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Inhibition of vitamin A synthesis by excess α-tocopherol in young rats

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CRABP	cellular retinoic acid-binding protein
CRBP	cellular retinol-binding protein
DPPD	N,N'-diphenyl-p-phenylenediamine
GIT	gastro intestinal tract
GSH-px	glutathione peroxidase
MCTG	medium chain triglycerides
NA	not applicable
PUFA	polyunsaturated fatty acids or lipids
RA	retinoic acid
RAL	retinal
RBP	plasma retinol-binding protein
RE	retinyl esters
ROL	retinol
SCO	tocopherol-stripped corn oil
SEM	standard error of the mean
αT	dl-α-tocopheryl acetate

INTRODUCTION

Vitamin A deficiency is still a worldwide problem, causing blindness in an estimated 50 to 100 thousand children annually (Olson, 1978). Various efforts have been and are being made by individual governments and international bodies to combat the problem of vitamin A deficiency. One such effort is the 6 monthly administration of large doses of vitamin A to children living in areas of endemic vitamin A deficiency in Indochina.

Empirical research has been active in numerous areas. Physiological effects of long or short term deprivations as well as excesses of vitamin A in experimental subjects are being studied. Concurrently, there has been renewed interest in the transport and metabolism of vitamin A following the isolation of a specific carrier protein for retinol. With the exception of the role of vitamin A in the visual process, the search still continues for specific biochemical reactions that are catalyzed by vitamin A in one form or another. Other areas of active research involve vitamin A precursors. Although it has been more than half a century since carotenoids were identified as precursors of vitamin A, information concerning the exact mechanism of the conversion process is still incomplete. Still further directions for research have been indicated by the increasing tendency of the general public towards megavitamin intakes. Megadoses of vitamins, their precursors and analogs are used increasingly for therapeutic purposes. For example, the use of retinoids for the prevention and therapy of certain types of cancer is being encouraged (Sporn and Newton, 1979). Large doses of β-carotene have been recommended

to help decrease sensitivity to sunlight in patients suffering from erythropoietic protoporphyria (Mathews-Roth et al., 1970).

The need for basic research into vitamin A function, metabolism, and conversion from precursors is still great in order to provide answers to long standing questions. Furthermore, investigations into the effects of megavitamin doses are becoming more important as increasing numbers of people are exposed to megavitamin therapy. Vitamins C and E are some of the most abused micronutrients by self-prescribing megavitamin enthusiasts. Megavitamin C is promoted as a prevention and cure for the common cold. Vitamin E, on the other hand, is believed to slow down aging and improve virility in men. Although a small amount of vitamin E is essential for adequate reproductive performance in rats, there is as yet no scientific proof of the utility of the vitamin in reproductive disorders in humans. Since there is no appreciable storage of water soluble vitamins in the body, megadoses of vitamin C pose little danger. Vitamin E, on the other hand, is fat soluble and very well stored in various tissues in the body but has proven harmless in large doses to experimental subjects. However, Arnrich (1978) has clearly shown that high doses (50 x requirement) of dl-α-tocopheryl acetate (αT) severely depressed hepatic deposition of retinol, when β-carotene was the sole source of vitamin A in the diets of young vitamin A depleted rats. Several postulations could be made about high αT intakes and vitamin A metabolism as follows:

(1) High αT interferes with β-carotene absorption.

(2) High αT interferes with the enzymatic processes of conversion of β-carotene to vitamin A.

(3) High αT enhances degradation and excretion of retinol stores in the body.

The present study was designed to examine in detail the nature of the interaction between vitamin E and β-carotene. Specifically the effects of (1) high tissue concentrations of vitamin E, and (2) the physical presence of excess tocopherol on the short term conversion of β-carotene to vitamin A were examined both in vivo and in vitro. The formation of retinol metabolites from ^{14}C-β-carotene was used as a measure of this extent of β-carotene conversion to vitamin A. Hepatic deposition of vitamin A derived from β-carotene during 28 or 56 day feeding of carotene was measured to assess the overall effect of excess αT on the utilization of the pro-vitamin.

REVIEW OF LITERATURE

Vitamin A

With the exception of carotenoids, compounds possessing the biological activity of retinol (ROL) are referred to as vitamin A. More recently, the term retinoids is being used to represent both the natural and synthetic analogs of vitamin A, with or without biological activity (Goodman, 1979).

In the diet, vitamin A occurs mainly as retinyl esters (RE) or carotenoids. Retinyl esters are hydrolyzed in the lumen of the small intestines by enzymes associated with the mucosal brush border. The newly released ROL or any ROL ingested as such is absorbed into the mucosal cell where it is esterified mainly as retinyl palmitate and to a lesser degree as stearate (Huang and Goodman, 1965; Goodman et al., 1966a; Ganguly, 1969). The retinyl esters (RE) are incorporated into chylomicrons and travel, via lymph, into the systemic circulation. The chylomicrons are partially cleared of triglycerides by extrahepatic tissues. The remnant particles containing cholesterol, RE and other lipids, are cleared from the blood by the liver (Redgrave, 1970). In the liver, hydrolysis of the RE and re-esterification (mainly as retinyl palmitate) occur. The newly formed RE are stored associated with lipid droplets (Lawrence et al., 1966). There has always been speculation as to the exact location(s) of vitamin A stores in the liver. Current research seems to implicate hepatic parenchymal cells under normal intakes of vitamin A, while perisinusoidal cells (also called Ito cells)

are thought to accommodate large doses of vitamin A (DeLuca et al., 1977). There is a strong suggestion that vitamin A is mainly stored as RE in 'vitamin A enriched globules (VAG)', which are contained in 'hepatic lipocytes' (Olson and Gunning, 1980).

Vitamin A is transported from the liver to extrahepatic tissues as free ROL bound to a carrier protein referred to as plasma retinol binding protein (RBP) (Kanai et al., 1968). Retinol binding protein has been isolated from the sera of various species including man, pig, cattle, dog, rabbit, monkey, chicken and rat. Since RBP is synthesized in the liver and catabolized in the kidney, plasma levels of RBP fall during liver disease and are elevated in patients with chronic renal diseases (Smith and Goodman, 1971).

The rough endoplasmic reticulum of the hepatocyte appears to be the major subcellular location for RBP but, so far, no particular organelle has been identified as the specific location for RBP synthesis, or for the synthesis of the RBP-ROL complex. According to Smith and Goodman (1979), most of the RBP in liver is associated with microsomes. These investigators postulate that, although the Golgi apparatus is involved in the secretion of RBP from the liver, it is not the major location for RBP in either normal or vitamin A deficient rats.

Human RBP has been characterized as a single polypeptide (M.W. ≈21,000), with α_1 electrophoretic mobility. Each polypeptide chain has a binding site for one molecule of ROL. Holo-RBP (RBP with ROL bound to it) travels in serum associated with prealbumin in a 1:1 molar ratio. Although both proteins are synthesized in the liver, it is believed that

they are synthesized and secreted independently and that they associate with each other in the blood (Navab et al., 1977). The affinity between RBP and prealbumin is reduced after RBP gives up its ROL to peripheral tissues and becomes apo-RBP. Because RBP is a small protein, free apo-RBP in the blood is quickly removed from circulation by glomerular filtration unless the kidneys are diseased and their filtration function is impaired.

Dowling and Wald (1960) have conclusively shown that serum ROL concentrations do not necessarily reflect the body's reserve of vitamin A because serum ROL concentration is maintained within a narrow range at the expense of hepatic ROL. This observation has been corroborated by many workers including Underwood et al. (1979). There have been several studies related to factors which control or regulate the release of vitamin A from the liver. Parameters which have been used include: serum ROL and RBP concentrations, and functional tests such as dark adaptation. Evidence for the essentiality of RBP to carry ROL out of the liver is well established. Any physiological condition that affects RBP synthesis or release would therefore indirectly affect serum ROL levels. At any one time, serum vitamin A could exist as ROL in holo-RBP and also RE incorporated into chylomicrons or lipoprotein fractions on their way to the liver. Serum vitamin A measured post prandially, is therefore partly dependent upon mobilization of ROL stores from the liver and also on RE of recent dietary origin. Most research on the subject has been focused on ROL mobilization from the liver.

Vahlquist et al. (1978) identified patients with liver malfunction and low plasma RBP concentrations (<20 μg/ml) who had impaired dark adaptation. Vitamin A therapy of these patients neither raised serum RBP concentrations nor improved dark adaptation. The deduction was made that the vitamin A deficiency observed in these patients was secondary to an inability to mobilize ROL from their diseased livers. In a series of papers, Smith and coworkers (1971, 1972, 1973) have shown that protein-calorie malnutrition, cystic fibrosis of the pancreas, some diseases of liver, kidneys and thyroid, all adversely affect RBP metabolism. In patients with acute hepatitis, serum ROL and RBP concentrations were initially low, but increased steadily in the absence of vitamin A supplementation as their condition improved. In contrast, patients with chronic renal disease showed elevated amounts of serum RBP and ROL reflecting the inability of their kidneys to clear RBP from the circulation. The authors also noted that low serum RBP and ROL could be raised in children suffering from protein-calorie malnutrition by supplementing their diets with protein and calories without supplemental vitamin A. Many other investigators, including Venkataswamy et al. (1977), provided further evidence in support of the contention that adequacy of dietary protein is essential for the synthesis of RBP, and in turn for normal transport of ROL out of the liver.

Smith and Goodman (1979) have shown that liver RBP concentrations rose while values for serum RBP fell during the development of vitamin A deficiency in rats. The observation indicated that RBP was secreted from the liver only when attached to ROL. Upon repletion of vitamin A

deficient rats with ROL, holo-RBP was rapidly secreted from the liver into the blood stream. Since the ROL-stimulated secretion of RBP from liver was not blocked by inhibitors of protein synthesis, it was suggested that the newly released RBP did not reflect de novo synthesis of RBP induced by ROL.

A different idea about serum ROL regulation was put forward by Underwood and coworkers (1979). Based on short and long term administration of retinoic acid (RA) to vitamin A deficient or sufficient rats, the authors concluded that the release of vitamin A stores from liver into blood is dependent on the need of extra hepatic tissues for ROL. This hypothesis was based on the finding that plasma ROL concentration was reduced when rats were given RA. It was suggested that RA fulfilled some of the rats' requirement for vitamin A in extrahepatic tissue, thus reducing the demand for ROL. If, as the authors hypothesized, in the presence of adequate liver stores of vitamin A, plasma ROL concentrations reflect the need for ROL by extrahepatic tissue, one might expect that physiological states which increase or decrease requirements for vitamin A would lead to noticeable increases or decreases, respectively, in serum ROL. If this hypothesis cannot be proven correct it could mean that RA depresses serum ROL for reasons other than those stated by the authors.

The manner of delivery of vitamin A to target tissues has been clarified with the discovery of specific receptor sites for RBP on these cells. It is believed that holo-RBP is relieved of its ROL at these sites. Cellular retinol-binding protein (CRBP) and cellular retinoic

acid-binding proteins (CRABP) are soluble proteins with high and specific binding affinities for ROL and RA, respectively. These two groups of proteins have been isolated from various tissues including rat testis cytosol (Ross and Goodman, 1979). It is hypothesized that the final fate of vitamin A in some target cells is dependent upon these types of two binding proteins acting as carriers from the cell membrane to the interior organelles.

Exactly how and in what forms vitamin A functions metabolically is still not known. The problem is being pursued with renewed vigor by researchers. The role of vitamin A in vision is by far the best understood and there is no attempt to review the subject here.

Beta-Carotene

Well over four hundred naturally occurring carotenoids have been identified and characterized by food chemists interested in using carotenoids as food additives and colorants. Chemical structure is of primary importance in determining the biopotency of a carotenoid. To be biologically active, a carotenoid must have at least one unsubstituted β-ionone ring with a polyene side chain attached to it, while the other end of the compound should have at least an eleven carbon polyene fragment. There can be some modifications in the basic structure with varying effects on biopotency (Bauernfeind, 1978). Carotenoids vary greatly in their biological activity. Beta-apo-12'-carotenal, from alfalfa, for example, is 20 percent more active than β-carotene, while homo-β-carotene and 5,6-epoxy-β-carotene have only 20 percent of the activity of

β-carotene. At doses barely large enough to result in ROL deposits in liver, β-carotene is considered only half as potent as ROL (Moore, 1957). At higher doses, the biopotency of β-carotene is less than 50 percent of the potency of ROL (Kamath et al., 1972).

Since carotenoids are naturally found inside plant cellular materials, their availability to animals is dependent on the digestibility of the plant material. Various factors have been shown to affect the efficiency of utilization of carotenoids by animals and man. Most investigators have used the response of animals to β-carotene administration. Some of the parameters commonly monitored were: growth, hepatic and renal retinol concentrations, amelioration of vitamin A deficiency symptoms and, sometimes, fecal excretion of intact β-carotene. Some animal species such as cattle, dog, and man are able to absorb β-carotene intact while others such as sheep, goat and rat have an effective barrier to the absorption of β-carotene (Huang and Goodman, 1965). Moore (1957) has reviewed information concerning factors influencing biological availability of carotenoids. Factors such as the functional integrity of the small intestine, digestibility of the plant material in which the carotenoid is encased, availability of fat (type and amount) in the diet, and the presence or absence of bile salts have all been shown to affect the utilization of carotenoids by animals. The absence of dietary fat or adequate bile flow both severely depress β-carotene utilization. With respect to dietary fat, the degree of saturation of the fat could be of importance as evidenced by growth depression in vitamins A and E depleted rats fed linoleates or linolenates and small

amounts of β-carotene daily (Sherman, 1941). This inhibition of β-carotene utilization could be due to oxidative damage to the β-carotene in the intestinal lumen in the absence of antioxidants since oxidative destruction of β-carotene in vitro is speeded up by the presence of polyunsaturated lipids (Sherman, 1942). Park (1975) has, however, shown to the contrary that increasing the vitamin E content of the diet of rats did not diminish the depression of β-carotene utilization due to dietary polyunsaturated lipids (PUFA). If the depression in β-carotene utilization by PUFA were solely due to oxidative destruction of the β-carotene, addition of increasing amounts of vitamin E should have ameliorated the problem. As this was not the case in Park's (1975) study, it can be concluded that PUFA depresses β-carotene utilization by means other than increased oxidative destruction of β-carotene.

The protein content of diets is important in the efficiency of conversion of carotene to vitamin A, thus rats fed low protein diets are unable to take full advantage of the β-carotene in their diet (Gronowska-Senger and Wolf, 1970; Kamath et al., 1972; Kamath and Arnrich, 1973). Even for in vitro assays of the enzyme responsible for the conversion of β-carotene to vitamin A, prefeeding high protein diets to rats before using them for an assay proved beneficial (Stoecker, 1970). Protein appears to interact with β-carotene at the intestinal level both directly and indirectly.

Site of conversion of β-carotene

After establishing the fact that β-carotene and some other carotenoids were precursors of vitamin A in animals, several researchers tried

to elucidate the site(s) and later the mechanism of the conversion of β-carotene to vitamin A.

In rats, orally administered β-carotene resulted in an increase in hepatic ROL, while β-carotene as such was undetectable in rat liver 24 hours after dosing (Mehl and Deuel, 1946). Parenteral or intrasplenic administration of β-carotene led to large β-carotene deposits in the liver. Based on growth rate data and related parameters, the authors concluded that rats were unable to derive maximum benefit from hepatic β-carotene as a source of vitamin A. The same group of researchers (Mattson, Mehl and Deuel, 1947; Wiese, Mehl and Deuel, 1947) established by both in vivo and in vitro experiments with rats that the small intestine was the major site for the conversion of β-carotene to vitamin A. This finding led to several studies intended to by-pass the small intestine by injecting β-carotene mixtures. Contrary to the data obtained by Mehl and Deuel (1946), different investigators have shown that β-carotene, injected in an aqueous medium containing suitable surfactants, was optimally utilized by various animal species including rats (Bieri and Sandman, 1951, Eaton et al., 1951; Tomarelli et al., 1946). Furthermore, Bieri and Pollard (1954) had evidence for a limited extent of conversion of β-carotene to vitamin A even after ligation of the bile duct, enterectomy, nephrectomy, and partial hepatectomy in rats. These authors concluded that sites other than the small intestines had the ability to convert β-carotene to vitamin A. Recognition of the existence of enterohepatic circulation and the presence of hepatic β-carotene-15,15'-dioxygenase (Olson and Lakshmanan, 1970) help to confirm these early

findings by Bieri and Pollard (1954).

It has been determined that the intestinal mucosa is capable of synthesizing more vitamin A from precursors than is required for growth and maintenance (Thompson et al., 1950). The amount of β-carotene converted to vitamin A, however, is apparently dose dependent up to a point of saturation of the enzyme system (Olson, 1961).

Biosynthesis of vitamin A

As stated earlier, β-carotene is converted to vitamin A primarily in the intestinal mucosa. The proximal two-thirds of the rat's small intestine is most active in the conversion of β-carotene to vitamin A, while the distal third has little activity (Olson, 1961; Stoecker, 1970). Two enzymes are involved in the conversion process. The first is known as β-carotene-15,15'-dioxygenase. It has been isolated and partially purified by many investigators including Goodman and fellow workers (1967).

Beta-carotene-15,15'-dioxygenase is a soluble enzyme that functions in vitro at a narrow pH range of 7.5 to 8.0. It requires molecular oxygen, a detergent, and a lipid to function at maximum capacity in vitro but the detergent and lipid requirements are nonspecific. The enzyme is stimulated by thiols, inhibited by sulfhydryl inhibitors and also by chelating agents. Goodman et al. (1966b) determined that the hydrogens attached to the central carbons of the β-carotene molecule are completely retained during cleavage of the molecule. From these and other characteristics, it was concluded that the enzyme cleaves β-carotene at the central double bond probably by a dioxygenase mechanism

yielding two molecules of retinal (RAL). The enzyme has been partially purified and characterized from hog and rabbit intestine as well as rat liver (Olson and Hayaishi, (1965).

The second enzyme, retinaldehyde reductase, catalyzes the reduction of RAL to ROL. This enzyme is also a soluble mucosal enzyme with a molecular weight of 60,000-80,000. It also has been isolated and partially purified from rat liver (Zachman and Olson, 1961). The enzyme has a requirement for NADPH or NADH and seems to be a relatively non-specific aldehyde reductase for the reduction of short and medium chain aldehydes (Fidge and Goodman, 1968). The newly formed ROL is quickly esterified mostly as retinyl palmitate. The fate of this new RE is the same as that of dietary RE.

Vitamin E

The term vitamin E is generic and refers to a series of fat soluble tocols and tocotrienols of which d-α-tocopherol is the most active. In most tests, the biopotency of vitamin E is assessed by the response of deficient animals to vitamin E intake. Parameters frequently used to ascertain vitamin E deficiency are: rat testes degeneration, fetal resorption, uterine color, muscular dystrophy, exudative diathesis, encephalomalacia and in vitro hemolysis of erythrocytes to oxidizing agents such as dialuric acid or hydrogen peroxide. The specific lesions are species dependent. If α-tocopherol is considered 100 percent active in vivo, then β, γ, and δ-tocopherols should be 40, 10, and 1 percent active, respectively (McLaughlin and Weihrauch, 1979). Ranking of the

antioxidant properties of tocopherols is in reverse to their biological activity; δ-tocopherol is a much more potent antioxidant than α-tocopherol. The reductions in biopotencies of the various tocopherols relative to d-α-tocopherol are based on decreased absorption and relatively rapid excretion (Bieri, 1976).

Food sources of vitamin E are widespread. Since animals do not synthesize the vitamin, the amounts contained in any animal tissue are influenced by diet. Generally, only small to moderate amounts of vitamin E are found in animal tissues. Alpha tocopherol tends to predominate in animal tissue but a diet high in foods containing γ-tocopherols would change this predominance.

Metabolism and function of tocopherols

Vitamin E absorption is poor and dose dependent. Estimates ranged from less than 10 percent when milligram amounts were fed to about 50 percent when microgram amounts were fed to rats (Losowsky et al., 1972). Fecal excretion, however, did not correlate with liver storage data. For example, dietary medium chain triglycerides (MCTG) favored vitamin E absorption and cholestyramine depressed absorption rate while liver storage of vitamin E was lower in rats fed MCTG compared to those fed cholestyramine. This discrepancy may be due to the usual inaccuracies inherent in estimating absorption based on fecal excretion and liver storage. Furthermore, liver is not the major storage site of vitamin E. In man absorption of small doses was estimated at 20 to 30 percent when lymphatic cannulation procedures were employed (Losowsky

et al., 1972). Based on the limited data available, it appears that the absorption of large amounts of vitamin E is limited relative to vitamin A. Adam and Korner (1968) observed that the appearance of vitamin A in the blood stream was twice as fast as that of vitamin E after an oral dose.

Alpha-tocopherol is transported in close association with plasma cholesterol and total lipids (Bjornson et al., 1976) and stored mainly in adipose tissue (Bieri, 1972). According to data from depletion studies, tissues other than adipose appeared to have a labile and a non-labile pool of α-tocopherol. The relative non-labile pool of α-tocopherol may represent α-tocopherol in cellular membranes (Molenaar et al., 1973).

Although cellular vitamin E appears to be closely associated with membranes, including mitochondrial (Csallany and Draper, 1960), a variety of enzymes related to the respiratory chain were unaffected by vitamin E status. This suggested that vitamin E is not directly involved in the electron transport mechanism (Green, 1972).

The function of vitamin E at the molecular level remains a subject of active research and controversy. Many water soluble vitamins are co-factors in enzymatic reactions but so far investigations into various metabolic steps or pathways affected by vitamin E deficiency have failed to reveal any reaction that is specifically α-tocopherol dependent.

Vitamin E is believed to play a two-fold role in the body. The role of α-tocopherol is generally attributed to its antioxidant properties and secondly, to specific physiological actions for which other

antioxidants cannot be substituted. Literature generated by researchers studying vitamin E action has been reviewed periodically (Green, 1969; Green and Bunyan, 1969; Molenaar, 1972; Bieri, 1976; Diplock, 1978).

The antioxidant theory of vitamin E action was proposed by Tappel (1962). According to this hypothesis, random, free radical-catalyzed peroxidation of polyunsaturated lipids is a continual biological process. The peroxidation products are damaging to cellular and intracellular structures, certain enzymes and other labile intracellular components. The biological role of vitamin E is to inhibit this peroxidation process. In the absence of vitamin E, the peroxidation process goes on uninhibited. Finally, membranes are damaged with a resultant sequelae of pathological conditions commonly associated with vitamin E deficiency. In their reviews, Green (1969) and Green and Bunyan (1969) questioned Tappel's hypothesis based on the following grounds:

1) Inability to isolate (conclusively at the time) lipid peroxides of endogenous origin.
2) Lack of increase in tissue lipid peroxides with progressive depletion of body vitamin E, or with increase in dietary PUFA, or significant decreases in tissue lipid peroxides upon vitamin E administration.
3) Inability to exacerbate damage to oxidizable components of intracellular structures, such as lysosomes, by increased PUFA in diets of vitamin E deficient animals.
4) Lack of evidence concerning a generalized loss of PUFA in vitamin E deficient animals.

Diplock (1978) generally agreed with Green and Bunyan (1969) and further pointed out the inability to demonstrate any acceleration in rate of loss of (^{14}C)-labelled tocopherol in nutritional situations known to lead to vitamin and/or selenium deficiency.

For several years the search for vitamin E function had been hampered by its apparent interaction with selenium. The discovery that glutathione peroxidase (GSH-px) is a selenium containing enzyme (Rotruck et al. 1973) which uses lipid peroxides as substrates has helped to resolve the confusion. This has also brought new credibility to parts of Tappel's hypothesis. If lipid peroxides are formed in vivo, two mechanisms appear to be available, theoretically, for their immediate discharge (Diplock, 1978). First, vitamin E could be involved in separate but complimentary reactions aimed at breaking a chain reaction involving formation of lipid peroxy radicals in membranes. The use of new methodology for measuring lipid peroxidation, namely, measurement of ethane and pentane evolution, has enabled Tappel (1980) to show that induced lipid peroxidation increased in vitamin E deficient rats compared to those on moderate or adequate vitamin E intakes. Secondly, vitamin E is believed to act as an antioxidant in the stabilization of membranes by maintaining the PUFA status of these membranes (Diplock, 1978). Maggio et al. (1977) demonstrated that vitamin E tended to associate with model systems containing PUFA. This observation, coupled with the knowledge that membranes tend to be high in PUFA lends further credibility to the notion that vitamin E plays an important role in the maintenance of membrane integrity.

Interactions of Vitamins A and E

A relationship between vitamins A and E has been recognized since the early 1940s. The nature of the relationship and the specific role of each vitamin has been difficult to ascertain.

Using doubly depleted rats, Hickman et al. (1944) found that vitamin E enhanced the growth promoting power of vitamin A. In addition, the vitamin A depletion period and the survival time, after vitamin A supplementation had ceased, were both increased in vitamin E supplemented compared to vitamin E deficient young rats. Similarly, Harrill et al. (1965) and Ames (1969, 1974) noted that addition of vitamin E to the vitamin A supplement of vitamin E deficient rats led to a dramatic increase in hepatic stores of vitamin A. Lemley et al. (1947) also observed that the synergistic effects of small amounts of both vitamins on rat growth was somewhat diminished when the two vitamins were fed separately. When animals were not deficient in either vitamin, small amounts of both did not appear to produce measurable effects. However, with high doses of both vitamins, interactions are readily demonstrable. At toxic doses of vitamin A, vitamin E has been shown to alleviate symptoms of hypervitaminosis A. Symptoms such as teratogenicity (Soliman, 1972) and growth depression in rats (Jenkins and Mitchell, 1975), mortality in chicks (McCuaig and Motzok, 1970), have been significantly alleviated in the presence of high amounts of vitamin E. Some authors (Irving, 1958; Pudelkiewicz et al., 1964; Combs and Scott, 1974) have shown that high doses of vitamin A proved detrimental to vitamin E utilization as measured by depression in vitamin E stores, or appearance of vitamin E

deficiency symptoms in animals receiving little or no vitamin E. In contrast, Green and others (1967) failed to observe a significant effect of relatively high doses of vitamin A on vitamin E utilization when (^{14}C) α-tocopherol was given by intramuscular injection.

The mechanism of the interaction between vitamins A and E has been difficult to elucidate. In chicks, high doses of vitamin A interfered with vitamin E absorption, as measured by appearance of tritium labeled α-tocopherol in serum (Combs, 1976). This effect was prominent only when the vitamin A was given as an oral supplement but not when the vitamin was mixed into the diet and consumed gradually over time. Few of the experiments cited have been designed to physically separate the administration of the two vitamins. Thus, it is difficult to distinguish between gut interactions and systemic interactions. It is probable that vitamin E acts to protect vitamin A against oxidative damage when the two vitamins are inside the gut lumen. Although vitamin E absorption is limited, it is unlikely that high doses of vitamin E interfere with the absorption of moderate doses of vitamin A. This is illustrated by Buckingham's (1974) observation that hepatic ROL storage was essentially the same when rats were fed daily 1 or 50 mg αT with equal amounts of retinyl acetate for 28 days.

In the antioxidant theory referred to earlier, Tappel (1962) assumed that oxygen-labile compounds such as vitamin A were co-oxidizable substrates and vitamin E was protecting them in vivo. This explanation of the mechanism of interaction of vitamin A and E has been severely criticized by Green et al. (1967). In addition to an extensive review of the

literature, these investigators reported 11 experiments, designed mostly to stress vitamin E status and its effect on vitamin A status in experimental animals. Vitamin E had a slightly depressing effect on the rate of depletion of hepatic vitamin A stores; otherwise, the authors could not find any increase in ROL utilization when vitamin E status was stressed by PUFA. The onset of encephalomalacia in vitamin E deficient chicks was not speeded up by administering moderately high doses of vitamin A. From their own data and others reviewed, Green and colleagues (1967) concluded that the antioxidant theory did not adequately explain vitamin A and E interactions in vivo. In view of recent information about the relationship of vitamin E and selenium through GSH-px, it might prove fruitful to repeat some of these earlier experiments with highly purified diets and a strict monitoring of selenium and vitamin E intakes. It is possible that under experimental conditions that would otherwise stress vitamin E, GSH-px activity might increase to compensate for the stress.

Vitamin E and β-carotene

Following the observation (Moore, 1940) that vitamin E enhanced liver storage of vitamin A from retinol or β-carotene in doubly depleted rats, investigations into the role of vitamin E in the metabolism of β-carotene and also preformed vitamin A became very active. Based on both in vivo and in vitro studies, investigators have concluded that vitamin E had a beneficial effect (Quackenbush et al., 1942; Herbert and Morgan, 1953), no effect (Burns et al., 1951; Bieri, 1955) or a detrimental

effect (Swick and Bauman, 1951; McGillivary and Worker, 1957; Buckingham, 1974) on the conversion of β-carotene to vitamin A. Parameters explored were: rate of growth of animals on suboptimal intakes of β-carotene, or storage of ROL in liver or in liver plus kidney. Upon closer scrutiny, it appears that vitamin E was beneficial to the utilization of β-carotene and even of preformed vitamin A provided comparisons were made between vitamin E deficient and sufficient status. The response was especially striking if the animals were previously depleted of both vitamins and resupplementation involved suboptimal amounts of both vitamins. Large doses of vitamin E have consistently been shown to be detrimental to β-carotene utilization even when enough β-carotene was being fed to result in appreciable amounts of liver stores of vitamin A (Park, 1975). At very low doses of β-carotene, the amounts of vitamin E necessary to depress vitamin A storage were correspondingly low also. When the daily β-carotene dose to young vitamin A depleted rats was around 1 μg, up to 1 mg of vitamin E per day did not show any significant adverse effect on rat growth while 2 mg or more of vitamin E per day proved detrimental (Harris et al., 1944; Burns et al., 1951). Upon raising the β-carotene dose to about 5 μg daily, Harris and coworkers (1944) reported that, up to 5 mg vitamin E no longer diminished β-carotene utilization for growth in rats. This apparent optimum ratio of vitamin E to β-carotene does not seem to hold at dose levels exceeding 5 mg vitamin E per day.

The mode of action of vitamin E and β-carotene utilization proved elusive to ascertain. Although some of the questions have been answered the problem is still far from resolved. The antioxidant theory proposed

by Tappel (1962) and discussed earlier did not prove very helpful in the interpretation of β-carotene and vitamin E interactions. It has been established experimentally, that carotenoids are subject to oxidative destruction in the intestinal lumen (Sherman, 1947; High et al., 1954). It is also known that antioxidants, both synthetic and natural, decrease the lability of carotenoids to oxygen (Milas, 1954). Therefore, the antioxidant effect of vitamin E operates most likely, under experimental conditions, where there is intragut interaction of vitamin E and carotenoids. This concept may be valid since vitamin E had to be administered simultaneously with β-carotene for optimal beneficial (Hickman et al., 1942, 1944) or detrimental effects (McGillivary and Worker, 1958), especially when animals were doubly deficient. In addition, α-tocopherol appeared to be more active than δ-tocopherol in suppressing retinol storage from β-carotene (Swick and Bauman, 1951), even though δ-tocopherol is a more potent antioxidant than α-tocopherol (Dugan, 1976).

Various antioxidants ranging from vitamin C (Mayfield and Roehm, 1956) to synthetic antioxidants have been compared to vitamin E in their behavior towards β-carotene utilization, but results are difficult to interpret. High and coworkers (1951, 1952, 1954 and 1956) compared effects of several antioxidants with those of vitamin E on β-carotene utilization. Among compounds tested were lutein, tert-butyl-hydroquinone, octyl-hydroquinone, 2,6,di-tert-butyl-4-methyl phenol and N,N'-diphenyl-p-phenylenediamine (DPPD). Whether used in small or large concentrations, all compounds behaved parallel to vitamin E in terms of β-carotene utilization. The only exception was DPPD which is less soluble in lipid

systems compared to the other antioxidants tested. Sherman (1942) found that catechol, hydroquinone and other antioxidants were as effective as vitamin E in stabilizing β-carotene in the presence of linoleate and linolenate esters in vitro. These antioxidants, however, were not as effective as vitamin E in promoting growth in rats fed β-carotene in the above mentioned fatty acid esters.

Whether a synthetic antioxidant shows any effect on β-carotene utilization by experimental animals is probably dependent on the choice of the antioxidant and the mode of administration of the antioxidant. Moreover, it is unclear whether synthetic antioxidants behave similarly to vitamin E in vivo. For example, Csallany and Draper (1960) found that the subcellular distribution of DPPD in rats differed from that of tocopherol; the former was found mainly in the supernatant of cell homogenates while the latter was associated with mitochondria and microsomes. It should be noted that there are three commonly used classes of synthetic fat-soluble antioxidants: phenols, amines, and aminophenols (Dugan, 1976). Alkyl substitution of the phenols at the ortho or para positions usually increases their potency as antioxidants. Functionally, the three classes are somewhat different. The phenolic antioxidants are free radical scavengers and are ideal for use with polyunsaturated lipids which tend to undergo autoxidation. They are low in toxicity, potency, and generate little color when used. The amines on the other hand are extremely potent, often toxic, and form intense colors when oxidized. They are especially useful in preventing heavy metal ion-catalyzed lipid peroxidations (Dugan, 1976).

Structurally and functionally tocopherols are similar to phenolic antioxidants. Some antioxidants provide increased protection as their concentrations increase. Others have optimum levels, and are pro-oxidant at high levels (Dugan, 1976). This behavior is more likely in antioxidants which function by being oxidized preferentially. Since tocopherols are potential free radical scavengers, it is unlikely that they turn prooxidant in high concentrations. Besides, vitamin E does not affect utilization of moderate doses of retinyl acetate in rats (Park, 1975). Hence the detrimental effects of high intakes of vitamin E on β-carotene utilization is probably not due to a direct pro-oxidant action on β-carotene.

PROCEDURE AND METHODS

Animals

Male albino rats of the Wistar strain bred in the Food and Nutrition Department at Iowa State University were used in all experiments. The animals were obtained at weaning wherever prefeeding was indicated in the experimental design (Experiments 1A,B, 2, 3A,B and 6). In Experiments 4 and 5 rats were taken from the stock colony when they weighed about 250 g. When prefeeding was indicated, weanling rats were assigned individually to suspended wire-mesh cages. Distilled water and feed were provided ad libitum, and the rats were weighed weekly. Litter mates were balanced in all groups. Cages were changed weekly, and water bottles were changed twice a week. The rats were kept in an environment controlled for temperature (approx. 23°) and humidity (45-55% R.H.). Lighting was restricted to a 12 hr light-dark cycle.

Diets and Supplements

Composition of the experimental diet used was kept constant throughout this study with the exception of Experiment 2, where a commercial source of corn oil was substituted for tocopherol-stripped corn oil (Table 1). Vitamins were provided daily in separate cups. Rats consumed these immediately after a 3-4 day learning period. Fat-soluble vitamins and β-carotene were dissolved in corn oil. The rats received 1 ml of water-soluble vitamin mixture (Table 2) and 5 drops (approx. 125 mg) of their appropriate fat soluble vitamin mixture daily. During the depletion phase, all rats received the basal vitamin A-free fat-soluble

Table 1. Diet composition

Ingredients	Percent by Weight
Casein (vitamin free test)[a]	22
SCO[a,b]	10
Salt Mix[c]	3.5
Corn Starch[d]	64.5

[a]Teklad Diets, Madison, Wisconsin.

[b]SCO = tocopherol-stripped corn oil. Substituted with commercial corn oil (Mazola, Best Foods Division, Commercial Products Company, New York, N.Y.) in Experiment 2.

[c]Williams et al. (1968).

[d]Clinton corn processing Co., 1251 Beaver Channel, Clinton, Ia.

vitamin mixture containing 50 µg vitamin K_1,[1] 0.65 µg vitamin D_3,[1] and 1 mg dl-α-tocopheryl acetate[1] (αT) daily. During the experimental period, all-trans β-carotene[2] was included in the daily vitamin dose, except in Experiment 2. Rats fed high αT received 50 mg αT per day during the experimental period while control rats continued to receive 1 mg αT.

[1]Sigma Chemical Company, St. Louis, MO.

[2]Eastman Kodak Co., Rochester, N.Y.

Table 2. Composition of water-soluble vitamin supplement in 1 ml of 20 percent ethanol

Vitamin	Dosage/day, µg
Biotin[a]	2.0
Calcium panthothenate[b]	97.0
Choline HCL[a]	4.8 mg
Folic Acid[a]	20.0
Inositol[a]	2.4 mg
Niacin[c]	64.0
Para-amino benzoic acid[d]	97.0
Pyridoxine HCl[d]	20.0
Riboflavin[a]	39.0
Thiamine HCl[a]	20.0
Vitamin B_{12}[a]	0.2

[a]General Biochemicals, Inc., Chagrin Falls, Ohio. (Known as Teklad Test Diets, Madison, Wisconsin, since 1975).

[b]Pharmaceuticals, Inc., Cleveland, Ohio.

[c]Teklad Test Diets, Madison Wisconsin.

[d]Grand Island Biological Company, Grand Island, N.Y.

Experiment 1

The purpose of Experiment 1 was to determine the effect of feeding excess αT daily on hepatic ROL storage (Fig. 1, 1A) and on the ability of the intestinal mucosa of rats fed high αT to convert β-carotene to vitamin A products (Fig. 1, 1B).

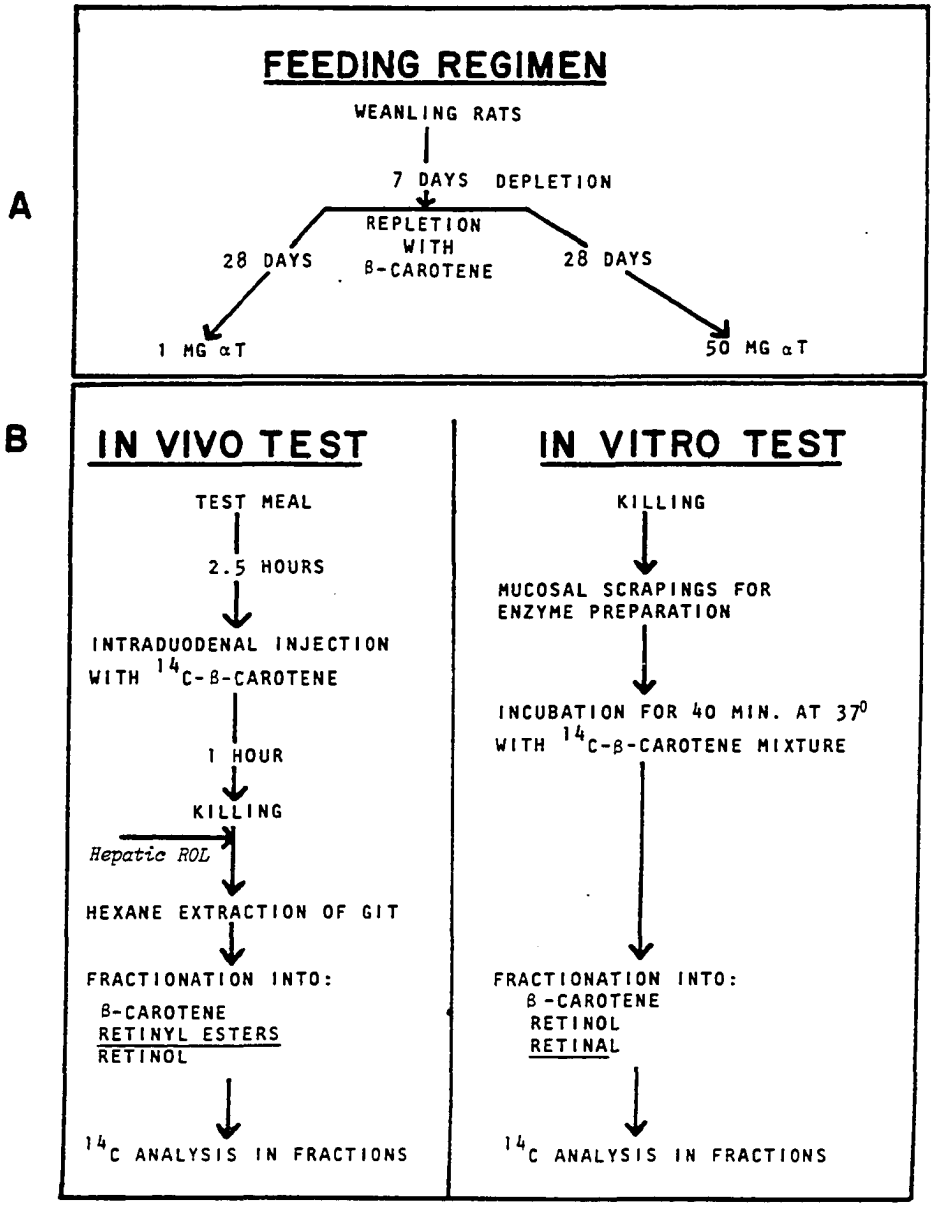

Figure 1. Experimental design

Twenty weanling rats were maintained on a vitamin A-free regimen for 7 days. Previous tests had indicated that a depletion period of 7 days was adequate to reduce hepatic stores of vitamin A to <5 μg/g. At the end of the depletion period, the rats were divided into two groups of 10 each. Litter mates were evenly matched between groups. Group I (control) received the basal diet and supplements plus 55 μg β-carotene daily, group II (50-αT group) received the same treatment but 50 instead of 1 mg αT. After 28 days the rats were fasted overnight. On the following morning they were fed 1 g basal diet plus their water soluble vitamins. Two and a half hours later, the rats were anesthesized with ether. An incision of approximately 2.5 cm was made in midventral portion of the abdomen. A dose of 10 μg of freshly chromatographed ^{14}C-β-carotene[1] (all-trans, 15-15' labelled with S.A. = 10 x 10^3 dpm/μg) was injected intraduodenally about 1.5 cm below the pylorus. The injection mixture contained 10 μg ^{14}C-β-carotene, 100 μl acetone, 100 μl Tween 20 (polyoxyethylene (20) sorbitan monolaurate),[2] and 800 μl Krebs Ringers bicarbonate solution. The incision was closed with wound clips and the animal was returned to its cage. Animals recovered from anesthesia 6 to 10 minutes after the start of the surgical procedure. One hour after the injection, the animal was reanesthesized with ether. Blood was drawn by heart puncture and left to coagulate at 4°. Serum was separated by centrifugation. The small intestine, (proximal 60 cm),

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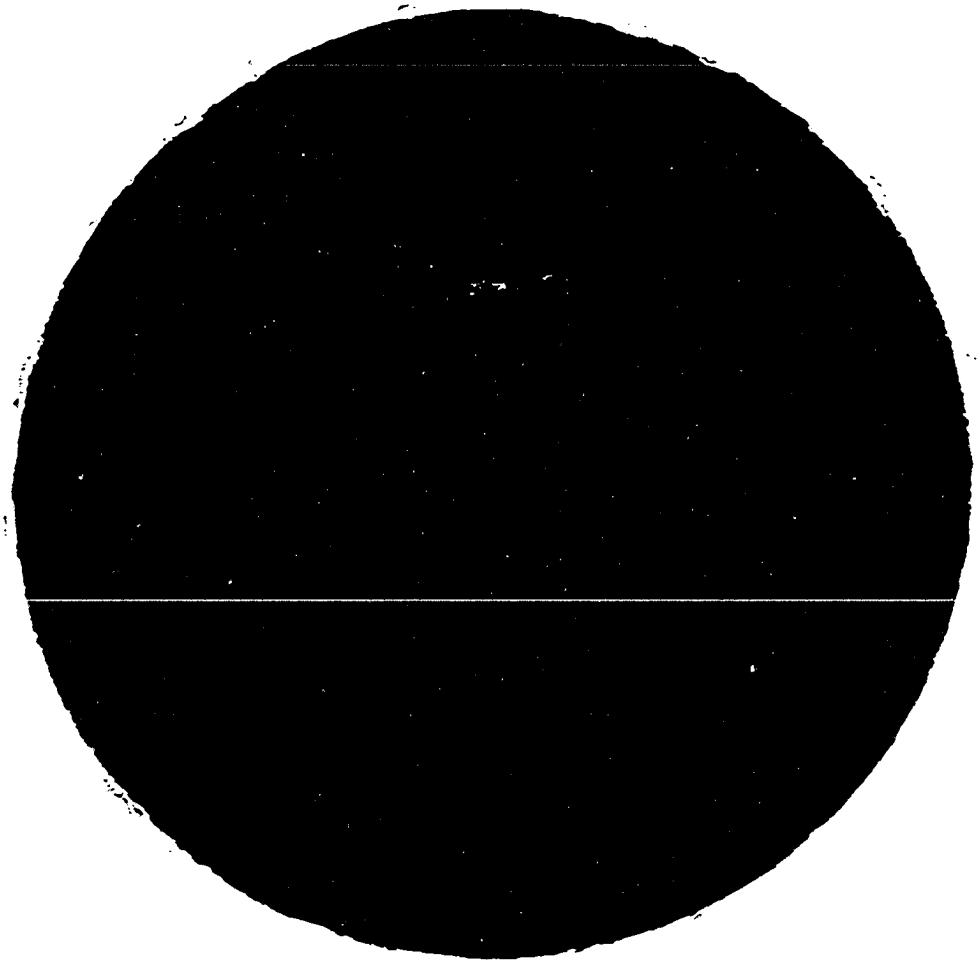
[1]Courtesy of Hoffman-La Roche, Basel, Switzerland.

[2]Courtesy of Atlas Chemical Industries, Inc., Wilmington, Delaware.



stomach, and liver were removed and prepared for chemical analyses. A detailed description of the methods used is given by Stoecker (1970). However, the extraction procedure of the small intestine, as described by Stoecker, was modified. The lumen of the small intestine was not washed with saline but the small intestine was extracted with contents in situ. Figure 1 summarizes the design and procedure of Experiment 1. For purposes of clarity, data from the determination of hepatic ROL are designated as 1A, while data on ^{14}C counts are referred to as 1B.

Experiment 2

The purpose of Experiment 2 was the same as that for Experiment 1B but instead of intraduodenal injections of β-carotene, crude enzyme preparations from the small intestine were incubated with β-carotene. Rats used in Experiment 2 had been prepared for another experiment designed to assess the depletion rate of relatively large hepatic stores of ROL. Thus, pretreatment of rats in Experiment 2 was similar to that in Experiment 1 except for a few changes: (1) Rats in Experiment 2 did not consume β-carotene during the experimental period but had adequate hepatic retinol reserves; (2) The source of dietary fat in Experiment 2 was a commercial source of corn oil[1] instead of tocopherol-stripped corn oil. The animals had received one large dose of approximately 1 mg retinyl acetate at the end of their 7-day depletion period. Following

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[1]Mazola, Best Foods Division. Commercial Products Company, New York, N.Y.

a 7-day equilibration period, they were given vitamin A-free supplements with either 1 or 50 mg αT during the subsequent 28-day experimental period. At the end of the feeding period the rats were fasted overnight to ensure empty stomachs and relatively clean small intestines.

Following the fast, rats received 1 g of basal diet to stimulate the digestive process. They were killed 2.5 hours later. The proximal 60 cm of the small intestine was quickly removed and washed with cold isotonic saline. The intestine was opened longitudinally, and the mucosa was scraped with a glass slide. The homogenized scrapings gave a crude preparation of β-carotene 15,15'-dioxygenase. The method of Goodman et al. (1967) modified by Stoecker and Arnrich (1973) was used for assay of the enzyme.

Experiment 3

The purpose of Experiment 3 was to provide additional data on hepatic ROL deposits (3A) and to study the distribution of radioactivity from ^{14}C-β-carotene in various tissues and excretory products. In Experiment 3, 20 weanling rats were treated identically to rats in Experiment 1. Daily β-carotene supplement was 75 μg per rat. At the end of the 28-day refeeding period, rats were fasted for 8 hours. At the end of the fast, each rat was given 20 μg of freshly chromatographed ^{14}C-β-carotene (S.A. = 6×10^3 dpm/μg) by gavage. The β-carotene emulsion was prepared as described for Experiment 1. Immediately after intubation, each rat received 3 g basal diet, which was consumed within 15 minutes. The animals were placed in individual stainless steel metabolism cages with provision for collection of urine and feces. Fourteen hours later the rats were killed. Blood was withdrawn by heart puncture and treated

as in Experiment 1. The bladder was drained of any remaining urine. The bottom of the metabolism cage was washed and the rinsings added to urine collections. The entire gastro-intestinal tract (GIT) was removed and divided into 2 portions. One portion consisted of stomach and small intestine. The remainder of the GIT was combined with fecal droppings and the composite was homogenized and extracted with hexane. Extraction procedures were identical to those used in Experiment 1. Livers and kidneys were removed and analyzed for radioactivity and ROL (liver only). Total radioactivity (from aqueous and solvent extracts) were determined in all samples collected. Hepatic ROL data are referred to as 3A. The data on ^{14}C counts are referred to as 3B.

Experiment 4

The purpose of Experiment 4 was to determine the effect and simultaneous presence of excess αT on β-carotene conversion process in vivo. Experiment 4 was essentially a repeat of Experiment 1B. Therefore, the 7-day depletion and 28 day repletion periods were omitted. Rats weighing about 250 g, were obtained from the stock colony and assigned to two groups with litter mates in each group. Following an overnight fast, they were treated as indicated in Figure 1 (Part B). The composition of the dose containing ^{14}C-β-carotene used for intraduodenal injections is given in Table 3.

An important modification in this experiment was that, in contrast to previous experiments, αT was included in the injection mixture. Since 10 μg β-carotene represented $1/5^{th}$ of the daily β-carotene dose in Experiment 1, the αT doses were also given at $1/5^{th}$ of 1 and 50 mg,

Table 3. Composition of mixtures injected into rats in Experiment 4

Ingredients	Groups 4A	4B
αT	.2 mg	10 mg
^{14}C-β-carotene[a]	10 µg	10 µg
Acetone	100 µl	100 µl
Tween 20	100 µl	100 µl
Sodium Glycocholate	12 µM	12 µM
Krebs Ringers Solution	500 µl	500 µl

[a]S.A. = 10 x 10^3 dpm/µg. Courtesy of Hoffman La Roche, Basil, Switzerland.

respectively. After injection, treatment of the rats, tissue collection, and analysis were identical to those in experiment 1B (Fig. 1).

Experiment 5

The purpose of Experiment 5 was the same as that for Experiment 4, but Experiment 5 was in vitro while 4 was in vivo. Experiment 5 was a repeat of Experiment 2, except that (1) prefeeding was omitted, and (2) the incubation mixture used in Experiment 5 contained αT in varying amounts.

Two groups of 4 rats weighing approximately 250 g each were obtained from the stock colony. They were fasted overnight, given a test meal following the fast, and killed 2.5 hours later. Scrapings of the proximal 60 cm of the small intestine mucosa were combined from all four rats

to obtain a sizeable pool of homogeneous enzyme preparation. Beta-carotene-15,15'-dioxygenase was assayed as in Experiment 2. The incubation mixture in Experiment 5 contained either 0.02 or 1.00 mg αT in addition to 1 μg ^{14}C-β-carotene. These amounts represented 1/50th of β-carotene and αT fed daily to control and 50-αT groups in Experiment 1.

Experiment 6

The main purpose of this experiment was to determine whether daily intakes of 50 mg αT for periods exceeding 28 days would have inhibitory effects on hepatic ROL storage similar to those accompanying a 28-day feeding period. Therefore, 20 weanling rats were depleted of vitamin A for 7 days as in Experiment 1. The rats were then kept on the experimental regimen of 100 μg β-carotene and 1 or 50 mg αT daily for 56 days. At the end of the 8 week feeding period rats were killed. Livers were analyzed for ROL content.

Summary of all experimental protocol is given in Table 4.

Analytical Procedures

All manipulations were carried out as rapidly as possible, in semi-darkness using amber glassware and under nitrogen whenever possible. All tissues (except liver) were extracted and analyzed on the day rats were killed. Details of extraction and chromatography procedures for intestine and stomach were identical to those of Kotecheri (1967) as modified by Stoecker (1970). The following changes were made:

Table 4. Summary of experimental designs

Experiment No.	Pre-feeding	No. Days Fed	Hepatic ROL Assay	Experimental Procedure	Duration
1A, 1B	55 µg β-carotene + 1 or 50 mg αT	28	Yes (1A)	Intraduodenal injection of ^{14}C-β-carotene (1B)	1 hr
2	Intubation of ≃1 mg vit. A. Start feeding 7 days later 1 or 50 mg αT	28	No	In vitro enzyme, incubation of ^{14}C-β-carotene	40 min
3A, 3B	75 µg β-carotene + 1 or 50 mg αT	28	Yes (3A)	Intubation of ^{14}C-β-carotene (3B)	14 hrs
4	None	—	No	Intraduodenal injection of ^{14}C-β-carotene + 0.2 or 10 mg αT	1 hr
5	None	—	No	In vitro enzyme incubation of ^{14}C-β-carotene + 0.02 or 1 mg αT	40 min
6	100 µg β-carotene + 1 or 50 mg αT	56	Yes	None	None

(1) Intestinal contents were not flushed out before extraction (Experiments 1B, 3B, and 4), and liver extracts were not chromatographed for assay of ROL or radioactivity; (2) In the β-carotene-15,15'-dioxygenase assays (Experiments 2 and 5), incubations lasted for 40 minutes instead of 30 minutes as used by Stoecker (1970).

Retinol analysis

Hepatic ROL was estimated by a modification of the method of Olson (1979). Estimation of ROL by correction of A_{330} readings were not useful because at 280 nm, readings were too high for liver extracts from rats fed 50 mg αT. Hepatic ROL concentrations were estimated by the Carr-Price reaction using 30 percent TCA in anhydrous chloroform as suggested by Olson (1979). By using a flow through cell[1] in a Beckman D.U. spectrophotometer[2] with a digital read out,[3] it was possible to take reliable readings in less than 12 seconds after the reaction had been started.

Liquid scintillation counting

In Experiments 1 through 5, suitable aliquots of all solvent extracts were transferred into scintillation vials, evaporated to dryness and made up to 10 ml with 7.5 percent butyl-P BD in toluene.

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[1]Precision Cells Inc., Hickville, N.Y.

[2]Beckman Instruments Inc., Fullerton, Calif.

[3]Update Instruments Inc., Madison, Wis.

Samples were counted in an ambient temperature scintillation counter.[1] It was unnecessary to correct for color quenching because carotene content of each scintillation vial was kept under 5 µg. Preliminary work, using internal standards, had shown that 10 ml of 7.5 percent butyl-PBD solution could take up to 10 µg β-carotene before any significant quenching occurred. Aqueous extracts and urine were counted in Brays'[2] solution. The external standard ratio method (Wang et al. 1975) was used for quench correction.

Statistical analysis

Data generated in all experiments were analyzed by computer using the generalized linear models procedure and F tests (Snedecor and Cochran, 1967).

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[1]Packard Tricarb Scintillation Spectrometer Model 2405.

[2]New England Nuclear, Boston, Mass.

RESULTS AND DISCUSSION

Data generated from each experiment were analyzed separately. However, groups of experiments will be discussed together whenever feasible. Even though all data were analyzed statistically, only significant differences ($P \leq .05$) are marked by asterisks.

Growth

In all experiments, growth was measured by increase in weight. Mean initial weights and weights at 28 days of rats in Experiments 1, 3, and 6 are shown in Table 5. Final weights of rats in Experiment 6 are also shown in Table 5. In addition, diagrammatic representation of growth of rats in Experiment 1 is given in Figure 2. The data show that rats fed either 1 or 50 mg αT in Experiment 1 grew at similar rates (Table 5, Fig. 2). Mean initial weights for both groups were identical by design. The difference between mean final weights of 243 g for the controls and 248 g for the rats fed 50 mg αT (50-αT group) was not statistically significant. The rats were still growing rapidly and at a constant rate. The decrease in growth rate, which is typical of maturing rats, had not yet occurred (Fig. 2). Hence, growth curves were still linear at the termination of Experiment 1. Also initial weights of rats in Experiment 3 were identical to initial weights in Experiments 1 (Table 5). But at 28 days, rats in Experiment 3 weighed only 194 (controls) and 206 g (for 50 αT group) compared to 243 g and 248 g, respectively, for comparable groups in Experiment 1. This apparent slow-down of growth in Experiment 3 was due to intermittent disruptions in

Table 5. Mean values for body weight of vitamin A-depleted rats fed β-carotene and two levels of αT[a] for 28 or 56 days (Experiments 1A, 3A and 6)

Experiment No.	αT/day mg	No. of Days Fed	Carotene/day μg	Initial Weight	Weight at 28 days	Weight at 56 days
1A	1	28	55	51 ± 1[b] (10)[c]	243 ± 7 (10)	N.A.
1A	50	28	55	51 ± 1 (10)	248 ± 6 (9)	N.A.
3A	1	28	75	50 ± 1 (10)	194 ± 7 (10)	N.A.
3A	50	28	75	49 ± 2 (10)	206 ± 5 (10)	N.A.
6	1	56	100	54 ± 2 (10)	169 ± 11 (10)	310 ± 15 (8)
6	50	56	100	53 ± 2 (10)	174 ± 8 (10)	323 ± 9 (8)

[a] dl-α-tocopheryl acetate.

[b] Mean ± SEM.

[c] N.

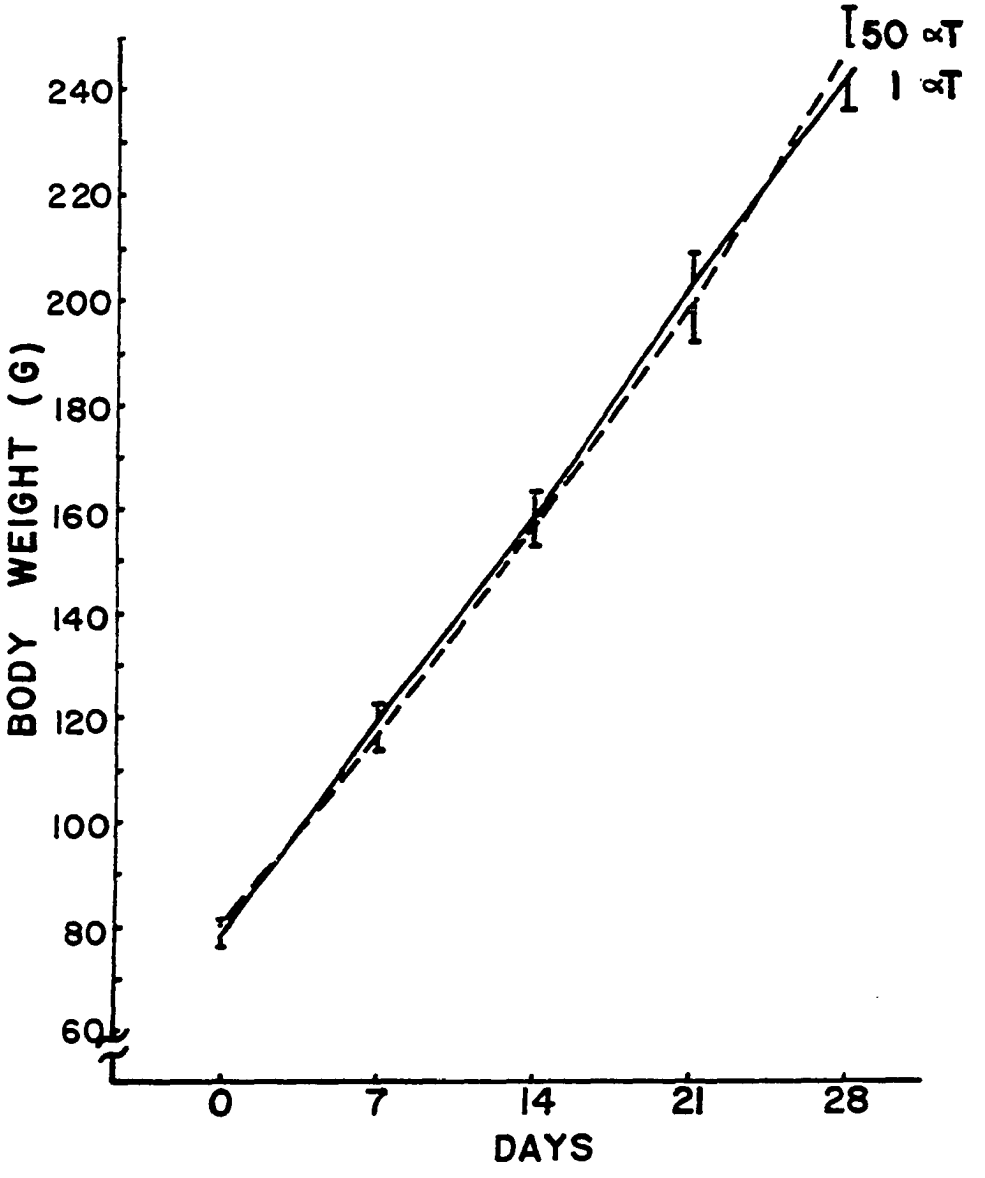

Figure 2. Growth curves of vitamin A-depleted rats repleted with 55 μg β-carotene and 1 or 50 mg dl-α-tocopheryl acetate daily for 28 days (Experiment 1)

temperature and humidity control during the progress of this experiment.

In spite of the slow growth rate of rats in Experiment 3, statistical analysis showed no significant difference between the final (28-day weights of both groups of rats in Experiment 3. Similarly, in Experiment 6, growth of the 50-αT group was not significantly different from that of controls. The same technical problems, responsible for the reduced growth rate of rats in Experiment 3, were encountered during the progress of Experiment 6. Consequently, 28-day mean weights of rats in Experiment 6 were only 169 g for controls and 174 g for the 50-αT group. Data from Table 5 and especially Figure 2 all suggest that in Experiments 1, 3, and 6, daily feeding of 50 mg αT to young, growing rats for 28 or 56 days had no effect on growth rate.

The effects of high doses of αT on rat growth have been investigated by other researchers with similar conclusions. Buckingham (1974) showed in one experiment that vitamin A-depleted rats refed β-carotene and 50 mg αT daily for 28 days attained final weights that were 15 percent lower than controls. In the same experiment, the αT effect did not occur when vitamin A-depleted rats were fed retinyl acetate together with 50 mg αT daily for 28 days. Rats used by Buckingham had been on a vitamin A-free diet for 21 days prior to the start of the feeding regimen with excess αT. The slight growth depression observed could, therefore, have been secondary to vitamin A insufficiency due to the limited ability of the 50-αT group to convert β-carotene to vitamin A. It must be stressed, however, that in a subsequent experiment Buckingham (1974) could not confirm the earlier observation. Corrick (1969) showed

conclusively that daily administration of 400 IU or more of α-tocopherol to growing rats significantly depressed growth. The evidence so far suggests, that in the presence of adequate amounts of all other nutrients, young growing rats can maintain their normal rate of growth on daily intakes of αT as high as 50X their requirement. This may or may not be valid for experiments lasting for several months.

Hepatic Retinol

Hepatic stores of ROL were determined at the end of Experiments 1A and 3A to confirm previous results from this laboratory (Buckingham, 1974; Park, 1975). In Experiment 6, hepatic ROL was determined mainly to investigate whether the effects of tocopherol seen in Experiments 1A and 3A would persist when the experimental feeding period was prolonged to 56 days.

The results of the three experiments indicate that with β-carotene as the sole source of vitamin A in the diets of young rats, simultaneous feeding of 50 mg αT daily resulted in marked decreases in hepatic ROL (Table 6). When feeding periods lasted for 28 days with a daily β-carotene dose of 55 or 75 μg, mean total hepatic ROL in rats fed 50 mg αT were 4 and 7 μg in Experiments 1A and 3A, respectively. These values represented only 4 and 7 percent of control values ($P < .001$).

In Experiment 6, the daily dose of β-carotene per rat was 100 μg compared to 55 μg in Experiment 1 and 75 μg in Experiment 3. Weight gain in Experiment 6 was also slower than in Experiments 1A and 3A, hence the metabolic need for ROL in Experiment 6 was presumably decreased.

Table 6. Mean hepatic vitamin A of vitamin A-depleted rats fed β-carotene and 1 or 50 mg αT[a] for 28 or 56 days (Experiments 1A, 3A, and 6)

Experiment No.	αT/Day mg	No. Days Fed	Carotene/Day µg	Vit. A/Liver µg	Vit. A/Liver as % of Controls
1A	1	28	55	92 ± 11[b] (10)[c]	Control
1A	50	28	55	4 ± 3*** (9)	4
3A	1	28	75	106 ± 16 (5)	Control
3A	50	28	75	7 ± 2*** (7)	7
6	1	56	100	560 ± 56 (10)	Control
6	50	56	100	63 ± 10*** (9)	11

[a] dl-α-tocopheryl acetate.

[b] Mean ± SEM.

[c] N.

*** Significantly lower ($P < 0.001$) than controls fed 1 mg αT/day.

Thus, a combination of high β-carotene doses, slow growth rate and long feeding periods (56 days vs. 28 days) all led to relatively high hepatic ROL deposits in Experiment 6 as compared to values in Experiments 1A and 3A. However, the inhibiting effects of excess αT on hepatic ROL deposition was still persistent in Experiment 6 (Table 6) with deposits amounting to 11 percent of controls.

The results confirm findings of Arnrich (1978) that high daily intakes of αT severely depressed hepatic deposition of ROL when β-carotene was the sole source of vitamin A in the diet. In an attempt to explain this effect, the possibility that excessive amounts of αT may act as pro-oxidants have been ruled out by Buckingham (1974) and Park (1975). In their studies high αT intakes did not depress hepatic deposition of ROL when retinyl acetate was fed in place of β-carotene. Furthermore, fecal excretion of β-carotene nearly doubled in the 50-αT group compared to controls (Park, 1975). The lack of inhibitory effects of high αT on retinyl acetate utilization suggests that αT acts on processes which preceed or lead to the synthesis of ROL from β-carotene rather than on mechanisms related to the utilization of the newly synthesized ROL.

The enzyme responsible for cleaving β-carotene into 2 molecules of RAL requires molecular oxygen (Goodman et al., 1967). Since vitamin E is an antioxidant, it could affect the conversion process by competing for available oxygen in the gut. High et al. (1951, 1952, 1953) and High (1954) have demonstrated that certain fat soluble antioxidants in large amounts behave like vitamin E by depressing hepatic stores of

vitamin A from β-carotene. Similar to the action of vitamin E, these antioxidants did not affect the utilization of dietary vitamin A.

The Effect of Prefeeding 50 mg αT on Functional Changes in Small Intestinal Mucosa

In vivo study (Experiment 1B)

In the experiments described so far, total hepatic ROL was measured after feeding a fixed amount of β-carotene to vitamin A depleted rats in the presence of high or low amounts of αT daily. Differences in values obtained reflect the effect of feeding high αT on storage of ROL derived from the daily feeding of β-carotene. However, the question was whether or not high tissue concentrations of tocopherol, achieved by feeding 50 mg αT daily for 28 days[1] would produce any relevant functional changes in the small intestinal mucosal tissue. The main point of course was whether these functional changes, if any, were related to the β-carotene conversion process and hence would help explain the vast differences obtained in hepatic ROL storage. Hence, only functional changes relevant to the conversion of β-carotene to vitamin A were investigated in Experiment 1B.

The last oral dose of αT and β-carotene was given about 24 hrs prior to the intraduodenal injection of ^{14}C-β-carotene. One hour after the injection, the proximal 60 cm of the small intestine was extracted

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[1]Supplementary data from our laboratory indicate a five-fold increase in αT from the GIT of rats fed 50 mg αT compared to controls.

into hexane[1] and separated, on a deactivated alumina column, into the following fractions:

1. β-carotene - uncoverted substrate
2. Retinyl esters - product
3. Retinol - product
4. Polar fractions - unidentified breakdown materials and polar metabolites, including retinoic acid.

Radioactivity recovered from each fraction was expressed as a percentage of total radioactivity recovered from the small intestine. This minimized errors due to reflux of materials from the duodenum into the stomach.

Results from experiment 1B are shown in Table 7. In rats fed 50 mg αT (50-αT) for 28 days, unconverted β-carotene amounted to 75 percent of total intestinal radioactivity recovered. This value was not significantly different from that of controls, 73 percent. The retinyl ester fraction was significantly higher ($P < 0.001$) in the 50-αT group than in controls. The mean for the ROL fraction was somewhat increased in controls though statistically not significant. When mean radioactivity recovered from retinyl esters and ROL fractions were combined, the data showed no significant difference between the two αT treatments (Table 7). Since the two fractions are indicative of β-carotene conversion, it appears that conversion was similar in both treatment groups though distribution between the free and esterified fraction varied. These

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[1]Aqueous layer contained insignificant amounts of radioactivity.

Table 7. In vivo intestinal conversion of ^{14}C-β-carotene to retinol and its metabolites following prefeeding rats 1 or 50 mg dl-α-tocopheryl acetate for 28 days[a] (Experiment 1B)

αT[b]/Day mg	N	Percent of Radioactivity Recovered				
		β-Carotene	Retinyl Esters	Retinol	Retinol + Retinyl/Esters	Polar Fractions
1	9	73 ± 3.5[c]	10 ± 0.7	10 ± 1.9	20 ± 1.9	7 ± 1.6
50	7	75 ± 0.6	17 ± 1.1***	6 ± 0.9	23 ± 0.7	2 ± 0.4*

[a]Rats were injected intraduodenally with approximately 10 μg ^{14}C-β-carotene and killed 1 hr later.

[b]dl-α-tocopheryl acetate.

[c]Mean ± SEM.

*Significantly different ($P < 0.05$) from control.

***Significantly different ($P < 0.001$) from control.

data suggest that daily feeding of 50 mg αT to rats for 28 days had no adverse effects on the capability of the intestine to synthesize ROL from β-carotene. Table 7 also shows that polar fractions were significantly lower ($P < 0.05$) in rats fed 50 mg αT than in controls. Since no attempt was made to establish the identity of compounds present in the polar fractions the data are difficult to interpret. It is possible that this fraction contains retinoic acid (Crain et al., 1967) and oxidation products of components from the other fractions (Olson, 1961).

In vitro study (Experiment 2)

Short term in vivo studies from experiment 1B, just discussed, led to the conclusion that high tissue concentrations of tocopherol do not adversely affect the capability of the intestinal mucosa to convert β-carotene to vitamin A. To confirm this hypothesis, crude β-carotene-15,15'-dioxygenase preparations were made from the mucosal scrapings of rats that had been fed either 1 or 50 mg αT daily for 28 days. The enzyme preparations were used to incubate ^{14}C-β-carotene. As in Experiment 1, the last oral dose of αT was given 24 hrs prior to killing the rats. Incubation mixtures were extracted into hexane and separated into the following fractions:

1. β-carotene - unconverted substrate.
2. Retinyl Esters - artefact?
3. Retinal - main product
4. Polar fractions - breakdown materials, retinoic acid.

Recovery of ^{14}C in each fraction was expressed as a percentage of the total. Percent radioactivity recovered from β-carotene and the main product, retinal, were similar whether the enzyme preparations came from rats prefed 50 mg αT or from controls (Table 8). Statistical analysis of the data showed no significant differences between groups. These results support the findings from experiment 1B.

Table 8. In vitro conversion of ^{14}C-β-carotene to retinol metabolites by mucosal homogenates from rats prefed 1 or 50 mg dl-α-tocopheryl acetate for 28 days (Experiment 2)

αT/Day mg	N	Percent of Radioactivity Recovered			
		Carotene	Retinyl Esters	Retinal	Polar Fractions
1	7	87 ± 1.0[a]	1 ± 0.2	7 ± 0.9	5 ± 0.2
50	7	85 ± 2.2	1 ± 0.3	10 ± 2.0	4 ± 0.3

[a]Mean ± SEM.

The retinyl ester fraction in Experiment 2 was treated as an artefact. Blank incubations of ^{14}C-β-carotene yielded about 1 percent of activity in the retinyl ester fraction. Polar fractions in the blank incubations amounted to about 2 percent of total activity. Hence, part of the polar fractions in Table 8 are considered to be artefacts or due to the oxidative degradation of β-carotene while the rest might possibly be due to oxidation of product during extraction and chromatographic procedure. Since the enzyme preparation was not purified, it is possible that small amounts of the RAL formed could have been converted

to ROL by other mucosal enzymes present in the preparation. Any ROL present would end up in the polar fractions under the procedure used for separating the fractions. Also, some of the RAL could have been converted to retinoic acid according to Crain et al. (1967). This metabolite would also contribute to the fraction designated as polar.

The overall yield of RAL in Experiment 2 was low compared to results from Goodman et al. (1966). They obtained yields as high as 54 percent RAL as compared with 7 and 10 percent in the present experiment. Part of the difference in results between the two laboratories is due to variations in length of incubation periods. Goodman and coworkers incubated for 60 minutes compared to 40 minutes used in Experiment 2. Different strains of rats were also used by Goodman and his group. Another contributing factor could be lack of technical expertise in our laboratory resulting in loss of enzyme activity during enzyme preparation.

Effect of feeding 50 mg αT on utilization of β-carotene (Experiment 3)

The finding that high tissue levels of tocopherol did not impair the ability of the rat's mucosa to convert β-carotene to ROL led to the exploration of other possibilities. The purpose of the next experiment was to determine whether the rate of catabolism of ROL was enhanced by feeding 50 mg αT. The results were essentially negative.

Young vitamin A-depleted rats which had been repleted with 75 μg β-carotene plus 1 or 50 mg αT daily for 28 days were fasted overnight. Following intubation with 20 μg ^{14}C-β-carotene, they were given small amounts of the basal diet and then placed in metabolism cages. Urine

and feces were collected during the next 14 hours. The entire gastrointestinal tract (GIT) was removed and separated into two portions. The caudal portion (from colon to anus) was combined with feces and extracted together. Liver, kidneys, urine, and serum were all appropriately treated for estimation of radioactivity. Total blood volume was estimated from the animals' body weight based on the assumption that blood constitutes 6 percent of the rat's body weight. Total serum volume was estimated from total blood volume based on a hematocrit value of 46 percent. Radioactivity determined from an aliquot of serum was extrapolated to activity in total serum volume.

Mean total radioactivity recovered, expressed as a percent of dose was only 29 and 21 percent in controls and rats fed excess αT, respectively (Table 9). Considerable variations within groups led to rather high standard errors of the mean. The high degree of variation within groups coupled with a mean total recovery of less than a third of the dose made it difficult to interpret the data with confidence.

Nearly one third of the radioactivity recovered was from liver in both groups of rats (Table 9). Expressed on the basis of dose (Table 10) there was a slight increase in radioactivity stored in the liver in control rats compared to the 50-αT group but the increase was statistically not significant. This result was unexpected since hepatic ROL data from the feeding studies (Table 6) led to an expectation of depressed storage in the excess αT group. The findings were, however, consistent with data from Experiments 1B and 2. Results from both Experiments 1B and 2 indicated that synthesis of ROL and its metabolites from β-carotene

Table 9. Mean percent radioactivity recovered from various tissues and fluids of rats intubated with ^{14}C-β-carotene and killed 14 hrs later (Experiment 3B)

αT[a] Fed mg	N	No. Days Fed	Total Recovery[b]	% of Label Recovered In						
				Liver	I GIT	II Colon + Feces	I + II	Kidney	Urine	Serum
1	9	28	29 ± 8	31 ± 5	16 ± 3	34 ± 6	50	3 ± 1	10 ± 2	5 ± 1
50	9	28	21 ± 5	29 ± 3	20 ± 3	27 ± 5	47	4 ± 1	10 ± 2	9 ± 2*

[a] αT = dl-α-tocopheryl acetate.

[b] Expressed as precent of dose.

[c] Significantly different ($P < 0.05$) from control.

Table 10. Mean radioactivity as percent of dose recovered from various tissues and fluids of rats intubated with ^{14}C-β-carotene and killed 14 hrs later (Experiment 3B)

αT[a] Fed mg	N	No. Days Fed	Total Recovery	% of Dose						
				Liver	I GIT	II Colon + Feces	I + II	Kidney	Urine	Serum
1	9	28	29 ± 8	7 ± .7	6 ± 2.2	13 ± 5.1	18	1 ± .2	2 ± .3	1 ± .1
50	9	28	21 ± 5	5 ± .6	4 ± .8	7 ± 3.4	12	1 ± .2	2 ± .4	2 ± .3*

[a]dl-α-tocopheryl acetate.

*Significantly different ($P < 0.05$) from control.

was not adversely affected by high content of tocopherol in tissues.

Of the total radioactivity recovered, an average of 50 percent was contributed by the gastrointestinal tract and feces of control animals. For the 50-αT group, mean recovery from similar sources was 47 percent. This value compared favorably with the control value. These amounts, however, represented only 18 percent of the dose in the control animals and 12 percent of the dose in the 50-αT group (Table 10). Only 2 percent of the dose was recovered from urine of both groups within the 14 hour collection period. Surprisingly, radioactivity recovered from serum was higher in rats fed 50 mg αT than in controls. Values of total radioactivity recovered in serum were 9 and 5 percent for the two groups, respectively (Table 9). This was the only statistically significant difference ($P < 0.05$) seen between treatments in Experiment 3. Since serum extracts were not separated into different fractions, it was impossible to tell whether the radioactivity recovered from serum was due to ROL, ROL metabolites, traces of β-carotene or a combination of these.

Most of the activity recovered from the GIT was unconverted β-carotene in the lower GIT and feces. When radioactivity recovered from urine was combined with that recovered from the GIT and feces, it became apparent that only 14 to 20 percent of the dose of ^{14}C-β-carotene was accounted for by remnants from digestion and absorption or excretory products. Thus 80 to 86 percent of the dose had disappeared but liver and serum could account for only a small portion of the loss. Since the ^{14}C-β-carotene was labelled in the 15,15' positions, some of the RAL

formed could have been coverted to retinoic acid and subsequently decarboxylated, eliminating $^{14}CO_2$ (Crain et al. 1967, DeLuca, 1979).

Although retinoic acid has been shown to be one of the metabolites of ROL (Emerick et al., 1967), there is no conclusive evidence yet that the conversion of ROL to retinoic acid occurs on a large scale at physiological doses of ROL or its precursors in rats.

It was expected that more radioactivity would be recovered from the fecal portion of extracts from rats fed 50 compared to 1 mg αT but this was not the case.

No attempt is made here to carry out a serious analysis of the data or make comparisons to other work published. To do that would imply confidence in the data of Experiment 3. The author has serious reservations about the validity of the data due to the great variability within treatment groups, the unusually low recovery of radioactivity from the lower GIT and feces, and the overall low recovery of the dose. A carefully controlled and executed repetition of Experiment 3 accompanied by CO_2 trapping during the 14 hour collection period might have provided additional information to help with interpretation. However, since there was no indication that a repeat would yield data which would help elucidate the overall problem of carotene-tocopherol interaction, this experiment was not repeated.

Effects of Simultaneous Administration of High αT on the Short-Term Conversion of β-Carotene to Vitamin A In Vivo and In Vitro

So far the data presented indicate that when β-carotene was fed to rats receiving high amounts of αT, hepatic deposits of ROL were severely depressed (Table 6). But short-term tests conducted in rats with high tissue levels of tocopherol showed no evidence of a depression of ROL synthesis from β-carotene. This was apparent both from the in vivo (Experiment 1B, Table 7) and the in vitro studies (Experiment 2, Table 8). It appeared that even over a 14 hour period, rats with high tissue levels of αT did not handle β-carotene differently from controls (Experiment 3B, Tables 9 and 10). The evidence from Table 6 was strong and corroborated previous findings (Park, 1975, Arnrich, 1978). Data in Table 6 resulted from simultaneous feeding of β-carotene and αT daily (Table 4). Therefore, Experiments 1B and 2 were repeated as Experiments 4 and 5, respectively, but this time β-carotene and αT were injected or incubated together.

In vivo study - Experiment 4

Experiments 4 and 5 were designed to investigate the effects of simultaneous administration of αT and β-carotene on the β-carotene conversion process. Summaries of procedures for experiments 4 and 5 are found in Table 4.

Intestinal extracts of rats injected with ^{14}C-β-carotene in the presence of 10 mg αT (excess αT) had 86 percent unconverted β-carotene compared to 73 percent for controls (Table 11, $P < 0.001$). With excess

Table 11. In vivo intestinal conversion of ^{14}C-β-carotene to retinol metabolites following intraduodenal injection of ^{14}C-β-carotene and 0.2 or 10 mg dl-α-tocopheryl acetate (Experiment 4)

αT[a] injected mg	N	% of Radioactivity Recovered				
		Carotene	Retinyl Esters	Retinol	Retinol + Retinyl Esters	Polar Fractions
0.2	8	73 ± 3.3[b]	18 ± 3.1	7 ± 0.3	25 ± 3.3	2 ± 0.2
10.0	18	86 ± 0.9***	8 ± 0.8***	4 ± 0.3***	13 ± 0.9***	2 ± 0.1

[a] αT = dl-α-tocopheryl acetate.

[b] Mean ± SEM.

*** Significantly different ($P < 0.001$) from control.

αT the retinyl ester fraction contained only 8 percent of the total radioactivity recovered from the intestine. This value was significantly lower ($P < 0.001$) than that of 18 percent for controls. Similarly, with excess αT, 4 percent of total radioactivity was recovered from the small intestine in the ROL fraction compared with a recovery of 7 percent for control rats. When the two principal fractions (i.e. retinyl esters and ROL) were combined, control rats had converted twice as much β-carotene to products as had the excess αT group. These findings were qualitatively consistent with those related to hepatic ROL storage (Table 6) but inconsistent with data obtained in Experiment 1B (Table 7). These results gave credence to the speculation that the inhibitory effect of high αT on β-carotene conversion is most pronounced when β-carotene and αT are administered simultaneously.

In vitro study - Experiment 5

Since results from Experiment 4 had indicated that simultaneous administration of excess αT was detrimental to the β-carotene conversion process, it was important to confirm these findings in vitro. It was crucial to use a homogeneous source of enzyme to eliminate errors due to variations in enzyme activity from different rats. Therefore, tissues from two groups of 4 rats each were pooled for the enzyme preparations.

Again unconverted β-carotene recovered was higher in samples treated with excess αT compared to controls (Table 12, $P < 0.001$). Eighty-six percent of the total ^{14}C recovered was unconverted β-carotene in the samples containing excess αT compared to 78 percent in controls.

Table 12. Effect of excess α-tocopheryl acetate on the in vitro conversion of ^{14}C-β-carotene to retinol metabolites by mucosal homogenates from rats (Experiment 5)

αT/Incubation mixture mg	N	Percent of Total DPM Recovered			
		Carotene	Retinyl Ester	Retinal	Polar Fractions
0.02	8	78 ± 1.6[a]	2 ± 0.3	15 ± 0.8	3 ± 0.3
1.00	9	86 ± 0.6***	1 ± 0.1	11 ± 0.4***	2 ± 0.2***

[a]Mean ± SEM.

***Significantly different ($P < 0.001$) from control.

The formation of RAL, the main product of incubation of ^{14}C-β-carotene, was depressed by 27 percent in excess αT samples compared to control samples. Here again the simultaneous presence of β-carotene with excess αT resulted in a significant depression of the conversion of β-carotene by RAL.

Recovery of ^{14}C in retinyl esters and polar fractions was considered to be mostly the result of artefacts since blank incubations of ^{14}C-β-carotene without enzyme yielded 1 percent in each of these 2 fractions. As mentioned earlier during the discussion of Experiment 2, some of the ^{14}C recovered from polar fractions could be due to ROL and possibly retinoic acid. In addition, any antioxidant activity of 0.02 mg αT would be considerably less than that of 1 mg αT under identical circumstances. Therefore, the significant decrease in polar fractions seen with the excess αT samples (Table 12) may be the result of a combination of factors.

The results of experiments 4 and 5 support qualitatively, conclusions based on hepatic ROL storage data in Table 6, but not in the same orders of magnitude. Rats fed 50 mg αT daily for 28 or 56 days had hepatic ROL deposits which were $\leq$ 11 percent of controls (Table 6). In contrast, data in Table 11 suggest a 50 percent reduction in β-carotene conversion while in Experiment 5 (Table 12) the reduction in activity was only 27 percent with relatively large amounts of αT in the medium.

In vivo experiments in our experimental protocol (Experiments 1B and 4) involve the formation of ROL and its derivatives over a relatively short period of time. The in vivo studies cover a short part of

a total process involving micelle formation, uptake of β-carotene into the mucosal cell, cleavage, reduction of RAL ro ROL and esterification of ROL. The in vitro studies (Experiment 5) on the other hand, eliminate some of the above steps and focus mainly on micelle formation and cleavage of β-carotene to RAL. Different kinetics might be operating during the various stages of the conversion process. The differences seen in Experiment 4 are probably due to a change in micelles brought about by the high concentration of αT used. We postulate that αT, when present in large amounts, influences the size of micelles formed, producing larger micelles and leading to a reduction in relative total micellar surface, and hence to a reduction in efficiency of micelles to carry β-carotene to the enzyme. This would also slow down the entry of β-carotene into the mucosal cell. In the in vitro situation in Experiment 5, however, the absorption factor is absent, hence the αT effect is less prominent.

It is proposed that data from Experiments 5 and especially 4 are more representative of the magnitude of inhibition of ROL synthesis from β-carotene than data based on hepatic ROL storage (Experiments 1A, 3A and 6) for the following reasons:

1) Hepatic ROL deposited over the 28 or 56 day feeding period represent only part of the total amount of ROL synthesized from β-carotene supplements since a certain amount must have been used in daily metabolism for growth and maintenance. There is evidence from our animal model to suggest that growing rats fed 1 or 50 mg αT daily use similar amounts

(≈10 µg/day) of ROL for daily metabolism and growth (Keller, 1979).[1] The amount of ROL stored in liver is thus over and above that which is used for daily metabolism. In effect, hepatic storage data exaggerate the differences between groups of rats fed either 1 or 50 mg αT, since amounts used in metabolism are not accounted for. For example, based on the assumption that rats in this model used an average of 10 µg ROL daily, a calculated total of 280 µg ROL would be used by each rat over a 28-day period. Adding this quantity to amounts of ROL actually determined from the liver (Table 6) would narrow the gap that exists between the group fed 50 mg αT and controls. Estimates of mean ROL formed by each group of rats over a 28-day feeding period then shows 372 µg for control vs. 284 µg ROL for the 50 αT group in Experiment 1A. Similar extrapolations in Experiment 3A show an estimated mean ROL formed to be 386 vs. 287 µg for the two treatment groups, respectively. Based on these estimates, rats fed 50 mg αT now show only a 25 percent decrease in their ability to convert β-carotene to ROL in both Experiments 1A and 3A.

2) Furthermore, in extrapolating data collected over a one-hour period (Experiment 4) to a 24 hour experimental period, the assumption is made that conversion of β-carotene to RAL occurs

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[1]Keller, J. A., Department of Food and Nutrition, Iowa State University, Ames, Iowa. Personal communication, 1979.

at a uniform rate and that observations made within one hour are representative of occurrences over the entire 24-hour period. These assumptions may not be valid hence quantitative differences in conversion between the 28-day feeding study and a 1-hour metabolic period can be expected. Yet qualitatively the two sets of data compliment each other.

Conditions influencing the in vivo conversion of β-carotene into vitamin A have been extensively reviewed by Olson (1961). He noted that the concentration of vitamin A derivatives in the intestinal wall reached a peak at one hour after injection of ^{14}C-β-carotene suspensions into ligated small intestinal loops. Liver radioactivity, on the other hand, increased more slowly and reached a maximum in 3 to 5 hours. He found relatively small amounts of β-carotene, retinal and retinol in the intestinal wall compared to retinyl esters present. This led to the conclusion that the uptake of β-carotene might be the rate limiting step in the over-all reaction. The requirement for bile appeared to be not solely related to the emulsifying qualities of bile since in the presence of a synthetic emulsifying agent (Tween 20), bile duct-ligated rats failed to convert appreciable amounts of β-carotene into vitamin A products. There also seemed to be a feedback inhibition since the presence of 2 mg ROL or RAL in a mixture containing 20 μg β-carotene severely depressed the formation of vitamin A products from the β-carotene by ligated small intestinal loops.

The β-carotene conversion process has also been studied in vitro by several investigators including Goodman et al. (1966a), Olson and Hayaishi

(1965), and Goodman et al. (1967). From these authors the general characteristics and requirements of β-carotene 15,15'-dioxygenase have been determined. Goodman et al. (1967) estimated that the enzyme required an appropriate combination of detergent plus lipid in order to function at maximum capacity in vitro. Contrary to Olson's finding in the in vivo system, Goodman et al. (1967) found that certain synthetic detergents were adequate substitutes for bile salts in the in vitro system. These investigators also noted that addition of 15 or 250 μg α-tocopherol in 25 μl acetone had no effect on the yield of RAL in an in vitro incubation of β-carotene plus enzyme. It should be pointed out that the highest amount of tocopherol used by Goodman et al. (1967) amounted to only 25 percent of the high level of αT used in Experiment 5. There are other differences between the two experiments. Goodman and coworkers (1967) added tocopherol in acetone separately to the incubation medium; in Experiment 5, αT was combined with the ^{14}C-β-carotene in the carrier before being introduced into the incubation medium. Thus, compared to the present experiments, amounts of tocopherol used by Goodman et al. (1967) were smaller and mode of administration was different. Therefore, the differences obtained in Experiment 5 compared to findings of Goodman et al. (1967) with respect to tocopherol would be expected.

Summary

In summary, data from the experiments reported here suggest that:

1) Hepatic ROL depression cannot be accounted for by growth differences between treatment groups.

2) The inhibition of the conversion process is not as great as hepatic ROL deposits would suggest.

3) High tissue levels of tocopherol do not seem to inhibit the conversion of β-carotene.

4) Excess αT must be simultaneously present with β-carotene in the digesta for this inhibition to occur.

These experiments may be useful in rekindling interest in the as yet unanswered questions involving the actual mechanisms of the conversion of β-carotene to RAL and consequently to ROL. In addition, even though caution must be taken in extrapolating results of animal research to humans, there may be some implications of interest to human nutrition from these data. For example, the vitamin A status of pure vegans, who might consume megadoses of vitamin E without concomitant intakes of preformed vitamin A, may need to be checked periodically. It might also be important to monitor the vitamin A status of patients who have to take therapeutic doses of vitamin E or at least to make such patients aware of the need to obtain some preformed vitamin A in their diets.

SUMMARY AND CONCLUSIONS

Effects of feeding excess α-tocopherol (αT) daily were assessed by measuring:

1) Growth (Experiments 1, 3 and 6),
2) Hepatic retinol (ROL) storage from β-carotene over extended feeding periods (Experiments 1A, 3A and 6),
3) The ability of the small intestinal mucosa of rats prefed high αT to convert β-carotene to vitamin A products (Experiments 1B and 2),
4) The utilization of β-carotene over a 14-hour metabolism period (Experiment 3B).

In another set of experiments, the effects of simultaneous administration of excess αT and β-carotene were assessed on the β-carotene conversion process (Experiments 4 and 5).

In Experiments 1, 3 and 6, weanling rats were fed vitamin A-free diets for 7 days. They were repleted with supplements of β-carotene in the presence of either 1 or 50 mg αT daily for 28 or 56 days. Growth was measured weekly. The extent of β-carotene conversion in the intestinal mucosa was assessed by measuring formation of metabolic products from ^{14}C-β-carotene following intraduodenal injections (Experiment 1B) or by in vitro incubations of ^{14}C-β-carotene with crude enzyme preparations (Experiment 2). In Experiment 3B, vitamin A depleted rats repleted with β-carotene were intubated with ^{14}C-β-carotene and metabolites and excretory products were checked within 14 hours. Hepatic ROL was determined after the rats were killed (Experiments 1A, 3A and 6).

In Experiments 4 and 5, rats weighing approximately 250 g each and with adequate hepatic ROL stores were obtained from the stock colony. The effects of high αT on the β-carotene conversion process were assessed by intraduodenal injections or in vitro incubations of β-carotene mixtures containing excess αT.

Growth

The daily consumption of excessive amounts of αT did not have any adverse effect on the growth rate of rats over feeding periods up to 56 days. Hence, any differences observed in other parameters measured could not be attributed to differences in body size between the two treatments.

Hepatic Retinol

Hepatic deposition of ROL was almost completely abolished when rats received daily supplements of 50 mg αT together with β-carotene as their sole source of vitamin A (Experiments 1A and 3A). The feeding period was extended from 28 to 56 days, yet the inhibitory effect of excess αT on hepatic ROL deposition persisted (Experiment 6). When hepatic ROL deposits were combined with an estimated use of ROL for metabolic needs, the apparent severity of inhibition became greatly reduced.

High Tissue Tocopherol and β-Carotene Conversion

Intraduodenally injected β-carotene was converted to vitamin A products equally well whether rats had been prefed with 1 or 50 mg αT (Experiment 1B). Similarly crude preparations of β-carotene-15,15'-dioxygenase from rats prefed 50 mg αT daily for 28 days were as active

as enzyme preparations from control rats (Experiment 2). Therefore, it was concluded that high tissue content of tocopherol produced by prefeeding large amounts of αT did not inhibit the β-carotene conversion process, at least not in the 40-60 minutes of observation used in Experiments 1B and 2.

In experiment 3B, excretion of radioactivity via feces or urine was not different between the two αT treatments. Hepatic radioactivity was slightly higher with 1 versus 50 mg αT but the difference was not significant. It was concluded that utilization of β-carotene by both treatment groups was essentially the same.

Simultaneous Presence of Excess Tocopherol and β-Carotene Conversion

When β-carotene was injected simultaneously with αT into the duodenum, the formation of product from the GIT of rats injected with excess αT was reduced by 50 percent (Experiment 4). In a subsequent in vitro experiment the presence of excess αT with β-carotene in the incubation medium led to a 25 percent decrease in the formation of RAL, the major metabolic product (Experiment 5). Thus, in order for a significant inhibition of the conversion of β-carotene to occur under these experimental conditions, αT must be simultaneously present with β-carotene. The magnitude of reduction of β-carotene conversion in the short term experiments were compared with hepatic storage data.

In Experiments 1A, 3A and 6, total hepatic ROL of rats fed excess αT constituted only 4-11 percent of control values. However, in Experiments 5 and 4, the observed inhibition of β-carotene conversion by excess αT was only 25 and 50 percent, respectively. Supplementary data

from our laboratory have indicated that both groups of rats in this experimental model used similar amounts of ROL for daily metabolism. Hepatic ROL fails to account for ROL already used for growth and maintenance. Therefore, it was concluded that measuring hepatic ROL alone, resulted in an exagerated assessment of the magnitude of the inhibition of β-carotene conversion by excess αT.

Since in previous studies from this laboratory, retinyl acetate intakes had not been affected by excess αT, it was concluded that αT might affect β-carotene conversion processes prior to the formation of ROL. The exact manner of the interference may not be known until the details of physical and biochemical steps for conversion of β-carotene to vitamin A are more fully understood. It is known that β-carotene-15,15'-dioxygenase requires molecular oxygen for maximum activity, hence excess α-tocopherol may reduce enzyme activity by competing for molecular oxygen. On the other hand, alpha-tocopherol may not have a chemical effect on the β-carotene conversion process but rather a purely physical effect, possibly through altering the physical characteristics of micelle formation, thus reducing the accessibility of β-carotene to the enzyme system.

BIBLIOGRAPHY

Adam, W., and W. F. Korner. 1968. Studies on the reabsorption of vitamins A and E. Int. Z. Vitaminforsch. 38:160-168.

Ames, S. R. 1969. Factors affecting absorption, transport and storage of vitamin A. Am. J. Clin. Nutr. 22:934-935.

Ames, S. R. 1974. Age, parity and vitamin A supplementation and the vitamin E requirement of female rats. Am. J. Clin. Nutr. 27:1017-1025.

Arnrich, L. 1978. Toxic effects of megadoses of fat-soluble vitamins. Nutrition and drug interrelations. Academic Press, Inc., New York.

Bauernfeind, J. C. 1978. Carotenoids: A class of natural pigments in food. AVI Encyclopedia of Food Science 3:113-126.

Bieri, J. G. 1955. Effects of tocopherol on carotene conversion. Proc. Soc. Exp. Biol. Med. 88:482-484.

Bieri, J. G. 1972 Kinetics of tissue-tocopherol depletion and repletion. Ann. N.Y. Acad. Sci. 203:181-191.

Bieri, J. G. 1976. Vitamin E. Vitam. Horm. 34:31-75.

Bieri, J. G., and C. J. Pollard. 1954. Studies of the site of conversion of β-carotene injected intravenously into rats. Br. J. Nutr. 8:32-44.

Bieri, J. G., and R. P. Sandman. 1951. Comparative utilization of carotene administered orally and parenterally. Proc. Soc. Exp. Biol. N.Y. 77:617-621.

Bieri, J. G., R. P. Evarts, and J. J. Gart. 1976. Relative activity of alpha-tocopherol and gamma-tocopherol in preventive oxidative red cell hemolysis. J. Nutr. 106(1):124-127.

Bjornson, L. K., H. J. Kayden, E. Miller, and A. N. Moshell. 1976. The transport of α-tocopherol and β-carotene in human blood. J. Lipid Res. 17:343-352.

Buckingham, L. E. 1974. Excessive alpha-tocopherol intake and the conversion of beta-carotene to retinol in young rats. M.S. Thesis. Iowa State University, Ames, Iowa.

Burns, M. J., S. M. Hauge, and F. W. Quackenbush, 1951. Utilization of vitamin A and carotene by the rat I. Effects of tocopherol, tween and dietary fat. Arch. Biochem. 30:341-346.

Combs, G. F. 1976. Differential effects of high dietary levels of vitamin A on the vitamin E-selenium nutrition of young and adult chickens. J. Nutr. 106:967-975.

Combs, G. F., and M. L. Scott. 1974. Antioxidant effects on selenium and vitamin E function in the chick. J. Nutr. 104:1297-1303.

Corrick, J. A. 1969. Growth and reproduction of albino rats as affected by various excessive levels of dietary vitamin E. Diss. Abstr. B29: 2249B.

Crain, F. D., F. J. Lotspeich, and R. F. Krause. 1967. Biosynthesis of retinoic acid by intestinal enzymes of the rat. J. Lipid Res. 8: 249-54.

Csallany, A. S., and H. H. Draper. 1960. Determination of N,N'-Diphenyl-p-Phenylenediamine in animal tissues. Proc. Soc. Exp. Biol. Med. 104:739-742.

DeLuca, H. F. 1979. Retinoic acid metabolism. Fed. Proc. 38(11):2519-2523.

DeLuca, L. M., J. Glover, J. Heller, J. A. Olson and B. Underwood. 1977. Guidelines for the eradication of vitamin A deficiency and xerophthalmia VI. Recent advances in the metabolism and function of vitamin A and their relationship to applied nutrition. (A report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation Inc., New York.

Dillard, C. J., and A. L. Tappel. 1979. Volatile hydrocarbon and carbonyl products of lipid peroxidation: A comparison of pentane, ethane, hexanal and acetone as in vivo indices. Lipids 14(12): 989-995.

Diplock, A. T. 1978. Biological function of vitamin E on the nature of the interaction of the vitamin with selenium. Wld. Rev. Nutr. Diet. 31:178-183.

Dowling, J. E., and G. Wald. 1960. The biological function of vitamin A acid. Proc. Nat. Acad. Sci. U.S.A. 46:587-608.

Dugan, L. R. 1976. Lipids. Pages 140-143. in O. R. Fennema ed. Principles of food science. Part I. Food Chemistry. Marcel Dekker Inc., New York.

Eaton, H. D., L. D. Matterson, L. Decker, C. F. Helmboldt, and E. L. Jungherr. 1951. Intravenous and oral administration of an aqueous suspension of carotene to calves depleted of their vitamin A stores. J. Dairy Sci. 34:1073-1078.

Emerick, R. J., M. Zile, and H. F. DeLuca. 1967. Formation of retinoic acid from retinol in the rat. Biochem. J. 102:606-611.

Fidge, N. H., and D. S. Goodman. 1968. The enzymatic reduction of retinal to retinol in rat intestine. J. Biol. Chem. 243:4372-4379.

Fidge, N. H., F. R. Smith, and D. S. Goodman. 1969. Vitamin A and carotenoids. The enzymatic conversion of β-carotene into retinal in hog intestinal mucosa. Biochem. J. 114:689-694.

Ganguly, J. 1969. Absorption of vitamin A. Am. J. Clin. Nutrition 22: 923-933.

Goodman, D. S. 1979. Vitamin A and retinoids: recent advances. Introduction, background, general overview. Fed. Proc. 38:2501-2503.

Goodman, D. S., R. Blomstrand, B. Werner, H. S. Huang, and T. Shiratori. 1966a. The intestinal absorption and metabolism of vitamin A and β-carotene in man. J. Clin. Invest. 45:1615-1623.

Goodman, D. S., H. S. Huang, and T. Shiratori. 1966b. Mechanism of the biosynthesis of vitamin A from β-carotene. J. Biol. Chem. 241: 1929-1932.

Goodman, D. S., H. S. Huang, M. Kanai, and T. Shiratori. 1967. The enzymatic conversion of all-trans-β-carotene into retinal. J. Biol. Chem. 242:3543.

Green, J. 1969. Vitamin E and the biological antioxidant theory. Pages 124-148 in DeLuca, H. F. and J. W. Suttie, eds. The fat soluble vitamins. University of Wisconsin Press, Madison, Wisconsin.

Green, J. 1972. Vitamin E and the biological antioxidant theory. Ann. N.Y. Acad. Sci. 203:29-44.

Green, J., and J. Bunyan. 1969. Vitamin E and the biological antioxidant theory. Nutr. Abstr. Rev. 39:321-346.

Green, J., F. R. Muthy, A. T. Diplock, J. Bunyan, M. A. Cawthorne, and E. A. Murrell. 1967. Vitamin E and stress. 7. The interrelationships between polyunsaturated fatty acid stress, vitamin A and vitamin E in the rat and chick. Br. J. Nutr. 21:845-846.

Gronowska-Senger, A., and G. Wolf. 1970. Effect of dietary protein on enzyme from rat and human intestine which converts β-carotene to retinal. J. Nutr. 100:300-308.

Harrill, I., G. Minarik, and E. D. Gifford. 1965. Effect of vitamins A and E on lipids in selected rat tissues. J. Nutr. 87:424-428.

Harris, P. L., M. Kaley, and C. D. Hickman. 1944. Covitamin studies II. The sparing action of natural tocopherol concentrates on carotene. J. Biol. Chem. 152:313-320.

Herbert, J. W., and A. F. Morgan. 1953. The influence of α-tocopherol upon the utilization of carotene and vitamin A. J. Nutr. 50:175-190.

Hickman, C. D., P. L. Harris, and M. R. Woodside. 1942. Interrelationship of vitamins A and E. Nature 150:91-92.

Hickman, C. D., M. Kaley, and P. L. Harris, 1944. Covitamin studies III. The sparing equivalence of the tocopherols and mode of action. J. Biol. Chem. 152:321-328.

High, E. G. 1954. Studies on absorption, deposition and depletion of vitamin A in rat. Arch. Biochem. 49:19-29.

High, E. G. 1956. Further antioxidant studies concerned with the metabolism of carotene and vitamin A. Arch. Biochem. Biophys. 60: 456-62.

High, E. G., and H. G. Day. 1951. Effects of different amounts of lutein, squalene, phytol, and related substances on the utilization of carotene and vitamin A for storage and growth in the rat. J. Nutr. 43:245-249.

High, E. G., H. C. Smith, H. H. Taylor, and S. Wilson. 1954. Antioxidant studies concerned with the metabolism of carotene and vitamin A. J. Biol. Chem. 210:681-686.

High, E. G., and S. S. Wilson. 1953. Effects of vitamin B_{12} on utilization of carotene and vitamin A by rat. J. Nutr. 50:203-212.

High, E. G., L. A. Woods, and S. S. Wilson. 1952. Effects of some alkyl hydroquinones on the utilization of carotene by the rat. J. Biol. Chem. 195:787-793.

Hoekstra, W. G. 1975. Biochemical function of selenium and its relation to vitamin E. Fed. Proc. 34:2083. (Abstr.)

Huang, H. S., and D. S. Goodman. 1965. Vitamin A and carotenoids. I. Intestinal absorption and metabolism of ^{14}C-labelled vitamin A alcohol and β-carotene in the rat. J. Biol. Chem. 240:2839-2844.

Irving, J. T. 1958. The actions of alpha-tocopherol and proteins upon the incisor tooth of the rat and the influence of vitamin A upon alpha-tocopherol activity. Br. J. Nutr. 12:196-202.

Jenkins, M. Y., and G. V. Mitchell. 1975. Influence of excess vitamin E on vitamin A toxicity in rats. J. Nutr. 105:1600-1606.

Kamath, S. K., and L. Arnrich. 1973. Effect of dietary protein on the intestinal biosynthesis of retinol from ^{14}C-β-carotene in rats. J. Nutr. 103:202-206.

Kamath, S. K., J. B. MacMillan, and L. Arnrich. 1972. Dietary protein and utilization of carotene or retinyl acetate in rats. J. Nutr. 102:1579-1584.

Kanai, M., A. Raz, and D. S. Goodman. 1968. Retinol-binding protein: the transport protein for vitamin A in human plasma. J. Clin. Invest. 47:2025-2044.

Keilson, B., B. A. Underwood, and J. D. Loerch. 1979. Effects of retinoic acid on the mobilization of vitamin A from the liver in rats. J. Nutr. 109:787-795.

Kotecheri, S. R. 1967. Studies on the biosynthesis of retinol and the effect of protein in rats. M. S. Thesis. Iowa State University, Ames, Iowa.

Lackshmanan, M. R., H. Chansang, and J. A. Olson. 1972. Purification and properties of carotene 15,15'-dioxygenase of rabbit intestine. J. Lipid Res. 13:477-482.

Lawrence, C. W., F. D. Crain, F. J. Lotspeich, and R. F. Krause. 1966. Absorption, transport and storage of retinyl-15-^{14}C palmitate-9, 10-^{3}H in the rat. J. Lipid Res. 7:226-229.

Lemley, J. M., R. A. Brown, O. D. Bird, and A. D. Emmett. 1947. The effect of mixed tocopherols on the utilization of vitamin A in the rat. J. Nutr. 34:205-218.

Loerch, J. D., B. A. Underwood, and K. C. Lewis. 1979. Response of plasma levels of vitamin A to a dose of vitamin A as an indicator of hepatic vitamin A reserves in rats. J. Nutr. 109:778-786.

Losowsky, M. S., J. Kelleher, B. E. Walker, T. Davies, and C. L. Smith. 1972. Intake and absorption of tocopherol. Ann. N.Y. Acad. Sci. 203:212-222.

Maggio, B. A., A. T. Diplock, and J. A. Lucy. 1977. Interactions of tocopherols and ubiquinones with monolayers of phospholipids. Biochem. J. 161:111-121.

Mathews-Roth, M. M., M. A. Pathak, T. B. Fitzpatric, L. C. Haber, and E. H. Kass. 1970. Beta-carotene as a photoprotective agent in erythropoietic protoporphyria. New England Journal of Medicine 282:1231-1234.

Mattson, F. H., J. W. Mehl, and H. J. Deuel. 1947. Studies on carotenoid metabolism. VII. The site of conversion of carotene to vitamin A in the rat. Arch. Biochem. 15:65-73.

Mayfield, H. L., and R. R. Roehm. 1956. The influence of ascorbic acid and the source of B vitamins on the utilization of carotene. J. Nutr. 58:203-206.

McCuaig, L. W., and I. Motzok. 1970. Excessive dietary vitamin E: its alleviation of hypervitaminosis A and lack of toxicity. Poult. Sci. 49:1050-1052.

McGillivary, W. A., and N. A. Worker. 1957. The utilization of aqueous dispersions of carotene by rats and of carotene and vitamin A by lactating goats. Br. J. Nutr. 11:47-56.

McGillivary, W. A., and N. A. Worker. 1958. The effect of tocopherol on the utilization of intravenously administered carotene. N. Z. J. Agric. Res. 1:273-280.

McLaughlin, P. J., and J. L. Weihrauch. 1979. Vitamin E content of foods. J. Am. Diet. Assoc. 75(6):647-665.

Mehl, J. W., and H. J. Deuel. 1946. Studies on carotenoid metabolism. VI. The relative pro-vitamin A activity of carotene when introduced orally and parenterally in the rat. J. Nutr. 31:299-304.

Milas, N. A. 1954. Vitamins A and carotenes. II. Chemistry and industrial preparation. Vitamins 1:4-58.

Molenaar, I., J. Vos, and F. A. Hommes. 1972. Effect of vitamin E deficiency on cellular membranes. Vitam. Horm. 30:45-82.

Molenaar, I., C. E. Hulstaert, and J. Vos. 1973. Membrane characteristics in vitamin E deficiency and the assessment of vitamin E status. Proc. Nutr. Soc. 32:249-254.

Moore, T. 1940. The effect of vitamin E deficiency on vitamin A reserves of the rat. Biochem. J. 34:1321-1328.

Moore, T. 1957. Vitamin A. Elsevier Publishing Company, Amsterdam.

Navab, M., J. E. Smith, and D. S. Goodman. 1977. Rat plasma prealbumin. Metabolic studies on effects of vitamin A status and on tissue distribution. J. Biol. Chem. 252:5107-5114.

Olson, J. A. 1961. The conversion of radioactive β-carotene into vitamin A by the rat intestine in vivo. J. Biol. Chem. 236-349.

Olson, J. A. 1978. Evaluation of vitamin A status in children. Wld. Rev. Nutr. Diet. 31:130-134.

Olson, J. A. 1979. A simple dual assay for vitamin A and carotenoids in liver. Nutr. Rep. Int. 19:807-813.

Olson, J. A., and D. Gunning. 1980. Cellular aspects of vitamin A storage in rat liver. Fed. Proc. 39:340. (Abstr.)

Olson, J. A., and A. O. Hayaishi. 1965. The enzymatic cleavage of β-carotene into vitamin A by soluble enzymes of rat liver and intestine. Proc. Nat. Acad. Sci. 54:1364-1370.

Olson, J. A., and M. R. Lakshmanan. 1970. Enzymatic transformations of vitamin A, with particular emphasis on carotenoid cleavage. Pages 213-226 in H. F. DeLuca and J. W. Suttie, eds. The Fat Soluble Vitamins. University of Wisconsin Press, Madison, Wisconsin.

Park, K. N. 1975. Beta-carotene utilization in young rats modified by excess tocopherol and dietary fat. M. S. Thesis. Iowa State University. Ames, Iowa.

Peake, I. R., and J. G. Bieri. 1971. Alpha- and gamma-tocopherol in the rat: in vitro and in vivo tissue uptake and metabolism. J. Nutr. 101:1615-1622.

Pudelkiewicz, W. J., L. Webster, and L. D. Matterson. 1964. Effects of high levels of dietary vitamin A acetate on tissue tocopherol and some related observations. J. Nutr. 84:113-118.

Quackenbush, F. W., R. P. Cox, and H. Steenbock. 1942. Tocopherol and the stability of carotene. J. Biol. Chem. 145:169-177.

Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. J. Clin. Invest. 49:465-471.

Rockley, N. L., B. A. Halley, and E. C. Nelson. 1980. Identification of a decarboxylation product of retinoic acid. Biochem. Biophys. A 627(3):270-275.

Ross, C. A., and D. S. Goodman. 1979. Intracellular binding proteins for retinol and retinoic acid: Comparison with each other and with serum retinol-binding protein. Fed. Proc. 38:2515-2518.

Rotruck, J. T., A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science 179:588-590.

Sherman, W. C. 1941. Activity of α-tocopherol in preventing antagonism between linoleic and linolenic esters and carotene. Proc. Soc. Exp. Biol. Med. 47:199-200.

Sherman, W. C. 1942. Influence of α-tocopherol and unsaturated fatty acids upon utilization of vitamin A. Fed. Proc. 1:134. (Abstr.)

Sherman, W. C. 1947. Relative gastro-intestinal stability of carotene and vitamin A and protective effect of xanthophyll. Proc. Soc. Exp. Biol. Med. 65:207-210.

Shinde, R., R. C. Das, K. Sarada, and J. Ganguly. 1980. Isolation of binding-proteins for ROL from cytosol, nucleosol and chromatin of rat testes. Int. J. Biochem. B, 17(2):135-138.

Smith, F. R., and D. S. Goodman. 1971. The effect of diseases of the liver, thyroid, and kidneys on the transport of vitamin A in human plasma. J. Clin. Invest. 50:2426-2436.

Smith, J. E. and D. S. Goodman. 1979. Retinol-binding protein and the regulation of vitamin A transport. Fed. Proc. 38:2504-2509.

Smith, F. R., D. S. Goodman, M. S. Zaklama, M. K. Gabr, S. ElMaraghy, and V. N. Patwardham. 1973. Serum vitamin A, retinol binding protein, and prealbumin concentrations in protein-calorie malnutrition. I. A functional defect in hepatic retinol release. Am. J. Clin. Nutr. 26:973-981.

Smith, F. R., B. A. Underwood, C. R. Denning, A. Varma, and D. S. Goodman. 1972. Depressed plasma retinol-binding protein levels in cystic fibrosis. J. Lab. Clin. Med. 80:423-433.

Snedecor, G. W., and W. G. Cochan. 1967. Statistical methods. 6th ed. Iowa State University Press, Ames, Iowa.

Soliman, M. K. 1972. High vitamin A dosage. Int. J. Vitam. Nutr. Res. 42:389-393.

Sporn, M. B., and D. L. Newton. 1979. Chemoprevention of cancer with retinoids. Fed. Proc. 38:2528-2534.

Stoecker, B. S. 1970. Biosynthesis of retinol as affected by dietary protein and feeding patterns in carotene-fed rats. Ph.D. Thesis. Iowa State University, Ames, Iowa.

Stoecker, B., and L. Arnrich. 1973. Patterns of protein feeding and the biosynthesis of vitamin A from carotene in rats. J. Nutr. 103: 1112-1118.

Swick, R. W., and C. A. Bauman. 1951. Effect of certain tocopherols and other antioxidants on the utilization of beta-carotene for vitamin A storage. Arch. Biochem. Biophys. 36:120-126.

Tappel, A. L. 1962. Vitamin E as the biological lipid antioxidant. Vitam. Horm. 20:493-498.

Tappel, A. L. 1980. In vivo lipid peroxidation: measurement via exhaled pentane and vitamin E protection. Fed. Proc. 39: (in press).

Thompson, S. Y., R. Braude, M. E. Coates, A. T. Cowie, J. Ganguly, and S. K. Kon. 1950. Further studies of the conversion of β-carotene to vitamin A in the intestine. Br. J. Nutr. 4:398-421.

Tomarelli, R. M., J. Charney, and F. W. Bernhart. 1946. Utilization of intramuscularly injected carotene. Proc. Soc. Exp. Biol. N.Y. 63: 108-110.

Underwood, B. A., J. D. Loerch, and K. C. Lewis. 1979. Effects of dietary vitamin A deficiency retinoic acid and protein quantity and quality on serially obtained plasma and liver levels of vitamin A in rats. J. Nutr. 109:796-806.

Vahlquist, A., K. Sjolund, A. Norden, P. A. Peterson, G. Stigmar, and B. Johansson. 1978. Plasma vitamin A transport and visual dark adaptation in diseases of the intestine and liver. Scand. J. Clin. Lab. Invest. 38:301-308.

Venkataswamy, G., J. Glover, M. Cobby and A Pirie. 1977. Retinol-binding protein in serum of xerophthalmic, and malnourished children before and after treatment at a nutrition center. Am. J. Clin. Nutr. 30: 1968-1973.

Wang, C. H., D. L. Willis, and W. D. Loveland. 1975. Radiotracer methodology in the biological, environmental, and physical sciences. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Wiese, C. E., J. W. Mehl, and H. J. Deuel. 1947. Studies on carotenoid metabolism. VIII. The in vitro conversion of carotene to vitamin A in the intestine of the rat. Arch. Biochem. 15:75-79.

Williams, M. A., L. C. Chu, D. J. McIntosh, and I. Hicenbergs. 1968. Effects of dietary fat level on pantothenate depletion and liver fatty acid composition in the rat. J. Nutr. 94:377-382.

Zachman, R. D., and J. A. Olson. 1961. A comparison of retinene reductase and alcohol dehydrogenase of rat liver. J. Biol. Chem. 236:2309-2313.

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APPENDIX

Hepatic β-Carotene

During the extraction of liver in Experiment 3 it was repeatedly noted that liver extracts from the 50-αT group were visibly more yellow than extracts from control rats. A pool of extracts from several rats was chromatographed on deactivated alumina and the hexane fraction from the column was scanned in a spectrophotometer.[1] A characteristic β-carotene spectrum was obtained (Fig. 3). Attempts to quantitate the β-carotene proved unreliable. The best estimates were about 0.5 μg β-carotene per liver. The definite presence of significant amounts of β-carotene in extracts of livers from the 50-αT group raised some interesting speculations, such as:

1) The absorption of β-carotene was enhanced by excess αT.
2) Gastrointestinal transit time was delayed by excess αT leading to a delay in the peak appearance of β-carotene in the liver.

Effects of excess αT on β-carotene absorption, and peak appearance in serum and liver may provide new directions for future study.

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[1] Perkin Elmer, model 552.

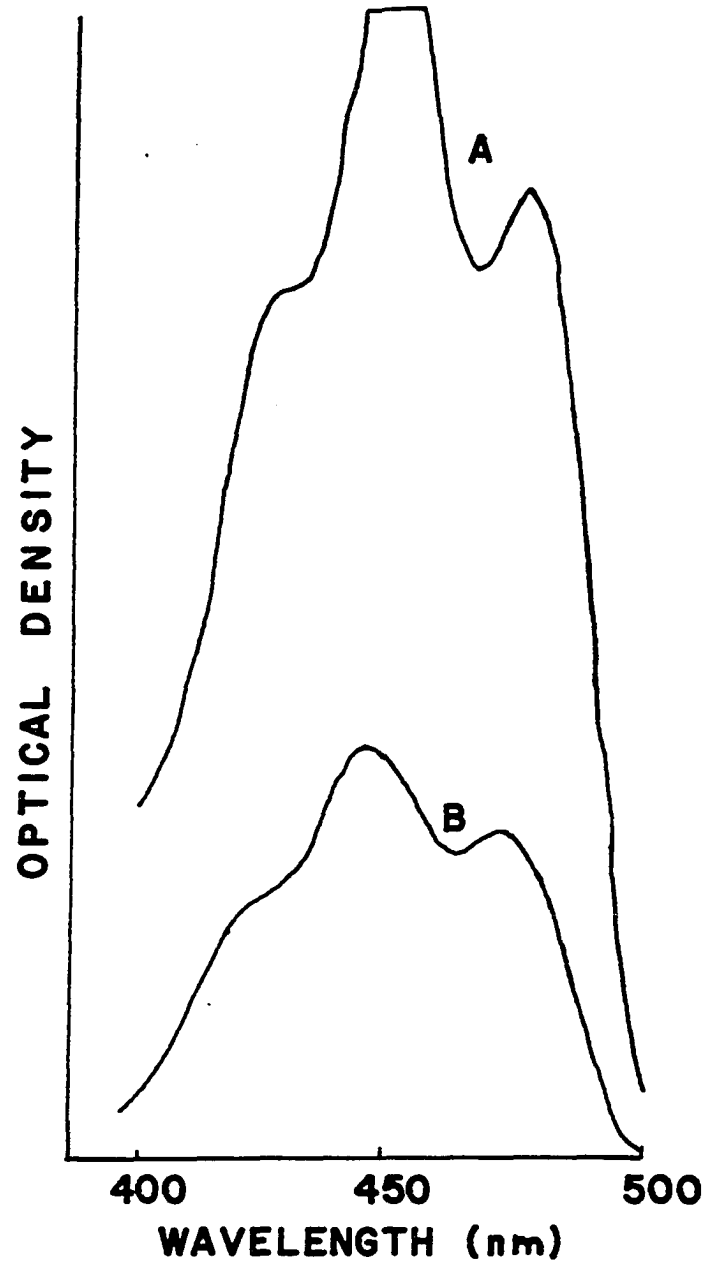

Figure 3. Beta-carotene spectra

A. Standard all-trans β-carotene.

B. Liver extracts from 50-αT group in Experiment 3.