KINETICS OF TISSUE α-TOCOPHEROL UPTAKE AND DEPLETION FOLLOWING ADMINISTRATION OF HIGH LEVELS OF VITAMIN E

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A linear relationship has been found between the tocopherol content of tissues and the logarithm of the dose administered when vitamin E is fed to experimental animals.[1-6] This relationship has been demonstrated for plasma,[1,2,4] platelets,[4,5] liver,[1-5] muscle,[1,3,4,6] heart,[1,6] testes,[1,6] spleen,[1] and lung[5] over a wide range of intakes. Some tissues, such as the testes and liver, were much more sensitive to changes in intake than were others, such as the muscle.

Larsson reported that the increase in tocopherol content of muscles of patients with intermittent claudication was proportional to their clinical improvement.[7] Haeger has reported a subjective improvement in such patients after 3-5 months of vitamin E administration and an improvement in arterial blood flow after 12-18 months of treatment.[8] It seems possible that the 12-18 month delay before improvement is observed results from the long time necessary to increase muscle tocopherol after beginning the administration of vitamin E. Bieri studied the effects of the addition of low levels of vitamin E to a diet deficient in vitamin E over a 25-week period in rats,[1] but the effects of higher levels added to an already adequate diet were not investigated. Yang and Desai reported that in rats the α-tocopherol content of the liver and plasma approximately doubled between 8 and 16 months over a wide range of vitamin E intakes,[2] suggesting that α-tocopherol continues to accumulate with time. However, no observations on muscle or other tissues were made. Thus, little information is available on the effects of long-term administration of high levels of vitamin E on tissue concentrations. Whether most tissues continue to accumulate tocopherol with prolonged ingestion of high levels of vitamin E is not known.

Materials and Methods

Experiment 1

Mature rhesus monkeys were fed a semipurified diet (casein, sucrose, cellulose, "stripped lard," vitamins and minerals). Groups of four monkeys were fed diets containing either no supplement or 5, 50, or 500 mg/kg of *all-rac*-α-tocopheryl acetate. Plasma vitamin E was determined every 1-2 months for 188 weeks.

Experiment 2

Mature female Sprague Dawley rats were used. Use of mature female animals reduces the influence of tissue growth on tocopherol concentration. At the start of

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the experiment and at 1, 2, 4, 8, 14, and 20 weeks of supplementation with 10,000 mg of vitamin E* per kg of laboratory chow,† groups of four animals each were sacrificed and tissues taken for vitamin E analysis. To study the rate of depletion after 8 weeks on the supplemented diets, a group of animals was transferred to the unsupplemented laboratory chow. Then at 1, 2, and 4 weeks after withdrawal of the supplement, four animals were sacrificed for tissue analysis. All animals were sacrificed after an overnight fast, and all tissues were immediately frozen and stored at −20°C until analyzed.

Plasma tocopherol levels were determined by the method of Storer.[9] Heart, muscle, lung, red blood cells, platelets, and brain were analyzed by the procedure of Taylor *et al.*,[10] while liver and adipose tissue were analyzed by a modification of this procedure as described by Fox and Mueller and Hansen and Warwick, respectively.[11,12]

Experiment 3

The protocol was the same as Experiment 2 except that only 1,000 mg of vitamin E per kg diet were added and groups of six animals each were sacrificed at 0, 1, 4, 9, and 14 weeks after the start of supplementation. After 14 weeks all animals were fed the laboratory chow, and animals were sacrificed for tissue analysis after 1, 2, and 4 weeks on the unsupplemented diet.

Data Summary for Experiments 2 and 3

Generally, there was a linear relationship between time and tissue levels of vitamin E (FIGURES 3, 4, and 5). Accumulation rates were estimated from a line drawn to "best fit" all of the data points. Similarly, depletion rate, calculated as half-life disappearance time, was estimated from a line drawn either through all of the points on the depletion curve or between the starting point and the first point that approximated presupplementation values. Although this calculation method is not completely precise, it still permits certain conclusions based on the large differences observed.

RESULTS

Monkey Studies

When 0 or 5 mg/kg of diet of vitamin E was fed, plasma tocopherol levels decreased to almost negligible values in about 50 weeks (FIGURE 2). With 50 mg/kg, plasma levels remained constant for 3½ years. With 500 mg/kg, there was a relatively rapid increase by 6 weeks, and then a slow but continuous increase for the duration of the experiment. The slope from 6 to 188 weeks was statistically significant.

*Added as *all-rac*-α-tocopheryl acetate (1 mg is equivalent to 1 IU). All diets were pelleted following addition of supplement.

†Purina Rat Chow, Ralston Purina, St. Louis, Mo. Contained 65 IU/kg vitamin E.

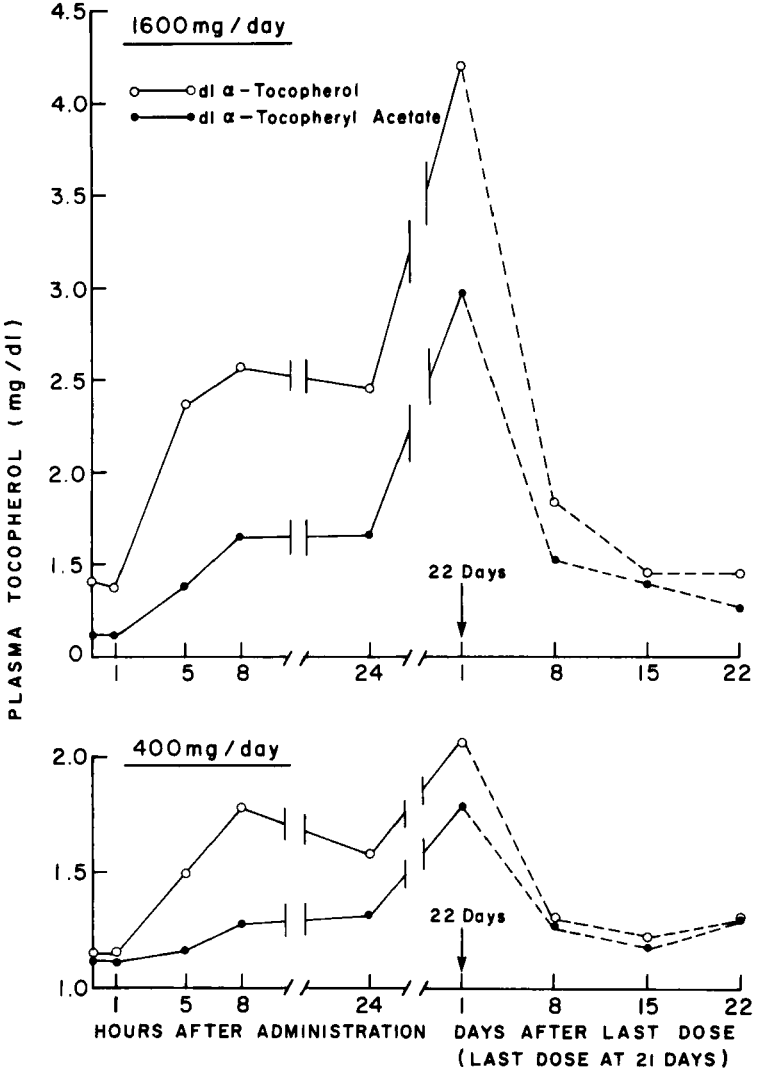

FIGURE 1. Plasma tocopherol levels in man after administration of vitamin E. Three male and three female normal adults were used for each treatment. They were given a single dose of either *all-rac-α*-tocopherol or *all-rac-α*-tocopheryl acetate, and plasma tocopherol was determined at 0, 1, 5, 8, and 24 hours after dosing. The subjects were continued on the same daily intake for 21 more days, and blood tocopherol was again determined at 1, 8, 15, and 22 days after the last intake of supplementary vitamin E. (Based on Baker *et al.*)[9]

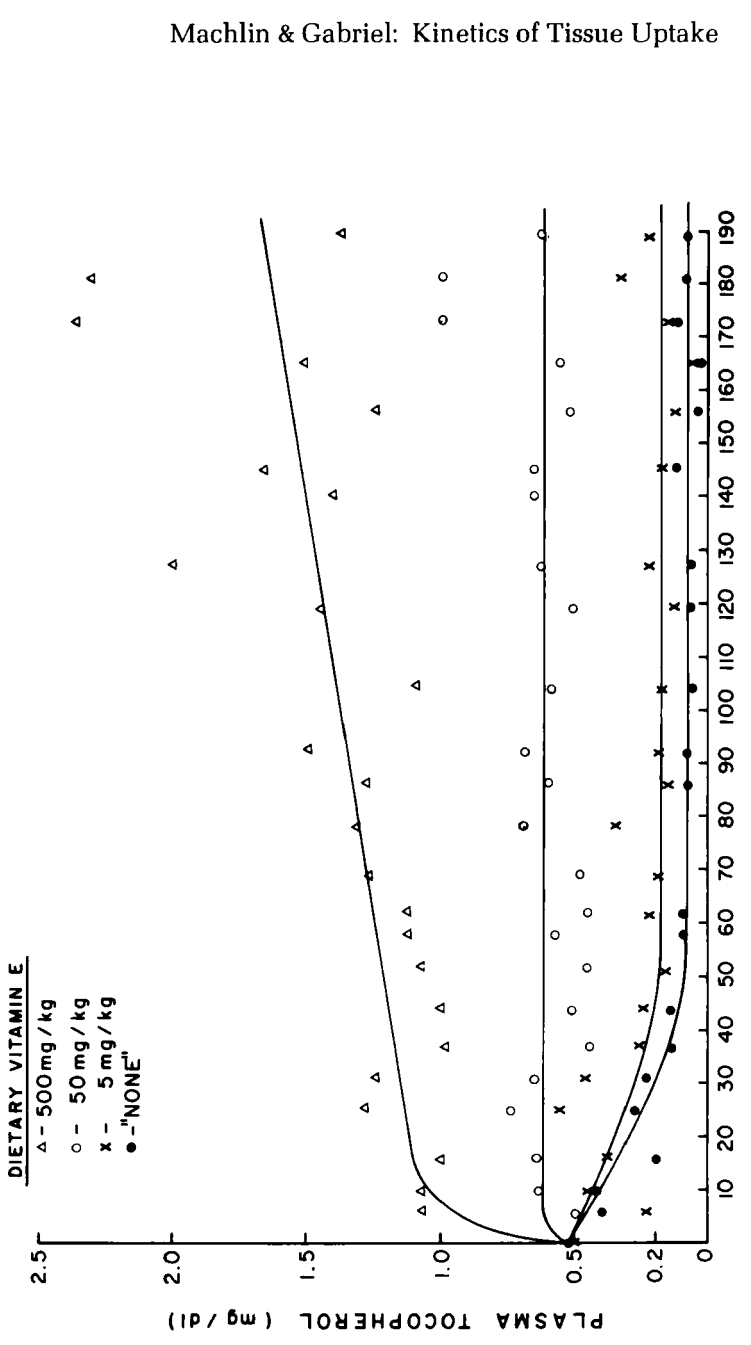

FIGURE 2. Effect of dietary vitamin E on plasma tocopherol levels in the rhesus monkey. Four mature monkeys (three males and one female), which had been fed a commercial diet, were transferred to a semipurified diet at zero time. After administration of ketamine, blood was taken from the cubital vein or femoral artery using EDTA (ethylenediaminetetraacetic acid) as an anticoagulant.

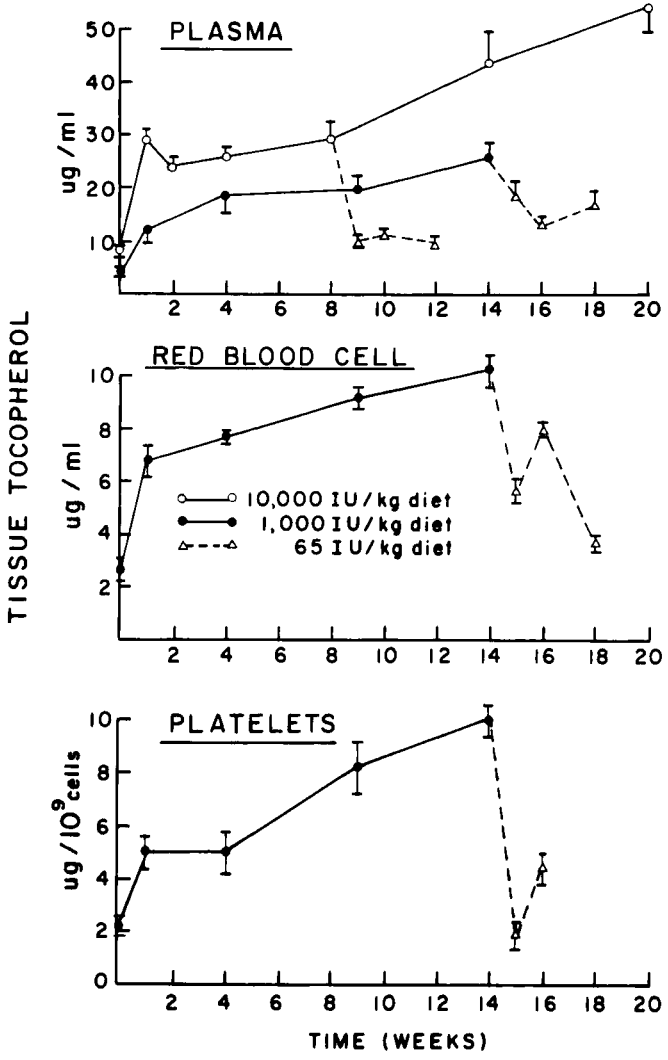

FIGURE 3. Effect of feeding two high levels of vitamin E to mature female rats on tocopherol concentration of plasma, red blood cells, and platelets and its depletion rate following withdrawal of vitamin E supplement from the diet. At the 10,000 mg/kg level (open circles), each point represents the average of four animals; at the 1,000 mg/kg level (filled circles), each point represents the average of six animals. Bar represents standard error.

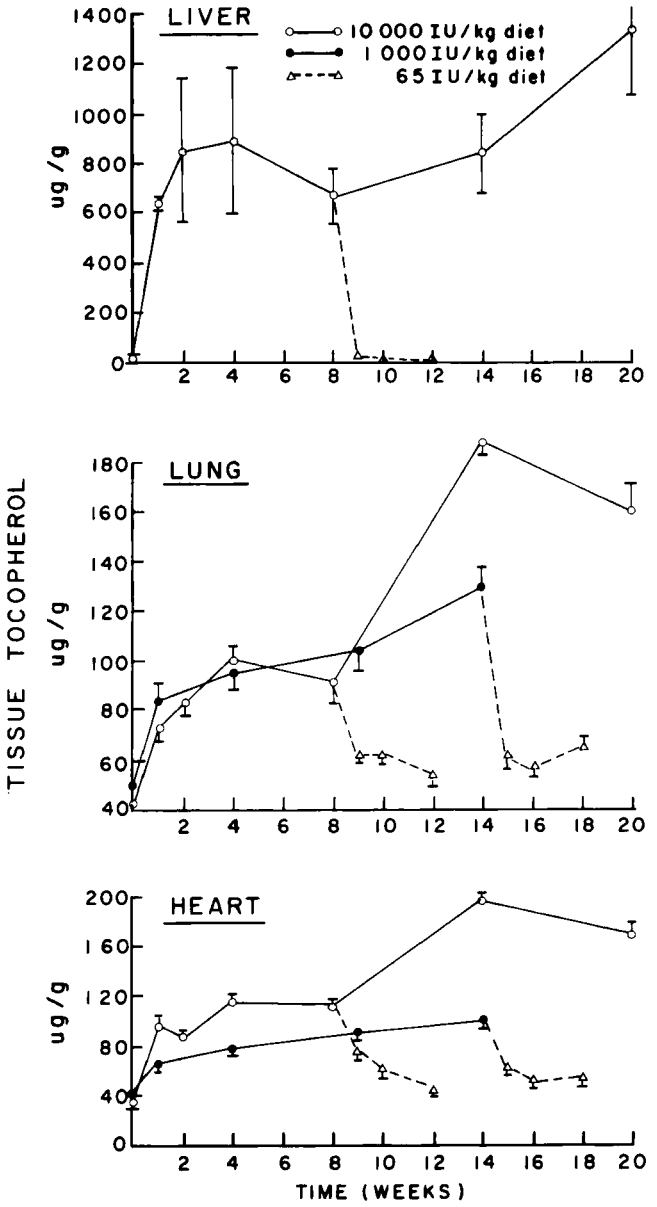

FIGURE 4. Uptake and depletion of tocopherol in liver, lung, and heart. For details, see FIGURE 3.

Rat Studies, Uptake of Tocopherol

One week after the animals were put on the supplemented diet, a rapid increase in the tocopherol level was observed in plasma, red blood cells, platelets, liver, heart, and lung (FIGURES 3 and 4). In contrast, this initial spurt was not as marked in muscle, adipose tissue, or brain (FIGURE 5). Nevertheless, in all tissues there was a progressive increase in tocopherol levels. The tissues of animals on

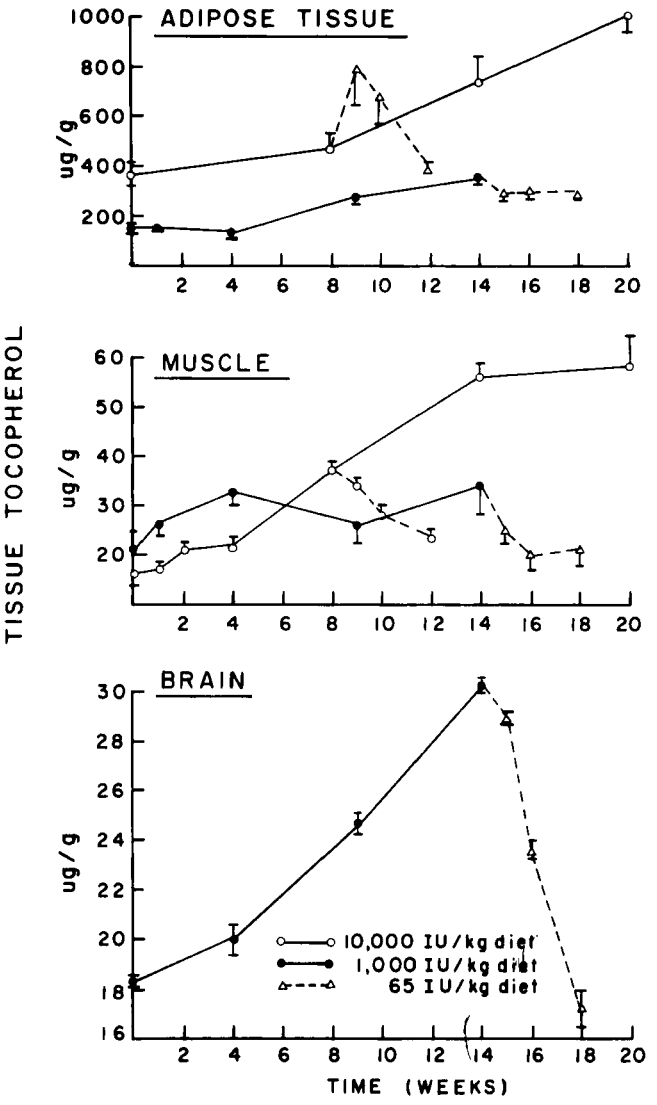

FIGURE 5. Uptake and depletion of tocopherol in adipose tissue, muscle, and brain. For details, see FIGURE 3.

TABLE 1

ACCUMULATION AND DEPLETION RATES OF TOCOPHEROL IN TISSUES

Tissue	Experiment 1 (10,000 mg/kg) Accumulation Rate (μg/g per day)	Experiment 1 (10,000 mg/kg) Depletion Rate $t_{1/2}$ (days)	Experiment 2 (1,000 mg/kg) Accumulation Rate (μg/g per day)	Experiment 2 (1,000 mg/kg) Depletion Rate $t_{1/2}$ (days)
Plasma	1.8*	1.2	1.0*	3.0
Red Blood Cells	—	—	0.4*	3.0
Platelets	—	—	0.5†	1.4
Liver	41.5	0.8	—	—
Heart	6.8	2.4	3.0	3.1
Lung	7.0	1.3	4.0	1.4
Muscle	2.2	8.2	0.5	6.2
Adipose	32.5	14.8	10.0	14.8
Brain	—	—	0.5	1.4

*μg/ml per day.
†μg/10^9 cells per day.

both levels of vitamin E supplementation showed the same pattern of uptake. Not unexpectedly, the rate of accumulation as well as the maximal levels reached was consistently and considerably higher in the group consuming 10,000 mg/kg of the supplement (FIGURES 3, 4, and 5 and TABLES 1 and 2) than in those fed 1,000 mg/kg. The relative increase in the tocopherol content was higher in liver than in any other tissue (TABLE 2). The accumulation rate of tocopherol was highest in the liver and adipose tissue and lowest in the muscle and brain (TABLE 1).

TABLE 2

RELATIVE SENSITIVITY OF TISSUE TO TOCOPHEROL ADMINISTRATION

Tissue	Tissue Tocopherol Concentration: Experiment 1 (10,000 mg/kg) Minimal*	Maximal*	Ratio of Max/Min	Experiment 2 (1,000 mg/kg) Minimal*	Maximal*	Ratio of Max/Min
Plasma†	8.2	54.0	5.9	4.7	26.4	5.6
Red Blood Cells‡	—	—	—	2.6	10.2	3.9
Platelets§	—	—	—	2.2	10.0	4.5
Liver	22	1,329	60.4	—	—	—
Heart	36	196	5.9	44	101	2.3
Lung	43	189	4.4	50	130	2.6
Muscle	16	58	3.6	21	34	1.6
Adipose	366	1,016	2.8	152	350	2.3
Brain	—	—	—	18	30	1.7

*μg/g.
†μg/ml.
‡μg/ml packed cells.
§μg/10^9 cells.

Rat Studies, Depletion of Tocopherol

With the exception of adipose tissue, the rate of depletion of tocopherol from all tissues was more rapid than the rate of accumulation (FIGURES 3–5). In general, depletion rates were similar in both experiments. The only exception was the plasma level, which decreased more rapidly at the higher level of supplementation (FIGURE 3, TABLE 1).

DISCUSSION

Human Studies

One of the few well-controlled studies with high levels of vitamin E was that of Baker *et al.* (FIGURE 1).[13] In all four treatments, blood levels peaked at 8 hours after administration and then remained the same or declined slightly by 24 hours. When administration was continued over a 21-day period, plasma levels increased substantially. Blood levels were consistently higher when the free tocopherol was administered rather than tocopheryl acetate. This difference has not been observed when low levels are administered.[14] The higher blood levels attained with tocopherol compared to tocopheryl acetate (FIGURE 1) suggest that hydrolysis of the acetate may be a limiting factor when high levels of the vitamin are administered. This is probably not very important in normal adults, but in subjects with pancreatic insufficiency this difference might be exaggerated. Clinicians may want to consider using free tocopherol for these conditions. The study also demonstrates that there is a considerable increase in plasma levels between 1 and 21 days of administration. Based on these results and those with monkeys (FIGURE 2), it would seem advisable to treat subjects with high levels of the vitamin for at least 1–3 weeks to attain maximal plasma levels.

Rat Studies

Liver and adipose tissue accumulate tocopherol at a much faster rate than do any of the other tissues (TABLES 1 and 2). However, while depletion rate in liver is extremely rapid, that of adipose tissue is extremely slow. This difference in the depletion rate between liver and adipose tissue has been observed previously in rats and guinea pigs.[15,16] In guinea pigs, the rate of release from adipose tissue is so slow that blood levels are not maintained and animals develop a myopathy even though adipose tissue stores are still high.[16] Thus, both studies suggest that liver is the major storage organ for tocopherol and helps maintain plasma levels when the intake of vitamin E becomes inadequate (at least for short time periods).

Because of the extremely slow rate of tocopherol release from adipose tissue, this tissue represents a large, but relatively unavailable, store of the vitamin. Although the vitamin continues to accumulate in adipose tissue with time, its low turnover suggests that analysis of this tissue might provide a useful index of relatively long-term dietary intake of vitamin E. Such an index could be useful in epidemiological studies that attempt to relate vitamin E status to some measure of health. In contrast, liver and plasma levels appear to reflect relatively recent intakes of the vitamin. Fortunately, a reliable procedure for measuring tocopherol by needle biopsy has been developed to study tocopherol kinetics in human adipose tissue.[17]

When 10,000 mg/kg vitamin E was fed (FIGURE 5), the muscle continued to accumulate tocopherol for the duration of the experiment. The trend was the same, but less pronounced, at the lower level of intake. These results suggest that if a significant increase in the tocopherol levels of tissues with slow accumulation rates, such as the muscle and brain, is desired, a prolonged treatment at high levels of intake is necessary. This would be of particular importance in clinical trials with the vitamin. For example, children with severe malabsorption had to be treated with high levels of vitamin E for 3, 6, or 16 months before improvement in neuromuscular symptoms was observed.[18-20] Even if studies are directed at tissues with more rapid accumulation rates, such as the lungs or heart, it would be desirable to continue treatment for at least a few weeks to insure that tissue levels of vitamin E had indeed been elevated.

In the present study tocopherol continued to increase in all tissues examined for the duration of the supplementation. In the study by Yang and Desai, there was a continued increase in both liver and plasma after 8 and 16 months of feeding high levels of vitamin E.[2] These observations suggest that saturation of the tissues with tocopherol is difficult to attain.

Generally, the release of a compound from a tissue is a fixed percentage of its content in the tissue. Therefore, as the tissue content increases, the absolute release rate continues to increase until it is equal to the entry rate, and at that point the tissue concentration remains constant. The observation that tissue concentrations of tocopherol do not plateau suggests that the release rate is limited to an absolute level and is not related to tissue content. The mechanism that controls the release rate must be quite sensitive to small changes in structure, since γ-tocopherol, lacking just one methyl group in the 5 position, is retained much less tenaciously in most tissues than is α-tocopherol.[21,22] Whether release rates are influenced by the presence of specific binding proteins[23-25] in a tissue remains to be determined.

SUMMARY

Following administration of high levels of vitamin E, plasma tocopherol levels in mature humans and monkeys continued to increase with time. In man plasma tocopherol levels were higher when tocopherol rather than tocopheryl acetate was given.

Mature female rats that had been maintained on laboratory chow were put on the diet supplemented with either 1,000 or 10,000 mg/kg of *all-rac*-α-tocopheryl acetate. All tissues analyzed (plasma, platelets, liver, red blood cells, adipose tissue, heart, lung, skeletal muscle, and brain) continued to increase in tocopherol content for the duration of the supplement (20 weeks). It was concluded that it is difficult to saturate tissue with tocopherol and that not only the level, but the duration, of supplementation with vitamin E influences the concentration of vitamin E in all tissues. Liver and adipose tissue both accumulated tocopherol at a very rapid rate compared to other tissues, but once the chow diet was resumed, liver tocopherol decreased very rapidly and adipose tissue decreased very slowly. The studies suggest that at least for a short time period, the liver is the major available storage organ for tocopherol. When animals were put back on the unsupplemented chow diet, platelet and plasma levels returned to baseline values within 1 week, and red blood cells, heart, muscle, lung, and brain within 4 weeks.

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DISCUSSION

M. K. HORWITT (*St. Louis Medical Center, St. Louis, Mo.*): Was the increase in plasma tocopherol levels with time in the monkeys related to the amount of lipid in the plasma?

L. J. MACHLIN: Plasma cholesterol and triglyceride levels were monitored continuously in the monkeys. There was no change with time, so I don't think the lipid levels influenced the results.

J. J. BARBORIAK (*Medical College of Wisconsin, Milwaukee, Wis.*): I was wondering whether at these higher doses of vitamin E, the absorption of vitamin E is somehow affected—or what the excretion part of it is—because not all of it was stored. Also, after giving high levels of vitamin E, does one see the production of deficiency at a faster rate because of an induced higher rate of metabolism?

L. J. MACHLIN: There's very little metabolism of vitamin to excretory metabolites. The Simon's metabolite has been identified as a glucuronate conjugate of tocopheronic acid. This metabolite generally represents less than 1% of the dose administered, even with high levels of intake. So then, since metabolism is minimal, I doubt whether you would see a rebound phenomenon, even after withdrawal of the vitamin. As you increase the intake, the efficiency of absorption goes down and more is excreted in the feces. Once it's absorbed, I don't think there's any significant alteration in metabolism.

L. HOWARD (*Albany Medical College, Albany, N.Y.*): What happens to those adipose tissue stores if you're not only vitamin E depleted but also starved?

L. J. MACHLIN: When we fasted animals for four days, the tocopherol concentration actually went up in the adipose tissue and there was no indication of any increase in the rate of loss upon fasting.

We speculated that tocopherol is primarily in the membrane of the adipocyte and not in the triglyceride part of the cell and that the turnover of membrane tocopherol in this cell is very low.

C. C. REDDY (*Pennsylvania State University, University Park, Pa.*): Regarding vitamin E in liver, do you have any comments on the two proteins that Mavis has recently published? One protein is involved in transfer of vitamin E from cytosol to the microsomes and has a molecular weight of about 34,000. The other one is a cytosolic binding protein with a higher molecular weight. Mavis showed that the binding protein is present in heart, lung, and liver in almost equal amounts, whereas the transfer protein is present only in the liver and absent in the lung and the heart.

L. J. MACHLIN: I would speculate that the importance of the binding proteins would only be apparent with low or nutritional levels of intake, and not when high levels are administered, such as in the present experiments.

P. M. THURLOW (*Duke University Medical Center, Durham, N.C.*): You noted the increased E levels only at the highest dietary intake. What is the relevant actual intake in these animals and which one of the intakes is the most relevant to a real dosage?

L. J. MACHLIN: There were accumulations of vitamin E at both the lower and the higher level of intake. The lower level would be a little more realistic, however. The blood levels with the high level were about 3 mg/dl. These levels are obtained when high levels of vitamin E are given to humans. Therefore, although both were quite high from a dietary standpoint, they may be relevant to humans consuming very high levels.

J. G. BIERI (*National Institutes of Health, Bethesda, Md.*): I think it should be pointed out that 10,000 international units per kilo of diet is 1% of the diet. I'm just wondering if this is really almost a toxic level. Other people have shown in rats and chicks interference with vitamin D or K utilization. With these very high amounts in the liver, did you see fatty liver? Did you look at the liver fat?

L. J. MACHLIN: No we didn't. However, there was no evidence of toxicity in rats fed 1% vitamin E.

UNIDENTIFIED SPEAKER: Are there any known products of vitamin E breakdown that are toxic?

L. J. MACHLIN: The main breakdown product is tocopheryl quinone. Massive amounts of quinone have an anti-vitamin K activity. However, I think these are levels that would never occur even with high level use of the vitamin.

M. P. CARPENTER (*Medical Research Foundation, Oklahoma City, Okla.*): Were there any differences between males and females in the phenomena that you've been seeing? And do you have any data on turnover of tocopherol in adipose tissue?

L. J. MACHLIN: There were no sex differences between blood levels of male and female monkeys. We only used female rats. We have observed a male-female difference in the development of myopathy in an E-deficient rat. We find that the male rats are much more prone to developing muscle degeneration than are the females. If you measure pyruvate kinase as an indicator of dystrophy, the enzyme levels in the male may be five times those of the female.

We have not carried out any turnover studies with the adipose tissue.

M. P. CARPENTER: What is the biological significance of the observation on adipose tissue?

L. J. MACHLIN: Guinea pigs became overtly deficient as manifested by muscle dystrophy even though adipose stores were not depleted. Clearly the rate of release from adipose tissue in this species was insufficient to prevent a vitamin E deficiency. We don't know, of course, whether that's true in humans.