamin E in Human Subjects

<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>Dose and Substance</th>
<th>Duration</th>
<th>Study Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 heart patients (38 treated, 37 placebo)</td>
<td>200 mg/day all-rac-alpha-tocopheryl nicotinate or placebo</td>
<td>4–6 weeks</td>
<td>No significant differences between the treatment and the placebo group (general clinical examination), general symptoms of vascular diseases frequent but not more than in the placebo group</td>
<td>74</td>
</tr>
<tr>
<td>202 healthy volunteers (104 treated, 98 placebo)</td>
<td>600 I.U./day all-rac-alpha-tocopheryl acetate or placebo</td>
<td>4 weeks</td>
<td>Serum thyroid hormones (T₃ &amp; T₄) lowered, serum triglycerides increased in women, cholesterol slightly increased but not statistically significant; no other effects on prothrombin time, leukocytes, etc.</td>
<td>69</td>
</tr>
<tr>
<td>30 healthy volunteers (15 treated, 15 placebo)</td>
<td>800 I.U./day vitamin E or placebo</td>
<td>16 weeks</td>
<td>No significant differences between the treatment and the placebo group</td>
<td>73</td>
</tr>
<tr>
<td>19 healthy subjects (14 treated, 5 placebo)</td>
<td>600 mg (900 I.U.)/day RRR-alpha-tocopherol or placebo</td>
<td>12 weeks</td>
<td>No objective or subjective side effects (clinical-chemical blood parameters; thyroid, liver and kidney function; serum lipids; prothrombin time, blood cells)</td>
<td>110</td>
</tr>
<tr>
<td>52 angina pectoris patients (crossover)</td>
<td>1,600 I.U./day RRR-alpha-tocopherol succinate or placebo</td>
<td>6 months</td>
<td>No significant differences between the treatment and the placebo group (clinical-chemical blood parameters, prothrombin time and urinary parameters were studied)</td>
<td>70</td>
</tr>
<tr>
<td>25 diabetics (crossover)</td>
<td>2,000 I.U./day all-rac-alpha-tocopheryl acetate or placebo</td>
<td>6 weeks</td>
<td>No side effects such as weakness, fatigue, thrombophlebitis (lipids, cholesterol, glucose, T₃, T₄ and blood coagulation were studied)</td>
<td>71</td>
</tr>
<tr>
<td>36 angina pectoris patients (18 treated, 18 placebo)</td>
<td>3,200 I.U./day RRR-alpha-tocopherol succinate or placebo</td>
<td>9 weeks</td>
<td>No side effects except for isolated cases of gastrointestinal disorders (intestinal cramps, diarrhea)</td>
<td>72</td>
</tr>
</tbody>
</table>
effect of vitamin E seems to be its anticoagulant effect which occurs if vitamin K-antagonists (warfarin) are given simultaneously. This is not observed in healthy subjects.

The intake of high doses of vitamin E increases the anticoagulant activity of vitamin K-antagonists like warfarin and is therefore contraindicated during oral anticoagulant therapy.30

3.3 Clinical symptoms (side effects) following the intake of high doses of vitamin E

The symptoms or side effects, which have been associated with the intake of high doses of vitamin E, are discussed in detail in the following sections and the relevance of each of these symptoms is evaluated with regard to vitamin E intake.

3.3.1 Breast symptoms. Roberts61 reported on 7 women (15% of a group with thrombophlebitis), who complained of sore breasts while taking <400 to >800 I.U. vitamin E/day over a period of more than 1 year. Breast pain disappeared in all 7 women when vitamin E administration was discontinued. No reference to breast pain was made in all the double-blind studies with vitamin E and in the more than 10,000 cases which were analyzed by Salkeld86 as discussed by Bendich and Machlin.80 Other authors reported that vitamin E treatment has a favorable effect on benign breast disease.77 Since breast pain did not occur as a side effect in the double-blind studies, the relationship between vitamin E intake and this symptom—suggested by Roberts—is questionable.

3.3.2 Creatine metabolism. With regard to creatine metabolism in relation to vitamin E intake, different findings have been described. Some authors report on creatinuria and an elevated serum creatine kinase activity; others report on the fact that an existing elevated creatine excretion was reduced when vitamin E was taken. No effects on creatine and creatinine metabolism were found by other authors.

3.3.2.1 Increased values. In the double-blind study described by Briggs in a letter to the editor,53 in which 800 I.U. RRR-alpha-tocopheryl acetate were given daily over 3 weeks, creatinuria and an increased level of serum creatine kinase were observed in 2 out of 8 healthy young men. These 2 subjects also reported fatigue and weakness. A 41-year old physician took 296 g alpha-tocopherol over a period of 93 days and developed a statistically significant, but reversible creatinuria.55 According to Salkeld,76,80 an increased creatine level in urine developed in 53 athletes after taking 50–300 mg alpha-tocopherol/day for 3 weeks; this level was, however, within the normal standard deviation.

3.3.2.2 Lowered values. In patients who suffered from creatinuria due to primary fibrositis, creatine excretion was reduced by the intake of vitamin E. A reduction in creatine excretion was also found in alcoholics, or in patients with malabsorption, after vitamin E intake (200 mg all-rac-alpha-tocopheryl acetate for 3 to 7 weeks).79,80

3.3.2.3 Unchanged values. Two large studies on the effects of vitamin E showed no significant effect on creatine or creatinine. In the studies of Farrell and Bieri63 on the health status of 28 adults, who had received large amounts of tocopherol (100–800 I.U./day) over a period of approximately 3 years, serum creatinine levels and plasma creatine phosphokinase activities were found to be normal. In Tsai’s double-blind study69 with 202 college students, who had taken 600 I.U. all-rac-alpha-tocopheryl acetate daily for 28 days, no changes in serum creatine phosphokinase activity were found.

Due to these varying results and the fact that several variables can influence the excretion of creatine, it must be concluded that vitamin E has no significant effect on these biochemical measurements. The sporadic observations of increased creatine levels could perhaps be due to the activity of the person tested, and analytical or statistical errors may also have contributed to the uncertainty of some of the results reported.

3.3.3 Emotional disturbances. Kligman56 observed an “unusually high prevalence of emotional disorders” in an unspecified number of women who had taken daily doses of 800 I.U. vitamin E. The general reactions included depression. “Tiredness” withdrawal, mood swings, loss of confidence, and other emotional symptoms. Kligman admits, however, that a causal connection between vitamin E intake and the observed symptoms is not proven and this opinion was given without the benefit of a controlled study. No similar effects were reported in any of the double-blind studies or in any of the other large studies that were designed to examine the effects and side effects of vitamin E intake, making relationships as suggested by Kligman very doubtful.

3.3.4 Fatigue, exhaustion and/or muscular weakness. Cohen52 reported in an uncontrolled study that severe weakness and fatigue occurred approximately 7 days after intake of 800 I.U. RRR-alpha-tocopherol/day in himself, his partner and several other healthy male subjects as well as in several patients with arteriosclerotic heart disease. These reactions
ceased, however, when the administration of vitamin E was interrupted. No symptoms of fatigue appeared in the same patients when 400 I.U. vitamin E were taken.

Kligman\textsuperscript{56} observed general fatigue in women who had taken 800 I.U. vitamin E per day. Briggs\textsuperscript{53} reported the same symptom in 2 out of 8 subjects who took the same dosage. Hillmann\textsuperscript{55} described muscle weakness which occurred in a male who had taken 296 g of all-rac-alpha-tocopheryl acetate over a period of 93 days.

Other authors who had treated hundreds of patients over a period of ten years, have not observed a single case of muscular weakness or fatigue in persons who had taken 400–1,600 mg vitamin E daily.\textsuperscript{81} Symptoms of fatigue were not observed in controlled double-blind studies with vitamin E except in the small study described by Briggs.\textsuperscript{53} According to the literature prior to 1975, which has been thoroughly reviewed by Salkeld, the incidence of symptoms of fatigue was very low. As reported by Bendich and Machlin,\textsuperscript{80} Salkeld\textsuperscript{86} noted in several studies on vitamin E that symptoms of tension rather than fatigue were described. He concluded that it is difficult to explain the reports on symptoms of fatigue and weakness after intake of vitamin E. He believes that there is no relation between vitamin E intake and these symptoms. Fatigue is a subjective feeling, which can stem from a large number of effects, including emotional and psychological factors.

3.3.5 Gastrointestinal symptoms. In a double-blind study with 18 subjects, who had received 3,200 I.U. vitamin E/day or placebo for 9 weeks, intestinal cramps were observed in one patient and diarrhoea was seen in three subjects.\textsuperscript{72} Gastrointestinal symptoms also occurred in a man who had taken a total of 296 g all-rac-alpha-tocopheryl acetate over a period of 93 days.\textsuperscript{55} Only one case of gastrointestinal disturbance was found among 55 subjects who received 2,000 mg vitamin E daily.\textsuperscript{71} Briggs\textsuperscript{54} reported 6 clinical cases of gastrointestinal irritation after the intake of vitamin E in wheat germ oil. Salkeld\textsuperscript{80} noted that minor attacks of vertigo or gastrointestinal disturbance occurred in 3.5% of 400 patients with intermittent claudication who had taken 400 I.U. RRR-alpha-tocopheryl acetate daily for 5 years. The symptoms disappeared when the medication was discontinued. Three out of 8 patients with angina pectoris who had been treated with 200–800 I.U. all-rac-alpha-tocopheryl acetate daily for 3 to 10 weeks complained of constipation and disturbance of the epigastric region, especially if the dosage was increased to 800 I.U. Three out of 126 heart disease patients, who received daily doses of 300 to 600 I.U. all-rac-alpha-tocopheryl acetate, developed diarrhoea. From these findings it can be concluded that a minor impairment of gastrointestinal activity can occur in a few individuals after intake of high doses of vitamin E, but lowering the dosage can usually be expected to eliminate this.

3.3.6 Leukocyte function. In a study lacking adequate controls,\textsuperscript{82} in which 18 male subjects participated, all-rac-alpha-tocopheryl acetate (300 mg daily for 3 weeks) caused lower cell-mediated immunity, as measured in vitro (mitogen-induced lymphocyte transformation test), and depression of the bactericidal activity in vitro of lymphocytes in less than half of the subjects examined. The test of cell-mediated immunity in vivo (delayed hypersensitivity of the skin) was, however, not affected by the intake of vitamin E in the same group of patients. The author had no explanation for the discrepancy between the unchanged results for the cell-mediated immunity in vitro and the changes in cell culture, using the same reagents in both test procedures. The delayed hypersensitivity is, however, regarded as a more relevant indicator of immune function than the in vitro mitogen test.\textsuperscript{83} Thus, it may be assumed that the intake of vitamin E does not affect immune reactions which are based on the function of leucocytes.

3.3.7 Pathologic hyperthermia. James\textsuperscript{60} reported in a letter to a journal that a single case of pathologic hyperthermia was related to the daily intake of 400 I.U. alpha-tocopheryl acetate. However, in a later communication James retracted his opinion and favored a genetic predisposition for the development of this disease. No other case exists in which pathologic hyperthermia was related to the intake of vitamin E.\textsuperscript{60}

3.3.8 Thrombophlebitis. Roberts\textsuperscript{49,61,85} has repeatedly reported on a possible relationship between the intake of vitamin E (<400 to >800 I.U./day) and the development of thrombophlebitis. He assumed that vitamin E can promote thrombosis in patients with predisposition. Other authors have not found any relationship between thrombosis and the intake of vitamin E, nor have symptoms of this type been seen in double-blind studies or in other open studies.\textsuperscript{63,71} In contrast, the intake of 1,600 I.U. vitamin E/day reduced the biosynthesis of thromboxane, thereby lowering the potential for thrombosis development.\textsuperscript{86}

3.3.9 Level of thyroid hormones. In a double-blind study with 202 college students who had received 600 mg all-rac-alpha-tocopheryl acetate daily for 28 days, a significant decrease of the serum thyroid hormones (T\textsubscript{3} and T\textsubscript{4}) was found in men, and in women who had not taken oral steroidal contraceptives. Women who
had taken oral contraceptives did not show this effect. In another double-blind study, in which much higher doses were given (2,000 I.U./day), no effects on the levels of thyroid hormones were found. The discrepancy between the two reports may be due to the fact that the second study involved fewer subjects, even though the vitamin intake was three times higher. The study by Tsai involved normal subjects, while in the study by Farrell, vitamin E was given to women in different amounts. In general, the combination of vitamin E and vitamin K has no effect on the concentration of I and II, but in most studies no significant effects were observed.

3.3.10 Mortality. The mortality rate of a population which was deliberately chosen to be apparently healthy was higher for that part of the population which had not received vitamin E supplements or received vitamin E doses of more than 1,000 mg/day, compared to a population which had received doses of 100–999 mg/day. If, however, a longer time period was considered, the mortality rate of those who had taken 1,000 I.U. per day was similar to or lower than the mortality rate of those who had taken daily doses of 100–999 mg vitamin E. While these findings cannot prove that the intake of vitamin E prolongs life span, there is nevertheless no doubt that intake of vitamin E does not decrease life span.

3.3.11 Serum lipids and lipoprotein levels. The effects of vitamin E on serum lipids and on lipoproteins vary, but in most studies no significant effects were observed. In 8 out of 10 studies, in which vitamin E was given in doses of 400–2,000 mg/day, no significant changes in the total cholesterol levels were noticed. In only one of three studies, there was a moderate increase in cholesterol which was statistically significant. The effects of vitamin E doses of 400–800 mg/day on lipoproteins have been examined in several studies. Five studies reported no effect on the high-density lipoproteins (HDL). Several others showed an increase. The concentration of triglycerides was not significantly increased by the daily intake of 400–2,000 mg vitamin E in 4 out of 7 studies. In several other studies triglycerides showed a trend to an increase. While in another study the increase was limited to women and was only slightly statistically significant (p < 0.05). Although the findings on serum lipids and on the lipoproteins vary and are, in part, contradictory, it cannot be entirely concluded that one or more lipid fractions may be affected by long-term intake of high doses of vitamin E if there is a pre-existing condition or disease (such as diabetes).

3.3.12 Blood coagulation and vitamin K-dependent coagulation factors. The oral administration of high doses of vitamin E did not cause changes in blood coagulation in healthy subjects. Intake of large amounts of vitamin E can, however, aggravate coagulation defects in persons with vitamin K deficiency. Vitamin K deficiency occurs as a complicating factor in malabsorption syndromes or if anticoagulants such as warfarin are administered. Warfarin is a vitamin K-antagonist which can change the coagulation of blood similar to vitamin K deficiency. In one study, either 100 or 400 mg/day of vitamin E were given for a period of 4 weeks to 6 patients with cardiac disease who had already taken warfarin for a longer period of time. The combination produced no statistically significant change in prothrombin time, no sign of ecchymosis and had no effect on the vitamin K-dependent coagulation factor II. The patients did not develop clinical bleeding. On the other hand, found a prolonged prothrombin time and ecchymosis in one older, debilitated patient, who, in addition to warfarin and clofibrate, took daily doses of vitamin E up to 1,200 I.U. The hemorrhagic symptoms disappeared after discontinuation of the vitamin E therapy.

In 19 patients with cystic fibrosis who received different amounts of vitamins to compensate for their nutritional malabsorption, it was shown that those who received vitamin E (100–200 mg/day), but not vitamin K, developed a more significant coagulopathy than those who received either both vitamins or none. It was suggested that vitamin E might inhibit the formation of the vitamin K-dependent coagulation factors. Zipursky et al. found in a randomized double-blind study that blood parameters of the vitamin K-dependent coagulation factors in premature babies could not be influenced by oral doses of vitamin E (25 mg/day). Detailed studies of the plasma coagulation factors were carried out after 1 to 6 weeks of therapy; none of the factors were significantly different between the treated children and a control group. Neuroblastoma patients who received vitamin E by intravenous infusion showed prolonged prothrombin and thromboplastin times. Blood levels of vitamin E had increased to 11.4–12.8 mg/dl which is approximately 10 times the normal value. The coagulation disturbance was shown to be reversed by administration of vitamin K. In all other reports on human trials, in which the vitamin K status was intact, oral application of vitamin E had no effect on coagulation. Accordingly, vitamin E only af-
fects the coagulation parameters if vitamin K deficiency is also present. This means that vitamin E must not be given under those circumstances or that administration of vitamin E must be combined with additional administration of vitamin K.

3.3.13 Assessment of clinical symptoms. In summary, only a small number of side effects were observed for oral intake of large amounts of vitamin E in those human studies which used the double-blind method as well as in other large studies that were less well controlled, even with large doses up to 3,200 USP-Units/day. While individual case reports or uncontrolled studies reported side effects, these observations could not be confirmed in larger or better controlled studies. Side effects such as breast symptoms, emotional depression, malignant hyperthermia and thromboembolism were only reported by single authors or test groups and only under uncontrolled conditions. Creatinuria was observed in a small double-blind study, but other, larger studies did not confirm this effect. Symptoms of fatigue and/or muscular weakness are rare; they were, however, noted once in a small double-blind study. Whereas a large double-blind study showed changes in the levels of the thyroid hormones, the same effect was not found in a second double-blind study in which higher doses were administered. Effects of vitamin E on serum lipids and on lipoproteins have also been reported, but the results are contradictory and depend, in part, on the initial values; some studies show positive effects, other studies negative effects, and yet others found no effect at all. Oral application of vitamin E can aggravate coagulation disorders which are due to vitamin K deficiency—either caused by malabsorption or by anticoagulant therapy—meaning that high doses of vitamin E are contraindicated in patients under these conditions. Vitamin E by itself has, however, not caused any coagulation abnormalities in persons without vitamin K deficiency. In conclusion, it can be said that vitamin E appears to be remarkably safe when taken orally by adults.

3.4 Clinical symptoms (side effects) during "therapeutic" application

Vitamin E is used prophylactically or therapeutically by some physicians to prevent or treat chronic muscle tension, muscle degeneration and rheumatic joint disease (chronic polyarthritis). Sometimes very large doses are used, leading to reported side effects which can provide information about the toxic effects of overdosage with vitamin E. Studies which were carried out with these patients may therefore be analyzed with respect to dosage and presence or lack of symptoms. The expected beneficial effects on the disease are disregarded in the present assessment.

Twenty-six patients with active arthroses were treated with vitamin E (400–1,200 I.U. RRR-alpha-tocopheryl acetate/day) in 6 centers as part of a multicenter placebo-controlled double-blind study.100 The tolerance to the vitamin E preparations was very good. Somewhat more side effects were found in the placebo group than in the group receiving vitamin E. Twenty-four patients with spondylitis ankylosa (Bechterew’s disease) were treated for 12 weeks with a vitamin E preparation (400 mg/day RRR-alpha-tocopheryl acetate) or diclofenac-sodium (50 mg/day) in a randomized double-blind fashion.101 Again the tolerance to the vitamin E preparation was very good.

Vitamin E has also been used for athletes with injured joints—mainly knee-joints—and in the treatment of acute irritating reactions or during rehabilitation.102 Athletes with injured joints were treated with 3 times 500 mg RRR-alpha-tocopheryl acetate during an entire summer season or initially for 10 days with 3 times two capsules (3,000 mg/day) followed by 3 times one capsule (1,500 mg/day). No side effects were reported in the 19 cases described. Twenty patients with recurrent spinal syndrome (spondylogenic irritative syndrome) received 375 mg alpha-tocopheryl acetate/day for 2 to 21 months. No subjective or objective side effects were noted.103 The tolerance to the vitamin E preparation (375–1,125 mg alpha-tocopheryl acetate/day) was studied in a multicenter pilot study in 8 centers with a total of 60 patients with degenerative spinal and joint disease and with extra-articular rheumatic disease.104 Tolerance was very good. Thirty-one patients with Scheuermann’s disease of stages I–III were treated for 6 weeks with high doses of all-rac-alpha-tocopheryl acetate (1,125 I.U. on day 1, 750 I.U. from day 2 to 6 and then 375 I.U. for 5 weeks). No side effects occurred and the tolerance was very good.105 It follows from these studies that relatively high doses of vitamin E are tolerated in most cases without apparent adverse reactions.

4. SAFETY OF A RANGE OF VITAMIN E INTAKES

There is a wide range of recommended human intakes of vitamin E because those making the recommendations have different nutritional goals. These range from the small amount of the vitamin needed to prevent deficiency, through the somewhat larger amount thought to be necessary to prevent degenerative diseases, to the very large amounts that are said to have “therapeutic” effects. Bearing in mind the contradictory, and often anecdotal, reports on side effects
of vitamin E, it is difficult to recommend with certainty, the level of vitamin E intake that will be optimal for metabolic processes. Intake and safety data, based on the scientific literature, have been merged in Figure 2 which allows conclusions to be drawn regarding the optimal human vitamin E intake.

Since tocopherols are vitally important substances, which by definition as vitamins cannot be synthesized in animals or in humans, they must be taken up with the food. It follows that there must be a minimum requirement and if this requirement is not met, deficiency symptoms may be expected. The recommendation of the German Society for Nutrition (DGE) with regard to the desirable amount of vitamin E intake is based on work by Schmandke and Proll who proposed a minimal daily requirement of 6 mg RRR-alpha-tocopherol, taking into consideration an intake of approximately 7 g polyunsaturated fatty acid (PUFA). However, the expected average daily consumption of PUFA is 16.2 g/day and the difference of 9.2 g PUFA must be compensated by 0.5 mg alpha-tocopherol equivalents/g PUFA. Since there is a loss of about 10% tocopherol in food preparation, a safety margin is added to the calculated amount. Thus, the minimal requirement (6 mg), the PUFA-compensation (4.6 mg) and the safety margin (> 1.1 mg) result in a value of approximately 12 mg RRR-alpha-tocopherol equivalents (alpha-TE) recommended by the DGE. The “arbitrary but practical allowance” of 8–10 mg alpha-TE/adult/day with an additional requirement of 2–4 mg/day during pregnancy and lactation as recommended in the USA by the Food and Nutrition Board of the National Research Council agrees very closely with the DGE and with the WHO recommendations. According to the WHO report of the 30th meeting of the FAO/WHO Expert Committee on Food Additives in 1986, the ADI-value (Acceptable Daily Intake) was established for human subjects as 0.15 to 2 mg alpha-tocopherol/kg body weight. If the lowest ADI-figure is used for an adult of 75 kg, a value of 11 mg results which is nearly identical to the recommendations cited above. The upper ADI-limit gives a value of 100–150 mg depending on body weight. Therefore, the range between 10 mg and 150 mg alpha-tocopherol is characterized as “absolutely safe” from a toxicological point of view as shown in Figure 2.

The daily dose which is not expected to cause side effects is also specified in the same WHO-report. Clinical studies have shown that intakes of up to 720 mg alpha-tocopherol/day are tolerated without side effects (“range without side effects” in Figure 2). Higher doses are also used for “therapeutic” purposes. Depending on the disease and on the purpose of the therapy, this “therapeutic” range starts at several hundred mg/day and ends at approximately 1,600 mg/day. At doses above 720 mg and in particular at doses of 1,600–3,000 mg/day, side effects can occur with extended intake and depend on the initial condition and disease of the patient; among them are gastrointestinal complaints, creatinuria and impairment of blood coagulation, which are, however, generally not severe and which subside rapidly on reducing the dosage or on discontinuing the admin-
istration of vitamin E. For very high doses, the physician must balance the "therapeutic" effects of vitamin E and the possible side effects. Thus, the entire range from the minimal requirement up to a dose of approximately 3,000 mg/day can be considered as a safe range. There is a risk of adverse effects above intakes of 3,000 mg vitamin E per day.

5. DISCUSSION

Studies on experimental animals have shown that vitamin E (alpha-tocopherol or alpha-tocopheryl esters and the corresponding homologs and isomers) has a very low acute and chronic toxicity when given orally. Tests for mutagenicity and carcinogenicity were negative; in contrast, anti-mutagenic effects and inhibitory effects of vitamin E on tumor-inducing properties of certain chemicals were reported but these effects must still be confirmed. In addition, examination for teratogenesis and studies on reproductive toxicology did not provide any indications for toxic effects of vitamin E. Vitamin E plays a special role as a protecting factor for membranes of cells and organelles; it protects lipids against oxidation and affects a variety of enzyme systems, although this may be an indirect effect via associated membranes. Whereas symptoms of vitamin E deficiency can be easily demonstrated in animal experiments, symptoms of overdosage in animals are rather unspecific except the antagonistic properties of vitamin E toward vitamin K. A prolonged prothrombin time and changes in blood coagulation as well as an interrelation between high doses of vitamin E and symptoms of vitamin K deficiency have been described in several animal species. Certain effects on the metabolism of lipids have also been observed at high vitamin E doses.

Different symptoms, which were unspecific in most cases, have been described in human subjects at high doses of vitamin E. In particular, in publications of single cases and in group reports, in which vitamin E preparations were taken under uncontrolled conditions, a variety of symptoms was attributed to the intake of vitamin E. These reports have to be evaluated critically, however, since in-depth analyses of these findings cannot demonstrate a clear causal relationship; the symptoms are contradictory and are frequently associated with the patient taking other medications or even with placebo.

In placebo-controlled studies, which were sometimes also conducted double-blind, side effects were seen much less frequently. Side effects were often observed in both the placebo group and in the group receiving vitamin E. Side effects were only rarely reported when administration of vitamin E was given "therapeutically" under chronic disease conditions of musculature and joints.

Roberts associated thrombophlebitis and disorders of thyroid function with the intake of high doses of vitamin E and Tsai et al. described an impairment of thyroid function. Other authors did not, however, observe these effects in controlled studies. Similarly, reports are contradictory on an increased excretion of creatine in urine; while several authors noted this effect, others made no such findings. The effect of vitamin E on the serum levels of lipids and lipoproteins is also a matter of discussion. Several authors observed an increase of certain lipid fractions during administration of high amounts of vitamin E, while others found no effects. With respect to the effect on the coagulation parameters (increased prothrombin time, delayed blood coagulation), no effect of high doses of vitamin E seems to exist if the vitamin K status is normal whereas blood coagulation is disturbed in experimental vitamin K deficiency or during simultaneous administration of anti-coagulants, e.g. warfarin. According to these findings, a synergistic effect between certain anti-coagulants and vitamin E, or an antagonistic effect between vitamin K and vitamin E, seems to exist.

Different international and national authorities have attempted to establish the amount of safe and adequate levels of vitamin E in the diet. The FAO/WHO-experts have given an estimate for a lower level of 0.15 mg and an upper level of 2 mg alpha-tocopherol per kg body weight as acceptable daily intake based on clinical experience in man. The Food and Drug Administration (FDA) has also set ranges for dietary supplements of vitamins in the U.S. Recommended Daily Allowance (U.S. RDA). For adults, the U.S. RDA is 30 I.U. vitamin E, with a lower and upper limit of 15 and 45 I.U. respectively. The National Research Council specifies a value of 8–10 mg alpha-TE (12–15 USP-Units) for adult women and men in its Recommended Dietary Allowance (RDA), similar to the German Society for Nutrition who set the recommended value to 12 mg alpha-TE.

Estimating the daily dose for human subjects, Böhles considers the following: the amount of tocopherol which is present in mother's milk (ca. 1.5 mg/100 ml milk) is considered harmless. A newborn of 3,500 g drinks approximately 500 g milk every day, corresponding to an uptake of approximately 2 mg tocopherol/kg/day. This corresponds to 140 mg/day for an adult who weighs 70 kg. In addition, the likely better bioavailability—due to the uptake from mother's milk—should also be considered. Based on this calculation, Böhles questions the validity of the
recommended amount of vitamin E for adult humans. Other experts also believe that an amount of 12 mg per day is too low.116,117

6. CONCLUSION

From studies in humans with vitamin E doses of 100–300 mg/day, it can be confidently concluded that these doses are well tolerated and cause no side effects.6,76,112-115 Other authors118 have even recommended daily doses of up to 200–400 mg vitamin E as food supplements under certain conditions. In studies in which doses of 400 to 2,000 mg vitamin E/day were given, no side effects were observed in most cases.63,71,100,110,119 At much higher doses (3,200 USP-Units/day) only, side effects and intolerance were increasingly noted.72

Based on these findings, a daily dosage of 100–300 mg can be considered entirely harmless from a toxicological point of view. These amounts of vitamin E have been particularly recommended by some physicians for groups considered to be at risk, such as smokers, persons performing heavy manual labor, athletes, recovering patients, alcoholics, patients with infections or metabolic disorders, post-surgical patients, persons performing heavy manual labor, athletes, recovering patients, alcoholics, patients with infections or metabolic disorders, post-surgical patients undergoing radiation therapy, as well as persons on a restricted diet.

Acknowledgement — The authors are indebted to Dr. W. Kästner for literature research and many helpful discussions, and to Dr. Nikoleit for help in designing Figure 2: “Relevant dose ranges of vitamin E” and for his assistance in preparing the manuscript for publication.

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