

THE EFFECT OF LYCOPENE ON THE UTILIZATION OF
BETA-CAROTENE AS MEASURED BY THE STORAGE OF
VITAMIN A IN THE LIVERS OF RATS

by

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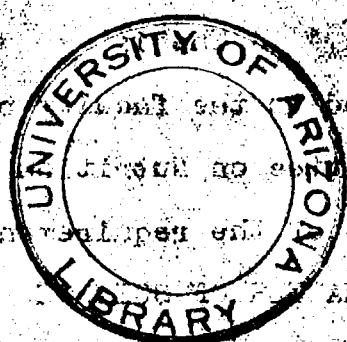
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THE EFFECT OF LYCOPENE ON THE UTILIZATION OF BETA-CAROTENE AS MEASURED BY THE STORAGE OF VITAMIN A IN THE LIVERS OF RATS

INTRODUCTION

Lycopene, the principal red pigment in tomatoes, is closely related to beta-carotene in chemical structure. Unlike beta-carotene, lycopene has no vitamin A activity. It has often been demonstrated that substances which do not have biological activity but have structures similar to that of the active substance will materially lower the activity of the latter substance. For instance Kemmerer et al. found that crude xanthophylls lower the biological activity of beta-carotene for rats (14) and chicks (14a). However, High and Day (8) reported that lycopene increased the amount of vitamin A stored in the liver of the rat when the lycopene was fed with either beta-carotene or vitamin A. This is not in agreement with the previous findings on xanthophylls. Either the lycopene used by High and Day contained traces of beta-carotene, or the lycopene did not perform like the xanthophylls in the experiments of Kemmerer and associates. This thesis reports an experiment on the utilization of beta-carotene for the storage of vitamin A in the livers of rats.

A REVIEW OF THE LITERATURE ON CAROTENOIDS

Description and Properties of Carotenoids

Carotene, lycopene, and xanthophylls are carotenoid pigments, yellow to red in color, and are widely distributed in the animal and vegetable kingdoms. Carotenoids have the following distinguishing features: They are generally composed of isoprene residues, usually eight, arranged so that the middle of the molecule has two methyl groups in respect to each other in 1:6 position and all other side-chain methyl groups are in 1:5 positions. The general structure on the carotenoids is of the aliphatic or aliphatic-alicyclic type, and their chromophoric systems contain conjugated carbon-carbon double bonds (11). All carotenoids are soluble in fats and fat solvents. Only the water-soluble carotenoids have acidic groups such as carboxyl or enol which can form water-soluble alkali salts, or have lyophilic properties from esterification with sugar residues.

At the present time between 70 and 80 carotenoids have been found in nature, and the chemical constitutions of about 35 are known. They are all closely related chemically; in fact they can all be derived from one carotenoid, lycopene, by simple chemical changes such as cyclization, double bond migration, partial hydrogenation, introduction of hydroxyl-,

keto-, or methoxyl-groups, or introduction of an oxygen bridge, and so forth (11).

Carotenoids in Plants and Animals

Although much investigation has been carried out and many hypotheses have been put forth, no definite conclusions have been reached as to the mode of formation and the function of carotenoids in plants. (11). More is known on the functions of carotenoids in the animal organism, but many important questions are yet to be answered. A number of carotenoids are converted into vitamin A in animals and therefore play the part of pro-vitamins.

Steenbock and his collaborators (23) were the first to suggest a connection between the yellow plant pigments carotene and vitamin A. Karrer (11) in 1937 observed the structural relation between beta-carotene and vitamin A and postulated that in the conversion of beta-carotene to vitamin A in the animal body, two molecules of vitamin A were produced from one molecule of beta-carotene. In 1948 Koehn (15) determined the relative biologic potencies of pure vitamin A alcohol, its acetate, and beta-carotene. His results showed that beta-carotene and vitamin A had equal activity on the weight basis. This supported the original theory of Karrer that conversion of beta-carotene into vitamin A is by fission of the molecule to yield two molecules of vitamin A aldehyde.

(11). This problem is still not solved as many workers have found that beta-carotene has only one-half the activity of vitamin A. It has been assumed that this conversion process depends on a ferment, carotenase.(11). In animals deficient in vitamin A, the conversion of pro-vitamins into vitamin A takes place rapidly and fairly completely (up to 70 or 80%). If the organism is saturated with vitamin A, however, or if high doses of pro-vitamins are given, only a small proportion is converted into the vitamin.(11).

The site in the animal body of this conversion is still under question. Moore (17) in 1931 found that rat livers were high in vitamin A content after the rats had been fed carotene, and that the alimentary tract was high in carotene but low in vitamin A. He interpreted this to mean that the site of conversion of carotene to vitamin A took place in the liver. Mattson, Mehl, and Deuel (19) in 1947 concluded that transformation of carotene to vitamin A took place in the small intestine of rats. By different experimental techniques Krause and Pierce (20) confirmed Mattson's findings. They partially ligated the portal vein, the portal artery and the common bile duct of rats. Carotene was fed and careful tests made. Their results indicated that hepatic circulation was not necessary for the conversion of carotene to vitamin A. In vitro conversion of carotene to vitamin A in the small intestine of the rat has recently been reported by Rosenberg and Sobel.(21).

Carotene was introduced by tube into the stomachs of vitamin A deficient rats; the rats were killed immediately and contents of the stomach squeezed into the small intestine which was tied with surgical thread and incubated for 2 hours in Ringers solution at 45° C. The intestinal contents were then flushed out and the gut tissue examined for vitamin A. These findings do not rule out the liver as an auxiliary site for the conversion of carotene to vitamin A.

As will be brought out later in this paper, many materials have substantial effects on the utilization of carotene and vitamin A storage. Factors within the animal body affect vitamin A storage. Johnson and Bauman (9) in 1948 showed that hypo- and hyperthyroidism exerted an effect, and Vavich and Kemmerer (26) that the size of the rat exerted an effect. In the latter work small rats (46 to 50 g.) stored more vitamin A than large rats (99 to 103 g.) when fed a given amount of carotene.

The Isolation of Carotenoids

The general method of Carter (2) for isolation of carotenoids consists usually of the following steps:

1. Extraction of carotenoids. The vegetable or animal source is dehydrated, then extracted with one of a number of suitable solvents. Those commonly used are benzene, petroleum ether, ethyl ether free from peroxide, carbon disulphide,

chloroform free from hydrochloric acid, ethanol, methanol and acetone. The extracts are concentrated in vacuum and protected from air.

2. Separation into hypophase and epiphase carotenoids. Carotenoids with two or more hydroxyl groups are obtained as hypophasic pigments. Mono-hydroxy compounds occupy an intermediate position and are found in the epiphase as well as in the hypophase.

3. Separation of the carotenoids within the hypo- and epiphases. The method usually used is Tswett's (25) chromatographic adsorption analysis. This analysis is carried out by percolating the solution of the carotenoids through a long column of suitable adsorbent. The amount of adsorbent used depends on the amount of pigments to be separated. The individual pigment zones are "developed" by washing the column with suitable solvents.

Constitution of Carotenoids and Their Absorption Spectrum

Carotenoids range in color from yellow to violet. Attempts have been made in the past to elucidate the relationships between the color and the constitution of carotenoids and to employ absorption spectra for the characterization and identification. Today it is possible to draw definite conclusions regarding the constitution of a carotenoid from its absorption spectrum.(11). Also certain changes in the spectrum can be predicted from a given change in structure.

Although carotenoids possess a relatively complex structure, the absorption spectra of these pigments are comparatively simple in character. In the visible region the spectrum usually consists of three or four absorption maxima and the position of the maxima depends upon the constitution of the pigments.

Distribution of Carotenoids in Nature

All green and yellow parts of plants contain carotenoids, mainly beta-carotene, xanthophylls, xanthophyll epoxides and small amounts of alpha-carotene. The carotenoids are found together with chlorophyll in the chromatophores and are present in either an amorphous or crystalline state. Carotenoids, especially xanthophylls, are present in etiolated leaves, but it is doubtful that they are responsible for the yellow color (11). In yellow autumn leaves it has been possible to recognize different stages in carotenoid metabolism. The carotenoids and also the anthocyanins remain in the leaves after the degradation of the chlorophyll in the autumn and produce the well-known striking colorations (11). Gradually the polyene pigments are degraded with carotene being decomposed more rapidly than xanthophylls. In the last phases of the necrobiosis, the brown pigments are produced.

Carotenoids are widely distributed in blossoms (11). About 35 different carotenoids, about half of all the known polyene pigments, have been isolated from blossoms up to the

present time. Nothing is known, however, concerning the function of the carotenoids in the blossoms. Many carotenoids are found in the fruits and seeds of plants.

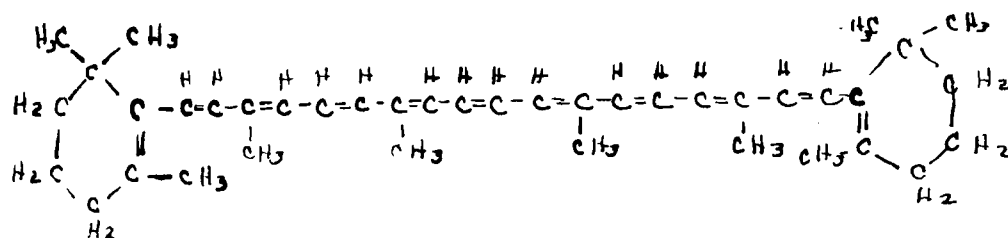
Although present information on carotenoids of cryptogams is scanty we know they are distributed in this group as well as in the phanerogams. In fact recent observations show that bacteria produce carotenoids not found in the higher plants.(11). This presents an interesting field for further study.

Carotenoids are present in a variety of invertebrates, though it is not known with certainty whether the pigments are synthesized by the animal or contained in the food. In contrast to the invertebrates a great deal of information is known of carotenoids in the vertebrates. Carotenoids have been found in nearly every part of the mammalian organism. It is almost certain that these are derived from vegetables.(11).¹⁾

Carotene

Wakenroder discovered carotene in the roots of carrots in 1831. For 100 years after that the substance he discovered was regarded as a homogenous compound. It wasn't until the chromatographic method were available that the complex nature of carotene was established.(11). In 1866 Arnaud established that carotene was a hydrocarbon. In 1907 Willstatter and Mieg proved the identity of carotene from leaves and carrots establishing the molecular formula as $C_{40}H_{56}$. In 1928

Zechmeister established the presence of 11 double bonds and 2 ring systems in carotene. In 1929-31 Karrer elucidated the constitution of beta-carotene, and in 1935 the formula was proved by Kuhn and Brockmann.

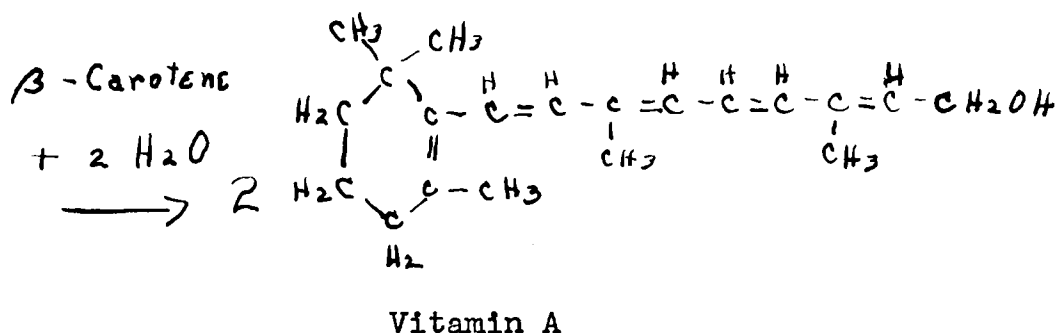


Beta-carotene

When a pigment is designated by its current name (beta-carotene, lycopene, etc.) it is usually considered the all-trans form in which each double bond possesses trans-configuration. The neo isomer contains cis configuration about some of the double bonds. Methyl groups along a chain introduce steric hindrance which limits the number of likely centers of rearrangement. Beta-carotene has 20 isomers. These include the natural all-trans-beta-carotene, 3 mono-cis-, 6 di-cis-, 3 tetra-cis-, and 1 all-cis-beta-carotene. Stereoisomers of carotene showing biological activity are all-trans-beta-carotene, neo-beta-carotene U, neo-beta-carotene B, all-trans-alpha-carotene, neo-alpha-carotene U, neo-alpha-carotene B, all-trans-gamma-carotene, pro-gamma-carotene, neo-gamma-carotene, and cryptoxanthin. Their relative potencies respectively are: 100, 38, 53, 53, 13, 16, 28, 44, 19, and 57.

Beta-carotene crystallized from benzene-methanol exists

as dark violet hexagonal prisms and as red rhombic plates from petroleum ether. Its melting point is from 181 to 187.5° It is soluble in carbon disulfide, benzene and chloroform, and slightly less soluble in ether and petroleum ether. It has absorption maxima of 457 mμ, and is optically inactive. Beta-carotene is fairly strongly adsorbed on calcium hydroxide from petroleum ether solution. It is found below gamma-carotene and above alpha-carotene on the chromatographic column, and it can be separated from other carotenoid hydrocarbons by this method and then identified by its absorption maxima. On standing, beta-carotene absorbs oxygen from the air.(11). Beta-carotene has high vitamin A potency and the change in formula takes place as follows:

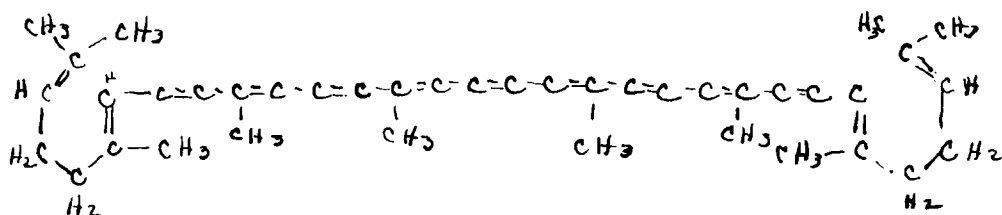


Lycopene

Hartsen isolated a dark red crystalline pigment in 1873, which was later identified as lycopene. In 1903, Schunck showed that the pigment from tomatoes, which he termed lycopene, had an absorption spectrum different from that of carotene. Willstatter and Echer in 1910 made a detailed investigation of

lycopene. They determined the corrected molecular formula as $C_{40}H_{56}$ and recognized that lycopene was an isomer of carotene. From 1928 to 1931, Karrer (11) and co-workers elucidated the constitution of lycopene. In 1932, Kuhn and Grundmann carried out the chromic acid oxidation of lycopene and obtained long-chain degradation products, the constitution of which confirmed the formula of lycopene(11).

Lycopene is widely distributed in nature in large amounts in ripe fruit and in smaller amounts in other parts of plants and in animal sources.



Lycopene

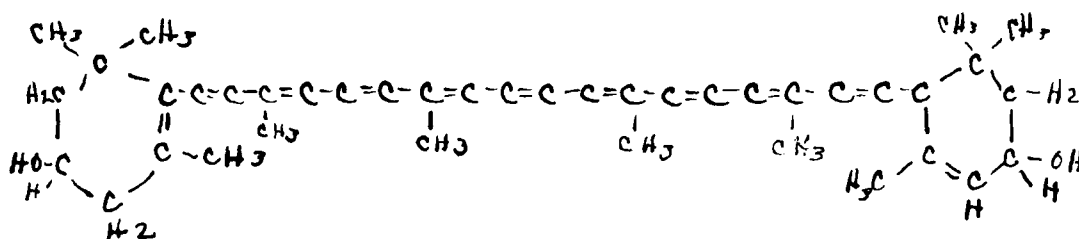
Lycopene crystallizes from carbon disulfide and ethanol in long red needles. From petroleum ether it crystallizes in felted, hair-like needles, and sometimes in long, dark, red-violet prisms. It melts from 170° to 175° C. and is soluble in carbon disulfide, chloroform, benzene, and slightly in hot ethanol. Lycopene is optically inactive. Lycopene dissolved in petroleum ether is adsorbed six times more strongly than carotene on alumina, calcium oxide or calcium hydroxide(11). It is best identified by chromatographic separation on calcium hydroxide, followed by a determination of the absorption

maxima. Lycopene has not yet been proved to have vitamin A potency.

Xanthophyll

In 1837 Berselius coined the term "xanthophyll" for the yellow pigment of autumn leaves. Wilstatter and Mieg in 1907 isolated xanthophyll from green leaves in crystalline state. In 1912 Wilstatter and Escher isolated lutein from egg yolk. The single recognized leaf xanthophyll of 30 years ago has since been shown to be a mixture of 12 or more pigments in some plant species. The principal xanthophyll in plants is lutein which is always accompanied by zeaxanthin and cryptoxanthin.(5).

Xanthophylls are very widely distributed in nature. They are found in all green parts of plants and in red and yellow blossoms. Also they are found in human fat and liver, egg yolk, in feathers and other animal and vegetable sources.



Lutein

Hydroxy, oxo, and hydroxy-oxo derivatives of carotene are xanthophylls. They crystallize from methanol in violet prisms which have a metallic lustre and dovetail shape(11). They are soluble in chloroform, benzene, acetone, ether and

carbon disulfide, and slightly in ethanol and methanol.

Xanthophylls are strongly optically active. They are separated from other phytoxanthins by adsorption on zinc carbonate and identification by determination of the absorption maxima. Lutein and most xanthophylls possess no vitamin A activity, however cryptoxanthin is a precursor of vitamin A(5) (6)

Present Knowledge of the Effect of Lycopene and Xanthophylls on the Absorption of Carotene and Vitamin A

By 1947 it was well known that when carotene of vegetables was fed to rats less vitamin A storage occurred than when carotene was fed dissolved in cottonseed oil. It was speculated then that the utilization of carotene in vegetables might be affected by substances that accompany it in such plants.

In 1947 Kemmerer et al. (14) found that xanthophylls and chlorophyll when fed with carotene decreased the utilization of carotene for liver storage of vitamin A about 20%. The findings of Kemmerer et al. were confirmed and extended in 1948 by Kelly and Day (12) who stated that relatively large amounts of xanthophyll (lutein) impair the utilization of vitamin A itself as well as of carotene. Johnson and Baumann (10) in 1948 also found that large amounts of lutein decrease the utilization of carotene. On the other hand using weight gain and mortality as criteria, Sherman (22) in 1947

observed that small amounts of lutein seemed to have a carotene-sparing action when given to rats on a vitamin E low diet. In 1950, Kelly and Day (13) reported further findings confirming earlier works which demonstrated that the adverse effect on carotene and vitamin A utilization was due, not to hastened depletion of liver and kidney stores, but to little if any absorption of the xanthophylls from the intestinal tract of experimental rats.

Owing to the structural similarities between lutein and beta-carotene it was thought by Kelly and Day at the beginning of their investigation that lutein might compete with carotene as a substrate in the enzymatic conversion of the latter to vitamin A, and thus impair the formation of vitamin A, but this was not confirmed.

The evidence available at that time offers no satisfactory explanation for the seemingly contradictory findings of Sherman. However, in the investigations reported by Kelly and Day the diet contained ample vitamin E, and the carotene to xanthophyll ratios were much larger than in Sherman's experiments.

In an attempt to answer their contradictory questions an experiment was carried out by High and Day (7) and reported in 1951. They studied the carotene to lutein ratio on the utilization of carotene. Also, they investigated the effect of various other structurally related compounds on the utilization of carotene and vitamin A. There was reason to suspect

that some of these compounds were antagonists of vitamin A or that they might impair the utilization of its precursors. Vitamin A depleted weanling rats were fed different levels of beta-carotene from 15 to 60 μ g. per day. Lutein was given in varying amounts from 5 to 500 μ g. with the different levels of beta-carotene. The addition of small amounts of lutein increased the quantity of vitamin A stored in the liver and kidneys, but increasing lutein from 25 to 500 μ g. per day without changing the beta-carotene resulted in a decrease of over 100% in the amount of vitamin A deposited. Also large amounts of squalene, phytol and alpha tocopherol acetate decreased the storage of vitamin A. Relatively small amounts of alpha-ionone, beta-ionone, and hydroquinone had no effect. The utilization of vitamin A by vitamin A depleted rats was not affected by large supplements of squalene or phytol. The rate of loss of vitamin A from the livers and kidneys of rats was not affected by any of the supplements given.

In the light of these experiments, High and Day (8) carried out an experiment reported in 1952 on the fate of lycopene in the rat and its effects on the utilization of carotene and vitamin A. Scarcely any data on the metabolic effects and the fate of lycopene in the rat were available. Due to the similarity of lycopene and carotene and their frequent occurrence together in nature, it was of interest to investigate the effects of lycopene on carotene and vitamin A utilization. The results of their experiments showed

that relatively small amounts of lycopene fed with either carotene or vitamin A to vitamin A depleted rats increased the utilization of both for tissue deposition of vitamin A. Large amounts of lycopene increased vitamin A deposition in vitamin A fed rats but had no effect on vitamin A deposition from carotene. Lycopene was absorbed and deposited as lycopene in the livers of rats in approximately 2-6% of the total amount ingested. No lycopene was detected in the kidneys.

The sparing effect of small amounts of lycopene on carotene and vitamin A may be due to its protection of the carotene and vitamin A from oxidative decomposition in the alimentary tract. High and Day speculated that the apparent failure of lycopene to exert a protective effect on carotene when the amount ingested is large may have been due to inhibition of the conversion of carotene to vitamin A which masked the sparing effect of the lycopene. The effect of other accompanying substances such as tocopherol in this respect has not been determined. Many interesting questions remain unanswered and suggest problems for further investigation.

OBJECTIVE AND DEFINITION OF PROBLEM

Because of the existance of the many unanswered questions in this field and because of the reported conflicting findings, it is of interest to investigate further in an attempt to confirm previous conclusions or perhaps shed some new light on the matter. The particular phase of this problem which

this paper reports concerns the influence of lycopene on the conversion of beta-carotene to vitamin A.

EXPERIMENTAL PROCEDURE

Preparation of Lycopene

The method for the isolation of lycopene follows in general the method for isolating carotenoids as described on page 5. One kilogram of canned tomato paste was dehydrated with 1.3 liters of methanol (2). The mixture was mixed in a Waring blender for five minutes and then allowed to stand for one hour. The mixture was mixed again in the Waring blender and filtered through a Buchner funnel fitted with filter paper. The yellow xanthophyll filtrate was discarded.

The dark red cake in the funnel was returned to the Waring blender and mixed with 650 ml. methanol and 650 ml. carbon tetrachloride. This mixture was again filtered through a Buchner funnel. The residue was re-extracted with more methanol and carbon tetrachloride as before and the suspension filtered. The filtrate consisted of a very dark red, carbon tetrachloride phase and an orange aqueous methanolic layer. All of the filtrate was put into a separatory funnel and one volume of water added. A white emulsion developed in the upper phase. A glass tube was introduced into the lower phase, and a small stream of distilled water was allowed to flow through the carbon tetrachloride layer for ten minutes to free it from any methanol or other impurities. The carbon tetrachloride solution was drained into an Erlenmeyer flask and dried over anhydrous sodium sulfate. The extract was then poured

through a fluted filter into a two liter round bottomed flask equipped with a standard taper joint. The solvent was evaporated to about 100 ml. in a vacuum distillation apparatus over a water bath at 60° C. The solution was then transferred quantitatively with a little carbon tetrachloride to a 200 ml. flask, and the solvent was removed completely in vacuum.

The dark oily residue was diluted with a few milliliters of benzene and evaporated again in order to eliminate the carbontetrachloride completely. The partially crystalline residue was transferred quantitatively with 25 ml. of benzene into a 125 ml. Erlenmeyer flask. The flask was warmed in hot water to obtain a clear solution. Fifteen ml. of boiling methanol was added slowly, stirring constantly. Crystals began to appear immediately. Crystallization was completed by placing the flask in the refrigerator over night. The crystals were then collected in a small fritted-glass funnel of medium porosity. In order to eliminate impurities, the suction was stopped, 10 ml. of boiling methanol poured on the crystals, and, after stirring with a glass rod, the suction was renewed before the methanol cooled. This was repeated.

Lycopene crystals were transferred to a 50 ml. centrifuge tube by dissolving from the funnel with boiling benzene. Benzene was added to the tube to make a volume of 25 ml. The lycopene was crystallized out with 20 ml. boiling methanol using the same method as before and allowing to stand in the

refrigerator for two hours. The crystals were separated by centrifuging at 2500 R.P.M. for 10 minutes. The liquid was decanted and discarded. The crystals were treated with 25 ml. boiling methanol, stirred, and the methanol removed by centrifuging. The methanol was decanted, and the washing was repeated twice more. The lycopene was dried in a vacuum desiccator.

A sample of the lycopene was dissolved in a 1:1 mixture of skelly solv and ethyl ether and poured into a lime-packed chromatographic column(24). The column was developed with a 10:1 mixture of the skelly-ether solution and acetone. Only one color zone developed which showed the crystals to be pure lycopene. However, to be absolutely sure the pigment was eluted from the lime, the solvent was evaporated, and the pigment redissolved in skelly solv. The solution was then examined on the Beckman spectrophotometer to determine the absorption maxima of the pigment. A graph of the light absorption was compared with that of pure lycopene. The two corresponded identically, being 506.0, 475.5, and 447.9 millimicrons in each case, proving the extracted lycopene to be of satisfactory purity.

Preparation of Supplement for Feeding to Rats

Samples of beta-carotene and lycopene were weighed and dissolved in very small amounts of chloroform. Cottonseed oil was added to the sample and each was heated and stirred until clear solutions were obtained. The two solutions were read on the Evelyn colorimeter to determine the concentration.

Aliquots of the two solutions were combined, with the addition of more cottonseed oil, in appropriate weighed portions so that 0.2 g. of each of the eight supplements gave the desired amounts of both beta-carotene and lycopene. An eye dropper was calibrated to deliver 0.2 g. Eight drops delivered from the dropper averaged 0.2 g. of oil varying only 0.004 g. more or less.

Procedure of Feeding Rats

Ninety-eight weanling albino rats were placed on a vitamin A free diet with water ad libitum. The vitamin A free diet consisted of 18% vitamin A free casein, 4% salt mixture, 6% brewers yeast, 2% irradiated yeast, 65% corn starch, and 5% cottonseed oil. The salt mixture in the vitamin A free diet was made up of the following ingredients: 84.42% calcium carbonate, 4.21% calcium citrate, 1.54% calcium biphosphate, 0.49% magnesium carbonate, 0.54% magnesium sulphate, 1.70% potassium chloride, 3.00% dibasic potassium phosphate, 1.06% sodium chloride, 0.007% cupric sulphate, 1.29% ferric ammonium citrate, 0.017% manganous sulphate, 0.008% ammonium alum, 1.72% potassium iodide, 0.044% sodium fluoride. When their gain in weight ceased, indicating a nearly complete depletion of liver stores of vitamin A, 84 of the rats were selected on the basis of litter, sex, and weight and were divided into eight groups of ten rats each, with four rats for controls.

Administration of Supplement

The eight groups of rats were given vitamin A free diet and water ad libitum plus the beta-carotene and lycopene supplement for twelve days. The supplement was administered with the calibrated eye dropper directly into the mouth of the rat to insure minimum loss. The first group of rats was fed 27.5 μ g. beta-carotene daily. The second group was given 55.0 μ g. beta-carotene. Groups 3, 5, and 7 were given the same amount of beta-carotene as group one with the addition of 25 μ g., 100 μ g., and 300 μ g. of lycopene respectively. Groups 4, 6, and 8 were given the same amount of beta-carotene as group 2 with the addition of the 25 μ g., 100 μ g., and 300 μ g. of lycopene respectively. Four control rats were continued on the vitamin A free diet and water exclusively. The thirteenth day all were given only the vitamin A free diet and water. On the fourteenth day they were weighed and sacrificed with chloroform. The livers were removed immediately, weighed and placed in stoppered Erlenmeyer flasks containing 30 ml. of 12% alcoholic potassium hydroxide. These were stored at 0° F. until analyzed.

Analysis of Livers for Vitamin A

Each liver was saponified by boiling in 12% alcoholic potassium hydroxide for 30 minutes on a hot plate. The liver was allowed to cool and 25 ml. of peroxide-free ethyl ether added. This mixture was transferred to a 500 ml. separatory funnel. The flask was washed with water and the washings added to the

funnel until an equal volume of water had been added. The two phases were allowed to separate and the lower aqueous phase drawn off. The upper phase was collected in the flask used for the saponification. The aqueous phase was returned to the funnel and another 25 ml. of ether added. The extraction was continued as before and repeated a third time. The ether phase was returned to the funnel and washed with an equal volume of water. This washing was carried out twice without shaking then repeated three times with gentle shaking. The last washing was tested with phenolphthalein to insure that the solution was free of alkali. The extract was transferred to a dry flask and dried over anhydrous sodium sulfate for 20 minutes. The ether solution was filtered into a 50 ml. graduate and made to volume with dry ether.

Five ml. of the extract was pipetted into an Evelyn Colorimeter tube. With the aid of a hot water bath and suction from a water pump the ether solution was evaporated to dryness. One ml. of dry chloroform was immediately added to the tube together with one drop of acetic anhydride. Control tubes contained only the chloroform and the acetic anhydride. The control tube was placed in the tube holder of the Evelyn Colorimeter and 9 ml. Carr-Price Reagent added with an automatic pipette. The galvanometer was adjusted to read 100% transmission and then each unknown tube was read immediately. A 620 light filter was used in the Evelyn Colorimeter (3) %

Ten ml. samples of each of two of the highly pigmented ether solutions of the livers were evaporated to dryness on the water pump in hot water. The two samples were redissolved in skelly solv and chromatographed through tubes packed with a 1:1 mixture of MgO and Hyflo Supercel. The filtrates were brought up to a volume of 25 ml., transferred to the Evelyn Colorimeter tubes, and read in the Colorimeter using a 440 light filter. Transmission readings were 99¹ and 99³. Comparing these readings with the beta-carotene conversion chart it was found to represent 0.00 g. beta-carotene. It was evident that all the pigment present in the liver solutions was lycopene and that no beta-carotene was present.

RESULTS

The data in tables 1 and 2 show that 25 µg. of lycopene significantly increased the storage of vitamin A in the rats on the 27.5 µg. beta-carotene supplement. The increase persisted in the higher levels of lycopene but with no significant difference between the various levels of lycopene. Lycopene did not significantly increase the hepatic vitamin A storage of the rats on the 55.0 µg. of beta-carotene supplement except at the 100 µg. supplement of lycopene.

TABLE I

The effect of varying amounts of lycopene on the utilization of beta-carotene as measured by hepatic deposition of vitamin A.

| LOW CAROTENE | | | | |
|--|----------------------|--------------------------------|-----------------------------|--|
| Dietary Supplement <u>μg. / day</u> | No. & Sex of Rats | Mean Weight Gain <u>gm.</u> | Vitamin A Deposited - g. | |
| 27.5 carotene | 10 5 M | 38.1 | 12.86 - 1.67 ¹ | |
| 27.5 carotene - 25 Lycopene | 9 4 M | 50.5 | 19.65 - 2.49 | |
| 27.5 carotene - | | | | |
| 100 lycopene | 10 5 M | 47.8 | 21.95 - 3.05 | |
| 27.5 carotene - 300 lycopene | 10 5 M | 48.6 | 22.80 - 3.19 | |
| HIGH CAROTENE | | | | |
| 55.0 carotene | 9 4 M | 50.5 | 21.96 - 3.42 | |
| 55.0 carotene - 25 lycopene | 10 4 M | 46.5 | 27.81 - 1.25 | |
| 55.0 carotene - 100 lycopene | 10 5 M | 41.9 | 32.93 - 2.19 | |
| 55.0 carotene - | | | | |
| 300 lycopene | 10 3 M | 37.2 | 27.32- 2.47 | |

¹ Standard error of the mean.

TABLE II
Statistical treatment of data.

| LOW CAROTENE | | | |
|---------------------------------|---------------------------|---|--------------|
| Dietary treatment μg. / day | Standard error of mean | t-value (for diff- erence between car- otene level & lycopene groups | Significance |
| 27.5 carotene | 1.67 | ---- | -- |
| 27.5 carotene - 25 lycopene | 2.49 | 2.41 | .05 |
| 27.5 carotene - 100 lycopene | 3.05 | 2.61 | .05 |
| 27.5 carotene - 300 lycopene | 3.19 | 2.79 | .05 |
| HIGH CAROTENE | | | |
| 55.0 carotene | 3.42 | --- | --- |
| 55.0 carotene - 25 lycopene | 1.25 | 1.49 | --- |
| 55.0 carotene - 100 lycopene | 2.19 | 2.71 | .05 |
| 55.0 carotene - 300 lycopene | 2.47 | 1.22 | --- |

DISCUSSION

The increased utilization of beta-carotene and resultant increased hepatic storage of vitamin A in these experimental animals corroborates the findings of High and Day in 1952 (8). Thus, it is confirmed that lycopene does not follow the pattern of the related carotenoid lutein in rats. Impairment properties of beta-carotene utilization manifested by lutein must be confined to action in the digestive tract, as it is not absorbed to any appreciable degree in rats. Lycopene is adsorbed and stored in the liver. It has been demonstrated that conversion of beta-carotene to vitamin A takes place in the small intestine, but the liver has not been ruled out as a possible auxiliary site of conversion. But the fact that little or no beta-carotene is present in plasma and liver tissue of rats fails to confirm the suggestion that vitamin A deposition is increased in the liver because of lycopene activity.

The sparing effect of lycopene on beta-carotene has been suggested as being due to protection of the provitamin from oxidative decomposition in the alimentary tract. As was previously mentioned very little work has been done on the metabolic effect of lycopene, but in none of the work has lycopene been shown to have any vitamin A potency. However, in a recent report (1) on new syntheses of vitamin A and related compounds it was stated that Meunire (16) had oxidized lycopene with MnO_2 to produce retinene (vitamin A_2) with biological activity which

was 40% that of vitamin A₁.

It is apparent that further investigation is necessary to answer the many remaining questions on this subject and to establish the mechanism by which lycopene increases beta-carotene utilization.

SUMMARY

The result of this experiment concerning the effect of lycopene on the utilization of beta-carotene as measured by the storage of vitamin A in the livers of vitamin A depleted rats shows that lycopene does have an increasing effect. The mechanism of this increase has not been established.

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