MONOGRAPH ON CAROTENES #118

Monograph on **Carotenes** #118 10/22/74

M O N O G R A P H

O N

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CAROTENES

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CAROTENES

Summary

The carotenes, pigments found widely distributed in both the plant and animal kingdoms, are important to man because of their provitamin activity. It has been estimated that normal dietary carotene provides 33-67% of the vitamin A activity required by man (626). Beta-carotene, which is the most common isomer and has a potency of 1.67 IU/mcg, provides the bulk of the vitamin A activity, while alpha- and gamma-carotene, which are prevalent in foods and have potencies of about one half that of beta-carotene, contribute relatively little vitamin A activity. None of the other carotenes express provitamin activity.

In spite of the provitamin activity of the carotenes, their primary function as food additives is that of a coloring agent. Only the beta isomer is used as a food additive.

Because of the relationship of the carotenes to vitamin A and the human requirements for and the known toxicity of vitamin A, considerable research has been conducted on the metabolism and toxicity of the carotenes.

The ingestion of carotene by man is followed by passage of the intact carotene into the intestine, whereupon it can be absorbed as such, converted to vitamin A, destroyed by enzymes, bacteria or oxidation, or excreted in the feces (604, 1216, 1554, 1716).

In man, the absorption of carotene varies widely, depending upon the source. Pure crystalline beta-carotene administered orally in an oil solution is almost completely absorbed, while carotene from natural sources is absorbed less readily (484, 1437).

A number of parameters have been reported to effect the absorption of carotene. Carotene has been found to be very inefficiently absorbed unless the diet contains a sufficient quantity of fat or oil (604, 1216, 1554, 1716). Furthermore, studies have shown that the amount of carotene absorbed is highest when low molecular weight fatty acids are given, and the percentage of absorbed carotene falls as the chain length of the fatty acids increases (604, 1216, 1554, 1716). Mineral oils such as liquid paraffin, which are not absorbed from the gastrointestinal tract but in which carotene is highly soluble, carry carotene into the feces, thereby greatly reducing absorption (307, 604, 1216). Dietary antioxidants, in particular vitamin E, apparently contribute in some manner either to the efficient absorption of carotene or to the protection of carotene or vitamin A from oxidative breakdown during storage in the body (604, 1216, 1554). Some evidence has been presented indicating that a diet low in protein, or containing poor-quality protein such as zein or gluten, may hinder carotene utilization (1554, 1716). The inclusion of raw soybeans in the diet interferes with the

utilization of carotene (604). Both sodium selenite (158) and DDT (1369) have been reported to inhibit the utilization of orally administered carotene.

Studies in which radioactive beta-carotene was administered to patients with cannulated thoracic ducts have indicated that the bulk of absorbed carotene is converted to vitamin A or related compounds. The intestinal wall has been established as the primary site of conversion (603, 165).

The actual mechanism whereby carotene is converted to vitamin A is as yet unresolved. Three mechanisms which enjoy popular support are:

1) Hydrolytic cleavage of the central double bond;

2) Oxidative cleavage of the central double bond;

3) Sequential terminal oxidative cleavage of two-carbon units (604, 1216, 1554).

In man, carotene is irreversibly accumulated in the fatty tissues of the body throughout life. Excessive consumption of carotene-rich foods has been observed to cause deposition of carotene in the skin, resulting in a yellow discoloration. Carotenoids have been reported to appear in human blood and organs in approximately the same ratio as that in which they occur in the diet (604, 1216, 1716). The normal range of human serum carotene has been reported as 70 to 300 mcg/100 ml (1492).

The excretion of carotene by man, like the absorption, varies with the source. Reported values for fecal excretion range from 2 to 99% of the ingested quantity. Carotene is never found in the urine and no carotenoids have been detected in human sweat (484, 604, 1437).

Studies in rats using radioactive carotene indicated that 2-12% of the administered dose was excreted as CO_2 in the 24 hours after dosing (506, 954, 1890).

The interrelationship of carotene and vitamin A has prompted a good deal of research into the toxicology of carotene.

The carcinogenicity of carotene was tested by Barry, et al. (90) through topical and subcutaneous administration (unspecified quantity). Observation of the animals throughout their lifespan, up to 488 days, disclosed the development of no epitheliomas nor papillomas.

In testing the teratogenicity of beta-carotene, Komatsu (935) found that the oral administration of 18-80 mg carotene/100 g body weight to rats from the 9th to the 12th day of pregnancy increased the number of resorptions and severely retarded sternebral ossification in the fetus.

Three studies of the effects of carotene on reproduction were found in the available literature. The administration of 0, 100, and 500 mg beta-carotene/kg/day for 34 weeks to 3 groups of 16 male rats was observed

by Scharer and Studer (1264) to produce no effect on fertility nor other parameters investigated.

Sherwood, et al. (1585, 1586) reported that oral or s.c. administration of 1500 or 3750 I.U. carotene/day to female albino rats for 5 to 32 days produced changes in the estrus cycle. Vaginal smears did not progress from the nucleated epithelial stage to the cornified cell stage and an excess of nucleated epithelial cells and leucocytes were observed regardless of the phase of the cycle. Examination of the uteri and ovaries showed that 21 of 26 animals were in late diestrus or early proestrus. Mating of the animals produced no pregnancies until the carotene effect had disappeared (about 20 days after termination of treatment).

Wells and Hedenburg (1863) reported that the intradermal injection of 0.2 ml of 10% carotene dissolved in olive oil into guinea pigs produced a little edema and swelling, while injections of 0.1 and 1% carotene were without effect.

In man, excessive consumption of carotene rich foods can give rise to hypercarotenemia, characterized by yellowish discoloration of the skin caused by deposition of the pigment in the epithelial tissue. It is generally agreed that hypercarotenemia is harmless; i.e., it is not accompanied by further pathological symptoms and it disappears within 2-6 weeks when ingestion of excess carotene is discontinued (3, 20, 293, 686, 691, 1320).

However, several authors have reported cases in which harmful effects may have resulted from excess dietary carotene. Page (1328) reported the cases of 6 women who, while on a carotene-rich diet, developed menstrual disturbances for which no other cause could be found. Henschen (704) reported that debility, weight loss, spleen and liver hypertrophy, and hypochromic anemia appeared in a 49-year-old woman after prolonged consumption of large amounts of carrots. The contention that these effects were due to the carotene content of the carrots has been disputed by other authors (686, 1320). The case of a man who supplemented his diet with carrots and carrot juice for 7 years was reported by Josephs (814). The man experienced weight loss and weakness, which were not attributable to any factor other than the carrots and which disappeared upon discontinuation of carrot consumption. Upon determining the liver and serum concentrations of carotene and vitamin A in a number of Ghanaians, Dagadu and Gillman (327) suggested that high carotene intake may inhibit mobilization of liver vitamin A stores. Finally, Loeckell (1035) suggested that the development of a pica for carotene-containing foods, particularly carrots, results from hypercarotenemia.

The acute toxicity of carotene has received little study and the lethal dose has not been determined. Upon testing 12 samples of beta-carotene, Banziger and Hane (1264) concluded that the LD_{50} of orally administered beta-carotene for mice is greater than 10,000 mg/kg. Follow-

ing the i.m. injection of beta-carotene into mice in doses of 1 g, Zbinden and Studer (1945) observed only a proliferating inflammation within the injection area, with a few giant foreign body cells, located at beta-carotene crystals. According to Wells and Hedenburg (1863), the presence of a considerable amount of fatty yellow material in the peritoneum was the only result of the i.p. injection of 0.1-0.2 g carotene in a single dose into male guinea pigs.

A large number of short-term feeding studies of carotene have been performed, primarily on rats and dosg.

Jurgens (1264) stated that, in growth tests with young vitamin A-deficient rats, synthetic beta-carotene was tolerated in oral doses of 20 mg/kg/day for 6 months without toxic symptoms. Also, normally growing rats were observed to tolerate 33.3 mg beta-carotene/day for 5 weeks without ill effects.

In a 13-week feeding study conducted by Bagdon (1264), five groups of Sprague-Dawley rats were given beta-carotene according to the following regimens: one group (20 M, 20 F) was given 100% pure beta-carotene at a dietary level of 1.0%; two groups (each 20 M, 20 F) received a mixture of 75% beta-carotene and 25% degradation products (pure beta-carotene was exposed to air at 45 degrees C for 96 hours, resulting in approximately 25% degradation) in dietary concentrations of 1.0% and 0.5%; one group (10 M, 10 F) received type 2.4S beta-carotene beadlets at a dietary concentration of 1.0%; a control group (20 M, 20 F) received only the basal diet of ground Purina Lab Chow Pellets. The only effect observed was the deposition of vitamin in the livers of the rats of the first group.

In male rats treated by stomach tube with 1 g beta-carotene/kg/day for 4 weeks (5 days/week), Scharer, et al. (1264) observed an increase in weight in the livers and, to a lesser extent, the kidneys. No other effects were noted.

Davies and Moore (340) reported the production of no adverse effects in one male and one female rat maintained for 34 days on a diet supplemented with 1 mg carotene/kg.

Lewis and Reti (1027) reported that the administration, by gastric catheter, of 0.04 g crystallized carotene to 5 rats daily for 10 days produced only the appearance of fat in the liver.

Vedder and Rosenberg (1794) fed several young rats 40,000 I.U. of carotene daily both alone and in solution in vegetable oil for 100 days without ill effects.

In a study by Zbinden and Studer (1945), 8 rats given 4.5 g injections of beta-carotene intramuscularly twice daily, 5 days per week for 17 weeks, exhibited fairly strong tissue irritation at the injection sites.

Skaloud (1611) reported the development of hypervitaminosis A in

rats treated with 24,000-72,000 IU vitamin A per day (unspecified duration), administered in the form of a carotene preparation. Other workers, however, doubt Skaloud's work (686).

The treatment of 15 male and 15 female rats for 238 days with an 0.1% dietary supplement of lycopene was found by Zbinden and Studer (1945) to result only in the storage of a brown, rather rough-grained pigment, presumably lycopene, in the liver cells.

Of 4 rabbits given, by Zbinden and Studer (1945), beta-carotene in oral doses of 500 mg/kg 5 times per week for 11 weeks and 1 rabbit maintained on this regimen for 2 years, two of the animals lost 140-160 g body weight while the other 3 gained 70-400 g.

Considerable storage of vitamin A was found in the liver and kidneys of 2 female dogs treated by Zbinden and Studer (1945) with 100 mg/kg doses of beta-carotene in gelatin capsules daily for 104 and 200 days, respectively. Histopathological examination of the livers and kidneys showed certain modifications which could possibly be interpreted as sequelae of increased vitamin A storage.

Bagdon, et al. (54) performed a series of 3 short-term tests on young beagles. Two dogs given beta-carotene orally in a dose of 250 mg/kg on the first day and progressively larger doses until 8000 mg/kg was administered on the 6th day manifested no signs of carotene toxicity. The occurrence of slight diarrhea was the only effect exhibited by 2 dogs given 2000 mg beta-carotene/kg/day for 21 days. Groups of 3 dogs each given beta-carotene in daily doses of 0, 1, 10, or 100 mg/kg 5 days a week for 13 weeks exhibited no effects.

Zbinden and Studer (1945) reported that the oral administration of lycopene to a female dog in daily doses of 100 mg/kg for 192 days resulted only in the pronounced storage of lycopene in the liver.

Spiesman (1641) reported that symptoms of toxicity - anorexia, loss of weight, and a general feeling of malaise - developed in 3 patients who received ca. 7000 I.U. vitamin A in the form of carotene daily for periods of 4 to 14 weeks. Other workers have suggested that the carotene solution used by Spiesman was probably contaminated with vitamin A (686, 1320).

Yellowish discoloration of the skin and gastrointestinal disturbances were observed by Korner and Hammer (1264) in a number of cases following the oral administration of 100 mg beta-carotene/day to 41 volunteers for 20 days.

No signs of toxicity were observed by Greenburg, et al. (630) among 41 patients given up to 180,000 IU of carotene daily for 1-2 months.

No adverse effects were found by Campbell, et al. (234) upon treating, orally, 17 patients with 100 mg beta-carotene/day for 12 months.

In the only long-term feeding study reported, Bagdon, et al. (54) maintained four successive generations of rats on a diet supplemented with 0.1% beta-carotene without the production of any symptoms of toxicity.

ALPHA-CAROTENE

Chemical Information

I. Nomenclature

A. Common Name

1. alpha-Carotene

B. Chemical Names

None

C. Trade Names

None

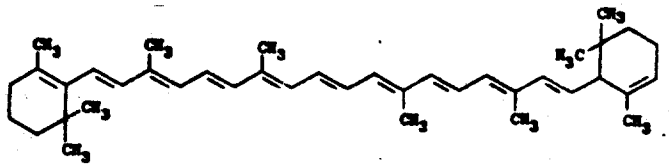
D. Chemical Abstracts Registry Number

000432-70-2

II. Empirical Formula

$C_{40}H_{56}$

III. Structural Formula



IV. Molecular Weight

536.85

V. Specifications

No Information Available

VI. Description

A. General Characteristics

Deep purple prisms, polyhedra from petroleum ether or from benzene + methanol.

B. Physical Properties

Melting point 187.5 degrees (evacuated tube). Specific optical rotation at 18 degrees C for Cd line +385 degrees (conc = 0.08 in benzene). Absorption maximum in chloroform: 485, 454 nm. More soluble than beta-carotene. Freely soluble in carbon disulfide and chloroform. Soluble in ether, benzene. Slightly soluble in petroleum ether and alcohol. 100 ml hexane dissolves 294 mg at 0 degrees. Practically insoluble in water, acids, alkalies.

C. Stability

Absorbs oxygen from the air, giving rise to inactive colorless oxidation products. The oxidation in light is autocatalytic.

VII. Analytical Methods

See beta-Carotene

VIII. Occurrence

About as widely distributed as beta-carotene, but in lesser amounts.

BETA-CAROTENE

Chemical Information

I. Nomenclature

A. Common Names

1. beta-Carotene
2. Carotene
3. Provitamin A

B. Chemical Names

None

C. Trade Names

None

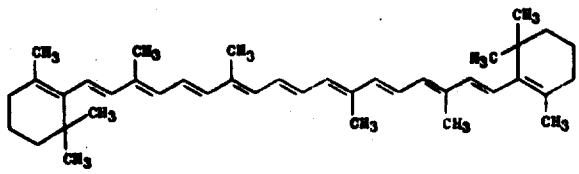
D. Chemical Abstracts Registry Number

beta-Carotene	000116-32-5
Carotene	000036-88-4

II. Empirical Formula

$C_{40}H_{56}$

III. Structural Formula



IV. Molecular Weight

536.85

V. Specifications

Food Chemicals Codex

Assay	Not less than 96.0% and not more than the equivalent of 101.0% of $C_{40}H_{56}$
Melting range	Between 176 degrees and 182 degrees, with decomposition

V. Specifications Con't.

Solution in chloroform	Passes test. (A 1 in 100 solution in chloroform is complete and clear.)
Limits of Impurities	
Arsenic (as As)	Not more than 3 ppm (0.003%)
Heavy metals (as Pb)	Not more than 10 ppm (0.001%)
Residue on ignition	Not more than 0.2%

VI. Description

A. General Characteristics

Deep-purple, hexagonal prisms from benzene + methanol. Red, rhombic, almost square leaflets from petroleum ether.

B. Physical Properties

Melting point 183 degrees (evacuated tube). Absorption maximum: 497, 466 nm in chloroform. Less soluble than alpha-carotene. Soluble in carbon disulfide, benzene, chloroform. Moderately soluble in ether, petroleum ether, oils. 100 ml hexane dissolve 109 mg at 0 degrees. Very sparingly soluble in methanol and ethanol. Practically insoluble in water, acids, alkalies. Dilute solutions are yellow.

C. Stability

Absorbs oxygen from the air giving rise to inactive, colorless oxidation products. Keep tightly closed and protected from light.

VII. Analytical Methods

In the A.O.A.C. official method of analysis of carotene in foods, the carotene is first extracted from the food sample by dissolution in an appropriate solvent (acetone-hexane, water-satd n-butyl alcohol, KOH-alcohol). The carotene is separated from other pigments (xanthophylls, chlorophylls, carotene oxidation products) by phase separation or column chromatography, and is then measured spectrophotometrically against a standard curve (1970).

VIII. Occurrence

beta-Carotene is widely distributed in the plant and animal kingdoms.

DELTA-CAROTENE

Chemical Information

I. Nomenclature

A. Common Name

1. delta-Carotene

B. Chemical Names

None

C. Trade Names

None

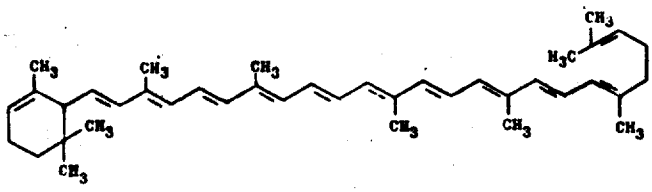
D. Chemical Abstracts Registry Number

None

II. Empirical Formula

$C_{40}H_{56}$

III. Structural Formula



IV. Molecular Weight

536.85

V. Specifications

No Information Available

VI. Description

A. General Characteristics

Long orange-red needles from carbon disulfide + hexane + ethanol.

B. Physical Properties

Melting point 140.5 degrees. Specific optical rotation +317 degrees.

C. Stability

No Information Available

VII. Analytical Methods

See beta-carotene

VIII. Occurrence

Some fruits, microorganisms.

EPSILON-CAROTENE

Chemical Information

I. Nomenclature

A. Common Name

1. Epsilon-Carotene

B. Chemical Names

None

C. Trade Names

None

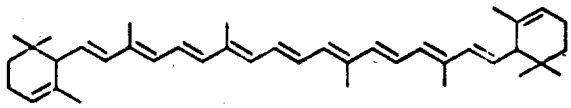
D. Chemical Abstracts Registry Number

None

II. Empirical Formula

$C_{40}H_{56}$

III. Structural Formula



IV. Molecular Weight

536.85

V. Specifications

No Information Available

VI. Description

A. General Characteristics

No Information Available

B. Physical Properties

Melting point 199-201 degrees.

Absorption max in petr ether

nm	$E^{1\%}_{1cm}$
266	672
416	1940
440	3120
470	3135

C. Stability

No Information Available

VII. Analytical Methods

See beta-carotene

VIII. Occurrence

epsilon-Carotene has been isolated from algae.

GAMMA-CAROTENE

Chemical Information

I. Nomenclature

A. Common Name

1. gamma-Carotene

B. Chemical Names

None

C. Trade Names

None

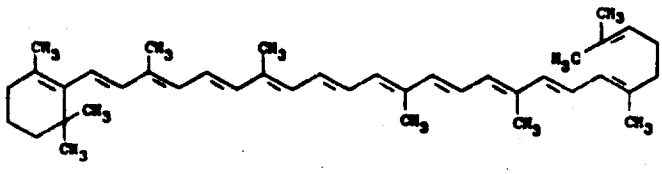
D. Chemical Abstracts Registry Number

000472-93-5

II. Empiricial Formula

$C_{40}H_{56}$

III. Structural Formula



IV. Molecular Weight

536.85

V. Specifications

No Information Available

VI. Description

A. General Characteristics

Probably crystallizes in polymorphous forms.

Natural form: minute, deep-red prisms with bluish luster from benzene + methanol.

Synthetic form: red plates.

B. Physical Properties

Natural form: melting point 152-153.5 degrees. Absorption maximum in petroleum ether: 437, 462, 494 nm (E 1%/1 cm 2055, 3100, 2720).

Synthetic form: melting point 177.5 degrees. Absorption maximum 508.5; 475, 446 nm in chloroform. Somewhat less soluble than beta-carotene.

C. Stability

No Information Available

VII. Analytical Methods

See beta-carotene

VIII. Occurrence

No Information Available

LYCOPENE

Chemical Information

I. Nomenclature

A. Common Name

1. Lycopene

B. Chemical Names

None

C. Trade Names

None

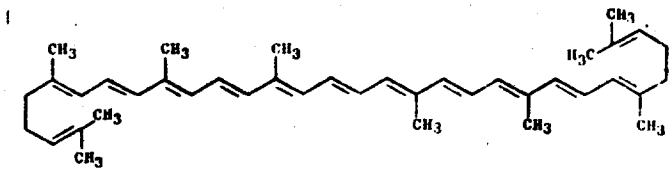
D. Chemical Abstracts Registry Number

None

II. Empirical Formula

$C_{40}H_{56}$

III. Structural Formula



IV. Molecular Weight

536.85

V. Specifications

No Information Available

VI. Description

A. General Characteristics

Long, deep red needles from carbon disulfide + ethanol, from methylene chloride + methanol.

B. Physical Properties

Melting point 172-173 degrees. Absorption maximum (trans-form) 446, 472, 505 nm (E 1%/1 cm 1110, 1280, 1660, 1280). One gram dissolves in 50 ml carbon disulfide, in 3 l boiling ether, in 12 l boiling petroleum ether, in 14 l hexane at 0 degrees. Soluble in chloroform, benzene. Almost insoluble in methanol, ethanol.

C. Stability

No Information Available

VII. Analytical Methods

See beta-carotene

VIII. Occurrence

Lycopene occurs in ripe fruits, especially in tomatoes. One kg of fresh ripe tomatoes yields 0.02 g lycopene.

Biological Data

I. Acute Toxicity

Mice

Banziger and Hane tested four samples of trans-beta-carotene for acute toxicity using CF-1 mice of both sexes weighing 17-22 g. Samples of carotene suspended in 5% gum acacia were administered orally. Ten mice were used per dose level and were observed 72 hours for mortality. The results were as follows (1264):

Trans-beta-carotene sample	LD_{50} ± S.E. (mg/kg, p.o.)
PP112866	>4000
PP081166	>4000
Lot 890	>4000
Lot 891	>4000

In a later study by Banziger and Hane, another eight samples of beta-carotene were tested for acute toxicity. CF-1 mice of both sexes weighing 17-25 g were orally administered the samples suspended in 5% gum acacia solution. Ten mice were used per dose level and were observed 72 hours for mortality. The results were as follows (1264):

Product	Dose (mg/kg, p.o.)	# Dead/ # Dosed	LD_{50} (mg/kg, p.o.)
Dry β-Carotene 2.4S yellow (cis) #40016	10,000	0/10	> 10,000
Dry β-Carotene 2.4S yellow (trans) #5751-114	10,000	0/10	> 10,000
Dry β-Carotene 2.4S orange (cis) #5751-157	10,000	1/10	> 10,000
Dry β-Carotene 2.4S orange (trans) M-313	10,000	0/10	> 10,000
Emulsion β-Carotene type 3.6 (cis) #313126	10,000	0/10	> 10,000
Emulsion β-Carotene type 3.6 (trans) #68-3-87	10,000	0/10	> 10,000
Dry β-Carotene 10%(cis)#352057	10,000	0/10	> 10,000
Dry β-Carotene 10%(trans)#354057	10,000	0/10	> 10,000

Zbinden and Studer reported that single i.m. injections of beta-carotene in doses of 1 g produced no toxic symptoms in 10 female rats weighing 145-165 g. Sacrifice and dissection of the animals between the 3rd and 48th test day disclosed no organic changes. Microscopically, a proliferating inflammation was observed within the injection area, with a few giant foreign body cells, located at beta-carotene crystals (1945).

Guinea Pigs

Wells and Hedenburg treated two pairs of male guinea pigs with carotin dissolved in olive oil through single i.p. injections according to the following schedule.

N 1. Wt. 339 gm., given 3 cc. oil with 0.100 gm. natural carotin.

P 1. Wt. 316 gm., given 3 cc. oil with 0.100 gm. chlorinated carotin.

N 2. Wt. 265 gm., given 5 cc. oil with 0.200 gm. natural carotin.

P 2. Wt. 245 gm., given 5 cc. oil with 0.200 gm. chlorinated carotin.

The animals were kept under close observation for 12 hours, and then after 4 days were sacrificed and autopsied. The animals appeared healthy throughout the test period and the autopsies showed no changes except the presence of a considerable amount of fatty yellow material in the peritoneum, which amounted to much less than the quantity injected (1863).

II. Short-Term Studies

Rats

In an unpublished memo, Dr. R. Jurgens stated that according to the experiments of Dr. Pfaltz (no reference given), in growth tests with young vitamin A-deficient rats, synthetic beta-carotene is tolerated for 6 months without toxic symptoms in daily oral doses of 20 mg/kg, corresponding to 30,000 I.U. of vitamin A. For the entire 6 months the rats maintained a weight of 240 g, which corresponded to the weight of normally fed animals. The general appearance of all the animals was good throughout. Jurgens also stated that according to the experiments of Dr. F. Studer (no reference given), after the daily administration of synthetic beta-carotene corresponding to 50,000 I.U. vitamin A for 5 weeks to normally growing rats the animals thrived and exhibited no hypervitaminosis; in contrast, rats given 30,000-50,000 I.U. vitamin A per os daily for 5 weeks manifested severe hypervitaminosis symptoms (1264).

In a 13-week feeding study conducted by Bagdon, five groups of Sprague-Dawley rats were given beta-carotene according to the following regimens: one group (20 M, 20 F) was given 100% pure beta-carotene at a dietary level of 1.0%; two groups (each 20 M, 20 F) received a mixture of 75% beta-carotene - 25% degradation products (pure beta-carotene was exposed to air at 45 degrees C for 96 hours, resulting in approximately 25% degradation) in dietary concentrations of 1.0% and 0.5%; one group (10 M, 20 F) received type 2.4S beta-carotene beadlets at a dietary concentration of 1.0%; a control group (20 M, 20 F) received only the basal diet of ground Purina Lab Chow Pellets. The animals were closely observed

daily and measurements of body weights and feed consumption were obtained at weekly intervals. Determinations of the hematocrit, hemoglobin, total and differential leukocyte counts were performed during the 0, 7th and 12th experimental weeks. At the completion of the 13 weeks treatment period, the animals were sacrificed and the tissue, and organs examined for evidence of gross pathology. Weights of livers and kidneys were obtained. Sections of brain, peripheral nerve, heart, lungs, liver, kidneys, adrenals, thyroid, pituitary, stomach, small and large intestines, bladder, pancreas, bone marrow and gonads were fixed and examined microscopically. In no case did any of the parameters studied reveal any toxic effects attributable to carotene administration. It was noted that the rats given pure crystalline beta-carotene exhibited vitamin deposits in the Kupffer cells of the livers, while those animals receiving the mixture of beta-carotene and degradation products exhibited no such deposits. From this information the authors concluded that either the breakdown products of beta-carotene are not absorbed from the gastrointestinal tract or cannot serve as precursors for the biosynthesis of vitamin A (1264).

Schaerer et al. treated 24 male rats, weighing initially 80-90 g, with beta-carotene (dissolved in peanut oil) by stomach tube in a dosage of 1 g/kg/day for a period of 4 weeks (5 days/week). Twelve rats were maintained as a control group. One third of the animals were killed on the 1st, 8th, and 15th day after completion of the treatment period. Records of weight gain showed no significant difference between the treated and control groups. Bromsulphalein tests on 5 treated animals and 4 controls revealed no indications of hepatic damage. Upon gross observation at autopsy all organs appeared completely normal, but the livers, and to a lesser extent the kidneys, of the treated animals were somewhat heavier than those of the controls. Histological examination of the liver, spleen, testes, heart, lungs, small intestine, large intestine and adrenals revealed no abnormalities. Chemical analysis of the organs revealed a deposition of 150 gamma of carotene/100 g tissue in the liver, but no deposition in the kidneys, spleen, lungs, heart or testes (1264).

Davies and Moore attempted to induce hypervitaminosis A in two rats by the administration of carotene for 34 days as a dietary supplement of 1 mg/g of diet. The male consumed 8.3 mg carotene daily and grew from an initial weight of 95 g to a final weight of 156 g. The female consumed 7.9 mg daily and increased in weight from 83 g to 129 g. At autopsy the animals were found to be perfectly normal. No indications of hypervitaminosis A or other adverse effects were observed (340).

Lewis and Reti administered, by gastric catheter, 0.04 g crystallized carotene to 5 rats daily for 10 days. Histological examination disclosed in some of the treated animals the appearance of fat in the liver, which was also seen, but somewhat less pronounced, in the controls. No other changes were observed (1027, 1298).

Vedder and Rosenberg fed several young rats 40,000 I.U. of carotent daily both alone and in solution in vegetable oil for 100 days without ill effect (1794).

In a study by Zbinden and Studer, 8 rats weighing 150-190 g were given 4.5 g injections of beta-carotene dissolved in oil intramuscularly twice daily, 5 days per week for 17 weeks. The treatment, however, had to be interrupted from time to time for a few days because of local irritation. All of the rats exhibited normal growth and their general condition was good. Post mortem examination revealed large cysts containing oil and beta-carotene in all animals at the site of injection. Foci of intensively yellow pigmentation were found in the fatty tissue of the retroperitoneum. Histopathological examination of the lungs, liver, spleen, epidermis, bones and marrow revealed fatty infiltration of the Kupffer cells of the liver and tubular epithelial desquamation at the transition between the marrow and cortex of the kidneys. A pronounced, productive inflammation was observed at the injection sites. The granulation tissue of fibroblasts, scarce capillaries, macrophages and sporadic giant foreign body cells was partially necrotic and infiltrated by numerous neutrophilic leukocytes and lymphocytes (1945).

Skaloud reported the development of hypervitaminosis A (see attached articles for details) in rats treated with 24,000-72,000 IU vitamin A per day (unspecified duration), administered in the form of a carotene preparation (1611). Other workers, however, doubt Skaloud's work (686).

For a period of 238 days, Zbinden and Studer treated 15 male and 15 female rats with an 0.1% dietary supplement of lycopene. In addition, 13 male and 18 female rats were maintained on the same regimen for a period of 50-80 days, during which time they were mated. All of the treated animals developed well, the treated males gaining somewhat more weight than the corresponding controls. Hematological tests showed no changes. No differences with respect to reproduction were found between the treated rats and the controls. Histopathological examination of 4 treated rats and 4 controls on Day 200 revealed in the treated females pronounced and in the treated males only slight storage of a brown, rather rough-grained pigment, presumably lycopene, in the liver cells. The kidneys, adrenals, spleen, pancreas, testicles, epididymis, ovaries, lungs, heart, epidermis, bones and marrow exhibited no adverse effects (1945).

Rabbits

Two male and two female rabbits were given, by Zbinden and Studer, beta-carotene in oral doses of 500 mg/kg 5 times per week for 11 weeks. In addition, one female rabbit was maintained on this regimen for 2 years. Two of the animals lost 140 and 160 g weight, respectively; 3 animals gained 70 to 400 g. No adverse effects were disclosed by hematological tests nor histopathological examination of the heart, lungs, liver, kidneys, spleen, pancreas, epidermis, stomach, small intestine, colon, bones, cartilage and bone marrow (1945).

Dogs

Zbinden and Studer gave two female dogs 100 mg/kg doses of beta-carotene in gelatin capsules daily for 104 and 200 days, respectively. Clinical tests disclosed the development of no disorders. Considerable

storage of vitamin A was found in the liver and kidneys of the dogs, while the beta-carotene content of these organs was not significantly changed. Histopathological examination of the liver and kidneys showed certain modifications which could possibly be interpreted as sequelae of increased vitamin A storage (for details, see attached article). Histological examination of the spleen, pancreas, stomach, colon, small intestine, suprarenal gland, hypophysis, cerebrum, cerebellum and trunk ganglia revealed no abnormalities (1945).

In a series of tests, Bagdon et al. examined the toxicity of beta-carotene through oral administration to young (9-12 months) beagles. In the first test, two dogs were given beta-carotene orally in an initial dosage of 250 mg/kg. The dosage was doubled on each successive day until dosages of 8000 mg/kg were given on the 6th day. Throughout the experimental period the dogs were closely observed and manifestations of toxicity were not detected at any time (54).

The second test consisted of the oral administration of 2000 mg beta-carotene/kg to 2 dogs daily for 21 days. The dogs tolerated this dosage without effect beyond the occurrence of slight diarrhea. No loss of weight was recorded throughout the 3-week period (54).

In the third test, groups of 3 dogs each were given beta-carotene in daily dosages of 0, 1, 10 or 100 mg/kg 5 days a week for 13 weeks. Body weight gain was reported to be uninfluenced by the treatment and there was a complete absence of toxic signs. Hematological findings indicated that beta-carotene had no adverse effect. Postmorten examination revealed no significant differences in organ weights between the controls and the treated animals. Histological examinations of the lungs, liver, spleen, kidneys, heart, adrenals, gastro-intestinal tract, gall bladder, pancreas, gonads, bone marrow, thyroid, pituitary, central nervous system and bladder indicated slight inflammatory changes in some tissues which were not related to the administration of beta-carotene. Dogs given beta-carotene showed some deposition of material in the Kupffer cells (54).

Zbinden and Studer reported that the oral administration of lycopene to a female dog in daily doses of 100 mg/kg for 192 days resulted in pronounced lycopene storage in the liver. Body weight, blood count, Takata-reaction, serum bilirubin, bromosulfalein test, non-protein nitrogen, blood sedimentation and histopathological examinations of the liver, kidneys, adrenals, heart, lungs, spleen, epidermis, stomach, small intestine, pancreas, cerebrum, cerebellum, trunk ganglia, hypophysis, ovaries and bone marrow revealed no other effects (1945).

Man

Spiesman observed symptoms of toxicity-anorexia, loss of weight, and a general feeling of malaise - in 3 patients who received ca. 7000 IU vitamin A in the form of carotene daily for periods of 4 to 14 weeks. Carotenemia of high degree, as evidenced by yellow discoloration of the skin and eyes, was present in all cases. Discontinuation of carotene administration relieved all symptoms (1641). Other workers have

suggested that the carotene solution used by Spiesman was probably contaminated with vitamin A (686, 1320).

In a study of the protective effect of beta-carotene against ultraviolet rays, Korner and Hammer treated 41 volunteers (both male and female students) for 20 days with daily doses of four capsules of the following preparations, corresponding to a daily intake of 100 mg beta-carotene.

1) Ro 01-8300/076 F

   Capsules containing 250 mg beta-carotene 10%, water soluble

2) Ro 01-8300/077 F

   Capsules containing 250 mg beta-carotene 10%, water soluble;
   50 mg DL-alpha-tocopherol acetate;
   100 mg ascorbic acid

While the treatment was found to offer some protection against UV rays and was generally well tolerated, some side effects were reported (see Table 1) (1264).

Table 1

Symptoms	Preparation	
	Ro 01-8300/076 F	Ro 01-8300/077 F
1) Yellowish coloring of skin of face and/or hands	8	10
2) Gastrointestinal disturbances		
a) pressure over stomach	3	1
b) belching	1	
c) nausea	1	1
d) diarrhea	5	1
3) colored feces	4	
4) Drowsiness		
5) Disturbed sleep	1	
6) No symptoms	5	10
N=	20	21

In addition to the dosages given, the serum carotene and vitamin A concentrations determined in 39 patients subjected by Greenburg et al. to chronic carotene ingestion are presented in Table 2. While the serum vitamin A concentrations were essentially unchanged by the treatment, the serum carotene was increased 1 to 2 fold in most cases. Two additional patients given, respectively, 10,000 and 180,000 IU of carotene daily exhibited 3 to 4 fold increases in serum carotene after 1-2 months. In these two patients a vitamin A-like fluorescent material was visualized within the sebaceous glands, in the sebum, and throughout the epidermis, while in the remaining 39 patients the vitamin A-like

material was visualized in the sebaceous glands only. It was noted the the rate of rise of the serum carotene was either markedly diminished or reversed in two thirds of the patients in the second and third months. In view of this, the authors suggested that a carotene tolerance was developed in these subjects. In no case were any manifestations of toxicity from carotene observed (630).

TABLE 2

Serum carotene and vitamin A concentration in gamma % in 44 subjects during chronic carotenoid ingestion

Dose Day Equivalent in Vit. A in I.U.[1]	No. of Subjects		Initial	1 Month	2 Months	3 Months
Roche Beta Carotene[2], 100,000	15	Serum Carotene	128 (±68)	308 (±116)	313 (±141)	314 (±184)
		Serum Vit. A	64 (±18)	71 (±9)	74 (±11)	82 (±17)
R.C.O.[1] 40,000.	5	Serum Carotene	113 (±17)	187 (±76)	194 (±101)	
		Serum Vit. A	69 (±16)	80 (±31)	74 (±21)	
Carotene concentrate in oil.[1] 40,000.	5	Serum Carotene	118 (±31)	211 (±95)	283 (±78)	
		Serum Vit. A	72 (±9)	76 (±38)	72 (±11)	
Carotene concentrate in oil.[1] 20,000	5	Serum Carotene	159 (±54)	262 (±69)	—	288 (±119)
		Serum Vit. A	65 (±19)	103 (±17)	—	88 (±44)
Carotene concentrate in oil.[1] 5000-10,000.	9	Serum Carotene	139 (±95)	139 (±67)	—	104 (±28)
		Serum Vit. A	72 (±17)	66 (±9)	—	60 (±7)
Roche Vit. A Acetate.[3] 100,000.	5	Serum Carotene	87 (±29)	87 (±12)	—	
		Serum Vit. A	85 (±28)	153 (±108)	—	

In the treatment of retinitis pigmentosa, Campbell et al. treated 17 patients with 100 mg beta-carotene (oral)/day for 12 months. The vitamin A in the blood rose from 97 to a maximum of 138 IU/100 ml. The effect on the cone and rod thresholds was only moderate. No adverse effects were reported (234).

III. Long-Term Studies

Rats

To test the chronic toxicity of beta-carotene, Bagdon et al. maintained four successive generations of rats on a diet supplemented with 0.1% beta-carotene. Initially 15 males and 15 females were placed on the test diet, while another group of 30 rats was maintained on the basal ration to serve as controls. These animals were kept on this regimen for up to 110 weeks. During the 16th test week, the first-generation rats were bred, resulting in a second generation of 21 test rats and 19 controls. These animals were fed their respective diets for 38 weeks. The third generation, 22 test animals and 21 controls, had been fed their respective diets for 96 weeks at the time of reporting. And the fourth generation, 27 test animals and 5 controls, had been maintained for 48 weeks at the time of reporting. Throughout all generations, the treated animals remained in good condition and exhibited normal growth and food consumption. Hematological tests disclosed no abnormalities. Repro-

duction of the carotene-fed rats was normal in all respects. Gross and histopathological examination of the liver, kidneys, spleen, lungs, skin, ovaries, testes, pancreas, adrenals, heart, gastric and intestinal mucosa, thyroid, bone, and bone marrow revealed no changes attributable to carotene consumption. However, it was observed that the Kupffer cells of the liver were filled with Sudan-staining material, indicating the storage of a moderate amount of vitamin A (54).

IV. Special Studies

Carcinogenicity

Using 10 mice, Barry et al. tested carotene for carcinogenic activity through topical and subcutaneous administration. A solution of 0.3 g carotene dissolved in 100 ml benzene was applied topically to the inter-scapular region twice weekly. For s.c. injection, the carotene (unspecified quantity) was dissolved in lard. Observation of the animals throughout their lifespan, up to 488 days, disclosed the development of no epitheliomas nor papillomas (90).

Teratogenicity

The teratogenic effects of beta-carotene were tested by Komatsu according to the regimen described in Table 3. The pregnant rats were sacrificed on the 21st day of pregnancy and the condition of the mothers and fetuses examined . Weight gain of the mother rats given beta-carotene was comparable to that of the controls. As shown in Tables 4-7, carotene administration increased the number of resorptions and severely retarded sternebral ossification, but produced none of the other effects associated with hypervitaminosis-A (935).

Table 3. Experimental methods

Group	Drug	Doses	Total number of female rats treated	Route of administration	Stage
Control	Physiological saline	1ml/100g	17	Oral	From 9th to 12th day of pregnancy
A	Vitamin A	30,000I.U./100g	15		
CAR—1	β-carotene	(18mg/100g 30,000I.U./100g)	10		
CAR—2	β-carotene	(36mg/100g 60,000I.U./100g)	12		
CAR—3	β-carotene	(180mg/100g 300,000I.U./100g)	10		

		Control	A	CAR-1	CAR-2	CAR-3
Total number of implantations		184	178	107	130	123
Mean number of per a mother		10.8	11.9	10.7	10.8	12.3
Number of fetal deaths	Resorptions	0	20	5	0	15
	Placental remnants	0	10	2	0	2
	Macerated fetuses	0	3	0	0	0
Percentage of fetal deaths (%)	Resorptions	0	11.2	4.7	0	12.2
	Placental remnants	0	5.6	1.9	0	1.6
	Macerated fetuses	0	1.7	0	0	0
Number of survived fetuses		184	145	100	130	106
Percentage of survived fetuses(%)		100	81.5	93.5	100	86.2
Sex ratio female/male		5.5/5.3	4.7/5.0	5.8/4.3	6.0/4.8	5.8/4.2
Mean weight of survivors(g)		4.07 ±0.14	3.79 ±0.36	3.95 ±0.41	3.89 ±0.29	3.83 ±0.27
Number of fetuses with growth retardation		4	1	0	2	1
Percentage of fetuses with growth retardation(%)		2.2	0.7	0	1.5	0.9
Weight of placenta(g)		4.20	3.99	3.98	3.99	4.50

Table 5 Incidence and types of malformations

		Control	A	CAR-1	CAR-2	CAR-3
Total number of survived fetuses		184	145	100	130	106
Percentage of abnormal fetuses	(%)	0	82.6	0	0	0
Microcephalia	(%)	0	5.5	0	0	0
Exencephalia	(%)	0	0	0	0	0
Auricular malformation	(%)	0	40.4	0	0	0
Open eyelid	(%)	0	14.7	0	0	0
Stomatic malformation	(%)	0	1.8	0	0	0
Pseudomacroglossia	(%)	0	20.2	0	0	0
Macrognathia	(%)	0	17.4	0	0	0
Cleft palate	(%)	0	61.5	0	0	0
Low jaw cleft	(%)	0	10.1	0	0	0
Cleft tongue	(%)	0	1.8	0	0	0
Micromelia	(%)	0	0.9	0	0	0
Anal atresia and ecaudate	(%)	0	0	0	0	0

Table 6 Ossification patterns

	Control	A	CAR-1	CAR-2	CAR-3
Fetuses with retardation of ossification of the occipital bone(%)	0	72.7	0	9.2	1.1
Fetuses with retardation of ossification of the sternebrae(%)	16.7	75.5	80.0	83.1	55.8
Mean number of cssified metacarpal bones per fetus	7.89 ±0.24	6.30 ±0.84	7.28 ±0.81	7.57 ±0.40	6.97 ±0.80
Mean number of ossified metatarsal bones per fetus	8.00 ±0.00	7.87 ±0.25	8.00 ±0.00	8.00 ±0.00	8.00 ±0.00
Mean number of ossified coccyges per fetus	4.95 ±0.46	5.00 ±0.64	4.40 ±0.39	4.76 ±0.37	3.97 ±0.96

Table 7 Deformities of the bone structures in fetuses

		Control	A	CAR-1	CAR-2	CAR-3
Formation of the 14th rib	left (%)	0	27.9	0	0	0
	right (%)	0	24.5	0	0	0
Brachygnathia	(%)	0	3.4	0	0	0
Abnormality of the humerus	(%)	0	8.2	0	0	0
Shortening of the ulna	(%)	0	8.8	0	0	0
Shortening of the radius	(%)	0	7.5	0	0	0
Shortening of the femur	(%)	0	4.1	0	0	0
Shortening of the tibia	(%)	0	1.4	0	0	0
Shortening of the fibula	(%)	0	8.2	0	0	0

Reproduction

To test the effect of beta-carotene on the fertility of male rats, Scharer and Studer treated, by gastric probe, 3 groups of 16 male rats (80-90 g) with 0, 100, or 500 mg beta-carotene/kg/day for 34 weeks. Starting after 10 weeks of feeding, 13-14 rats from each group were mated each month with untreated virgin females (4 females per male). The percentage of fertile males per group, the number of pregnant females per fertile male, and the number of progeny per litter were determined. Three weeks after termination of treatment the animals were sacrificed. In addition to fertility, the following parameters were also tested: weight gain; blood counts; kidney function; liver function; organ weights - heart, liver, kidney, adrenal, spleen, testicles; histology - heart, lungs, liver, kidneys, spleen, bone marrow, thyroid, adrenals, testicles, esophagus, stomach, jejunum, rectum, pancreas, skeletal muscle system, skin, brain, cerebral membrane, and nervus ischiadicus. In no case were any changes attributable to carotene treatment observed (1264).

In a study by Sherwood, et al., a group of 18 female albino rats, 150 days of age, were monitored through 8 complete estrous cycles and then given a dietary supplement of 1500 I.U. of carotene daily for 15 days. After discontinuation of the carotene treatment, the rats were examined until 8 normal estrus cycles had again been observed. A second group of 16 animals was treated as above, but was given 3750 I.U. carotene/rat/day. Vaginal smears from the treated animals were observed to be abnormal within 2 days following carotene administration. The smears did not progress from the nucleated epithelial to the cornified cell stage and an excess of nucleated epithelial cells were seen regardless of the phase of the estrus cycle. Leucocytes were also observed continuously but were not as numerous as were the epithelial cells. Occasionally a few scattered cornified cells were observed which might have been obtained from the orifice of the vagina. The estrus cycle did not return to normal until 20 days after the carotene feeding was terminated. In most instances the animals did not show a desire to copulate, no pregnancy resulted until the carotene effect had disappeared. The authors concluded that the carotene must have been converted into vitamin A in excessive amounts, resulting in hypervitaminosis A (1585).

In a follow-up study by Sherwood, et al., 26 female rats were treated with 1500 I.U. of carotene per day, per os or subcutaneously, for a period of 5 to 32 days. Some of the animals were sacrificed after various periods of treatment for examination of the uteri and ovaries. Vaginal smears revealed disturbances similar to those in the previous study, the effects being more pronounced in those treated subcutaneously. Examination of the uteri and ovaries showed that 21 of the 26 animals were in late diestrus or early proestrus (1586).

Local Irritation

To examine the local effects of carotene, Wells and Hedenburg injected carotene, dissolved in varying concentration in olive oil, intradermally in parallel sites in 0.2 ml quantities into two guinea pigs. Carotene dissolved in olive oil to the strength of 10% had a very slight toxicity, manifested in a little edema and swelling. At

1% the results were very little different from those of olive oil alone. No appreciable differences were observed between chlorinated and natural carotene. The authors noted that olive oil containing very small amounts of carotene (0.1%) seemed to cause slightly less protracted swelling than olive oil alone and suggested that a slight irritant effect from the carotene may hasten absorption (1863).

Hypercarotenemia

Since first being recognized in the early 1900's, it has become well known that in man excessive consumption of carotene-rich foods can give rise to hypercarotenemia, the increased carotene content of the blood being normally accompanied by a yellow, or even orange, discoloration of the skin, called xanthosis (cutis), psuedo-icterus, or hyperlipochromia, and resulting from the deposit of pigment in the skin. The discoloration initially appears in the nasolabial folds and palms and gradually extends over the entire body, with the exception of the sclera. As hypercarotenemia is not accompanied by further pathological symptoms and it disappears within 2-6 weeks when ingestion of excess carotene is discontinued, it is generally agreed that such hypercarotenemia is harmless (3, 20, 293, 686, 691, 1320).

Hypercarotenemia has also been reported in cases of diabetes mellitus, hypothyroidism, lipoid nephrosis, and hereditary disfunction even when no excessive amounts of carotene were ingested. Such reports, while not feeding studies, indicate the innocuousness of unusually high concentrations of carotene in the body (293, 686, 1570).

While most authors agree that hypercarotenemia is harmless, several authors have reported cases in which harmful effects may have resulted from excess dietary carotene.

Page reported the cases of 6 women who, while on a carotene-rich diet, developed menstrual disturbances for which no cause could be found. Four of the patients, who ranged in age from 35 to 58 and who were well nourished with respect to protein intake, exhibited irregular menstrual bleeding or postmenopausal bleeding. It was proposed that these women converted an excessive amount of carotene to vitamin A and consequently suffered from vitamin A intoxication. The other two patients, who were 22 and 23 years of age and who were undernourished with respect to protein intake, exhibited oligo-amenorrhoea. It was preposed that this condition was a result of the protein insufficiency which led to insufficient vitamin A conversion (1328).

Henschen reported that debility, weight loss, spleen and liver hypertrophy, and hypochromic anemia appeared in a 49-year-old women after prolonged consumption of large amounts of carrots (704). As pure carotene was shown not to have any effect in this case and as previous studies have suggested the possibility that carrots and other carotene-rich foodstuffs may contain other toxic factors, Henschen's contention that the observed effects resulted from excess carotene has been disputed by other authors (686, 1320).

Josephs reported six cases of carotenemia taken from the records of the Johns Hopkins Hospital. The specifics of the cases are presented in Table 8. In the first 3 cases the patients were symptomless, other than skin discoloration, and entirely unharmed by the carotenemia. In case 5 the entire clinical picture may have been caused by the extremely inadequate diet, and in case 6 the impression of hypothyroidism was particularly strong and in addition the diet was inadequate. Case 4, however, indicates that carotenemia may not be entirely harmless. In this case there was no apparent cause for the observed symptoms other than the carotenemia. According to Josephs it is the abnormal intake of carotene rather than the amount in the blood that is the cause of trouble. In conditions such as diabetes and nephrosis, in which carotenemia may occur without excessive intake, none of the symptoms observed in the preceeding cases are present. Josephs concluded that under ordinary conditions carotene-rich foods must be ingested in excessive amount for a long time or must form the major part of the diet to the exclusion of other constituents in order to be harmful (814).

TABLE 8 —*Laboratory Data for Patients with Carotenemia*

Case No.	Age, Sex	Source of Carotene	Date	Serum Carotene, Micrograms/100 Cc.	Serum Vitamin A, U. S. P. Units/100 Cc.	Serum Lipids, Mg/100 Cc.	Serum Cholesterol, Mg/100 Cc.	Basal Metabolic Rate	Loss of Weight	Weakness	Hypotension	Leukocytes, No./Cu. mm.	Comments
1	16 mo. F	Oranges	2/8/20	610	80	1,100	...	..	0	0	...	.....	
2	Child F	Unknown	Jan. 1920	220	55	610	...	..	0	0	...	.....	
3	37 yr. M	Carrots	Oct. 1936	...	...	.....	...	..	0	0	...	8,500	Diagnosis made on appearance of skin
4	36 yr. M	Carrots and carrot juice for 7 years	9/12 9/23 12/10	600 430 180	60 100 180	1,410 970 720	441	—19	+	+	Slight	4,100	Symptomatic improvement on discontinuance of carrots and carrot juice
5	18 yr. M	Vegetables, especially spinach, principal source of food for 2 years	12/22	500	...	1,230	470	—45	++	++	++	6,800	Rapid improvement on high calory diet
6	47 yr. F	Vegetables and fruit principal source of food for 4 years	....	...	...	.....	Elevated	—45	+++	++	+	4,100	Considered to be hypothyroid on 2 occasions, but improved rapidly on high calory diet

With the knowledge that carotene-rich foods, such as palm oil, mangoes, nkontomire (a green leafy vegetable), tomatoes, and pawpaws, are extensively consumed in some parts of West Africa, Dagadu and Gillman examined a number of inhabitants of Accra, Mali for possibly hypercarotenemia. Carotene and vitamin A were determined in the sera of 67 pregnant women (aged 15-44) and 15 soldiers (aged 22-45), as well as in 49 livers of patients coming to necropsy at the Korle Bu Hospital. As shown in Table 9, while the serum vitamin A levels appeared to be close to standards previously reported, the serum carotene levels were abnormally high. It was noted that very low levels of vitamin A in the serum were associated with hypercarotenemia. While varying widely, vitamin A content of the livers was found to be considerably higher than in previous reports from other countries. Carotene concentrations in the liver, however, were found to be relatively low. It was suggested that high carotene intake may inhibit mobilization of liver vitamin A

stores. No further manifestations of carotene toxicity were reported (327).

Table 9

CONCENTRATION OF VITAMIN A AND CAROTENE IN THE SERUM

Subject	Total cases	Vitamin A (μg. per 100 ml. serum)			Carotene (μg. per 100 ml. serum)		
		Range	Median	Mean	Range	Median	Mean
Pregnant women	67	6-292	40	66	268-1950	604	681
Soldiers	15	20-131	51	56	185-1881	758	850

In a review of cases of hypercarotenemia resulting from the excess consumption of foods containing carotene, Loeckell suggested that the development of a pica for carotene-containing foods, particularly carrots, results from hypercarotenemia. No explanation for such a development was offered. Other than yellow discoloration of the skin and mucous membranes, this pica was the only recurrent symptom of carotene intoxication in the cases discussed (1035).

Biochemical Aspects

I. Breakdown

No Information Available

II. Absorption-Distribution

Under the proper conditions man and cattle absorb carotene fairly well, while other mammals generally absorb carotene very poorly. As they occur in natural food sources, carotenes are confined within the plant cells, necessitating breakdown of the cells before the carotenes are available for absorption. As food additives, however, the carotenes are in a free state and are more readily absorbed. It is believed that, when ingested, carotenes pass for the most part into the intestines intact, whereupon they can be absorbed as such, converted into vitamin A, destroyed by enzymes, bacteria or oxidation, or excreted in the feces (604, 1216, 1554, 1716).

Recent studies by Goodman, et al. (598) and Blomstrand and Werner (165) using radioactive carotene have disclosed the relative rates of absorption of intact carotene and of conversion of carotene to vitamin A compounds. Earlier studies which attempted to determine the degree of absorption of carotene by subtracting the measured fecal excretion from the amount ingested obviously gave unreliable figures for absorption, but did provide accurate measurement of fecal excretion. The degree of destruction of the carotene in the intestines can be determined by correlation of these studies.

Radioactive beta-carotene was employed by Goodman, et al. in studying the absorption of carotene in two patients in whom polyethylene cannulae had been inserted in the thoracic duct in the neck. Following oral administration of 46-47 mcg of beta-carotene-15,15'-^{3}H, serial samples of lymph were collected and analyzed. The time course of the absorption of radioactivity into the lymph, as shown in Figure 1, appeared to coincide with the absorption of fat, showing a peak of absorption between 3 and 7 hours and, essentially, discontinuation of absorption after 12 hours. The total amount of radioactivity recovered in the thoracic duct lymph during the 20-21-hour test period amounted to 8.74% of the ingested radioactivity in study I and 16.76% in study II. Table 1 reveals the percentage of absorption of carotene intact and the percentage of conversion to vitamin A and related compounds (598).

TABLE I

Distribution of radioactivity in human lymph after β-carotene-15,15'-^{3}H

Alumina column fraction	Percentage distribution of absorbed ^{3}H			
	Study I		Study II	
	Chylo-microns	Bottom fraction*	Chylo-microns	Bottom fraction*
1. β-Carotene	23	22	30	27
2. Retinyl esters	67	71	61	65
3. Retinal	3	2	3	2
4. Retinol	3	3	3	3
5+6. Polar; acids	4	2	3	3

* Lymph from which chylomicrons had been removed by centrifugation.

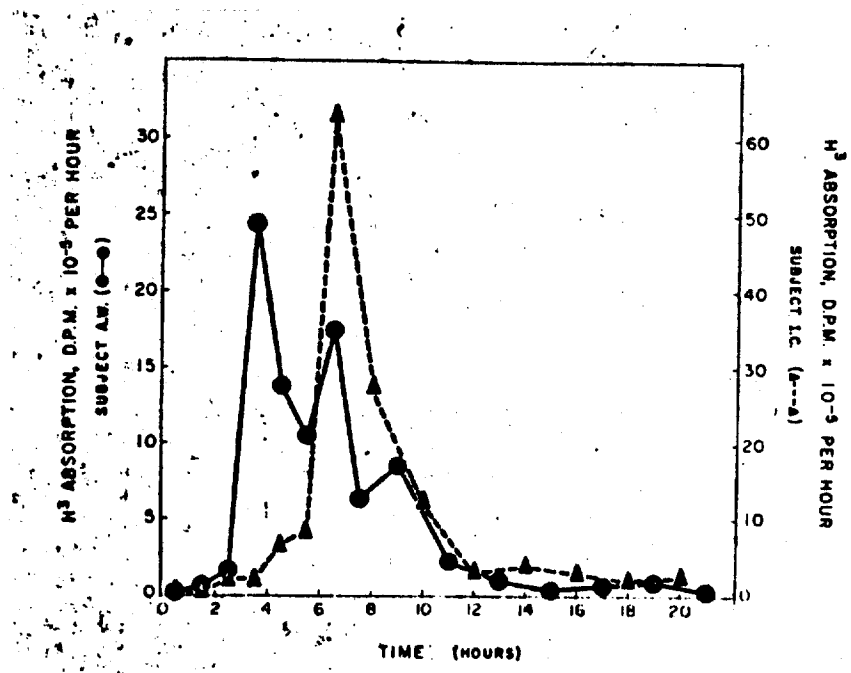


FIG. 1. THE TIME COURSE OF THE ABSORPTION OF RADIOACTIVITY INTO THORACIC DUCT LYMPH IN TWO HUMAN SUBJECTS FED β-CAROTENE-15,15'-^{3}H.

During radioactive beta-carotene, Blomstrand and Werner studied the intestinal absorption of carotene in man. Four patients with cannulated thoracic ducts were given, orally in vegetable oil, 0.4-45 g of labelled beta-carotene. Analysis of the lymph collected over the following 18-22 hours revealed that the subjects had absorbed 8.7, 14.6, 16.8 and 52.3%, respectively, of the radioactivity administered. The distribution of the absorbed radioactivity in the thoracic duct lymph is summarized in Table 2. The results show that during the intestinal absorption of dietary beta-carotene in man, while some of the carotene is absorbed intact, that which is absorbed is primarily converted to vitamin A or related compounds. Since Patient 3, who absorbed 52.3% of

the administered radioactivity, converted only a small portion of the carotene to vitamin A compounds, the authors assumed a metabolic disorder was involved and discounted the data obtained on said patient (165).

Table 2 *Percentage distribution of radioactivity in human lymph after ingestion of radioactive β-carotene*

Alumina column fraction	Patient 1	Patient 2	Patient 3	Patient 4a
β-carotene	1.7	11.3	89.6	27.9
Retinyl esters	87.9	73.2	2.1	68.2
Retinal	5.5	6.3	7.0	2.3
Retinol	4.9	9.2	1.3	1.6

Four healthy young men were employed by Rao and Rao in testing the absorption of carotene from various sources. The test results presented in Table 3, obtained by subtracting fecal excretion from amount ingested, indicate that pure crystalline beta-carotene was almost completely absorbed, while the carotene from food sources was absorbed less readily. Among the various carotene fractions present in the foods, beta-carotene was better absorbed than the others (1437).

TABLE 3

Absorption of carotene from different sources

Source	Ingested, μg		% Absorption	
	Total carotene	β-Carotene	Total carotene	β-Carotene
Amaranth	5,151	2,926	58.1 (44.1–79.6)	75.7 (52.4–99.8)
Papaya	9,840	3,319	45.8 (19.3–63.5)	90.3 (73.3–99.4)
Carrot	7,288	1,381	35.5 (15.9–67.3)	81.1 (73.2–96.8)
Crystalline β-carotene	4,500	3,679[a]	97.7 (93.2–100.0)	98.8 (95.4–100.0)
Diet with mixed sources[b]	4,253		33.4 (15.7–59.2)	

Values are means of four subjects with range of values in parentheses. [a] Determined by column chromatography on calcium hydroxide (5). [b] Contained yellow maize, spinach, orange, and peas as sources of carotene.

A 1967 FAO/WHO report summarized the results of 19 reports on the availability of carotene from various sources (see Table 4) (484).

Table 4

AVAILABILITY OF CAROTENE FROM VARIOUS SOURCES

Number of subjects	Source of carotene	Intake mg/day[1]	"Absorbed"[2] percentage of intake	Reference
33 infants	Carotene in oil	36–54	[3]72 (31–95)	126
3 infants	Carotene in oil	0.4–2.6	55 (30–70)	127
1 infant	Carrot meal	3.8	50	
7 infants	Carotene in oil	1.1–1.5	36 (25–40)	128
2 "	Carrot, cooked, mashed	0.9, 1.9	2, 3	
2 "	Carrot, raw, mashed	0.4, 0.8	26, 33	
2 "	Spinach, cooked, mashed	0.3, 0.7	12, 2	
2 "	Spinach, raw, mashed	0.3, 0.4	25, 45	
4 "	Tomato juice	0.26	8 (7–11)	
5 infants	Carotene in oil	6–14	68 (59–81)	129
10 infants	Carrot, cooked, pureed	[4]26.7	60 (46–75)	130
4 children	Carotene in oil	8–15	52 (30–75)	127
1 child	Carrot puree	8	30	
1 "	Carrot meal	38	45	
1 "	Spinach, cooked, minced	5	30	
1 child	Carotene in oil	0.9	52	128
6 children	Sweet potato, cooked, mashed	3.6	28 (5–58)	131
5 children	Carrot, raw, grated	19	less than 5	132
5 "	Above + oil	19	about 25	
4 "	Carotene in oil	28	" 45	
1 adult	Carrot, raw	5.5, 15.5	78,87	133
1 "	Spinach, cooked	2.6	88	
1 "	Above, fat-free diet	2.6	52	
2 adults	Carotene in oil	1.1	59	—
2 "	Carrots, cooked	5.7	1	134
2 "	Spinach, cooked	1.1	6	
2 "	Above + fat	1.7	5	
2 adults	Carrot, raw, finely grated	7.9	21 (20,22)	135
2 "	Carrot, raw, coarse	10	2 (2.3,2.4)	
2 "	Carrot, cooked, cubed	26	4 (1.6,5.4)	
5 adults	Carotene in oil	3	52 (46–56)	136
2 adults	Carotene in oil	2	55 (40,70)	127
2 "	Carrot, puree	11, 14	55 (50,60)	
1 adult	Spinach, minced	2	35	
1 adult	Carrots, raw	20	1	137
1 "	Carrots, cooked	13	19	
1 "	Spinach, raw	32	45	
1 "	Spinach, cooked	33	58	

Table 4 (Cont.)

Availability of carotene from various sources

Number of subjects	Source of carotene	Intake mg/day[1]	"Absorbed"[2] percentage of intake	Reference
2 adults	Carotene in oil	1.9–19.6	40 (30–51)	138
3 adults	Carotene in fat	1.6–2.7	38 (35–41)	128
3 adults	Carrot, raw, finely grated	6.7	10 (8–13)	139
3 "	Carrot, raw, blended in oil	7.2	47 (32–50)	
3 adults	Carrots, fresh, raw	8.3	13 (11–15)	140
2 "	Carrots, fresh, cooked	8.6	9 (8,9)	
1 adult	Above + oil	8.6	13	
3 adults	Carrots, stored, raw	7.6–30.3	4 (2–7)	
1 adult	Carrots, stored, cooked	32	1.4	
1 "	Above + oil	8	4	
2 adults	Spinach, fresh	9.6	9 (9,10)	
2 "	Spinach, dried	6.3	7 (7,8)	
1 adult	Above + oil	27	7	
1 "	Above, extracted, in oil	6	45	
2 adults	Beet greens	4.5	8 (5,11)	
1 adult	Above, extracted, in oil	22	46	
5 adults	Carotene in oil or fat	3.1–3.6	73 (69–82)	28
2 "	Carrots, canned, sliced	2.2	24 (16,31)	
4 "	Carrots, cooked, puree	3.0	25 (6–36)	
4 "	Above + fat	3.0	33 (0–60)	
2 "	Carrots, cooked, homogenized	3.0	56 (46,63)	
3 "	Spinach, canned, puree	2.5	41 (28–62)	
2 "	Spinach, canned, homogenized	4.0	43 (35–47)	
3 "	Cabbage, dried, outer leaves	1.9	41 (13–60)	
2 "	As above	3.8	28 (28,27)	
3 adults	Carrots, fresh, raw	2.9–6.9	42 (37–46)	141
2 "	Carrots, stored, raw	9.2	43 (36,49)	
3 "	Carrot meal, coarse	6.0–7.5	37 (34–42)	
2 "	Carrot meal, fine	1.2	55 (54,56)	
8 adults	Sweet potato, cooked, mashed	3.5	54 (41–50)	142
1 adult	Carotene in oil	1.7	98	143
1 "	Carrot, cooked	2.6	29	
1 "	Carrot, grated, raw	2.1	21	
1 "	Carrot, grated, cooked	2.1	48	
1 "	Above + fat	2.1	46	
1 "	Pumpkin, strained	0.9	53	
1 "	Above + fat	0.9	66	
1 "	Squash, strained	3.2	35	
1 "	Above + fat	3.2	38	
1 "	Spinach, strained	2.4	33	
1 "	Above + fat	2.5	42	
1 "	Spinach, strained	3.4	37	
1 "	Above + fat	3.2	37	
1 "	Seaweed, dried	2.1	8	

[1] When intakes were expressed in international units, they were recalculated on the basis 1 IU = 0.6 µg β-carotene. In some instances, the methods employed did not differentiate between the various carotenes. — [2] For the present purposes the "absorption" is taken as the difference between intake and fecal content (often corrected for carotene excretion on carotene-free diets); see page 23. — [3] Values in parentheses indicate the reported range. — [4] Value represents total dose administered; number of days not known.

A number of parameters have been reported to effect the absorption of carotene.

The level of dietary fat is important in absorption. Carotene is very inefficiently absorbed unless the diet contains a sufficient quantity of fat or oil. The nature of the fat is also important. Studies have shown that the amount of carotene absorbed is highest when low molecular weight fatty acids are given, and the percentage of absorbed carotene falls as the chain length of the fatty acids increases (604, 1216, 1554, 1716).

Mineral oils such as liquid paraffin seriously reduce carotene absorption. These oils, in which carotene is highly soluble, are not absorbed from the gastrointestinal tract and consequently carry carotene into the feces (307, 604, 1216).

Dietary antioxidants, in particular vitamin E, also appear to have an important effect in the utilization and perhaps on the absorption itself of dietary carotene. It is not clear, however, whether the antioxidants contribute directly to the efficient absorption of the carotene, or whether they protect either the carotene or vitamin A from oxidative breakdown during storage in the body (604, 1216, 1554).

Relatively little research has been performed on the effect of dietary proteins on the utilization of carotene, but some evidence has been presented indicating that a diet low in protein, or containing poor-quality proteins such as zein or gluten, may hinder carotene utilization (1554, 1716).

Soybeans contain an enzyme capable of oxidizing fats, which in turn oxidize carotene. As a result, the inclusion of raw soybeans in the diet interferes with the utilization of carotene (604).

Cis-trans isomerism of the carotenes is another important factor determining their absorbability. It was noted that the absorption of these forms of the provitamins was studied by measuring the liver storage of vitamin A, so that in effect what was being done was the measurement of the ability of the rat to convert them to vitamin A. Thus the relative biopotencies of the various isomers may be the result of the specificity of the enzyme mechanisms converting carotene to vitamin A in the small intestine. The relative biopotencies of a number of cis-trans isomers are presented in Table 5.

TABLE 5

THE RELATIVE POTENCIES OF *cis-trans* ISOMERS

	Potency
All-*trans* β-carotene	100
Neo-β-carotene B	53
Synthetic 15-15′ mono-*cis* β-carotene	50
Neo-β-carotene	38
All-*trans* α-carotene	53
Neo-α-carotene B	16
Neo-α-carotene U	13
All-*trans* γ-carotene	28 or 43
Pro-γ-carotene (poly-*cis*)	44
Neo-γ-carotene P	19
Mixed neo-γ-carotenes	16
All-*trans* cryptoxanthin	57
Neo-cryptoxanthin	42
Neo-cryptoxanthin U	27

Phillips reported that feeding a dietary supplement of 10-100 ppm DDT to rats for periods of up to 72 days resulted in decreased utilization of orally administered carotene, as evidenced by a reduction (up to 26%) in storage of vitamin A in the liver (1369).

On the basis of a study using rats, Bieri, et al. reported that sodium selenite impairs the utilization of carotene given either orally or intravenously (158).

While it has been established that the intestinal wall is the primary site of conversion of carotene to vitamin A, there is considerable disagreement as to the actual mechanism whereby this conversion takes place. Three mechanisms which enjoy popular support are:

1) Hydrolytic cleavage of the central double bond, resulting in the formation of 2 molecules of vitamin A from each molecule of carotene;

2) Oxidative cleavage of the central double bond resulting in the formation of two molecules of retinal which are subsequently reduced to vitamin A;

3) Terminal oxidative cleavage which proceeds with sequential removal of two-careon units until a single molecule of vitamin A is formed.

Recent work by Goodman, et al. (598, 603), using radioactive carotene has lent strong support to the second mechanism (604, 1216, 1554). (The metabolism of vitamin A will be discussed in detail in a forthcoming monograph.)

In a 1967 FAO/WHO report, a discussion of the absorption of carotene was concluded with the following remarks (484):

> "The group recommends that, in the absence of more specific data for foods, the availability of beta-carotene be taken as one third and that the efficiency of conversion in the body be accepted as one half of the available beta-carotene: hence the utilization efficiency in the human is taken as one sixth. Thus, in the human, 1 mg beta-carotene in the diet is taken to have the same biological activity as 0.167 mg retinol."

III. Metabolism and Excretion

Mammals can be divided into two groups with respect to the accumulation of carotene in the body. Members of the first group, which include man, accumulate carotene in the fatty tissues of the body throughout life, accounting for the fact that infant fat is almost white and that adult fat is yellow. This accumulation is apparently irreversible as indicated by the fact that when body fat is mobilized, for any reason, the carotene remains in the depot. This accounts for the characteristic yellowness observed in cases of emaciation. Other members of this group include cattle, horses, sheep, dogs, deer, antelope, Indian buffalo, water buffalo, elephants, hedgehogs, fox, fitchets, badgers, and roedeer (604, 1216, 1716).

The members of the second group do not accumulate carotene in the fatty tissues. This group includes goats, swine, rats, rabbits, hares, giraffes, and reindeer (604, 1216, 1716).

In man, excessive consumption of carotene has been observed to cause the deposition of carotene in the skin, invoking a yellow discoloration known as hypercarotenemia (see Biological Data, Special Studies, Hypercarotenemia). Carotenoids have been reported to appear in human blood and organs in approximately the same ratio as that in which they occur in the diet (604).

Ross and Parker reported that the normal range for serum carotene in 227 apparently healthy individuals on a normal mixed diet was found to be 70 to 300 micrograms per 100 ml with a mean of 153 and a standard deviation of ± 57. No statistical variation was found according to sex, age, or season. In their report, Ross and Parker also tabulated the serum carotene values reported by other investigators (see Table 6). All of the reported values were in essentially the same range (1492).

TABLE 6 Serum carotene values (in micrograms per 100 ml. of serum) as reported by other investigators

Investigator	Range	Mean	Standard-Deviation	Comment
Murrill *et al.*[11]	100 to 348	199	...	25 males
	136 to 420	227	...	20 females
Harris *et al.*[8]	80 to 370	210	±31	70 subjects
Haworth, Moschette, and Tucker[14]	49 to 202	120	±34	40 females 15 to 22 years of age
Kimble[4]	50 to 300	166	±59	30 males
	90 to 340	187	±77	34 females
Yudkin[9]	117 to 241	168	...	11 subjects, summer
	50 to 118	85	...	12 subjects, winter
Wenger, Kirsner, and Palmer[1]	70 to 282	123	±47	110 subjects
Caveness, Satterfield, and Dann[10]	18 to 278	138	±62	71 healthy persons, chiefly college students on ordinary diet
Yarbrough and Dann[6]	34 to 422	131	...	197 subjects 5 months to 68 years
Kirk and Chieffi[9]	200 to 320	..	...	155 middle-aged and old individuals; 47 younger subjects
Highman[6]	110 to 370	207	...	20 European males
	113 to 340	244	...	20 European females
	100 to 310	162	...	11 Indian males
	30 to 260	131	...	8 Bantu males

The excretion of carotene by man was discussed in the previous section in conjunction with absorption. Depending upon the source of carotene, fecal excretion of intact carotene by man was reported to range from 2 to 99% of the ingested quantity (484, 1437). Carotene is never found in the urine and no carotenoids have been detected in human sweat (604).

Several studies have been reported in which radioactive carotene was employed in studying the metabolism of carotene in the rat.

In a study by Fishwick and Glover, two vitamin A-deficient rats were given orally 7.8 and 4.3 mg, respectively, of crystalline ^{14}C-beta-carotene dissolved in 1.0 ml arachis oil. Upon sacrifice, 24 hours after dosing, it was found that 50-70% of the doses were not absorbed. Just over 2% of the radioactivity was eliminated as CO_2 in the 24-hour test period. The percentage radioactivity in the total tissue lipids of the second animal were as follows: liver 30; intestine 13; and kidney 0.3. Determination of the radioactivity of the beta-carotene, vitamin A, sterol, and fatty acid (ether-soluble) fractions of the liver lipids revealed values of 3.0, 14.4, 1.3 and 1.4%, respectively, indicating that the carotene had been degraded to yield intermediary metabolites as well as vitamin A (506).

The distribution of radioactive carbon in the rat was determined by Willmer and Laughland following the administration, by stomach tube, of randomly labelled C^{14}-beta-carotene (about 0.2 mg). The total activity recovered per organ at various times after dosing is shown in Table 7. The total activity in the non-saponifiable fractions of tissues from eight rats over the 28-hour period was greatest in the liver followed in descending order by the intestines, adrenal glands, blood, kidneys, lungs, heart, hypophysis, stomach, and spleen. Barely detectable amounts

of C^{14} were found in the pancreas, testes, and thymus. $^{14}CO_2$ production amounted to an average of about 12% of the administered carotene and 47.7% of the radioactivity was recovered from the excreta (1890).

TABLE 7

TOTAL ACTIVITY PER ORGAN,* C.P.M.

Tissue	Fraction	Hours after dosing							
		1	2	4	6	10	16	22	28
Adrenals	N.S.	N.a.‡	3600	4896	6952	15000	6132	3536	2652
	Sap.	–	+	195	1272	1047	770	1760	1677
Heart	N.S.	188	209	363	327	174	307	186	–
	Sap.	195	137	139	175	279	227	177	128
Hypophysis	N.S.	117	110	120	615	135	105	120	200
	Sap.	+	197	180	1007	+	307	+	+
Intestines	N.S.	N.a.‡	7750	12334	13354	8575	1673	1626	–
	Sap.	2000	–	2750	–	–	–	–	–
Kidneys	N.S.	293	460	558	889	731	911	879	907
	Sap.	–	781	–	–	159	–	–	158
Liver	N.S.	2700	2700	8750	6650	13150	12450	13750	8800
	Sap.	1341	1177	1221	1302	1224	2436	679	1060
Lungs	N.S.	–	–	+	+	553	1038	855	667
	Sap.	–	–	–	–	–	–	–	–
Spleen	N.S.	150	+	116	119	204	128	+	+
	Sap.	–	–	–	–	–	–	–	–
Stomach	N.S.	546	275	144	–	–	–	–	–
	Sap.	–	–	–	+	+	+	–	–
Blood†	N.S.	1137	2920	1441	553	705	668	574	717
	Sap.	+	+	+	+	+	+	+	+

*+ Trace of radioactivity. – No radioactivity.
†Total activity calculated on basis of 6.0 ml. blood per 100 g. body weight.
‡Result not available.

Krause and Sanders investigated the metabolism of carotene through the oral administration of C^{14}-labelled beta-carotene to rats. Analysis of the feces, urine and intestinal contents 24 hours after feeding disclosed approximately half of the total radioactive carbon fed, 30% of the fed dose remaining as intact carotene. Therefore, 70% of the carotene was assumed to be absorbed. The distribution of the absorbed radioactivity is summarized in Table 8. About 40% of the absorbed C^{14} remained unaccounted for (954).

TABLE 8 Distribution of C^{14} in Rats after Oral Administration of C^{14} Labelled β-Carotene.

	Total c.p.m. in sample		% of dose absorbed*	
	Rat 1	Rat 2	Rat 1	Rat 2
Fed	65,000	35,000		
Feces, urine, and intestinal contents	35,000	16,000		
Non-saponifiable	21,000	10,000		
Fatty acids	14,000	6,000		
H_2O soluble	—	—		
Total extrahepatic tissue	13,200	10,850	44.0	57.1
CFNS	9,700	7,050	32.3	37.1
Fatty	3,500	3,800	11.7	20.0
H_2O soluble	—	—	—	—
Total liver	1,425	872	4.8	4.6
CFNS	765	599	2.6	3.1
Vit. A	425	310	1.4	1.6
Sterols	60	39	.2	.2
Rest	280	250	.9	1.3
Fatty acids	510	173	1.7	.9
H_2O soluble	150	100	.5	.5
Expired CO_2	1,420	1,020	4.7	5.4
Recovered	51,045	28,742		
Non-saponifiable	31,465	17,649		
Fatty acids	18,010	9,973		
H_2O soluble	150	100		
CO_2	1,420	1,020		
Unaccounted for	13,955	6,258		

* Absorbed dose = Administered dose − (amt in feces, urine, and intestinal contents).

IV. Effects on Enzymes and Other Biochemical Parameters

No Information Available

V. Drug Interaction

No Information Available

VI. Consumer Information

The average daily consumption of carotene and vitamin A, in terms of vitamin A potency, in Great Britain during the period from 1942 to 1964 was presented in graphic form (Figure 2) in a report by Greaves and Tan. A divisor of 3 was used in determining the vitamin A potency of carotene, so the actual amount of carotene consumed was 3 times the value shown in Figure 2. A 1957 survey set the daily intake of carotene in the United States at 5870 IU/person (626).

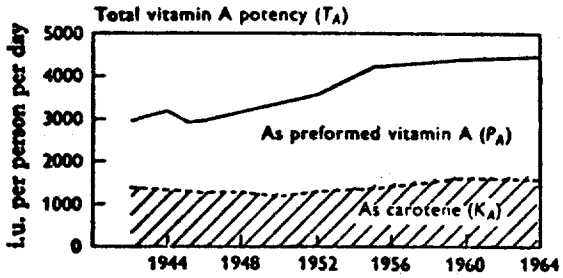


Figure 2

Table 9 presents the carotene content of numerous plant greens, fruits, seeds and tubers (604).

Table 9.

Carotene Content of Plants

Note : Only values obtained using well-authenticated chemical methods of analysis are tabulated. Values for processed foodstuffs are not included.

(*A*). *GREEN TISSUES.*

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Agathi	*Sesbania gangeticus*	14·3–	1		
Alkali drop-seed	*Sporobolus aeroides*	2·7		4·1–	
		7·5b	86	18·9b	86
Amaranth	*Amaranthus gangeticus*	11·0	1		
		2·5–11·1	79		
Andropogon	*Andropogon annulatus*	4·4–11·8	2		
Asparagus (Green)	*Asparagus acutifolius*	0·58	3	3·29–7·47	3
		3·58	80	7·25	4
		1·12	82	7·47	82
Aster	*Aster indicus*	6·30	91		
Australian beard-grass	*Andropogon intermedius*			14·0	5
Bahia grass	*Paspalum notatum*			27·1	5
Bajri (Indian Millet)	*Pennisetum typhoideum*	4·5–5·6	2		
Banana (Plantain)	*Musa paradisiaca*	0·7	1		
Barley (grass)	*Hordium vulgare (sativum)*	9·6	83	41·5	8
				37·6	83
„ (whole plant)		3·5	7	17·1	7
Beet (tops)	*Beta vulgaris*	6	8	32·9–44·2	10
		5–10	9	30–60	9
		10·5	11	57·2	12
		5	80		
Bengal gram	*Cicer arietinum*	8·1	1		
		18·4	76		
Bermuda grass	*Cynodon dactylon*			18·7–41·3	13
Berseem (Egyptian clover)	*Trifolium alexandrinum*	8·4	2	28·6	5
				18·8–36·2	10
				15–40	14
Big blue stem	*Andropogon provincinalis*	0·3—7·7	83	0·3a	5
		0·54–10·7b	86	10·3	5
				0·4–22·1	83
Biri	*Biri* spp.	13·0	2		
Black finger	*Chloris evcullata*			11·9–16·5	13
Bird's foot trefoil	*Lotus corniculatus*			52·6	75
Black grama	*Bouteloua eripoda*			0·54–12·57b	15
Blue grama	*Bouteloua gracilis*	0·48–		0·51	
		1,·9b	86	–26·9b	86
Blue grass	*Poa arachnifera*	12–18	16	1·14a	5
Blue joint	*Calamagrostis canadensis*	0·44		0·54	
		–5·3b	86	–16·1b	86
Bottle gourd	*Lagenaria vulgaris*	9·7	76	52·9	5
Bracken	*Pteridium aquilinum*	0·8	91		
Bristle grass	*Setaria macrostachys*			0·25a	5
				15·8	5
Broad red clover	*Trifolium pratense*			22·8–59·4b	19
				16·0–41·1	20
				49·2	75
				5–92	34
Broccoli		0·6–1·1b	17	21·3	3
		4·65	80	45·3–51·7	13
		1·58	82	10·67	82

Table 9 (Cont.)

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Brome grass	*Bromus* spp.			28·9	13
				72·6	20
				36·2–57·5	84
Brussel sprouts	*Brassica oleracea*	0·4	21	3·69	82
		0·12–0·36	22		
		0·58	82		
Buffalo grass	*Buchloe dactyloides*	0·7–12·6	23	9·9–25·4	5, 13
		0·55–7·8b	86	1·55a	
Buttercup	*Ranunculus* spp.			0·9–28·2	83
Cabbage (outer leaves)				45·13	5
(heads)		3·9	79	14·6	10
Cactus (spineless; Bokhara clover)	*Melilotus alba*	0·032	79	0·5	10
Canadian Brome				41·3	13
Carpet grass	*Axonopus affinis*	4·2–10·4	83	7·7–53·3	83
Carrot (tops)	*Daucus carota*			9·4–25·7b	23
		17·4	24		
		11·2	21		
Cauliflower (leaves)	*Brassica oleracea*	14·8	76		
(head)				40	10
Celery leaves	*Apium graveolens rapaceum*	0·038	79		
		5·76	79		
		0·21	85		
Cenchrus	*Cenchrus ciliaris*	0·05–7·35	25	0·06–41·3b	25
Chinese cabbage	*Brassica chiensis*	7·8	35		
Chloris	*Chloris babata*	4	2		
Chrysanthemum	*Chrysanthemum coronarium*	3·8	91		
Cocksfoot (orchard grass)	*Dactylis glomerata*	3·8–6·8	83	13·7–59·6b	19
				38·5b	73
				14·2–37·7	75
				7·5–15·4	83
Collards	*Brassica oleracea*	3·28–4·64b	17	34·4–57·5	84
	var. *acephala*	2·86–7·92b	11	29–46	17
		1·74	80	51·1	12
Combs' Paspalum	*Paspalum album*				
Coriander	*Coriandium sativum*			19·8	5
		30	1		
		11·6	76		
Cow pea (plants)	*Vigna catjang*	12·6	79		
Crab grass	*Digitaria sanguinalis*	11·4–16·1	2		
				1·68a	5
Cress				16·9–23·1	5
		4·5	21		
Cryptotaenia	*Cryptotaenia canadensis*	0·9	22		
Curly Mesquite	*Hilaria belangeri*	5·6	91		
Curry leaf	*Murraya koenigi*			11·2–22·2	13
		24	1		
		12·63	79		
Cynodon	*Cynodon plectostachyum*	5·3	85		
				0·59a	5
Dakota brome				7·2	
Dallis grass (Italian blue grass)	*Paspalum dilatum*	3·8–8·6	83	6·6–33·4	83
		26	2	22·7–49·5	13
				18·7	5
Dandelion	*Taraxacum officinale*	8·7	26	11·3–46·4b	23
		9·66	82	69	82
Dhub grass	*Cynodon dactylon*	11·1	1		
		24·6	6		
Digitaria	*Digitaria eriantha* v. *stolonifera*	0·12–10·9b	25	0·13–39·2b	25
Dock	*Rumex crispus* *R. obtusifolius*			85·6	12
				58·3	75
Dolichos (Broad bean)	*Dolichos lablab*	13	2		
Drumstick grass	*Moringa oleifera* (*aptera*)	32	1		
Early white clover	*Trifolium repens*			27·3–67·0b	19

Table 9 (Cont.)

Common Name	Botanical Name	Carotene content (mg./100 g.).			
		Fresh wt.	Ref.	Dry wt.	Ref.
Earthnut	*Conopodium denudatum*			48·8	75
Eastern grama	*Tripsacum dactyloides*			3·19[a]	8
				18·9–22·5	5
Egyptian clover (*see* Berseem)					
Elephant grass	*Pennisetum purpureum*	2·8–10·0	2	20·4	6
Eragrostis	*Eragrostis plana*	0·05–8·06[a]	25	0·05–14·7	25
Erichloa	*Erichloa sericea*			19	5
Fahli clover				33·7	10
Flax	*Linum usitatissimum*	0·65	27		
Garagway grass		12·6	2		
Garlic	*Allium sativum*	4·2	76		
		0	79		
Goosegrass	*Eleusine indica*			10·3	5
Goosefoot	*Chenopodium album*	12·4	76		
Gordura grass	*Melinis minutiflora*	8·8	2		
Grapevine mesquite	*Panicum obtusum*			13·6	13
Green clover				10·8–33·4	28
Groundnut (stem and leaves)	*Arachis hypogaea*	1	2		
Guajillo	*Acacia berlandieri*			10·5	13
Guinea grass (Hairy "buffels-gras")	*Panicum maximum*	4·9	2		
		0·07–7·0	25	0·11–30·85	25
Hairy grama	*Bouteloua hirsuta*	0·2–14·9b	86	12·2	13
Hamburg parsley		12·5	21		
Horse grama	*Dolichos biflorus*	13·0	2		
Indian grass	*Sorghastrum nutans*	1·0–13·2b		4·1[a]	5
				13·2	5
				37·3	13
Indigofera	*Indigofera annaphylla*	5·2	2		
Ipomea	*Ipomoea hispida*	8·8	2		
	I. sepiaria	10·8	76		
Italian blue grass (*see* Dallis grass)					
Italian rye grass	*Lolium italicum* (*multiflorum*)			11·9–45·6b	19
Japanese peppers	*Xanthoxylum piperitum*	4·4–13·7	91	37·2	13
Jews mallow	*Kerria japonica*	7·9	91		
Johnson grass	*Sorghum halepense*	3·2	2	36·6	13
Jowar	*Andropogon sorghum*	4	2	21·4	6
June grass	*Koeleria cristata*	3·7–8·3b	86	6·1–18·3	86
Jute	*Corchorus capsularis*	10·8	76		
Kachnar ka patta	*Bauhinia variegata*	8·4	76		
Kalai	*Phaseolus radiatus*	4·6	2		
Kale	*Brassica oleracea*			87	29
Karki	*Celtis caucasica*	4·6	2		
Kentucky blue grass	*Poa pratensis*	3·6–7·2	83	4·1–21·4	83
				19·5–43	84
Knotgrass	*Paspalum distichum*			22	13
Kohlrabi (leaves)				36–38·8	30
Kollukatti grass	*Pennisetum cenchroides*			14·9	6
Lang's paspalum	*Paspalum langei*			0·85[a]	5
				23·5	14
Lathyrus	*Lathyrus sativus*	15·5	76		
Leek	*Allium porrum*	0·63	85		
Lettuce	*Lacuta sativa*	0·77–2·23b	11	74	12
		6.8	76		
		3·7	85		
		2·1–2·4	79	21–36	10
		0·61	80		
Lindheimer's Panicum	*Panicum lindheimeri*			11·0	5
Little blue stem	*Andropogon scoparius*	2·7–6·7	83	4·3–15·6	83
Lofa	*Malva verticillata*	7·5	76		
Love grass	*Eragrostis curvula*	0·44–5·7b	86	12·9	5
Love lies bleeding	*Amaranthus caudatus*	14·8	76		

Table 9 (Cont.)

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Lucerne	*Medicago sativa*	6·7–10	30	15·3	5
				21·7–28·3	84
		8·5	4	11–35·5	10
		29·6	1	80 (max.)	31
		3·2–8·8	83	30–53	30
		16·8	2	30·1–43·2	18
				8·3–29·2	83
Maize (leaves)	*Zea mais*	9	72	70	7
		12·2–18	32	56·3	20
		3·7	2		
" (whole plant		0·9	7	3·4	7
Mallow	*Malva* spp.			49·7	10
Mangold leaves				111·3	10
Mautitius	*Panicum muticum*	10	2		
Meadow fescue	*Fescuta elatior*			28·6–54·7	84
Mesa dropseed	*Sporobolus flexuosus*			0–13·86b	15
Mesquite leaves	*Prosopis chilensis*			4·4	13
Methi (Fenugreek)	*Trigonella foenum-graecum*	5·8	2		
Mint	*Mentha viridis*	9·1	76		
Mugwort	*Artemisia vulgaris*	8·7	76		
Mustard		6·7	91		
Napier (see Elephant grass)		1·93	80		
Needlegrass	*Stipa spartea*	1·2–11·1b	86	1·6–23·6b	86
Neem	*Azadirachta indica* (*Melia azadirachta*)	14·3	1		
		4·50	79		
Nettle	*Urtica dioica*	14	21	38·4	19
				34·8	10
Northern reed grass	*Calamogrostis inexpansa*	0·57–4·8b	86	0·79–12·3	86
Oak	*Quercus* spp.			5·5–11·4	13
Oats	*Avena sativa*			22·6–64·7	10
				38·7	20
				33·3–49·8	33
Onion (leaf)	*Allium cepa*	2·0	76	52·5	18
Onion	*A. fistulosum*	0·3	91	17·5	10
Onion	*A. odoratum*	2·4	91		
Orchard grass (see Cocksfoot)					
Pan (Betel)	*Piper betel*	13·0	1		
		7·27	79		
		13·7	85		
Panicum	*Panicum fasiculatum*			49·5	13
Panicum	*Panicum milare*	10·4	2		
Parsley	*Petroselum sativum*	10–11	16	38·8	10
		5·6	85		
		9·4	21		
		3·2	79		
Parwar	*Trichosanthes dioica*	13·4	76		
Paspalum	*Paspalum stramineum*			40·7	13
Pea (leaves)	*Pisum arvense*	8·0	2		
		15·5–24·4	76		
(stem)		6·8	76		
Penicillaria	*Penicillaria* Spp			8·5–11·8	10
Peppers	*Capsicum annuum*	10·2	91		
Perennial rye grass	*Lolium perenne*			14·4–43·0b	19
Perilla	*Perilla ocimoides*	6·3			
		12·4	91		
Petastines	*Petastines japonica*	8·6	91		
Plantain	*Plantago lanceolata*			40·6	19
Pooin	*Basella viridis*	4·8	76		
Portulaca				19·1	10
Potato (leaves)	*Solanum tuberosum*	12·2	76		
Prarie dropseed	*Sporobolus heterolepis*	0·26–8·2b	86	0·33–15·3	86
Prickly pear	*Opuntia* spp.			0·6	13

Table 9 (Cont.)

Common Name	Botanical Name	Carotene content (mg./100 g)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Pumpkin (leaves)	*Cucurbita maxima*	10·1	76		
Purple top grass	*Sieglingia (Triodia) flava*			10·8	5
Quack grass		12	26		
Radish	*Raphanus sativus*	7·4–9·8	76, 91	69·8	12
Ragi	*Eleusine coracana*	9·6	2		
Ragwort	*Senecio jacobea*			39·9	19
Ranmatki	*Phaseolus trilobus*	8·8	2		
Rape	*Brassica napus*	8·8	76	79·2	12
Reana	*Reana luxurians*	5·4	2		
Red Pigweed	*Amaranthus rubrum*	11·5	76		
Red top	*Agrostis alba*	3·4–5·7	83	5·4–24·1	83
Rescue	*Bromus catharticus*			40·6	13
Rhodes grass	*Chloris gayana*	3·0	2	28·3	13
			25	16–21	5
					25
Rhubarb (leaves)	*Rheum officinale*	6	8		
" (stalks)		0·1	21		
Rye		9·5–10·7	33	25·8–37·8	3
		5·5–8·4	83	25·1–30·6	83
Sainfoin	*Onobrychis cristagalli*			20·6–40·6	35
St. Augustine	*Stenotaphrum secundum*			3·5[a]	5
Salt grass	*Distichlis stricta*	0·75–17·0[b]	86	1·1–17·2	86
Sand dropseed	*Sporobolus cryptandrus*	0·48–11·5[b]	86	0·53–30·4[b]	86
Sand reedgrass	*Calamorilfa longifolia*	0·93–14·1[b]	86	1·1–43·3[b]	86
Sandhill bluestem	*Andropogon halii*	1·0–13·4[b]	86	1·3–51·2[b]	86
Seresia	*Seresia lespedeza*			28·1	20
Sesbania	*Sesbania grandiflora*	20·2	2		
Setaria	*Setaria italica*	12·0	2		
"	*Setaria lindbergiana*	0·4–9·41[b]	25	0·53–33·7[b]	25
Sheeps fescue	*Fescuta ovina*			44·7	75
Shevari		11·2	2		
Side oats grama	*Bouteloua curtipendula*	0·75–13·0[b]	86	0·80–28·3[b]	86
Slough grass	*Spartinia pectinata*	1·36–9·6[b]	86	1·7–27·1[b]	86
Sorghum	*Sorghum versicolor*	7·6	2		
	S. purpureo-sericeum	9·0	2		
Sotol (leaves)	*Dasylirion texanum*			4·2	13
Soya bean (leaves)	*Glycine soja*	15–29	36	43·2	32
		11·4	2		
Spear grass	*Heteropogon contortus*	3·4	2		
Sowa	*Peucedanum sowa*	15·0	76		
Spear grass	*Stipa leucotrida*			27·4	13
Speedwell	*Veronica chamaedrys*			35·6	19
Spinach	*Spinacia oleracea*	3·2	11	12·25–13·0	16
		5·0	37	65	12
		6·1	76	12·4–44	10
		2·63	79	44–56[c]	3
		3·12	70	41·4–57·4	38
		7·58	82	94·6	82
		4·8	85		
Spring onion	*Allium* spp.	5·6	83		
Stick grass	*Panicum antidotale*			1·86[a]	5
				21·8	5
Sudan grass	*Holcus (Sorghum) sudanensis*	5·8	2	43	32
		2·8	7	7·2–18	10
				13·5	7
Sugar beet (leaves)		6	8		
Swede (leaf)	*Brassica campestris*	2·6	91		
Sweet clover				24·7	32
Sweet potato (leaves)	*Ipomoea batatas*			60.8	10
Swiss chard	*Beta vulgaris. v. rapa*	5·9	8	46–62[c]	8, 12
		3·4	80		

Table 9 (Cont.)

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Switch grass	*Panicum virgatum*	0·2–9·0b	86	0·21–33·3b	86
Tall dropseed	*Sporobolus asper*	0·85–13·1b	86	1·1–33·8b	86
Tall oatgrass	*Avena elatior*			10·6–67·0b	19
Tea, black	*Thea sinensis*	2·54	79	47·3	39
" green				71·5	40
				76·1	39
" Oolong				150·7	40
" Touchang				78·4	40
Teosinte	*Euchlaena mexicana*	3·8	2	168·1	40
Thistle	*Carduus* (*Cirsium*) spp.	1·33	27	32·7	19
Thorny pigweed	*Amaranthus spinosus*	28·8	76		
Timothy	*Phleum pratense*			11·0–58·2b	19
				11·2–27·5b	73
Tufted hair grass	*Aira caespitosa*			44·4	75
Tobacco leaves		13·8	1	59·88	11
Tulip	*Tulipa gesneriana*	4·3	91		
Turnip	*Brassica rapa*	2·9–5·1	16	83·7	12
		5·10	9		
		3·26	80	30–60	9
Vetch	*Vicia sativa*			0·02	35
				13–43	10
Vite	*Vitis quadrangularis*	0·27	41		
Water hyacinth	*Eichhornia crassipes*	5·8	2		
Watercress	*Nasturtium officinale*	3·2	85		
Western needle grass	*Stipa comata*	0·74–9·1b	86	0·94–13·9b	86
Western wheat grass	*Agropyron smithii*	4·3–10·2b	86	5·1–32·5b	86
Wild rye	*Elymus canadensis*	1·5–6·2b	86	4·7–21·0b	86
Wheat		6·6	83	17·9	83
Wild white clover	*Trifolium repens*			27·4–66·9b	19
				14·3–55·2	73
				36·1	75
Yarrow	*Achillea millefolium*			37·9	19
Yorkshire fog	*Holcus lanatus*			13·9–60·4b	19

(B). *FRUITS, SEEDS AND TUBERS*

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Actinida	*Actinidia chiensis*	0·035	41	0·190	41
Apple	*Pyrus malus*	0·05	41	0·311	41
		nil	79		
		0·08	80		
Apricots		3·5	41	24	11
		10·0	80		
		1·05	11		
Asparagus	*Asparagus officinalis*	0·6	91		
Avocado		0·710	80		
Bamboo	*Dendrocalamus flagellifer*	0	85		
Bananas	*Musa sapientum*	0·05	41		
		0·1	78		
		0·8	80		
		1·0	85		
Barley	*Hordeum vulgare* (*sativum*)			0·17–0·24	35
Beet	*Beta vulgaris*	0	85	0·11	43

Table 9 (Cont.)

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Bengal gram (husks)	*Cicer arietinum*	0·316	79		
Black Bengal gram	*Phaseolus mungus*	1·93–3·1	83	0·310	41
Blackberry	*Eugenia jambolana*	0·064	79		
		0·03–0·24	42		
Blimburg	*Averrhoa bilimbi*	0·32	85	0·11	43
Blueberry		0·06	87		
Canary grass seed	*Phalaris canariensis*	0·28	27		
Canistel	*Lucuma nervosa*	0·12–96	78		
Carambola	*Averrhoa carambola*	0·95–7·4	42		
Cantaloupe (yellow)		0·12	85		
" (green)		4·56	80		
Cape gooseberry	*Physalis peruviana*	0·39	80		
Carrot [d]	*Daucus carota*	3·4	76		
		13·6	32	111	32
		2·0	79	30–130	44
		8·5	44	82–130	45
		6·6	11	73–86	46
		16·2	1	17·5–160·5	77
		3·2–22·6	77	98–104	89
		2·7–73·8[c]	21		
		7·7	76		
		0·8	85		
		0·02–11·7	47		
		6·5–14·4	48		
		0·7–17	49		
		10·8–12	22		
		14	72		
		4·8	50		
Cherries		4·13	80		
		0·39	42		
Chinese persimmon	*Diopyros kaki*	0·30	41		
Cluster bean	*Cyamopsis psoralioides*	0·21	85		
Coconut	*Cocos nucifera*	1·2	76		
		nil	42, 79, 85		
Colocasia	*Colocasia antiquorum*	0·04	79		
Coriander (seeds)	*Coriandrum sativius*	0·157	79		
		0·045–(0·055)[e]	24		
Cow peas	*Vigna catjang*	0·3–0·7	76	17·0–20·5	10
		0·025–0·040[e]	52		
Cranberry		0·6	76		
Cucumber skin	*Cucumis sativus*	0·24	11		
flesh		0·10	91		
Custard apple	*Anona squamosa*	nil	91		
Dolichos (Broad beans)	*Dolichos lablab*	nil	76		
Egg plant, skin	*Dolichos melanogena*	0·32	85		
flesh		0·10	91		
Elephant apple	*Feronia elephantum*	nil	91		
Fenugreek (seeds)	*Trigonella fornicumgraecum*	trace	76		
Figs	*Ficus hispida*	0·24	11		
Four-angled bean	*Psophocarpus tetragonolobus*	0·3	76		
		0·052	80		
French beans	*Phaseolus vulgaris*	0·53	85		
		0·19–0·42	54	4–4·9[c]	3
		1·1	85		
		0·22	79	2·36	53
		5·31	80		
		2·28	80		
Ginger	*Zingiber officinale*	0·1	85	1·9–3·8	54
Gourd (bitter)	*Momordica charantia*	0·21	79		
		0·16	85		
Grape, skin	*Vitis vinifera*	5·0	1		
flesh		0·1–0·4	91		
		0·01	91		

Table 9 (Cont.)

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Grape fruit	*Citrus decumana*	nil	42		
Green gram	*Phaseolus radiatus*	0·158	79		
Ground nut seeds	*Arachis hypogoea*	0·063	79		
Guavas	*Psidium guajava*	0·18–0·28	13		
Hemp seeds	*Cannabis sativa*	0·98	27		
Hog plum	*Spondias mangifera*	0·4	76		
		0·16	85		
Horse gram	*Dolichos biflorus*	0·158	79	55·3–73	10
Indian plum	*Zizyphus jujuba*	0·5	78		
Jackfruit	*Artocarpus polyphemia*	0·55	85		
Kang-kang	*Ipomea aquatica*	8·4	85		
Kwini	*Mangifera odorata*	2·45	85		
Ladies finger	*Hibiscus esculentus*	0·06	79		
		0·16	85		
		0·3	76		
Latarus	*Dioscorea alata*	0·4	76		
Lemon		0·120	41	1·0	41
Lentils	*Lens esculenta*	0·45	79		
Lima beans		4·3–6·6	55	45·4	20
Lime	*Citrus medica*	0·07–0·23	85		
Loquat	*Eriobotrya japonica*	0·4	76		
Mace		0·172 (1·98)[j]	81		
Maize (yellow)	*Zea mais*	0·3	4	0·72	4
		0·32	85		
		0·11	44	0·20–0·94[c]	57
,, (white)		0·9–1·1	59	0·039–0·73	35
		0·042	79		
		0·039	80		
Mamey (S. Domingo)	*Mammea americana*	1·17–5·2	42		
(red)	*Achras zapota*	0·14–0·24	42		
		0·16	85		
Mandarin	*Citrus aurantium deliciosa* (*C. nobilis*)	0·40	81	3·201	41
		0·25	91		
Mangoes[f]	*Mangifera indica*	1·2–14[c]	58		
		2·6–6·0	76		
(green)		3·4–11·0[c]	60		
		trace	76		
Mangosheen	*Garcinia mangostana*	0·15	79		
Marking nut (orange cups)	*Semecarpus anacardium*	0	85		
		0·9	76		
Millet	*Andropogon (Holcus) sorghum*			0·165–0·22	35
Mulberry	*Morus indica*	0·2	76		
Musk, skin	*Cucubita moschata*	0·1–2·6	91		
flesh		0·2	91		
Musk melon (Gourd)	*Cucumis melo*	0·76	42	82·7	39
		0·5	76		
Mustard seeds	*Brassica juncea*	0·27	79		
Oats	*Avena sativa*	1·0	27	0·09	43
Okra	*Hibiscus esculentus*	0·24	42	0·065–1·41	35
		0·35	80		
Olives		0·23	80		
Onion	*Allium*	0·025	79		
Orange	*Citrus* spp.	0·150	81	1·15	41
,, (skin)		0·14–0·33	85		
Palm flesh	*Elaeis guineensis*	2·57	79		
Palmyra (mesocarp)	*Borassus flabellifera*	33	91		
Papayas	*Carica papaya*	7·6	76		
		0·128 (1·158)[k]	61		
(skin)		1·07	85		
(pulp)		2·4–4·7	76		
Parsnip		0·4–2·8	76	1·84	79
Passion fruit	*Passiflora laurifolia*	0·03	79		
		0	85		

Table 9 (Cont.)

Common Name	Botonical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Peach	*Prunus persica*	1·0	41	3·5	41
		0·35	80		
Pear	*Pyrus communis*	0·01	41	0·06	41
		0·014	79		
Peas (English)[a]	*Pisum sativum*	0·5	4	3·1–5·1	9
		0·47–0·71	63	2·0	82
		0·14	79		
		0·3	21		
		1·2	76		
Peas (country)	*Pisum arvense*	3·1	76		
		1·56	80		
Peppers	*Capsicum annum*	3·76–16·7[c]	62		
		0·26	85		
		2·18	80		
(green)		2·74	42		
		4·8	85		
Pineapple	*Ananas comosa*	0·26–0·74	79		
		0·42	85		
Poppy (seeds)	*Papaver somniferum*	0	27	0	27
Potato		0·056	79		
Pumpkin	*Curcurbita maxima*	0·084	79		
		0·16	85		
		1·27	80		
Radish	*Raphanus megalantha*	0·003	79	23–28	64
		0·0	85		
Ragi	*Eleusine coracana*	0·037–0·090	79		
Rambutan	*Nephelium lappaceum*	0	85		
Raspberries		0·35	41	0·54	41
Red cashew nuts	*Anacardium occidentale*	0·44–1·55	42		
Red gram (dhall)	*Cajanus indicus*	0·22	79		
		0·16–0·32	85		
Red sorrel (fruit)	*Hibiscus sabdariffa*	0·4	76		
Rice	*Oryza sativa*	nil	85		
Ridge gourd (loofah)	*Luffa acutangula*	0·4	76	11·9	64
		trace	85		
		0·06	79		
Rose apple	*Eugenia malaccensis*	0·11	85		
Rose hips	*Rosa spaldingi R. acicularis*	4·5	90	15	41
Sapodilla (*see* Red mameys)					
Snake gourd	*Trichosanthes anguina*	0·16	79		
		1·6	85		
Sotol bulbs	*Dasylerion texanum*			0·2	13
Soya beans	*Glycine soja*	0·018–0·705[b]	51		
		0·11	85		
		0·45–0·97	79		
Strawberries	*Fragaria* spp.	0·06	41	2·0	41
Sugar beet				0·05–0·15	31
Sweet potatoes	*Ipomoea batatas*	0·13–3·94[c]	65	42·3	12
		3·85–4·95	70	14	66
		0·05	85		
		0·06–0·15	91	13	28
				4–36·2[c]	73
Sword bean	*Canavalia ensiformis*	0·2	76		
Tamarind	*Tamarindus indicus*	0	85		
Tangerine	*Citrus nobilis*	0·75	47		
Tomatoes	*Lycopersicum esculentum*	1·4	44		
		0·58	42		
		0·9	1		
		4–5	67		
		0·1–19·1[c]	68, 69		
		0·51	80		
		1·0	85		
	L. pimpinellifolium	0·66–1·92	68, 69		
	L. peruvianum	0·07–0·36	68, 69		
	(*L. esculentum*, × *L. hirsutum*), × *L. esculentum*	0·07–6·75	68, 69		

Table 9 (Cont.)

Comman Name	Botanical Name	Carotene content (mg./100 g.			
		Fresh wt.	Ref.	Dry wt.	Ref.
Vegetable marrow	*Luffa aegyptiaca*	0·2	76		
Vetch	*Vicia faba*	0·05	91		
Water chestnut	*Trapa bispinosa*	nil	76		
Water melon	*Citrullus vulgaris*	1·8	76		
		0·19	80		
		0·0	85		
Wheat		0·16–0·38	71	0·08	43
		0·108	79		
				0·24–0·84	35
White gourd	*Benincasa hispida*	0·5	91		
White turnips	*Brassica campestris*	nil	79	0·05–0·15	31
Wood apple	*Aegle marmelos*	0·1	76		
Yellow mulanga	*Xanthosoma sagittifolium*	1·08	42		
Yam	*Dioscorea* spp.	0·434	79		
Yellow swedes	*Brassica campestris*			2·4–4·3	31
Yellow turnips	*Brassica campestris*			0·6–3·0	31

FOOTNOTES

a. Dormant forages.
b. According to season.
c. According to variety.
d. See Table 12 (p. 82) for further information.
e. mature plants.
f. See Table 11 (p. 81) for further data.
g. See Table 6 (p. 44) for further data.
k. Cryptoxanthin content.
l. Lycopene content.

The following tables were compiled in 1972 with data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

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TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
CAROTENE NAS 0063 FEMA 3548	01	BAKED GOODS(R)	4	.00163	.00178
	03	OTHER GRAIN(R)	6	.01072	.01287
	04	FATS OILS(R)	10	.00101	.00267
	07	FROZN DAIRY(R)	*	.00222	.00500
	08	PROCSD FRUT(R)	8	.01882	.01385
	10	MEAT PRODS(R)	*	.00500	.00750
	14	PROCSD VEGS(R)	*	.00282	.02350
	19	SWEET SAUCE(R)	*	.01244	.02613
	20	GELATIN PUD(R)	*	.00180	.00180
	21	SOUPS(R)	*	.00240	.00300
	22	SNACK FOODS(R)	*	.03000	.03000
	27	GRAVIES(R)	*	.00392	.07843
	28	IMIT DAIRY(R)	5	.00012	.00014

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TABLE 3 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP II) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(B)

NAS SURVEY NO.	SUBSTANCE NAME	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0063	CAROTENE	63	FORMULAS(B)	*	.00002	.00002

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TABLE 11, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
CAROTENE NAS 0063 FEMA 3548	27/ 37	15,200	32,371	40,276	*	2,333	42,609

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
CAROTENE NAS 0063	01 BAKED GOODS(R)	4	0-5 MO.	.055420	.073350	.060520
			6-11 MO.	.414020	.844340	.452120
			12-23 MO.	.888350	1.463740	.970100
			2-65+ YR.	2.236360	3.321940	2.442160
CAROTENE NAS 0063	03 OTHER GRAIN(R)	5	0-5 MO.	.053600	.182240	.064350
			6-11 MO.	1.039840	3.065920	1.248390
			12-23 MO.	1.758080	4.062880	2.110680
			2-65+ YR.	2.980160	6.582080	3.577860
CAROTENE NAS 0063	04 FATS OILS(R)	10	0-5 MO.	.005050	.005050	.013350
			6-11 MO.	.028230	.075750	.074760
			12-23 MO.	.063630	.121200	.168210
			2-65+ YR.	.176750	.319160	.467250
CAROTENE NAS 0063	07 FROZN DAIRY(R)	*	0-5 MO.	.022200	.091020	.050000
			6-11 MO.	.210900	.586030	.475000
			12-23 MO.	.319680	.750360	.720000
			2-65+ YR.	.568320	1.369740	1.280000
CAROTENE NAS 0063	08 PROCSD FRUT(R)	8	0-5 MO.	.884540	2.371320	.885950
			6-11 MO.	9.748760	24.277800	9.764300
			12-23 MO.	18.932920	37.583540	18.963100
			2-65+ YR.	22.264060	47.162920	22.299550
CAROTENE NAS 0063	10 MEAT PROCS(R)	*	0-5 MO.	.055000	.145000	.082500
			6-11 MO.	1.035000	2.790000	1.552500
			12-23 MO.	1.510000	2.595000	2.265000
			2-65+ YR.	3.920000	6.505000	5.880000
CAROTENE NAS 0063	14 PROCSD VEGS(R)	*	0-5 MO.	.039480	.118440	.329000
			6-11 MO.	.676800	1.579200	5.640000
			12-23 MO.	1.099800	1.841460	9.165000
			2-65+ YR.	2.397000	4.038240	19.975000
CAROTENE NAS 0063	19 SWEET SAUCE(R)	*	0-5 MO.	.037320	.049760	.078390
			6-11 MO.	.111960	.385640	.235170
			12-23 MO.	.323440	.945440	.679380
			2-65+ YR.	.845920	2.226760	1.776840

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
CAROTENE NAS 0063	20 GELATIN PUD(R)	*	0-5 MO.	.036000	.048600	.036000
			6-11 MO.	.230400	.698400	.230400
			12-23 MO.	.248400	.604800	.248400
			2-65+ YR.	.367200	.945000	.367200
CAROTENE NAS 0063	21 SOUPS(R)	*	0-5 MO.	.004800	.036000	.006000
			6-11 MO.	.559200	1.744800	.699000
			12-23 MO.	.835200	2.306400	1.044000
			2-65+ YR.	.760800	2.028000	.951000
CAROTENE NAS 0063	22 SNACK FOODS(R)	*	0-5 MO.	**********	.030000	**********
			6-11 MO.	.120000	.330000	.120000
			12-23 MO.	.330000	.930000	.330000
			2-65+ YR.	.390000	1.110000	.390000
CAROTENE NAS 0063	27 GRAVIES(R)	*	0-5 MO.	.003920	.011760	.078430
			6-11 MO.	.054880	.152880	1.098020
			12-23 MO.	.141120	.399840	2.823480
			2-65+ YR.	.325360	.834960	6.509690
CAROTENE NAS 0063	28. IMIT DAIRY(R)	5	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.001680	.002760	.001960
			12-23 MO.	.000960	.004080	.001120
			2-65+ YR.	.001080	.001800	.001260
CAROTENE NAS 0063	83 FORMULAS(B)	*	0-5 MO.	.067140	.123000	.067140
			6-11 MO.	.013680	.065180	.013680
			12-23 MO.	.004400	.001240	.004400
CAROTENE NAS 0063	ALL CATEGORIES ********** ********** **********	39	0-5 MO.	1.264470	3.285540	1.751630
			6-11 MO.	14.245400	36.598750	21.605300
			12-23 MO.	26.455980	53.609980	39.492870
			2-65+ YR.	37.233010	76.445600	65.917810

CAROTENES

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Hypercarotenemia

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With all the progress and advancement in medical education of the lay public, we were amazed to find the occurrence of hypercarotenemia in this modern age. Since the condition is rare and was seen in 2 patients in less than 24 hours, we thought it was worth reporting in the medical literature.

Carotenodermia, xanthosis cutis, pseudoicterus, or hyperlipochromemia, are all different terms used to describe a yellowish, or even orange-colored pigmentation of the skin, developing after excessive consumption of carotene-rich foods, such as carrots or carrot juice.[1] Intake of large quantities of these products has been practiced by some diabetic patients and has erroneously been thought by many people to be the cure for many eye diseases, such as night blindness or cataracts. The yellowish pigment starts from the nasolabial folds and palms of the hands and gradually extends over the entire body, including the soles of the feet, although it does not extend to the sclera. The sclera is NOT icteric, thus differentiating this condition from hepatitis, other liver diseases,[2] gallbladder disease, heart failure, or abnormal hemoglobin metabolism. The yellowish discoloration of the skin is the result of hypercarotenemia or increased β-carotene content in the blood, with a deposit of carotene in the dermis, but without any other manifestations or pathological symptoms occurring.

While vitamin A excess may be harmful, producing toxic effects, it is agreed that hypercarotenemia is harmless, and the pigmentation of the skin disappears when ingestion of excess carotenoids is discontinued. This is due to the fact that carotene is less readily absorbed than vitamin A and that the conversion ratio of carotene to vitamin A, is not rapid enough to allow toxic quantities of vitamin A to accumulate and thus produce the hypervitaminosis A syndrome. Thus there are no toxic effects induced by the vitamin A originating from carotene; however, Henschen[3] in 1941 reported that emaciation, fatigue, and hyperchromic anemia appeared in a 49-year-old woman after consumption of large quantities of carrots.

The cases to be reported below are not those of the hypervitaminosis A syndrome, but are the result of ingestion of large quantities of carrot juice by misinformed patients who were under the illusion that this product would improve their eyes.

Report of Cases

CASE 1.—A 50-year-old white male, a laborer, was told that carrot juice would improve his eyesight, which had been impaired by nuclear cataracts. For 1½ years he had his wife grind up carrots daily to make 2 quarts of carrot juice, which he regularly consumed. He was seen in our office for the first time on Aug. 10, 1961, at which time his appearance resembled a good "suntan." His skin was 3+ icteric, the palms of his hands and the soles of his feet were quite yellow, but his sclera was not icteric. Dense bilateral, cortical, and nuclear cataracts were present, reducing his vision to: right eye 20/200 and left eye 20/400. On Sept. 13, 1961, a successful intracapsular cataract extraction with peripheral iridectomy was performed for the removal of the dense cataract in his left eye. No untoward effect such as bleeding was noted. To date the postoperative course has been uneventful, and he is making a successful recovery (Figs. 1-3). The skin pigmentation gradually disappeared in the ensuing 3 weeks.

CASE 2.—To our surprise and amazement, a 74-year-old white female housewife was seen the same day the first case was seen. She presented herself at our office because of poor vision. The yellowish appearance of her skin immediately prompted the question, "How much carrot juice have you been drinking?" She said, "One and a

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Fig. 1.—Case 1: full body.

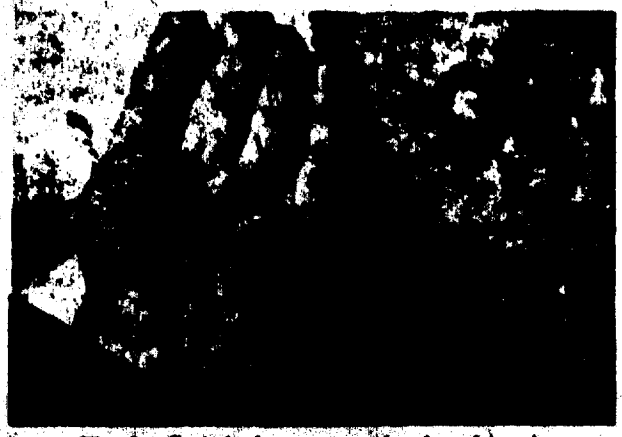

Fig. 2.—Case 1: face, eye, and palm of hand.

Fig. 3.—Case 1: sole of foot.

half pints a day, for 2 years." Her scleras were white, but the palms of her hands as well as the soles of her feet and the rest of her skin were quite yellow. She stated that she had been told by "somebody" that carrot juice would improve her sight. Examination revealed 4+ icterus of the skin and dense cataracta complicata in her right eye. Hypertensive retinopathy with scattered exudates was present in the left fundus, but the right fundus could not be visualized.

Although not the most expensive form of therapy, drinking carrot juice is probably the most useless, and it should be condemned. Both patients were informed that after they discontinued the use of this product, the skin would lose some of the yellowish pigmentation. To date the discoloration has completely disappeared.

We acknowledge gratitude to Leon Goldman, M.D., Professor of Dermatology, for encouraging us to publish this paper, because of the rarity of this condition.

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Addendum

Since submitting this paper, another case presented itself which could be added to the 2 already described in our paper. This was a patient seen on Dec. 11, 1961.

While examining a child with strabismus, we observed her 9-month-old sister in the mother's arms. The infant's face and arms were markedly yellowish-orange in color. Upon questioning the mother, we learned that the child had been given 1 pint of carrot juice daily for the past 8 months because she was able to tolerate this better than other vegetable juices, and the mother thought this would prevent the development of crossed eyes like those her older daughter had.

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CAROTINAEMIA

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We have recently observed 5 cases showing pigmentation of the skin pronounced enough to warrant provisional diagnoses, by the attending practitioners, of "jaundice," "pernicious anaemia," "haemolytic anaemia," and "cholelithiasis." This pigmentation can only be properly described as "carroty," and while in the mild cases it is most obvious in the naso-labial folds and the palms of the hands, in the more advanced cases it is a generalized body tanning. The condition is due to the excessive consumption of carrots, though it has been recorded when other lipochrome foods are eaten in quantity.

The Literature of Carotinaemia

The term "carotinaemia" for the orange pigmentation of the skin observed after the excessive consumption of carrots and other vegetables was first applied by Hess and Myers in 1919. The observation of this skin discoloration in diabetics whose diet consisted mainly of foods rich in yellow lipochrome—fruit, vegetables, butter, and eggs—was made by von Noorden (1907), who considered the condition to be a diabetic manifestation. Moro (1908) noted that normal infants developed a similar pigmentation when carrots were added to the diet. Van den Bergh and Snapper (1913) showed that there were lipochrome pigments circulating in the blood of diabetic patients with the orange pigmentation, and that these pigments were identical spectroscopically with those of carrots and other vegetables. Salomon (1919) referred to this pseudo-icterus in non-diabetic patients whose diet largely consisted of the foods mentioned, and the gradual disappearance of this pigmentation with abstinence from such foods. Stoeltzner (1919), commenting on Kaupe's observation (1919) that nurslings lost their yellow coloration when their high carrot diet was stopped, observed that the number of similar children had increased during the last war. He called it a "pseudo-icterus" and thought a high tomato diet might produce a similar pigmentation.

Greene and Blackford (1926) and Stoner (1928) discussed the condition under the heading of "carotinaemia." Boeck and Yater (1929) used the term "xanthaemia" for the circulation in the blood of lipochrome pigments (thereby excluding bile pigments) and "xanthosis" for the visible presence of these yellow pigments in the skin. They found xanthaemia in all their nephritics and in most diabetics, and even in most "normal" cases examined. However, xanthosis was present in only 10% of the renal and diabetic cases and in only 3% of other cases. All these patients with stained skin had eaten excessively of lipochromic foods.

Stannus (1929) called the discoloration "hyperlipochromia," and concluded that it resulted from the failure to oxidize the carotinoid pigments in the body. He first reported carotinaemia in myxoedema, and Wendt (1935) noted the condition in some cretins. Bomford (1938) confirmed the presence of carotinaemia in some cases of myxoedema on a normal diet. On thyroid medication the lipochrome index fell to normal. He remarks that as the colour of cream is due to lipochromes, the description of the myxoedematous facies as a "strawberry and cream" complexion is not without justification.

The Present Series of Cases

Our interest in carotinaemia was first aroused by the visit of a clerk's wife on account of "jaundice," though her sclera was contrastingly white. In the spring months further cases were referred as "pernicious anaemia" and "haemolytic anaemia"; the woman with "gall-stones" was visiting her husband, an in-patient with acute nephritis, when the ward sister drew our attention to her carroty complexion. None of these patients had symptoms of ill-health, though the curious discoloration was a source of anxiety.

Careful histories were extracted to find the actual pounds of carrots consumed per week (see Table). As kitchen standards of quantity are given vaguely in pinches, spoons, and cups, we sought and obtained the number and size of the carrots, from which a correct estimate was made. These weights apply only to the raw carrots, as the other vegetables and cooked carrots were always eaten in pre-war helpings. During the

Table showing Amount of Carrots consumed Weekly

Occupation of Husband	Provisional Diagnosis (owa Doctor)	Raw Carrots: lb. per Week	Raw Carrots: Threshold Months	Other Veg. and Eggs	Icteric Index	Serum Bilirubin (mg./100 c.cm.)	On Normal Diet Fading: First Noticed (Weeks)	On Normal Diet Fading: Complete (Weeks)	Other Medical Conditions
Lorry-driver	Gall-stones	8	8	—	10	0·1	3	6	Cholecystectomy, 1932
Clerk ..	Jaundice	6	6	+++	8	0·3	2	4	Microcytic anaemia
Shop-keeper	Pernicious anaemia	5	7	+	12	0·6	2	4	Mitral stenosis
Corporal	Haemolytic anaemia	4	8	+	8	0·1	2	5	Microcytic anaemia
Baby ..		? breast-fed	2	—	—	—	4	8	Healthy

winter carrots were cheap and easily obtainable, and "besides, they were so nice to munch." The lorry-driver's wife had been told "carrots were full of vitamins." The clerk grew so many carrots on his allotment that his wife felt she had to eat them. She liked the clean taste left in her mouth so much that she invariably carried one about in her pocket. Since June of last year the corporal's wife used to munch carrots, especially while preparing the family meals, in order to increase her vitamin intake during her period of gestation. A healthy baby was born at Christmas and thrived on the breast. It had no other treatment. In February the corporal noticed his wife and baby were carroty and sought his doctor's advice. Jaundice was diagnosed and the breast dethroned by the bottle. On a light diet and milk feeds the pigmentation gradually faded.

The time taken for saturation before the carroty skin was first noticed is tabulated as the threshold months. Each patient was thoroughly investigated for diseases which may have provided other factors; but they were quite healthy women, apart from one moderate case of mitral stenosis and the almost invariable hypochromic anaemia so prevalent in this area. These patriots of the "Kitchen Front" were congratulated on their heroic consumption both of carrots and of propaganda, and were asked to return to their basic helpings of vegetables, including carrots. On this moderation (it was not abstinence) the times in weeks of initial and then complete fading were noted.

Our threshold value corresponds approximately to that of a patient, quoted by Wood and Agnor (1941), who ate two carrots daily for a year. Kaupe's (1919) finding of carotinaemia in babies weaned on carrot soup, the lipochrome content of which must necessarily have been small, is in harmony with our baby's susceptibility in developing skin pigmentation after only two months on the breast milk of its carotinaemic mother. This is believed to be the first case reported of carotinaemia from the breast milk of an affected mother.

Laboratory Investigations

The routine estimations of icteric index and serum bilirubin were carried out in all cases. These revealed an obviously "jaundiced" serum of a high icteric index (on colour comparison with the potassium dichromate standard 1/10.000 dilution) but a serum bilirubin within normal limits. Obviously the orange colour was not due to bile pigment. However, the lipochromes are soluble in petroleum ether and the bile pigment in absolute alcohol, which will also precipitate the proteins in the serum.

This fact forms the basis of the test of Greene and Blackford, which we refer to as the three-layer test, to differentiate carotinaemia from jaundice. Equal quantities of serum, alcohol, and petroleum ether are shaken up in a tube. This may be left to settle, as the volatile petroleum ether, with its dissolved lipochromes, rises to the top as a distinct layer. If lipochromes are present in abnormal amount this top layer will show a definite yellow tinge which is pathognomonic of carotinaemia. The depth of colour may be compared with the potassium

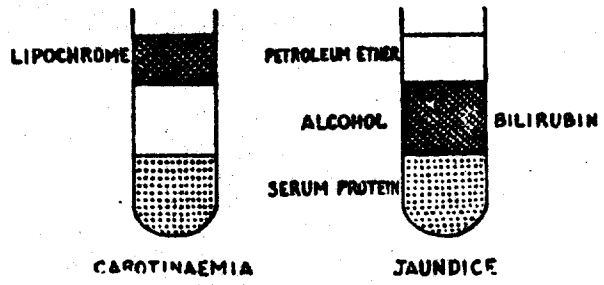

Diagram illustrating the three-layer test for the differentiation of carotinaemia from jaundice.

dichromate standard to give its lipochrome index. However, it is better to centrifuge the mixture in the tube. The precipitated proteins spin down to the bottom as a white curd, covered by the middle band of absolute alcohol with its dissolved bile pigments, on which the "true icteric index," or bile index, can be estimated against the dichromate standard. If this middle alcohol band is excessively yellow and is covered by a clear petroleum layer, then the sandwich is jaundiced and the bile index will equal the icteric index.

Icteric index (on whole serum)	=	*Bile index* (on alcohol layer)	+	*Lipochrome index* (on ether fraction)

In a hypothetical case of jaundice and carotinaemia both layers will be quite yellow, and the degree of each pigmentation can then be estimated separately.

Carotene is the yellow colouring matter—the lipochrome—of carrots, spinach, palm oil, and nettles. It is a highly unsaturated hydrocarbon, $C_{40}H_{56}$, which exists in three forms: alpha-, beta-, and gamma-carotenes. These can be separated by filtering a light petroleum solution through a Tswett column of calcium hydroxide. Each form adsorbs out of solution in a different layer in the column. All three forms are precursors of vitamin A, but owing to its internal pattern one molecule of beta-carotene yields two molecules of the vitamin while the alpha and gamma forms produce only one molecule. Thus the beta form has twice the physiological activity of alpha- and gamma-carotenes. Natural products, however, contain much more beta- than alpha-carotene and little, if any, of the gamma form. The isomer of carotene is lycopene, which is the colouring matter of tomatoes, rose hips, and bitterswee berries, but is inactive physiologically in not forming vitamin A. Carotene is the principal though not the only lipochrome in human serum. It is always associated with its hydroxy derivative, a xanthophyll. Lutein is the xanthophyll of alpha-carotene, and is the lipochrome in egg yolk and green leaves. Thus the term "xanthaemia," of Boeck and Yater (1929) is more accurate than the popular carotinaemia.

The absorption of the lipochromes from the bowel is stated by Wood and Agnor (1941) to depend on the presence of dietary fat and the bile acids with which it is absorbed as a water-soluble diffusible complex. Curtis and Ballmer (1939) have shown that liquid paraffin interfered with this transport of carotene across the epithelium of the intestine. In the plasma the carotene is probably adsorbed to the albumin molecule and carried in colloidal suspension. The plasma proteins can be precipitated down by absolute alcohol, which itself dissolves bile pigments, while the lipochromes are soluble in petroleum ether, which floats lightly as a separate layer above the alcohol. This provides the basis for the three-layer test of Greene and Blackford. The conversion of carotene into vitamin A probably takes place in the liver.

Ralli *et al.* (1936) showed that the liver of a carotinaemic diabetic contained an increased amount of carotene. Pariente *et al.* (1936) theorized that in diabetes the conversion of carotene to vitamin A was impeded and that this resulted in a damming up of carotene in the liver and a diminished absorption from the blood. They neglected the additional factor of the high lipochromic diet usual in diabetics.

According to Wood and Agnor (1941), any excess of carotene is either destroyed or excreted via the sweat. They state that when excretion is unusually heavy the carotene is reabsorbed by the horny layer of the skin. Palmer and Kempster (1919) found the lipochromes in fowls in the rete malpighii and cornified layers of the skin. Greene and Blackford (1926) observed the concentration of pigment in the naso-labial folds, where sebaceous glands abound, and in the palms and soles where cornified skin is thickest. We find that the soles of Lancashire housewives are undistinguished, but that the palms of our hospital cooks, after scraping carrots for dinner, were stained like T.N.T. workers. However, this direct staining from handling carrots could be washed off almost completely—in contrast with that from excessive intake, which was undiminished by washing. In our cases, in addition to the naso-labial folds and palms, the rest of the skin was almost uniformly tanned, but the sclera was contrastingly white. This immunity of the sclera in carotinaemia is the chief clinical sign in differentiating it from jaundice. The colour of the skin and the details of food intake are additional factors in clinical diagnosis.

Several of our patients noticed a deep orange colour in the urine. Attempted extraction of the colour with petroleum ether was unsuccessful, and no typical absorption bands were found spectroscopically.

Boeck and Yater (1929) discuss the wide variation in the appearance of xanthosis and xanthaemia in individuals of

similar diets. To account for this, they believe that the rates of oxidation and excretion must vary widely. Bernheim (1926) fed large quantities of carrots to 3 patients, who quickly produced a high icteric index, which, however, fell to normal within 12 hours. In our cases the rate of metabolism seemed to be remarkably constant in the different individuals. The threshold quantity appeared to be a minimum of 4 lb. of excess carrots per week for a minimum period of seven months before skin pigmentation became visible. The rate of fading appeared to be equally constant once they returned to an average diet. Fading was first noticed in a minimum of two weeks and was complete within eight weeks. In view of Bernheim's finding of the rapid disappearance from the blood of excess lipochromes within 12 hours, it would seem that above the threshold quantity the body is unable to oxidize or excrete all the lipochrome, so that some pigment is slowly accumulating. The capacity of the body to eliminate lipochromes must be well above the normal requirements, as it takes a minimum of six months' accumulation before the high threshold is reached on such abnormal intake and before skin pigmentation is noticeable.

The findings of the remarkably constant threshold in the accumulation of pigment and of the steady rate of fading in normal individuals support the theories of diminished conversion of carotene to vitamin A in the liver of diabetics and poor urinary excretion in nephritics, as in both diseases carotinaemia occurs more frequently than in health.

Summary

Carotinaemia is a carroty pigmentation of the naso-labial folds and palms when mild and, when marked, a general body tanning except in the sclera, which distinguishes it clinically from jaundice. It is due to the excessive consumption of carrots and other lipochromic foods. The patients are healthy and do not require treatment apart from reduction to average consumption.

Four cases of carotinaemia are described in normal women after eating more than the threshold quantity of 4 lb. of raw carrots per week for a minimum period of seven months.

A baby fed only on the breast of its carotinaemic mother developed the condition within two months. This is believed to be the first case described.

The simple three-layer test of Greene and Blackford to differentiate between carotinaemia and jaundice is described.

We are indebted to Drs. S. Smithson and M. C. Welsh for allowing us to see and investigate their cases. We wish to thank especially Dr. M. L. Thomson for permission to investigate the case of the infant of the carotinaemic mother.

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Chronic Toxicity Studies of β-Carotene

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β-Carotene is a naturally occurring pigment widely found in edible plants and in animal tissues and is, therefore, a normal constituent of the human diet (Karrer and Jucker, 1950; Goodwin, 1952). Man commonly ingests β-carotene over his entire life span, apparently without the occurrence of toxicity. The development of a total chemical synthesis of β-carotene by Isler *et al.* (1956 a–c) has led to its extensive use by the food-processing industry as a source of vitamin A and as a yellow food color. The availability of adequate supplies of chemically pure β-carotene permitted long-term experiments to be undertaken in animals for the first time. Accordingly, extensive toxicologic investigations were conducted in these laboratories and the results obtained have been reported (Zbinden and Studer, 1958). These chronic toxicity studies have been continued, and the additional information obtained since the appearance of the earlier publication is included in this communication.

METHODS

The rats employed in these studies were bred in these laboratories and are derived from the Wistar strain. At the initiation of the feeding experiments, the animals were 30–40 days old and weighed 40–45 g. The rats were maintained on a prepared diet consisting of casein 20%, cocofat (Pura) 10%, sucrose 61%, salt mixture 4%, cellulose meal 5%, and a vitamin supplement; the complete composition of this diet is given in the previous publication (Zbinden and Studer, 1958). At various intervals, a ration consisting of ground Purina Lab Chow Pellets was fed in place of the synthetic diet. The β-carotene used in these investigations was 96% all-*trans* isomer having a melting point of 182° C. The absorption spectrum of this material in hexane, expressed as $E_{1cm}^{1\%}$, was found to be: 338 mμ = 200, 370 mμ = 250, 453 mμ = 2450, 481 mμ = 2150. Chro-

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matography on alumina yielded a single zone of all-*trans*-β-carotene. The minimal vitamin A activity of the substance was 1,600,000 IU per gram. The diet containing β-carotene was prepared at intervals of 3 days and stored under refrigeration until used.

Registered young beagles, 9–12 months of age and immunized against distemper and hepatitis, were used for toxicity measurements in dogs. The animals were maintained on a diet consisting of Friskies dog ration supplemented with beef liver. β-Carotene was administered by the oral route in gelatin capsules.

Hematologic determinations were obtained by standard laboratory procedures. Following sacrifice, the tissues were fixed in Bouin's solution or formalin, embedded in paraffin, and stained with hematoxylin-eosin. Also, frozen sections were prepared from tissues fixed in formalin and stained with fat Red 7 B; determination of vitamin A was made by means of ultraviolet-fluorescence microscopy.

RESULTS

Studies in Rats: First Generation

A group of 30 rats, 15 males and 15 females, was given β-carotene as a 0.1% dietary admixture for 110 weeks; another group of 30 rats was maintained on the basal ration and served as controls. Results obtained after 57 weeks of administration were described previously (Zbinden and Studer, 1958). During the two-year feeding experiment, 19 rats in the control group expired or were sacrificed because of the occurrence of pneumonia, otitis, or other spontaneous diseases. Four rats were sacrificed after one year and tissues taken for chemical analysis (Zbinden and Studer, 1958).

At the 110th experimental week the remaining 7 control rats were sacrificed and complete histopathologic examination of the tissues was performed. In the group administered β-carotene, 13 rats were sacrificed or succumbed owing to respiratory or middle ear infections, and 4 rats were also sacrificed after one year for determination of vitamin A and β-carotene in the tissues. The 13 surviving animals were sacrificed at the completion of the 110th week and tissues taken for microscopic examination.

Although some individual variation was observed, the rate of growth of rats receiving this carotenoid did not differ significantly from that of controls. Weight generally increased during the first year of the experiment and then this body weight was maintained at constant level until

the 90th experimental week was reached. At this time, a commercial ration was substituted for the synthetic basal ration, and this produced weight loss in some rats in both the treated and control groups.

Hematologic determinations, consisting of measurements of hemoglobin, erythrocytes, and total and differential leucocytes, were obtained during the 70th experimental week. These results, shown in Table 1, indicate that chronic administration of β-carotene does not exert deleterious effects on circulating blood cells or hemopoietic tissues. Similarly, β-carotene did not influence food consumption; this was determined during the 96th experimental week and the data obtained are shown in Table 2. Normal blood counts and unimpaired food consumption before the 70th week have been reported earlier (Zbinden and Studer, 1958).

Following sacrifice of 4 animals from each group after one year and of all surviving rats after two years' administration of β-carotene, the tissues were examined for pathologic changes. The livers of both β-carotene-treated and control rats occasionally showed a slight peripheral fatty infiltration, but other cytoplasmic changes were not observed. The Kupffer cells of the β-carotene group were filled with material which gave a Sudan-positive staining reaction and an intense green-yellow fluorescence when exposed to UV-light. The fluorescence faded almost completely after exposure to UV-light for 20 minutes. Thus, it is highly probable that the Kupffer cells stored vitamin A.

The sections of kidneys of both control and treated animals were found to have partly calcified concretions in the tubules and especially in the lumens of Henle's loops. A few cells lining the convoluted tubules contained scattered fat droplets, and some animals showed slight diffuse or focal lymphocytic infiltration of the interstitial tissue. Both treated and untreated animals contained deposits of hemosiderin in the spleen; some sections also exhibited some decrease in lymphoid tissue. There were no histopathologic changes found in the lungs, skin, ovary, testes, pancreas, adrenals, heart, gastric and intestinal mucosa, thyroid, bone, and bone marrow. In this experiment, as in all the other studies in rats, the only change which can be attributed to chronic administration of β-carotene was the storage of Sudan-positive material in the Kupffer cells. No signs of hypervitaminosis A were present in these rats.

Second Generation

During the 16th experimental week, first-generation rats were bred and the resulting second generation was also maintained on a 0.1% β-carotene

TABLE 1

AVERAGE BLOOD COUNTS OF RATS (FIRST GENERATION) TREATED WITH 0.1% β-CAROTENE IN THE DIET FOR 70 WEEKS[a]

Treatment	Sex	HGB (g/100 ml)	RBC (millions)	HBE	WBC (thousands)	Differential					
						SN	NSN	L	M	E	B
0.1% β-Carotene	F	15.2 ± 1	7.8 ± 0.3	19.5 ± 1	11.1 ± 2	27.9 ± 11	0.1	66.4 ± 12	1.2	4.4	0
	M	15.1 ± 1	8.3 ± 0.6	18.2 ± 1	10.1 ± 2	24.8 ± 7	0.1	69.8 ± 7	2.5	2.8	0
Controls	F	14.8 ± 0.6	7.9 ± 0.4	18.8 ± 1	7.4 ± 1	24.4 ± 7	1.4	70.4 ± 9	1.6	2.2	0
	M	15.4 ± 0.5	8.0 ± 0.6	19.3 ± 3	12.0 ± 4	27.7 ± 8	0.5	65.6 ± 10	1.8	4.4	0

[a] Abbreviations: HGB, hemoglobin; RBC, red blood cells; HBE, hemoglobin per erythrocyte; WBC, white blood cells; SN, segmented neutrophiles; NSN, nonsegmented neutrophiles; L, lymphocytes; M, monocytes; E, eosinophiles; B, basophiles.

diet. Second-generation rats receiving the carotenoid as a dietary admixture consisted of 16 males and 5 females; the controls included 6 males and 13 females. The duration of this feeding experiment was 38 weeks. These results, as reported previously (Zbinden and Studer, 1958), indicated that β-carotene was tolerated without the occurrence of untoward reactions. General health remained excellent and growth was regular. The incidence of spontaneous deaths was very low; 2 treated rats and 4 control animals expired during this period. Food consumption did not differ significantly from controls and hematologic determination remained within normal limits. Following sacrifice after 38 weeks administration, the tissues were examined for pathologic changes. As in the

TABLE 2
DAILY FOOD CONSUMPTION (GRAMS) OF FIRST-GENERATION RATS GIVEN 0.1% β-CAROTENE DURING THE 96TH WEEK

Groups and sex		Food consumption
β-Carotene	F	10.2
β-Carotene	M	11.0
Controls	F	11.1
Controls	M	11.6

rats of the first generation, storage of Sudan-positive fluorescent material in the Kupffer cells of the liver was the only change observed in β-carotene-treated rats, but not in the control rats.

Third Generation

This generation of rats was obtained by breeding treated and untreated rats constituting the second generation during the 16th experimental week. As with the previous groups, the third generation was maintained on a diet containing 0.1% β-carotene. The results obtained for the first 16 weeks are contained in the earlier publication (Zbinden and Studer, 1958); the third generation has now received β-carotene for 96 weeks and this experiment is continuing.

Ten females and 12 males were initially given the carotenoid, and the control group for this series of experiments consisted of 10 females and 11 males; 5 rats of the treated group have expired whereas 7 control rats have expired as a result of otitis, pneumonia, or other spontaneous diseases. Thus, when compared with the corresponding control animals, the third generation of rats given 0.1% β-carotene exhibited a low mortality. The gain in weight of both groups was regular, and β-carotene did not produce an impairment of growth.

As observed in the first two generations, substitution of a commercial ration for the synthetic diet during the 43rd to 53rd experimental weeks resulted in weight loss in some rats in both the β-carotene and control groups. However, food consumption of the two groups measured during the 24th, 59th, and 95th experimental weeks did not differ significantly; these data are shown in Table 3.

TABLE 3

DAILY FOOD CONSUMPTION (GRAMS) OF THIRD-GENERATION RATS GIVEN 0.1% β-CAROTENE

Group and sex		Food consumption (g)		
	Experimental week:	24	59	95
β-Carotene	F	10.1	9.9	11.4
β-Carotene	M	10.7	10.7	9.4
Controls	F	9.7	11.3	11.3
Controls	M	11.0	12.3	9.6

In agreement with hematologic findings previously obtained during the 9th experimental week (Zbinden and Studer, 1958), similar determinations taken during the 28th and 74th week indicate that β-carotene did not depress the hemopoietic tissues of the third-generation rats.

Breeding experiments conducted with the first and second generations indicated that β-carotene does not adversely influence the process of reproduction (Zbinden and Studer, 1958). These reproduction studies were extended and the third-generation β-carotene and control rats were bred during the 40th experimental week. These results are tabulated in Table 4.

TABLE 4

REPRODUCTION STUDIES OF THIRD-GENERATION RATS

Group	Number of females bred	Litters obtained	Days to parturition	Number per litter	Weight at birth (g)
β-Carotene	10	6	23	9	5.3
Control	7	1	23	5	6

The cause for the decrease in the number of litters obtained in the control group is not apparent; possibly this is related to the relatively advanced period at which the animals were bred, viz., 40th experimental week.

Fourth Generation

Fourteen females and 13 male rats of the fourth generation have been maintained on 0.1% β-carotene in the diet for 48 weeks; a group of 3 females and 2 male rats are receiving the basal ration to serve as controls. During this interval, 3 rats receiving 0.1% β-carotene have expired due to pneumonia and otitis and no deaths have occurred among the 5 control animals. The fourth-generation rats have remained in good condition and signs of toxicity have not been observed in this group. Weight gains have been regular and food consumption, shown in Table 5, and hematologic

TABLE 5
FOOD CONSUMPTION OF FOURTH-GENERATION RATS GIVEN 0.1% β-CAROTENE

Group and sex	Food consumption (g)		
Experimental week:	12	24	47
β-Carotene F	14.8	10.2	9.7
β-Carotene M	11.4	12.6	11.8
Control F	14.4	10.6	10.2
Control M	14.0	14.7	11.0

findings obtained in the 32nd week (Table 6) are normal. The fourth-generation rats are being maintained on 0.1% β-carotene.

The results of these long-term toxicity studies in rats extending through four generations reveal that large doses of β-carotene are well tolerated by this species.

Studies in Dogs

Carotenoid metabolism in the dog closely resembles that of man in that a significant amount of β-carotene can be absorbed and transported by the portal circulation to the liver where it can be transformed to vitamin A (Zbinden and Studer, 1958; Urbach *et al.*, 1952). In contrast, rodents appear to metabolize β-carotene to a great extent in the intestinal wall, although the liver and other organs also have the capacity to degrade the compound to vitamin A and other products (Ganguly *et al.*, 1953).

The acute oral toxicity of β-carotene was determined in 2 dogs by administering an initial dose of 250 mg/kg. The dose was increased twofold on successive days until a single dose of 8000 mg/kg was administered on the sixth day. Toxic manifestations were not detected at any time during the entire observation period. These results clearly indicate the essentially nontoxic nature of β-carotene when given in single massive doses.

TABLE 6

AVERAGE BLOOD COUNTS OF RATS (FOURTH GENERATION) TREATED WITH 0.1% β-CAROTENE IN THE DIET FOR 32 WEEKS[a]

Treatment and Sex		HGB (g/100 ml)	Hematocrit (vol %)	WBC (thousands)	Differential					
					NSN	SN	L	M	E	B
0.1% β-Carotene	F	16.3 ± 0.6	44.2 ± 2.5	12.3 ± 1.3	0.1	23.1 ± 11	72.9 ± 12	0.6	3.3	0
	M	17.5 ± 1	44.2 ± 3	12.5 ± 2.7	0	26.7 ± 10	70.7 ± 10	0.4	2.2	0
Controls	F[b]	17.1	44.0	5.9	2.3	19.7	75.0	0.3	2.7	0
Controls	M[c]	16.9	43.5	8.1	0	23	66.5	0	2.0	0

[a] Abbreviations as in Table 1.

[b] Only 3 animals.

[c] Only 2 animals.

To assess the toxic potentialities of β-carotene when the material was given repeatedly, two dogs received 2000 mg/kg daily for 21 days. The dogs tolerated this high dosage without effect beyond the occurrence of slight diarrhea. No loss of weight or other untoward reactions were recorded throughout the 3-week experiment; these results give further weight to the nontoxic nature of β-carotene.

A further effort was made to induce toxic effects in dogs by administering high dosages of β-carotene 5 days a week for 13 weeks. The dosage levels employed were 100 mg/kg, 10 mg/kg, and 1 mg/kg. Three dogs per dosage level together with one group of 3 dogs serving as controls were used in this study. Although changes in weight were variable among individual animals, β-carotene is judged to have no influence on growth. There was a complete absence of toxic signs, and the hematologic findings, shown in Table 7, indicate that β-carotene has no effect on these tissues.

Following sacrifice, gross examination of the tissues of these animals did not reveal any relevant pathology that could be attributed to carotene. There were no significant differences in organ weights between controls and dogs given the food additive. Microscopically, liver sections of both treated and control dogs showed a moderate amount of granulation in the cytoplasm of the parenchymal cells; animals given β-carotene also showed deposition of material in the Kupffer cells. A few kidney sections showed slight hyperemia and many of the connecting tubules showed a small amount of amorphous deposits in the lumen; cells lining the tubules showed no abnormalities. The lungs in both treated and control showed slight thickening of the trabeculae, hyperemia, and occasionally an area of chronic inflammation characterized by infiltration with plasma cells and lymphocytes. This is judged to be unrelated to the administration of β-carotene. The heart, spleen, adrenals, gastrointestinal tract, gallbladder, pancreas, gonads, bone marrow, thyroid, pituitary, central nervous system, and bladder did not exhibit abnormalities. Thus, histologic findings obtained in dogs treated chronically with the β-carotene closely resembled those seen in rats; the only significant change induced by administration of massive doses of β-carotene for prolonged periods was the deposition of material in the Kupffer cells of the liver.

The results obtained following administration of single doses, 21-day subacute and 13-week chronic toxicity studies in dogs, strongly indicate that β-carotene is nontoxic even when given in high dosages to this species.

TABLE 7

AVERAGE BLOOD COUNTS OF DOGS GIVEN β-CAROTENE FOR 13 WEEKS

Weeks on experiment	Number of dogs	Dosage (mg/kg)	WBC (thousands)	Hematocrit (% Vol.)	HGB (g/100 ml)	Differential							Nucleated RBC
						NSN	SN	L	M	E	B	Pl.[a]	
0	3	1	12.4	50.8	16.5	1.6	51	43	1.6	2	0	0.3	0
4			11.2	48.3	15.5	1.6	53	42	5.3	1.3	0	0.3	0.3
8			14.0	49.5	16.2	0.7	57	35	3.6	2.6	0.3	1.0	0
12			13.0	49.0	16.1	1.0	57	34	3.6	3.3	0.3	0	0
0	3	10	11.1	50.3	16.6	2.3	51	37	3.0	7.0	0	0	0.6
4			11.9	49.3	16.3	0.6	54	35	3.3	6.0	0	0.6	0
8			14.5	50.0	15.9	0	57	33	3.3	4.6	0.3	1.0	0
12			13.4	52.3	17.0	1.0	54	35	4.0	4.6	0.6	0.3	0.3
0	3	100	8.9	54.8	18.0	1.6	49	39	4.6	6.3	0	0	0
4			9.7	53.2	17.0	1.6	58	32	4.6	2.6	0	0.6	0
8			10.9	51.8	17.0	1.0	54	43	5.0	3.0	0	0.6	0
12			10.6	52.5	17.2	0.6	63	30	3.3	2.3	0	0.6	0.3
0	3	Control	12.9	44.2	14.4	2.6	53	37	3.6	3.3	0.3	0	0
4			10.7	46.2	14.6	1.3	62	29	5.6	2.3	0	0	0
8			11.7	49.3	15.5	1.3	57	31	4.6	4.3	0	1.0	0
12			11.7	48.9	15.9	0.6	58	35	8.3	1.3	0	0	0

[a] Pl. = plasma cells; other abbreviations as in Table 1.

DISCUSSION

The results of intensive toxicologic investigations clearly indicate the absence of toxicity in rats and dogs chronically treated with β-carotene. Administration of this carotenoid to four generations of rats for periods including the entire life span of some groups did not produce toxic manifestations. In particular, there was a complete absence of the signs of hypervitaminosis A syndrome in these animals such as retardation of growth, bone fractures, thickening of epithelial tissue, hemorrhage, and congenital abnormalities, reported by others following overdosage of vitamin A to rats (Moore and Wang, 1945; Rodahl and Moore, 1943; Studer and Frey, 1949; Studer and Winkelmann, 1954; Wolbach and Maddock, 1951).

The findings obtained in these animal studies, revealing the innocuous nature of β-carotene, have been substantiated by the clinical observations of Greenberg *et al.* (1959). These investigators administered crystalline β-carotene to 15 human subjects at a daily oral dose equivalent to 100,000 IU of vitamin A for a period of 3 months. Serum carotene values rose from an initial level of 128 μg % to 308 μg % after 1 month but did not rise above this figure for the remainder of the observation period; serum vitamin A levels of these subjects were not elevated by β-carotene administration. Throughout this study, there were no clinical signs of vitamin A toxicity. These data indicate that excessive amounts of β-carotene are not absorbed from the gastrointestinal tract following oral ingestion. Thus, while this nutrient is found in common food materials and can serve as a precursor of Vitamin A, β-carotene does not have deleterious effects following consumption of high doses for extended periods. It is evident that β-carotene can be considered to have no hazard associated with its use as a food additive.

SUMMARY

1. β-carotene was administered at a dietary concentration of 0.1% to four successive generations of rats. The duration of these feeding experiments extended to 110 weeks in some groups. This dosage schedule represents continuous administration of daily dosages of β-carotene equivalent to 16,000 IU of vitamin A per rat over the life span of these animals.

2. In rats, β-carotene had no deleterious effects on growth, food consumption, hemopoietic tissues, or reproduction. Gross or histopathologic tissue changes attributable to feeding of this carotenoid were not detected in these animals. Microscopically, the Kupffer cells of the liver were filled with Sudan-staining material, indicating the storage of a moderate amount of vitamin A.

3. A three months' chronic toxicity study of this carotenoid in dogs at doses as

high as 100 mg/kg indicates the innocuousness of β-carotene. In terms of vitamin A activity, this dose represents daily administration of 160,000 IU of vitamin A per kilogram.

4. In the dog and rat toxicity experiments, signs of hypervitaminosis A were completely absent in all groups of animals.

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The Production of Cancer by Pure Hydrocarbons—Part III

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(*Received December* 22, 1934)

[Plates 20 and 21]

The carcinogenic properties of 1 : 2 : 5 : 6-dibenzanthracene, 5 : 6-*cyclo*-penteno-1 : 2-benzanthracene and some closely related compounds have already been described (Part I, Cook, Hieger, Kennaway and Mayneord, 1932; Part II, Cook, 1932, *a*; Burrows, Hieger and Kennaway, 1932; Burrows, 1932 and 1933; Barry and Cook, 1934). The present communication records the results of a systematic examination for carcinogenic activity of tetracyclic and pentacyclic aromatic hydrocarbons, and also deals with the extension of this work along lines suggested by the results obtained.

Technique

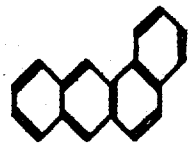
(1) Tests for the production of cancer of the skin were carried out by applying the substance in solution to the interscapular region of mice twice weekly. In the great majority of cases the compound was applied in benzene (0·3 gm in 100 cc), whether this gave a saturated solution or not. The advantages and disadvantages of this method have been discussed in an earlier paper (Part I). Any other concentrations or solvents used are stated in the tables below. The results are shown in Tables I–IX.

(2) Tests for the production of sarcoma were made by injecting the compound *sub cutem* in a fatty medium, generally lard; details are given in the various sections on next page.

Tetracyclic Compounds

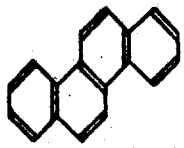
Tests have now been completed (Tables I and III) with the six possible hydrocarbons which have molecules consisting of four condensed aromatic rings; the formulæ of these are given below. Evidence of any considerable cancer-producing properties was obtained in only one case, namely, with 3 : 4-benzphenanthrene. This compound is of particular interest, in that it is the simplest hydrocarbon yet shown to have pronounced

carcinogenic activity (7 epitheliomas and 5 papillomas in 20 mice. fig. 1). Moreover, it was the only one of six compounds under discussion which was not already known, and it is the only actively carcinogenic compound yet encountered which is not derived from, or related to, 1 : 2-benzanthracene. This latter circumstance clearly indicates that it is undesirable to confine the search for cancer-producing activity to compounds which contain the ring system of 1 : 2-benzanthracene.

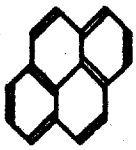


1 : 2-Benzanthracene

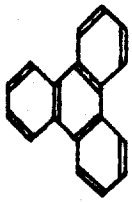
2 : 3-Benzanthracene



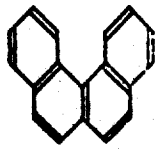
Chrysene



Pyrene



Triphenylene



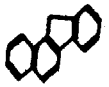
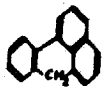
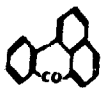
3 : 4-Benzphenanthrene

Entirely negative results were obtained with triphenylene and 2 : 3-benzanthracene (naphthacene), and also with 9 : 10-dihydronaphthacene. In order to make more complete the comparison of the two benzanthracene ring systems, 6-*iso*propyl-2 : 3-benzanthracene was also tested, with negative results. The analogous 6-*iso*propyl-1 : 2-benzanthracene has moderately potent cancer-producing properties (Cook, 1932, *a*; Table III).

Evidence of very slight carcinogenic power was obtained with chrysene, pyrene and 1 : 2-benzanthracene. 1 : 2-benzanthracene when applied to the skin gave only one epithelioma in 80 mice. This compound was injected also *sub cutem* (4 mg in 1 cc lard) into 10 mice, of which six were alive after 1 year, when the experiment was discontinued, the result having been negative. An extensive investigation of the action of chrysene has yielded 3 papillomas and 1 epithelioma in 290 mice (Table I). The chrysene used in these experiments, although satisfying the usual criteria of purity (*cf.* Barry and Cook, 1934), was presumably obtained from coal tar, and it is impossible to say that these four tumours were actually produced by chrysene and not by a trace of a persistent impurity, for in our experience it is a particularly difficult task to remove traces of certain other hydrocarbons from chrysene. Hence the question whether

TABLE I—TETRACYCLIC COMPOUNDS NOT RELATED TO 1 : 2-BENZANTHRACENE

Compound	Method of preparation	Mice: Initial number	Mice: Alive after 6 months	Mice: Alive after 1 year	Mice: Day of death of last mouse	Tumours: Epithelioma	Tumours: Papilloma
Naphthacene (2 : 3-benzanthracene) ..	Fieser (1931)	10	10	8	508	0	0
β-*iso*Propylnaphthacene (6-*iso*Propyl-2 : 3-benzanthracene)	Cook (1934)	10	10	10	819	0	0
9 : 10-Dihydronaphthacene	Fieser (1931)	10	10	7	466	0	0
Triphenylene	Diels and Karstens (1927)	10	10	4	548	0	0
Pyrene—							
(1) Crude, from coal-tar (1% in benzene)	—	5	3	3	634	0	1
(2) Sample (1) above after purification through picrate	—	20	15	8	600	0	1
(3) " Pure pyrene " supplied by Gesellschaft für Teerverwertung ..	—	20	18	5	717	0	1 and ? 1
†2-Methyl pyrene	Cook and Hewett (1934) ..	10	10	5	3 alive day 490*	0	0
unsym. Hexahydropyrene	Cook, Hewett and Hieger (1933)	10	8	1	461	0	0
sym. Hexahydropyrene	Cook, Hewett and Hieger (1933)	10	9	7	664	0	0
3 : 4-Benzphenanthrene	Cook (1931, *c*)	20	18	14	676	7	5
Chrysene—							
(1) Sample A (kindly given by Mr. Gordon Adam) 0·3% in benzene	—	50	30	24	775	0	2
(2) Sample A, 7·5% in comml. oleic acid	—	100	77	44	797	0	0

(3) Sample B, from Rütgerswerke A-G., 0·3% in benzene	—	100	290	74	30	704	1	1
(4) Sample B, 0·3% in mouse fat ..	—	20		12	5	695	0	0
(5) Sample C, synthetic	—	20		11	6	5 alive day 440*	0	0
1 : 2-Diphenylchrysene	Cook and Galley (1931) ..	5		4	1	525	0	1
Fluoranthene	—	10		3	3	501	0	0
"	—	10		4	1	379	0	0
Chrysofluorene 	Cook and Hewett (1934) ..	10		9	0	350	0	0
Benzanthrene 	Clar and Furnari (1932) ..	20		14	9	608	0	0
1 : 9-Benzanthrone 	—	10		7	0	294	0	0

* On November 1, 1934.

† This compound was originally regarded as 1-methylpyrene, but has now been shown to be 2-methylpyrene by comparison with a sample supplied by Dr. G. Kränzlein, and synthesized by him by a method which establishes its constitution (private communication). The system of numbering which we adopt is that suggested by the International Committee on Organic Nomenclature, and differs from that used in continental publications.

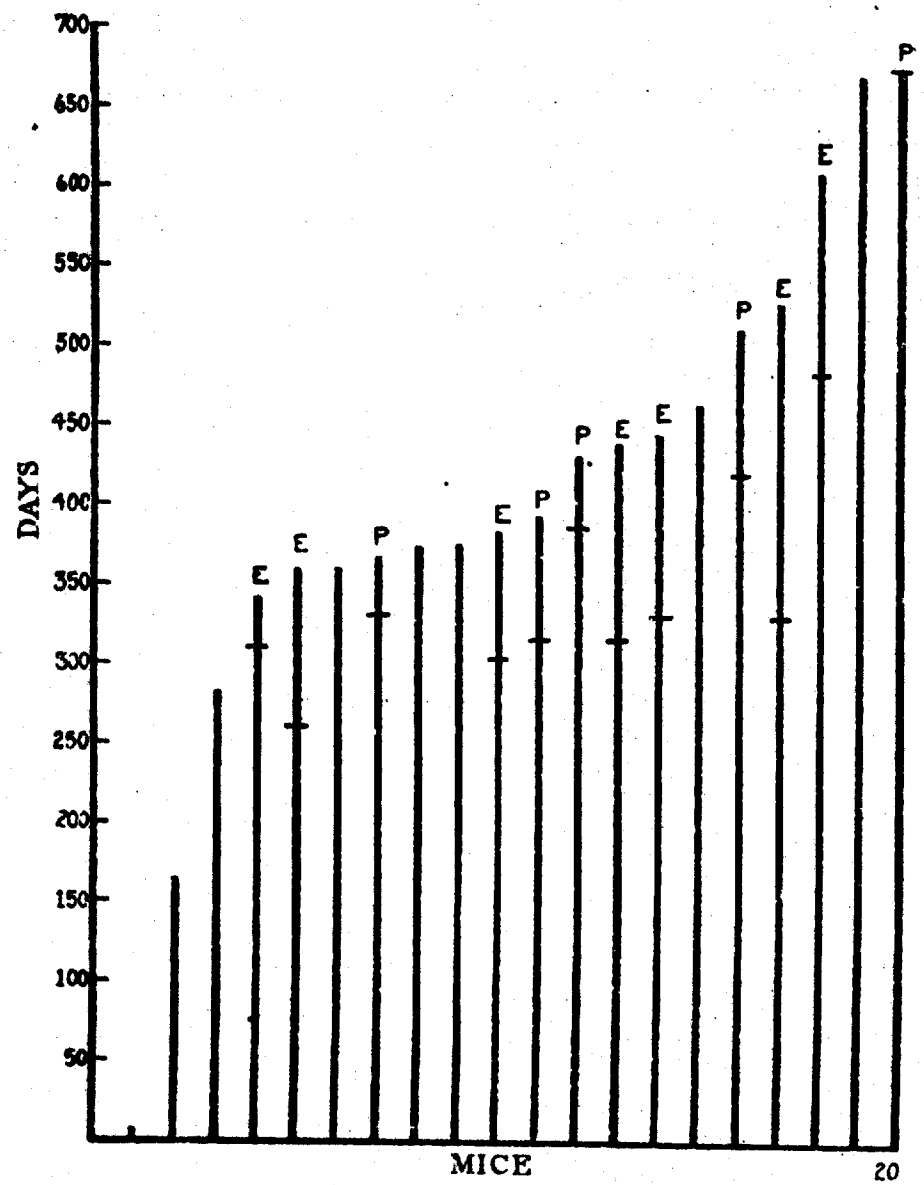


FIG. 1—3:4-Benzphenanthrene. 0·3% in benzene. (In this and figs. 2 to 7, each vertical line represents the duration of the life of one mouse after the commencement of painting. The transverse mark on some of the lines denotes the day when a tumour was first noticed. The letters at the tops of the lines show the results of microscopical examination of the tumours. E = epithelioma, P = papilloma. K indicates that the mouse was killed to terminate the experiment.)

chrysene has any carcinogenic activity must remain in abeyance until the conclusion of experiments now in progress with synthetic chrysene. In our opinion, the results obtained with chrysene by Bottomley and Twort (1934), who claimed to have produced a considerable number of skin tumours in an unspecified number of mice, would have been more convincing had they been obtained with pure synthetic chrysene.

With pyrene also, occasional papillomas (3 in 45 mice) have been obtained; we are not prepared to assert that these were definitely due to the pyrene itself, as our earlier samples of this hydrocarbon which were used in these experiments were tinted yellow with impurities which we had been unable to remove. No tests have yet been completed with our later samples of pure, colourless pyrene. Table I shows also the negative results obtained with fluoranthene, a somewhat analogous hydrocarbon which is present in coal tar.

Derivatives of 1 : 2-Benzanthracene

Tests have now been completed with a considerable number of simple homologues of 1 : 2-benzanthracene. Positive results are summarized in Table III and negative results in Table IV. From these results it is abundantly clear that the ring system of 1 : 2-benzanthracene gives a potentially carcinogenic molecule and that cancer-producing properties are developed by substitution in suitable positions. The activity of the 6-*iso*propyl compound in contrast with the negative results obtained with the 3-, 7-, and 10-*iso*propyl derivatives of 1 : 2-benzanthracene, the frequency with which tumours have arisen with other derivatives bearing substituents at positions 5 or 6, and the almost entire absence of tumours with numerous other methyl (3-, 7-, 2′-, 3′-) and dimethyl (2′ : 6-, 2′ : 7-, 3′ : 6-, 3′ : 7-) derivatives, all lend strong support to the view that attachment of alkyl groups to positions 5 or 6 (or both) of the 1 : 2-benzanthracene molecule gives molecular conditions which are favourable for cancer-producing activity.

All simple derivatives of 1 : 2-benzanthracene which have no substituent in positions 5 or 6 have so far given negative results, with the exception of the 4-methyl derivative, which gave one papilloma. It should be remarked, however, that no compounds with substituents at position 8 have yet been examined. Experiments described below with 1 : 2 : 7 : 8-dibenzanthracene and 3 : 4 : 5 : 6-dibenzacridine (pp. 327, 334) suggest that simple 8-alkyl derivatives of benzanthracene would be worthy of attention.

Although 6:7-*cyclo*penteno-1:2-benzanthracene is not strictly a homologue of benzanthracene, it may be classified among these for comparative purposes. The production of tumours with this compound has been definitely slower than with the isomeric 5:6-*cyclo*penteno-1:2-benzanthracene (Cook, 1932, *a*). This is exemplified in Table II.

TABLE II—TIME OF APPEARANCE OF TUMOURS OF SKIN

Produced by

5:6-*cyclo*Penteno-1:2-benzanthracene (50 mice)			6:7-*cyclo*Penteno-1:2-benzanthracene (10 mice)		
Day	Number of tumours	%	Day	Number of tumours	%
143 to 184 (41 days) ..	12	50·0		—	—
185 to 285 (100 days)..	10	41·7	240 to 285 (45 days)	2	28·6
286 to 305 (19 days) ..	2	8·3	286 to 353 (67 days)	5	71·4
	24	100·0		7	100

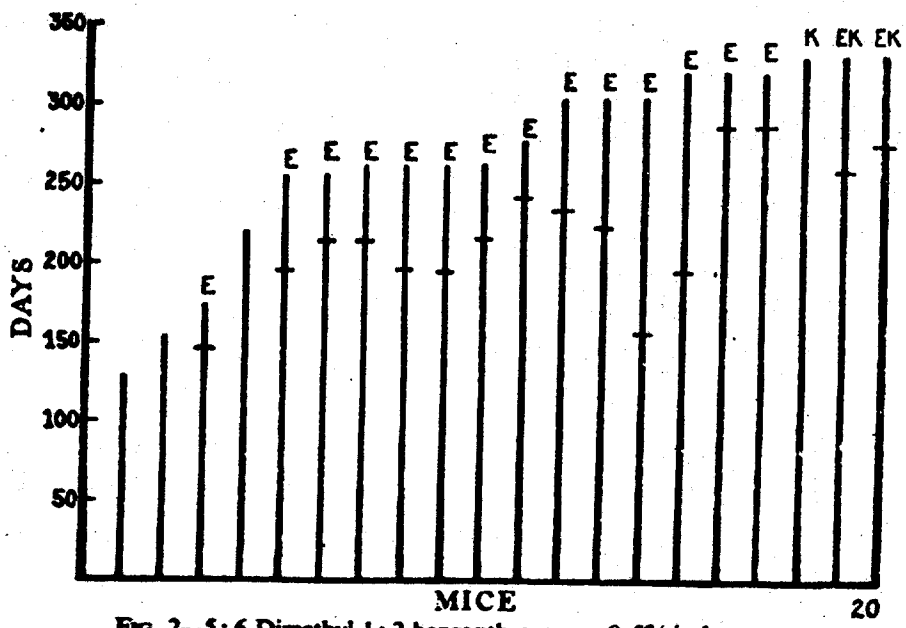


FIG. 2—5:6-Dimethyl-1:2-benzanthracene. 0·3% in benzene

Comparisons of 5-methyl-1:2-benzanthracene with the 6-methyl compound, of the 5:6-dimethyl homologue, fig. 2, with the 6:7-dimethyl compound, and of 5:6-*cyclo*penteno-1:2-benzanthracene with 6:7-*cyclo*penteno-1:2-benzanthracene point to the indubitable conclusion

TABLE III—1 : 2-BENZANTHRACENE AND ITS TUMOUR-PRODUCING HOMOLOGUES

Compound	Method of preparation	Mice: Initial number	Mice: Alive after 6 months	Mice: Alive after 1 year	Mice: Day of death of last mouse	Tumours: Epithelioma	Tumours: Papilloma
1 : 2-Benzanthracene—							
(1) Not specially purified (2% in benzene)	Barnett and Matthews (1925)	50	13	2	574	0	0
(2) Purified through the picrate		30	10	7	584	1	0
4-Methyl-1 : 2-benzanthracene	Cook (1933)	10	3	0	312	0	1
5-Methyl-1 : 2-benzanthracene	"	10	8	6	433	5	2
6-Methyl-1 : 2-benzanthracene	Cook (1932, *b*)	10	8	5	717	2	1
5 : 6-Dimethyl-1 : 2-benzanthracene ..	Cook and Haslewood (1934)	20	18	—	331	16	0
6 : 7-Dimethyl-1 : 2-benzanthracene—							
(1) Crude sample, m.p. 166-168° ..	Cook (1932, *b*)	10	10	5	588	3	1
(2) Pure sample, m.p. 173-174°		20	16	5	486	0	2
6-*iso*Propyl-1 : 2-benzanthracene—							
(1) Pure, m.p. 131-132°	"	10	8	0	348	5	1
(2) Purified through quinone, m.p. 132-133°		7	6	0	275	5	0
5 : 6-*cyclo*Penteno-1 : 2-benzanthracene—							
(1) 0·3% in benzene	Cook (1931, *d*)	40	20	0	358	14	5
(2) 0·1% in benzene		10	6	0	339	5	0
(3) 0·03% in benzene		20	18	14	795	1	1
6 : 7-*cyclo*Penteno-1 : 2-benzanthracene	"	10	9	5	559	6	1

TABLE IV—NON-CARCINOGENIC SIMPLE DERIVATIVES OF 1 : 2-BENZANTHRACENE

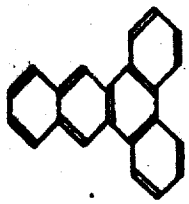
Compound	Method of preparation	Mice				Tumours	
		Initial number	Alive after 6 months	Alive after 1 year	Day of death of last mouse	Epithelioma	Papilloma
Dodecahydro-1 : 2-benzanthracene	Cook and Hewett (1934)	10	9		7 alive day 351*	0	0
3-Methyl-1 : 2-benzanthracene	Cook (1930)	14	9	4	458	0	0
7-Methyl-1 : 2-benzanthracene	Cook (1932, *b*)	10	5	4	563	0	0
2′-Methyl-1 : 2-benzanthracene	,,	10	6	4	666	0	0
3′-Methyl-1 : 2-benzanthracene	,,	10	3	3	924	0	0
2′ : 6-Dimethyl-1 : 2-benzanthracene	,,	10	7	6	674	0	0
2′ : 7-Dimethyl-1 : 2-benzanthracene	,,	10	8	6	786	0	0
3′ : 6-Dimethyl-1 : 2-benzanthracene	,,	10	9	6	707	0	0
3′ : 7-Dimethyl-1 : 2-benzanthracene	,,	10	9	8	612	0	0
3-*iso*Propyl-1 : 2-benzanthracene	,,	20	9	7	554	0	0
7-*iso*Propyl-1 : 2-benzanthracene	,,	10	9	6	794	0	0
10-*iso*Propyl-1 : 2-benzanthracene	,,	10	6	3	611	0	0
6-Phenyl-1 : 2-benzanthracene	Cook (1930)	20	12	6	651	0	0
9 : 10-Diphenyl-1 : 2-benzanthracene	,,	20	11	7	652	0	0
10-benzyl-1 : 2-benzanthracene (1·0, 0·5% in benzene)	,,	10	8	3	466	0	0
Dimeride of 7-*iso*Propenyl-1 : 2-benzanthracene	Cook and Hewett (1933, *b*)	10	10	4	709	0	0

* On November 1, 1934.

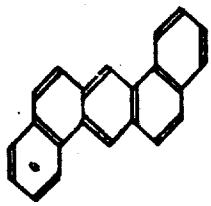
that a substituent at position 5 is more efficacious in stimulating carcinogenic action than is the same substituent at position 6. The effect of introducing a single methyl group into a suitable position of the benzanthracene molecule is strikingly shown in the 5-methyl compound which has given 7 tumours (5 epitheliomas and 2 papillomas) in a series of 10 mice. It is also noteworthy, in connection with the feeble but definite activity of 6-methyl-1 : 2-benzanthracene, that both the 2′ : 6- and 3′ : 6-dimethyl derivatives of 1 : 2-benzanthracene have given negative results. This is in harmony with the observations previously recorded in the 1 : 2 : 5 : 6-dibenzanthracene series, that a methyl group at positions 2′ or 3′ has a depressant effect on the carcinogenic activity (Parts I and II).

Pentacyclic Compounds

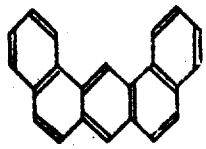
Of the 15 possible hydrocarbons containing systems of five condensed benzene rings in the molecule (formulæ shown below), 12 have now been tested for carcinogenic action, and of these only 2 have shown any pronounced activity. These are 1 : 2 : 5 : 6-dibenzanthracene and 1 : 2-benzpyrene. Tumour production by 1 : 2 : 5 : 6-dibenzanthracene has been described in previous communications, and the results with 1 : 2-benzpyrene are discussed below. In experiments carried out here 1 : 2 : 5 : 6-dibenzanthracene, when applied to the skin in eight different solvents, has given 126 epitheliomas and 41 papillomas in 413 mice. The three compounds (1 : 2 : 3 : 4-dibenzphenanthrene, 1 : 2 : 5 : 6-dibenzphenanthrene, 1′ : 2′-naphtha-1 : 2-anthracene) not yet tested are still unknown. Of the remaining 10 hydrocarbons, suggestions of very faint carcinogenic activity have been given in certain cases. Thus, 1 : 2 : 7 : 8-dibenzanthracene has given 1 epithelioma and 3 papillomas in 20 mice, in an experiment extending over 2 years, Table V. The tumours were very slow in making their appearance. Somewhat analogous results with 1 : 2 : 3 : 4-dibenzanthracene were recorded in Part I; as was pointed out, the material used in those experiments was not quite pure, and a further test in which pure 1 : 2 : 3 : 4-dibenzanthracene was applied to 20 mice has given no tumours. In this series the last mouse died on the 487th day, so that we cannot say that no tumours would have been obtained had the mice lived exceptionally long, as in the earlier series with the impure material (Part I, p. 466). 3 : 4 : 5 : 6-Dibenzphenanthrene gave only 2 transient papillomas. One epithelioma was also obtained in a series of 10 mice to which was applied a specimen of 4 : 5-benzpyrene isolated from coal tar (Table VII). We are reluctant to attribute this tumour, which first appeared after 13 months, to 4 : 5-benzpyrene, as the



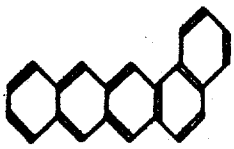
1 : 2 : 3 : 4-Dibenz-anthracene



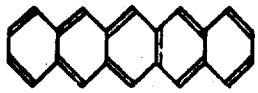
1 : 2 : 5 : 6-Dibenz-anthracene



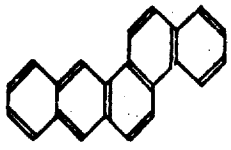
1 : 2 : 7 : 8-Dibenz-anthracene



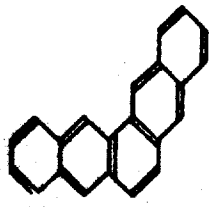
2′ : 3′-Naphtha-2 : 3-phenanthrene



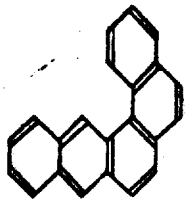
2 : 3 : 6 : 7-Dibenz-anthracene



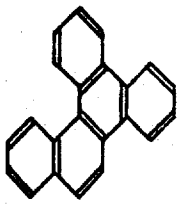
2′ : 1′-Naphtha-1 : 2-anthracene



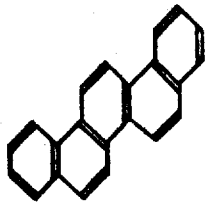
2′ : 3′-Naphtha-1 : 2-anthracene



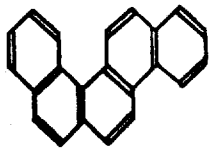
1′ : 2′-Naphtha-* 1 : 2-anthracene



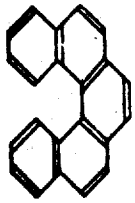
1 : 2 : 3 : 4-Dibenz-* phenanthrene



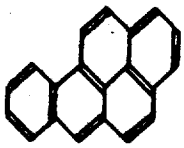
Picene [1 : 2 : 7 : 8-Dibenz-phenanthrene]



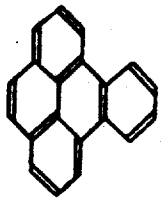
1 : 2 : 5 : 6-Dibenz-* phenanthrene



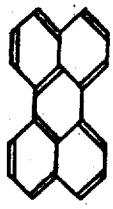
3 : 4 : 5 : 6-Dibenz-phenanthrene



1 : 2-Benzpyrene



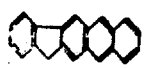
4 : 5-Benzpyrene



Perylene

* These three compounds are still unknown.

TABLE V—PENTACYCLIC AND HEXACYCLIC HYDROCARBONS

Compound	Method of preparation	Mice: Initial number	Mice: Alive after 6 months	Mice: Alive after 1 year	Mice: Day of death of last mouse	Tumours: Epithelioma	Tumours: Papilloma
1:2:7:8-Dibenzanthracene	Cook (1932, *c*)	10	10	5	627	1	1
"		10	9	7	722	0	2
1:2:3:4-Dibenzanthracene	Clar (1929)	20	11	2	487	0	0
2:3:6:7-Dibenzanthracene	Clar and John (1930)	10	6	4	553	0	0
"		4	2	0	313	0	0
3:4:5:6-Dibenzphenanthrene	Cook (1933)	10	7	6	613	0	0
2′:3′-Naphtha-2:3-fluorene	Barnett, Goodway and Watson (1933)	10	2	0	231	0	0
							
2′:3′-Phenanthra-1:2-anthracene (0·16% in benzene)	Cook (1931, *b*)	10	7	4	754	0	0
2′:3′-Phenanthra-2:3-phenanthrene (0·25% in benzene)	"	5	4	2	517	0	0
Do. do. (0·17% in xylene)	"	5	5	3	787	0	0

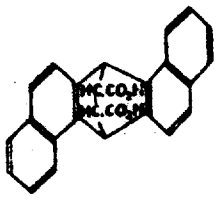
specimen used was certainly contaminated with other substances and a carcinogenic impurity, possibly 1:2-benzpyrene, may well have been present. No tests have been carried out with pure synthetic 4:5-benzpyrene.

Compounds Related to 1:2:5:6-Dibenzanthracene

The tests on some 20 simple derivatives of 1:2:5:6-dibenzanthracene are now almost complete, Table VI and Parts I and II, and the results fully justify the preliminary conclusion (Cook, 1932, *a*) that substitution, particularly in the *meso* positions, tends to diminish the carcinogenic potency of the 1:2:5:6-dibenzanthracene molecule. Reference to Table VI shows that, of the 20 mice to which the pure sample of phenanthra-acenapthene was applied, 7 developed tumours (4 epitheliomas and 3 papillomas). This is a simple derivative of 1:2:5:6-dibenzanthracene in which the *meso* positions are unsubstituted, and somewhat similar results were obtained with the 4-methyl, Table VI, and 2′-methyl, and 3′-methyl (*see* Parts I and II) derivatives of 1:2:5:6-dibenzanthracene. No compounds have been examined in which a single alkyl group is attached to a *meso* position, and it is impossible to assess the influence of the extraneous atoms introduced in the other 9-monosubstituted derivatives which have been examined (for example, the 9-methoxy and 9-amino compounds, which both gave positive results, and the 9-acetoxy compound, which gave negative results). It is perhaps not without significance, however, that the 9:10-dialkyl derivatives which have been examined, dimethyl, di-*n*-butyl, dibenzyl, Table VI and Part II, have given a comparatively small yield of tumours. For example, in an experiment which lasted more than 2 years, 9:10-di-*n*-butyl-1:2:5:6-dibenzanthracene gave only one epithelioma in a series of 10 mice. Similar loss of activity was observed with 9:10-dihydro-1:2:5:6-dibenzanthracene, which has given 2 tumours in a series of 10 mice; entirely negative results were obtained with *cis*-9:10-dimethyl-9:10-dihydro-1:2:5:6-dibenzanthracene (Part II). Thus any disturbance of molecular structure which affects the *meso* positions of 1:2:5:6-dibenzanthracene (either replacement of hydrogen, or reduction) seems to be attended by marked diminution of carcinogenic power. A comparison of 1:2:5:6-dibenzanthracene with its 9:10-dialkyl derivatives suggests that the property of carcinogenesis is facilitated (although not determined) by the presence of unsubstituted hydrogen atoms in the *meso* positions. These hydrogen atoms are readily removed by oxidation. It is certainly true that the active carcinogenic compounds are all extremely susceptible to the action of oxidizing agents, and the

role of these carcinogenic substances in producing tumours may be associated with some sort of interference with the normal oxidation processes of cells, occasioned by this ready oxidizability of the cancer-producing agents. That this cannot be the complete explanation of the cancer-producing action of the compounds is clear, for then the property of cancer-production would be much more common among many classes of easily oxidized substances. Attempts have been made to find some measure of explanation of carcinogenesis by hydrocarbons on lines such as these (Boyland, 1933; Pourbaix, 1933), but so far these attempts have not led to any conclusive results.

Most of the compounds tested in this Institute for carcinogenic action have been complex hydrocarbons and hence practically insoluble in water. Preliminary attempts have been made to find water-soluble carcinogenic agents by introducing into the 1:2:5:6-dibenzanthracene molecule groups which confer solubility in water. The biological effects of such compounds, administered intravenously, for example, would doubtless be of considerable interest. Inseparable mixtures of sodium salts of disulphonic acids of 1:2:5:6-dibenzanthracene were applied to the skin of mice and gave no tumours. Negative results were also obtained with a mixture of the sodium salts of 1:2:5:6-dibenzanthracene 4:8-dicarboxylic acid and 3:4:5:6-dibenzphenanthrene-1:8-dicarboxylic acid. More successful results were obtained with the sodium salt of 1:2:5:6-dibenzanthracene-9:10-endo-αβ-succinic acid (Cook, 1931, *e*)



. Subcutaneous injection of an aqueous solution of this salt has given 6 tumours, mostly spindle-celled, in 60 mice. One of the tumours thus initiated has now reached the thirtieth transplanted generation, and is accompanied by leukæmia. These biological experiments were carried out by Mr. Burrows and extended by Mrs. Parsons, and will be described in detail in a subsequent communication.

In connection with the factor of oxidizability at the *meso* positions, it seemed of interest to examine compounds related to 1:2:5:6-dibenzanthracene in which one or both of the *meso* carbon atoms are replaced by nitrogen. Such compounds are 1:2:5:6-dibenzacridine and 1:2:5:6-dibenzphenazine.

1:2:5:6-Dibenzacridine proved, in fact, to be a cancer-producing compound and in the course of experiments extending over 482 days it

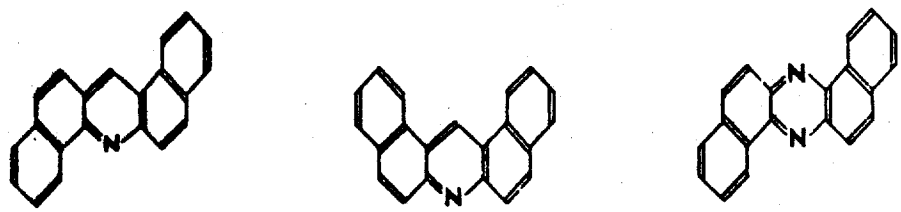
TABLE VI—DERIVATIVES AND HETEROCYCLIC ANALOGUES OF 1:2:5:6-DIBENZANTHRACENE

Compound	Method of preparation	Mice: Initial number	Mice: Alive after 6 months	Mice: Alive after 1 year	Mice: Day of death of last mouse	Tumours: Epithelioma	Tumours: Papilloma
4-Methyl-1:2:5:6-dibenzanthracene	Cook (1933)	10	9	0	291	4	1
9:10-Di-*n*-butyl-1:2:5:6-dibenzanthracene	Cook (1931, *a*)	10	8	1	742	1	0
9:10-Dihydro-1:2:5:6-dibenzanthracene	Cook (1933)	10	7	1	426	2	0
9-Acetoxy-1:2:5:6-dibenzanthracene	Cook (1931, *e*)	10	9	5	678	0	0
9-Methoxy-1:2:5:6-dibenzanthracene	,, ...	10	3	0	298	1	0
,, ,,		20	17	2	441	6	5
1:2:5:6-Dibenz-9-anthranol (not pure)		10	5	4	623	0	0
,, ,,		10	8	7	786	0	0
1:2:5:6-Dibenzanthraquinone	Clar (1929)	10	6	6	570	0	0
9-Nitro-1:2:5:6-dibenzanthracene ..	Cook (1931, *e*)	10	4	4	538	0	0
9-Amino-1:2:5:6-dibenzanthracene	,, ...	10	7	5	632	3	1
9-*n*-Butyrylamino-1:2:5:6-dibenzanthracene	,, ...	10	3	2	476	0	0
N-1:2:5:6-Dibenzanthranylsuccinimide	,, ...	10	5	2	569	0	0
1:2:5:6-Dibenzanthracene-9:10-*endo*-αβ-succinic acid	,, ...	10	8	8	724		

Phenanthra-accnaphthene (pure)	Cook (1931, *b*)	5	3	2	577	2	0
"		5	3	2	677	0	1
"		10	9	6	539	2	2
1 : 2 : 5 : 6-Dibenzacridine	Ullmann and Fetvadjian (1903)	10	7	0	349	1	0
"		30	27	12	482	4	2
3 : 4 : 5 : 6-Dibenzacridine	Möhlau and Haase (1902)	10	6	3	597	2	0
"		30	28	18	551	9	2
1 : 2 : 5 : 6-Dibenzphenazine	Reitzenstein and Andre (1913)	20	16	8	660	0	0
*Iso*naphthathioxin ..	Cohen and Smiles (1929)	10	9	—	5 alive day 295*	—	—

* On November 1, 1934.

yielded 7 tumours (5 epitheliomas and 2 papillomas) in 40 mice, fig. 3. Of considerable interest, also, is the fact that an allied substance, 3:4:5:6-dibenzacridine was also carcinogenic, fig. 4. Indeed, it seemed more active than the 1:2:5:6-compound, for 13 tumours (11 epitheliomas and 2 papillomas) were obtained in two series com-



1:2:5:6-Dibenzacridine 3:4:5:6-Dibenzacridine 1:2:5:6-Dibenzphenazine

prising 40 mice in all. The action of these two compounds is slow; only 5 of the 17 tumours represented in figs. 3 and 4 appeared before the 300th day.

This 3:4:5:6-dibenzacridine bears the same relationship to 1:2:5:6-dibenzacridine as 1:2:7:8-dibenzanthracene does to 1:2:5:6-dibenzanthracene (*cf.* formulæ on p. 328), at least so far as molecular

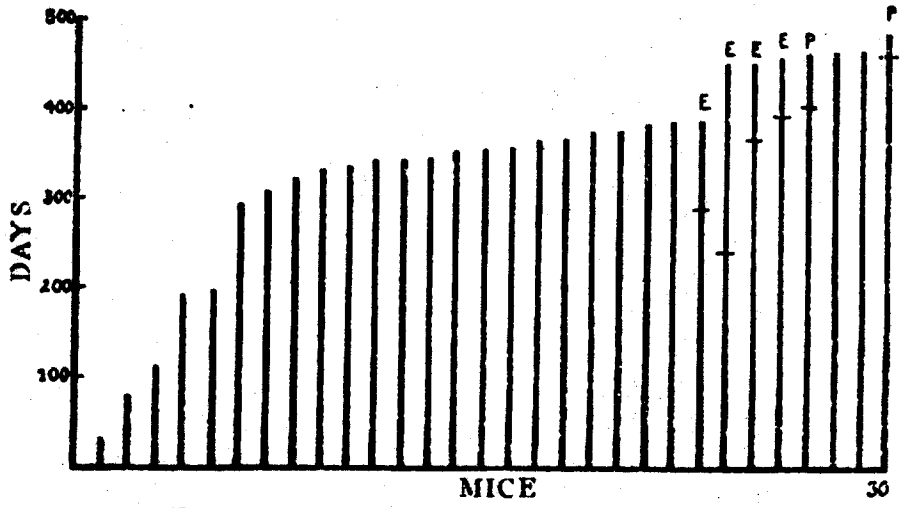


FIG. 3—1:2:5:6-Dibenzacridine. 0·3% in benzene.

structures are concerned, and it will be recalled that 1:2:7:8-dibenzanthracene had very little cancer-producing activity (p. 327). Relationships such as these indicate the difficulties in arriving at any complete generalization regarding the molecular conditions which are necessary for cancer-producing activity. The parent compound acridine has been

tested on the skin of 200 mice with negative result (Kennaway, 1924). Proflavine (diaminoacridine sulphate) and acriflavine (diamino-methylacridinium hydrochloride) have given negative results in painting experiments of 488 days' duration.

1 : 2 : 5 : 6-Dibenzphenazine was also examined for comparison with the corresponding anthracene and acridine derivatives. It has been applied to the skin of mice in the usual way, but so far with completely negative results. In this connection, one may point out that in this dibenzphenazine there are no longer the possibilities of ready oxidation at

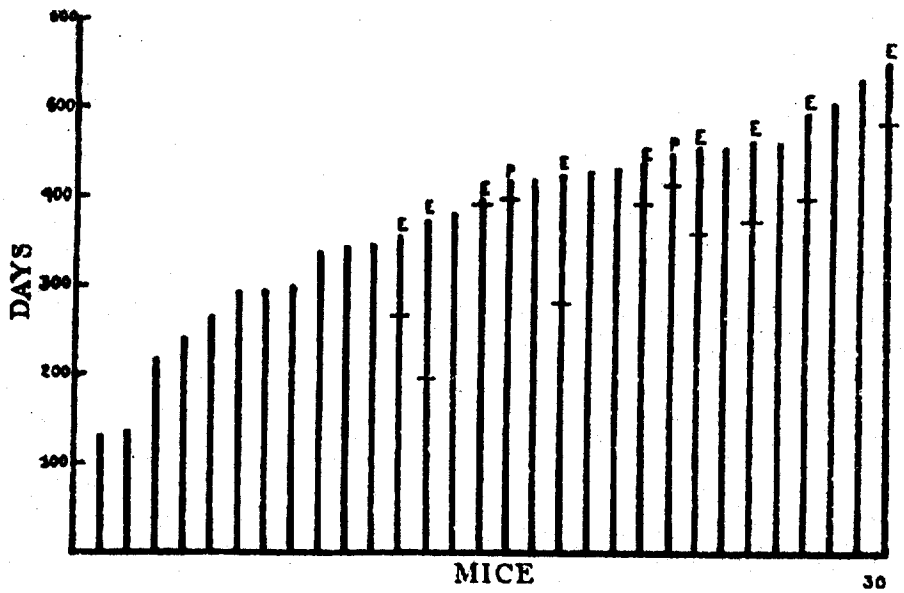


FIG. 4—3 : 4 : 5 : 6-Dibenzacridine. 0·3% in benzene.

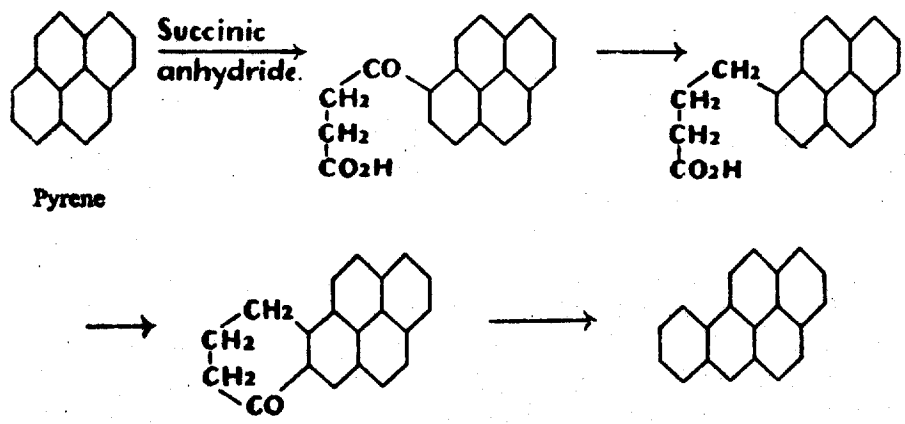
the *meso* positions as is the case with 1 : 2 : 5 : 6-dibenzanthracene and the dibenzacridines.

A sulphur compound related to 1 : 2 : 5 : 6-dibenzacridine, namely, *iso*naphthathioxin has so far given no tumours in a series of 10 mice of which 5 are still alive after 295 days.

1 : 2-BENZPYRENE

This hydrocarbon is the only one of the carcinogenic substances so far examined which has been shown to be present in coal tar. It was first isolated from coal tar pitch and was afterwards prepared synthetically (Cook, Hewett and Hieger, 1933) from pyrene by the sequence of changes shown below.

It will be seen from its formula that 1 : 2-benzpyrene contains the ring systems of pyrene, chrysene and 1 : 2-benzanthracene. The actual "fine structure" of the molecule is probably more closely related to 1 : 2-benzanthracene than to the other two hydrocarbons, for the fluorescence spectrum is of exactly the same type as that of 1 : 2-benzanthracene, but is appreciably different from the spectra given by chrysene and pyrene (Hieger, 1930). Comparison of the absorption spectrum of 1 : 2-benzpyrene with those of the three parent hydrocarbons under discussion



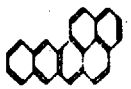
Pyrene

1 : 2-Benzpyrene

shows also that the benzpyrene spectrum is definitely of the benzanthracene type, although some of the features of the pyrene spectrum may also be detected. These spectroscopic comparisons have been made by Dr. W. V. Mayneord and Miss Roe, B.Sc., and will be published subsequently.

The results obtained by the application of 1 : 2-benzpyrene to mice, figs. 9 and 10, Plate 20, are summarized in Table VII, which shows that the material isolated from pitch gave results quite parallel with those obtained with the synthetic sample. In all, 58 tumours (47 epitheliomas and 11 papillomas) have been obtained in 100 mice, and from this high incidence as also from the early appearance of tumours, figs. 5 and 6, it is clear that 1 : 2-benzpyrene is a considerably more potent carcinogenic agent than 1 : 2 : 5 : 6-dibenzanthracene, or, in fact, than any compound already discussed. In recent publications, Maisin and Liégeois (1934) and Maisin and De Jonghe (1934) stated that they had examined a sample of 1 : 2-benzpyrene prepared synthetically by the method of Cook Hewett and Hieger (1933) and that they had found it to be much more active in producing tumours than the material which we used. Since

TABLE VII—1 : 2-BENZPYRENE AND RELATED COMPOUNDS

Compound	Method of preparation	Mice: Initial number	Mice: Alive after 6 months	Mice: Alive after 1 year	Mice: Day of death of last mouse	Tumours: Epithelioma	Tumours: Papilloma
1 : 2-Benzpyrene—	Cook, Hewett and Hieger (1933)						
A. *Synthetic*—							
Series 1		10	3	0	206	4	1
Series 2		10	4	1	368	3	0
Series 3		10	2	0	221	5	2
*Series 4. 1 : 2-Benzpyrene picrate		10	6	0	353	5	0
*Series 5. Mixture of 1 : 2-benzpyrene (3 parts) and 1 : 2-benzpyrene picrate (1 part)		10	8	1	368	5	1
B. *Isolated from coal-tar pitch*—							
Sample 1, m.p. 177·5–178·5°		10	10	0	295	6	3
Sample 2, m.p. 175·5–176·5°		10	3	0	202	6	1
Sample 3, m.p. 175·5–176·5°		10	5	0	225	8	2
Sample 3, (0·03% in benzene)....		10	10	9	638	3	0
" "		10	9	3	503	2	1
4 : 5-Benzpyrene (isolated from coal tar pitch, not completely purified)	Cook, Hewett and Hieger (1933)	10	9	5	660	1	0
1-Phenyl-2 : 3 : 4 : 5-dibenzpyrene	Clar (1930)	10	10	3	635	0	1
2′-3′-Naphtha-1 : 2-pyrene 	Cook, Hewett and Hieger (1933)	20	20	15	713	0	0

* Results show that addition of picric acid did not affect tumour production.

we have not hitherto published any details of our experiments, it is difficult to understand the basis of this claim. Actually, the data which we now record are in fair agreement with those published by Maisin, Liégeois and

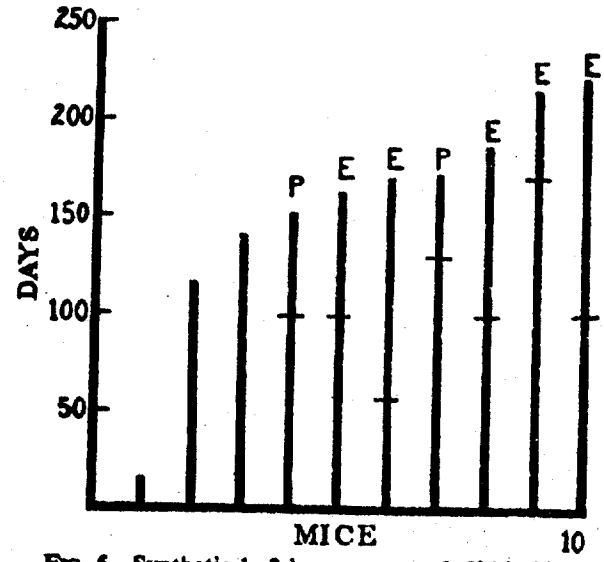


FIG. 5—Synthetic 1 : 2-benzpyrene. 0·3% in benzene

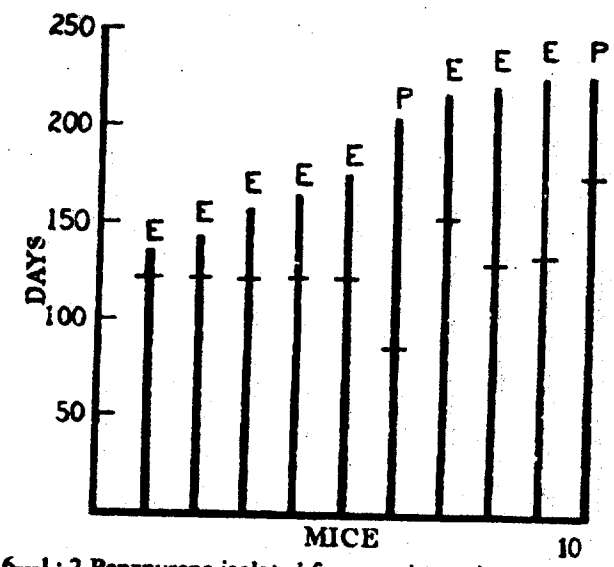


FIG. 6—1 : 2-Benzpyrene isolated from coal-tar pitch. 0·3% in benzene

De Jonghe. The differences in physical properties between their 1 : 2-benzpyrene and our own highly purified synthetic samples are clearly due to the presence of a small amount of impurity in the material which

they used. Their suggestion that they were dealing with " une variété particulière de cette substance " is completely meaningless.

2′ : 3′-Naphtha-1 : 2-pyrene, in which an additional benzene ring is condensed with the 1 : 2-benzpyrene molecule, has given no tumours in a series of 20 mice, of which 15 lived for more than one year, Table VII.

Tumours of Connective Tissue Produced by 1 : 2-Benzpyrene

No subcutaneous injections of the purest samples of 1 : 2-benzpyrene have been made. A rat which had received *sub cutem* 0·1 gm of moderately pure 1 : 2-benzpyrene (m.p. 168–170°), from pitch, in 3 cc sesame oil in the course of tests by Professor E. C. Dodds for œstrogenic action (Cook, Dodds, Hewett and Lawson, 1934), developed a spindle-celled tumour at one of the sites of injection. An autograft made on the 165th day showed growth when the rat was killed on the 181st day. Grafts of the tumour died out in the second generation.

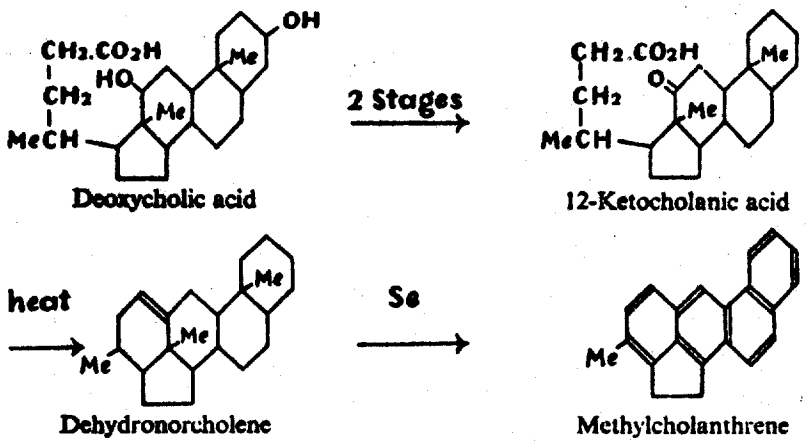
Two series of 10 mice each received *sub cutem*, usually at weekly intervals, crystalline fractions, which consisted very largely of 1 : 2-benzpyrene, obtained in the course of isolation from pitch. Series A received a fraction of m.p. about 160°, series B one of m.p. 164–168° (both 0·3% in lard). Series A yielded 4, and series B 5, spindle-celled tumours, the 9 mice being killed between the 115th and 191st days (average 146th day); grafts made from one of these tumours did not persist beyond the second generation. These figures indicate a rapid growth of the tumour. Thus, in earlier experiments (Burrows, Hieger and Kennaway, 1932; Barry and Cook, 1934) 31 mice bearing primary tumours caused by 1 : 2 : 5 : 6-dibenzanthracene were killed between the 144th and 243rd days (average 186th day) and 4 mice bearing primary tumours caused by 5 : 6-*cyclo*penteno-1 : 2-benzanthracene were killed between the 196th and 280th day (average 250th day).

Maisin and Coolen* (1934) have produced tumours by subcutaneous injection in rats of 1 : 2-benzpyrene dissolved in lard. " Ces tumeurs ont une évolution extrêment rapide, 15 à 25 jours après l'apparition du 1er nodule les animaux meurent du développement énorme du néoplasme qui peut donner des métastases multiples (rein, poumon, pancréas, cœur, etc.). Ces tumeurs sont très anaplasiques et certaines aussi semblent bien être des myosarcomes. Elles naissent plus tôt qu-après injection de 1-2 5-6 dibenzène anthracène."

* These authors credit us with experiments which we have never performed on the production of sarcomas in rats by 1 : 2-benzpyrene.

METHYLCHOLANTHRENE

The investigations relating to pure synthetic carcinogenic compounds of known molecular structure have assumed an entirely new aspect with the recognition and proof of the true molecular structures of the group of natural products which comprise the sterols and bile acids. For it is now clear that there is a definite similarity of molecular structure between these natural products and the cancer-producing hydrocarbons. In particular, the sterols and bile acids contain in their molecules condensed carbon-ring systems to which is attached a long side-chain in such a position that a new six-membered ring can be formed so as to give the 1 : 2-benzanthracene ring system without molecular rearrangement or group migration. The purely aromatic hydrocarbon, methyl-



cholanthrene,* obtained thus from the bile acid, deoxycholic acid, by the above series of changes, is a benzanthracene hydrocarbon with substituents in positions 5 and 6. This is a favourable type of molecular structure for the development of carcinogenic properties (p. 323).

* Wieland and Dane (1933) have suggested the name "cholanthrene" for the hypothetical anthracenoid hydrocarbon from which such aromatic hydrocarbons related to the cholane group are derived.

[*Note added in proof, March* 19, 1935.—The synthetic preparation of methylcholanthrene has recently been announced by L. F. Fieser and A. M. Seligman (J. Amer. Chem. Soc., vol. 57, p. 228, (1935)), and the parent hydrocarbon cholanthrene, m.p. 170°, has now been synthesized in this Institute by J. W. Cook, G. A. D. Haslewood, and Mrs. A. M. Robinson.]

The structure of methylcholanthrene has been established by the synthesis of a degradation product (5: 6-dimethyl-1: 2-benzanthraquinone) (Cook and Haslewood, 1934). Moreover, methylcholanthrene has proved to be a potent cancer-producing hydrocarbon, the order of activity of which is at least equal to that of 1: 2-benzpyrene, and possibly slightly greater. The high activity of methylcholanthrene is shown not only by the early appearance of tumours, but also in the very high proportion of animals which develop tumours, fig. 7 and Table VIII. In one series of 20 mice, 18 tumours (17 epitheliomas and one papilloma) were obtained in an experiment which was ended in 180 days, and in another series the first tumour appeared after 31 days. Fig. 11, Plate 20, illustrates a metastasis obtained in another series of mice, in which a dilute solution of very highly purified methylcholanthrene was employed. Methylcholanthrene thus establishes a clear connecting link between the carcinogenic hydrocarbons and the sterols and bile acids, and it is of great interest that the changes by which it is obtained from deoxycholic acid are all reactions of the type which are known to occur normally in the animal body, although there is no evidence that this particular sequence of changes involved in the formation of methylcholanthrene does actually occur in nature.

Tumours of Connective Tissue Produced by Methycholanthrene

Four earlier papers from this Institute have described spindle-celled tumours of connective tissue produced by 1: 2: 5: 6-dibenzanthracene (Burrows, Hieger and Kennaway, 1932; Burrows, 1932 and 1933; Barry and Cook, 1934), and by 5: 6-*cyclo*penteno-1: 2-benzanthracene and chrysene (Barry and Cook, 1934). Methylcholanthrene was dissolved in lard liquid at 37° (2 or 3 mg in 1 cc) by heating in boiling water, and injections of 1 cc of this solution into the right groin of 15 rats were made at intervals, usually of 1 week. Tumours arose, fig. 8, in each one of the 9 rats out of the original 15 which lived for more than 70 days, the tumour-bearing animals being killed between the 123rd and 193rd day (average 162nd day); the comparable average figures obtained here for duration of life in experiments on rats with other hydrocarbons are:—

	Days
1: 2: 5: 6-dibenzanthracene (2 series)	210, 193
5: 6-*cyclo*penteno-1: 2-benzanthracene	422
Chrysene.....	449

Hence methylcholanthrene produces tumours of this type more rapidly than does any other hydrocarbon which we have tested as yet.

The structure of these tumours is spindle-celled, figs. 12–15, Plate 21, and they have on the whole a more fibromatous appearance than those

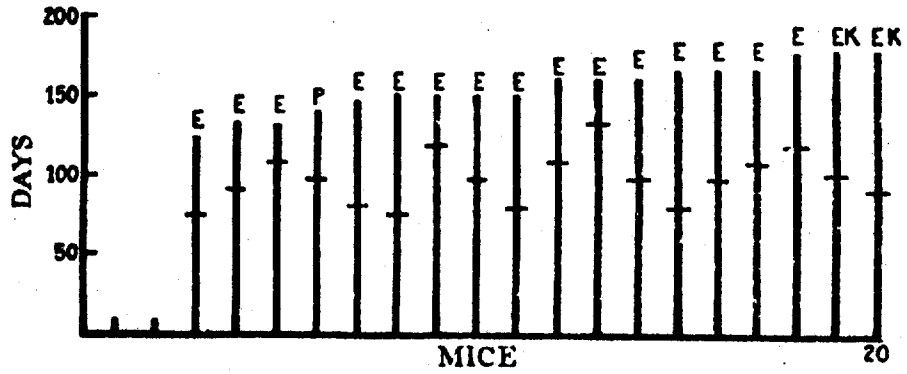


FIG. 7—Methylcholanthrene. 0·3% in benzene

produced by other hydrocarbons. Numerous giant cells, fig. 14, Plate 21, are present in some of them (*cf.* Burrows, 1932, fig. 9). The invasive powers of these tumours appear to be rather low in spite of their great size,

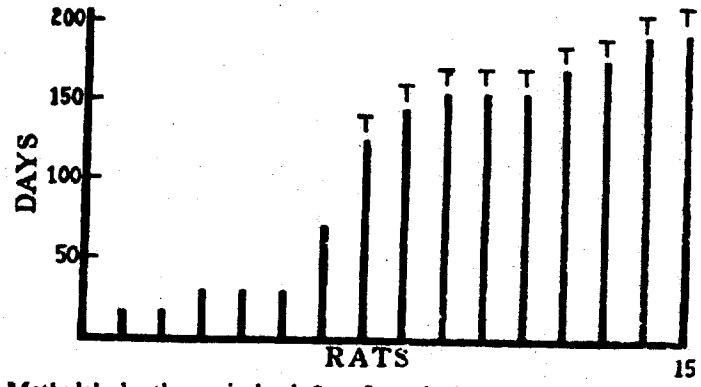


FIG. 8—Methylcholanthrene in lard, 2 or 3 mg in 1 cc. Connective tissue tumours in rats. Each vertical line represents the duration of the life of one rat from the beginning of the experiment. T = tumour present at death

but they have given more vigorous autografts than have any of the earlier series, fig. 13, Plate 21. The only metastases found have been single nodules—one of these 30 mm diameter—in the left flank of two rats. Four heterografts were carried out; three were negative and the fourth failed after the third generation (*cf.* a rat tumour, LR 10, produced in

TABLE VIII—METHYLCHOLANTHRENE

Compound	Mice				Tumours	
	Initial number	Alive after 6 months	Alive after 1 year	Day of death of last mouse	Epithelioma	Papilloma
Methylcholanthrene	20	0	0	179	17	1
Methylcholanthrene (0·06% in benzene)	20	12	0	268	7	4
Methylcholanthrene (very highly purified)	10	5	0	198	6	1
" "	5	0	0	164	2	3
Methylcholanthrene (very highly purified, 0·06% in benzene)	10	6	0	252	5	1

this laboratory by 1:2:5:6-dibenzanthracene, which has now reached the 71st transplanted generation).*

It is noteworthy that the various attributes of a malignant mesodermal neoplasm (rapid growth; cellular structure; invasive power; formation of metastases; growth in autograft, and in heterograft) appear to be by no means uniformly distributed among the tumours produced by the five hydrocarbons which have been used (1:2:5:6-dibenzanthracene, 5:6-*cyclo*penteno-1:2-benzanthracene, chrysene, methylcholanthrene, 1:2-benzpyrene). Those due to methylcholanthrene show rapid growth and good development of autografts but are more or less deficient in the other characters.

The same solution of methylcholanthrene in lard was injected *sub cutem* in 10 mice; some ulceration occurred and there was a high death rate. Eight of the 10 mice died, showing no tumours, before the 148th day. The remaining 2, killed on the 152nd and 154th days, bore tumours at the site of injection which were spindle-celled and similar in microscopic appearance to those produced in mice by 1:2:5:6-dibenzanthracene and 5:6-*cyclo*penteno-1:2-benzanthracene. Grafts of these tumours gave negative results.†

Sterols, Œstrins and Related Compounds

Certain sterols and their derivatives (ergosterol, calciferol, neo-ergosterol, cholestene) and keto-hydroxyœstrin (œstrone) and some derivatives and allied compounds (tri-hydroxyœstrin, keto-methoxy-œstrin, equilin, equilenin, pregnandiol) were tested for carcinogenic action by painting on the skin of mice, Table IX. These experiments have been entirely negative, but one must bear in mind that with œstrin and some of its derivatives the life of the animals, and hence the time of exposure to the compound in question, is shortened by various obstructive effects upon the genito-urinary tract (pyometra, hydronephrosis, abscess of the coagulating gland) (Lacassagne, 1932; Burrows and Kennaway, 1934). We have not yet used solutions so dilute that these effects are absent, or of slight degree.

* The use of heterografts as a test for malignancy in tumours of this type requires to be considered in the light of the recent work of Andervont (1934), who finds that spindle-celled tumours produced by 1:2:5:6-dibenzanthracene in pure-strain mice will grow only in mice of the same strain.

†[*Note added in proof, March* 19, 1934.—Three more spindle-celled tumours have been obtained from 10 mice injected *sub cutem* with methylcholanthrene, the tumour-bearing animals being killed on the 116th, 116th and 120th days. One of these tumours is growing in the 3rd grafted generation.]

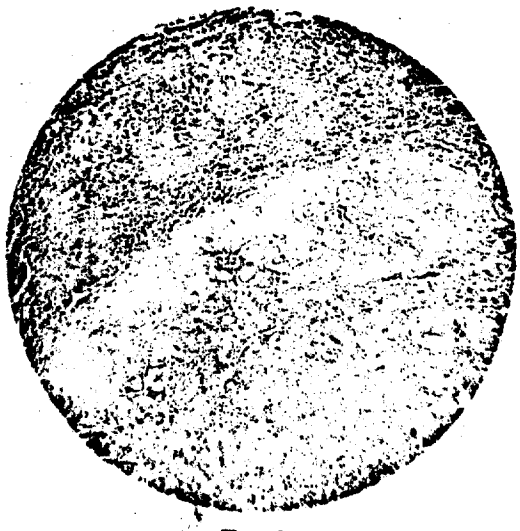


FIG. 9

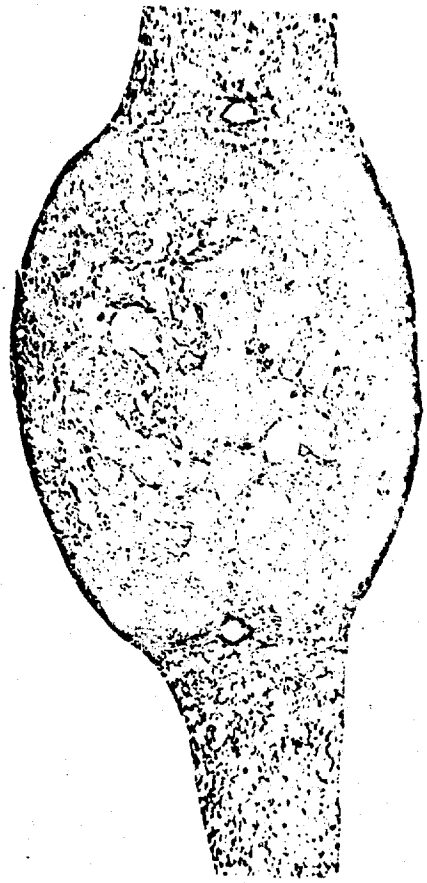


FIG. 10

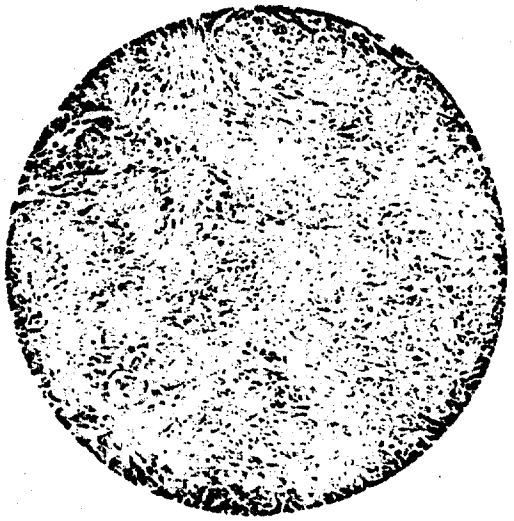


FIG. 11

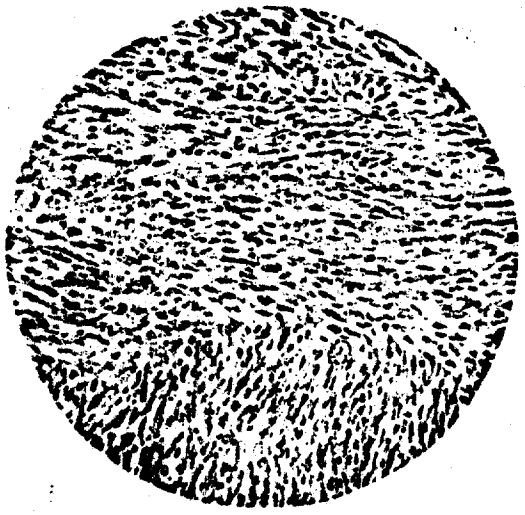


FIG. 12

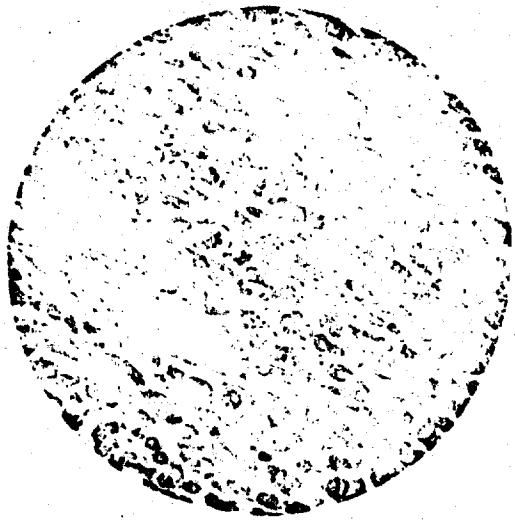


FIG. 13

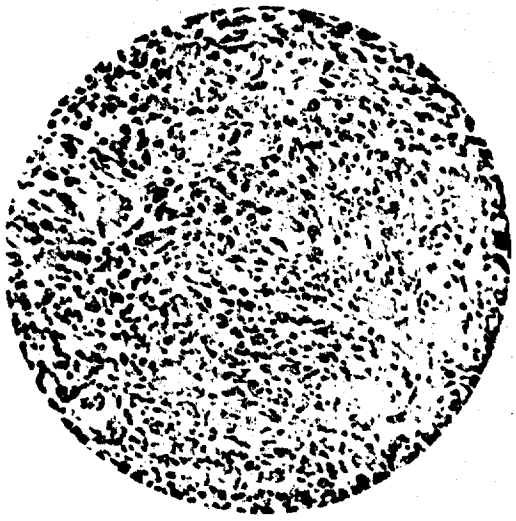


FIG. 14

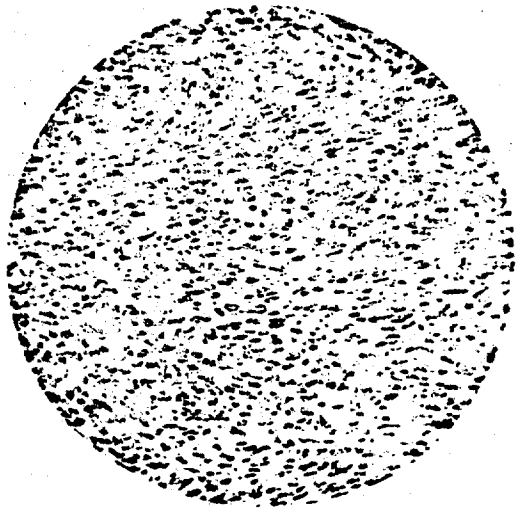


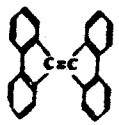
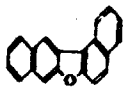
FIG. 15

TABLE IX—STEROLS, ŒSTRINS AND RELATED COMPOUNDS

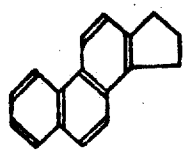
Compound	Method of preparation	Concentration % in benzene	Mice: Initial number	Mice: Alive after 6 months	Mice: Alive after 1 year	Mice: Day of death of last mouse	Duration of experiment on 1.11.34 (in days)	Tumours: Epithelioma	Tumours: Papilloma
Ergosterol		0·3	50	34	11	1 alive	597	0	0
"		0·3	20♀	18	18	4 alive	583	0	0
Calciferol		0·3	50	29	11	518	—	0	0
"		0·3	20♀	15	11	5 alive	583	0	0
Neoergosterol	Inhoffen (1932)	0·3	20	10	6	2 alive	511	0	0
Cholestene		0·3	20	14	7	587	—	0	0
Ketohydroxyœstrin—									
(1)		0·1	30	7	1	372	—	0	0
(2)		0·01	20♂	3	1	378	—	0	0
(3)		0·01	20♀	18	11	5 alive	422	0	0
(4)		0·01	11♂ C	10	6	6 alive	391	0	0
Ketomethoxyœstrin—									
(1)		0·1	20♀	15	—	6 alive	321	0	0
(2)		0·1	10♀ C	5	—	5 alive	282	0	0
(3)		0·1	20♂	12	—	5 alive	296	0	0
(4)		0·1	20♂	2	0	245	—	0	0
(5)		0·1	6♂ C	4	—	2 alive	258	0	0
Trihydroxyœstrin (0·1% in alcohol)		—	5	3	0	3 alive	236	0	0
Equilin		0·1	5♂	4	0	246	—	0	0
Equilenin		0·1	5♂	5	0	245	—	0	0
Pregnandiol (0·3% in alcohol)		—	20	16	9	8 alive	369	0	0
1 : 2-*cyclo*Pentenophenanthrene	Cook and Hewett (1933, *a*)	0·3	20	15	7	439	—	0	0
"		0·3	30	24	21	8 alive	468	0	0
1 : 9-Dimethylphenanthrene	Haworth and Mavin (1932)	0·3	20	15	6	2 alive	490	0	0
1-Keto-1 : 2 : 3 : 4-tetrahydro-phenanthrene	Haworth (1932)	0·3	10♂	10	6	535	—	0	0
" "		0·3	10♀	9	9	5 alive	583	0	0

C = castrated.

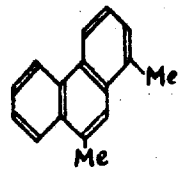
TABLE X—OTHER COMPOUNDS

Compound	Method of preparation	Mice: Initial number	Mice: Alive after 6 months	Mice: Alive after 1 year	Mice: Day of death of last mouse	Duration of experiment on 1.11.34 (in days)	Tumours: Epithelioma	Tumours: Papilloma
Synthetic anthracene		50	34	16	5 alive	615	0	0
bis-Diphenylene ethylene ..		10	8	6	553	—	0	0
								
7 : 8-Dihydrophenalyl-7-*spirocyclo*pentane	Cook and Hewett (1934)	20	15	4	3 alive	492	0	0
" "		10	10	10	5 alive	478	0	0
Carotene		10	7	1	488	—	0	0
βββα-Dinaphthafuran		10	4	2	486	—	0	? 1
								

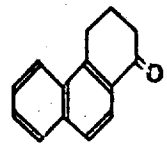
1 : 2-*cyclo*Pentenophenanthrene may be regarded as the fundamental aromatic hydrocarbon from which are derived not only the sterols and bile acids, but also the œstrus-producing hormones keto- and trihydroxy-œstrin, equilin, and equilenin. It has been tested on the skin in series of 50 mice in all for now nearly 18 months, with negative results, and it has been injected *sub cutem* (2 mg in 1 cc lard), usually at weekly intervals, in 10 rats of which 8 are now alive (326th day) and show no tumours. This compound has shown no œstrogenic action (Cook, Dodds, Hewett and Lawson, 1934). Thus various biological effects brought about by



1 : 2-*cyclo*Penteno-phenanthrene



1 : 9-Dimethyl-phenanthrene



1-Keto-1 : 2 : 3 : 4-tetrahydrophen-anthrene

compounds which may be regarded as derived from 1 : 2-*cyclo*penteno-phenanthrene must depend upon modifications of the parent molecule. 1 : 9-Dimethylphenanthrene and 1-keto-1 : 2 : 3 : 4-tetrahydrophenanthrene have given no epithelial tumours. The latter compound gave, on sub-cutaneous injection in 5 rats, 2 tumours of polymorphous cellular structure; these will be described by Mr. Burrows in another communication.

Discussion

We have now examined for cancer-producing activity 71 compounds which are not related in molecular structure to 1 : 2-benzanthracene, and 65 of these have given entirely negative results. Of the 6 compounds which gave positive results only 3 : 4-benzphenanthrene had considerable activity, fig. 1. The remaining 5 comprised pyrene (p. 323) and chrysene (p. 319) and their derivatives 4 : 5-benzpyrene, 1-phenyl-1 : 3 : 4 : 5-dibenzpyrene and 1 : 2-diphenyl chrsyene. With the exception of chrysene (which gave 1 epithelioma and 3 papillomas in 290 mice) and an impure specimen of 4 : 5-benzpyrene (which produced 1 epithelioma in 10 mice, *see* p. 327), these compounds have given only occasional papillomas. βββα-Dinaphthafuran gave one doubtful papilloma.

In all, 69 compounds related to 1 : 2-benzanthracene have now been tested and 25 of these have given positive results. With very few

exceptions, the active compounds are all derivatives of 1 : 2-benzanthracene containing substituents at positions 5 or 6, or both, these substituents being (*a*) alkyl groups, as in 5-methyl- and 6-*iso*propyl-1 : 2-benzanthracene, (*b*) saturated rings, as in 5 : 6-*cyclo*penteno-1 : 2-benzanthracene and methylcholanthrene, or (*c*) an additional aromatic ring, *i.e.*, in 1 : 2 : 5 : 6-dibenzanthracene and its derivatives. Analogous to the latter group is 1 : 2 : 5 : 6-dibenzacridine. The active compounds related to 1 : 2-benzanthracene which do not come within these categories are 1 : 2-benzpyrene and 1 : 2 : 7 : 8-dibenzanthracene, and its analogue 3 : 4 : 5 : 6-dibenzacridine. 1 : 2-Benzpyrene is more active than any other compound examined except methylcholanthrene, and forms an exception to the generalization that substituents in the 5 and 6 positions of the benzanthracene molecule are necessary for a high order of carcinogenic activity. However, 1 : 2-benzpyrene is not of the same type as the other benzanthracene derivatives under discussion, as it contains in its molecule a more highly condensed aromatic ring system than these.

The 44 compounds related to 1 : 2-benzanthracene which have given negative results are for the most part derivatives in which the substituents have been attached to the molecule at positions other than 5 and 6, or else compounds related to 1 : 2 : 5 : 6-dibenzanthracene by the attachment of additional benzene rings. The lack of activity of such hexacyclic, heptacyclic and octacyclic derivatives of 1 : 2 : 5 : 6-dibenzanthracene is in keeping with the view that there is an optimum state of molecular complexity for carcinogenic activity. In this connection it is noteworthy that an additional benzene ring destroys the activity of the 1 : 2-benzpyrene ring system, Table VII. Attention has been drawn (p. 330) to the suppression of carcinogenic activity brought about by reduction of 1 : 2 : 5 : 6-dibenzanthracene or by substitution in positions 9 and 10.

The particular compounds which we have tested for cancer-producing action are mostly polycyclic aromatic hydrocarbons or closely related compounds. Our attention was especially directed to this group of compounds in the course of investigations on the chemical nature of the carcinogenic constituents of coal tar and other carcinogenic mixtures of like nature. We fully recognize that there may be other classes of carcinogenic substances of quite different chemical type from those with which we have been concerned. In connection with such a possibility, we may observe that Browning, Cohen, Cooper, Ellingworth and Gulbransen (1933) have recorded the development of a sarcomatous growth following the injection into mice of 2-(*p*-aminostyryl)-6-(*p*-acetylaminobenzoylamino) quinoline methoacetate, a substance which they had prepared and tested for trypanocidal action.

Table XI summarizes the number of mice used and the number of tumours obtained in tests for carcinogenic activity carried out in this Institute with 140 compounds.

TABLE XI

Classes of compounds	Number of compounds tested	Number of mice	Tumours produced	
			Epithe-lioma	Papil-loma
Not related to 1 : 2-benzanthracene—				
Negative	65	2026	—	—
Positive	6	380	9	13
Related to 1 : 2-benzanthracene—				
Negative	44	619	—	—
Positive	25	1220	335	102
	140			

Thus, the 6 compounds not related to 1 : 2-benzanthracene which have given positive results gave 22 tumours (9 epitheliomas and 13 papillomas) in 380 mice. Twelve of these tumours (7 epitheliomas and 5 papillomas) were produced by 3 : 4-benzphenanthrene. On the other hand, the 25 carcinogenic compounds which are related to 1 : 2-benzanthracene have given 437 tumours (335 epitheliomas and 102 papillomas) in 1220 mice.

We are indebted to the British Empire Cancer Campaign and to the International Cancer Research Foundation for grants which have enabled two of us (I. Hieger and G. A. D. Haslewood, respectively) to take part in this investigation. We wish to thank Miss Knox, B.A., for preparing the graphs illustrating this paper and for tabulating the numerous records of experiments. We are also much indebted to the following for gifts of valuable material:—Dr. A. Girard (œstrin, equilin, equilenin); Messrs. Parke Davis & Co. (trihydroxyœstrin); Mr. W. Gordon Adam (chrysene); Dr. F. L. Pyman, F.R.S. (ergosterol and calciferol); Dr. E. de Barry Barnett (*bis*. diphenylene ethylene and 2′ : 3′-naphtha-2 : 3-fluorene); the Dyestuffs Group of Imperial Chemical Industries. Ltd. (ββ3α-dinaphthafuran); Professor S. Smiles, F.R.S., and Dr. A. Cohen (*iso*-naphthathioxin); Dr. R. A. E. Galley (1-phenyl-2 : 3 : 4 : 5-dibenzpyrene); Messrs. British Drug Houses, Ltd. (pregnandiol); Glaxo Laboratories (calciferol). We wish also to express our thanks to all our laboratory

assistants, whose good work has in various ways helped these investigations.

Summary

Tests for cancer-producing action have been carried out upon a large number of compounds, chiefly tetracyclic and pentacyclic aromatic hydrocarbons. Two derivatives of 1 : 2-benzanthracene which have considerable cancer-producing power are 1 : 2-benzpyrene, a compound isolated from coal tar pitch, and methylcholanthrene, a compound derived from the deoxycholic acid of bile. Substitution in the 5- and 6-positions of the 1 : 2-benzanthracene molecule is important in the development of carcinogenic properties. The only compound not related to 1 : 2-benzanthracene which has shown any considerable carcinogenic power is 3 : 4-benzphenanthrene.

Description of Plates

Plate 20

Figs. 9–11—Metastases from cancer of skin in mice after painting with pure hydrocarbons dissolved in benzene.

Fig. 9—1 : 2-Benzpyrene, 0·3%. Axillary gland. Day 199. × 26. 7/33.

Fig. 10—1 : 2-Benzpyrene, 0·3%. Lung. Day 143. × 26. 25/33.

Fig. 11—Methylcholanthrene, 0·06%. Right axillary gland. Day 230. × 108. 106/34.

Plate 21

Figs. 12–15—Tumours of connective tissue in rats receiving methylcholanthrene *sub cutem* in lard, fig. 8.

Fig. 12—Primary tumour. Day 144. × 144. LR 215.

Fig. 13—Autograft from tumour shown in fig. 12, made 16 days before death. × 400.

Fig. 14—Primary tumour showing giant cells. Day 191. × 144. LR 226.

Fig. 15—Primary tumour. Day 175. × 144. LR 222.

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Utilization of Vitamin A and Carotene by Selenium Poisoned Rats.* (22932)

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A previous study has shown that aqueous dispersions of carotene given intravenously to rats are readily converted to vit. A(1). In further experiments designed to obtain information about the site of conversion of carotene injected intravenously, attempts were made to block the reticuloendothelial system and also to damage the liver.

Methods. Weanling male rats of Holtzman strain were placed on synthetic, vit. A-free diet as follows: purified casein 18, salts 4(2), sucrose 74, cottonseed oil‡ 2, vit. mixture in sucrose 2(3). Half of the animals in each experiment received 12

* Part of this work was done at University of Texas Medical Branch, Galveston.

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‡ Wesson oil.

TABLE I. Storage of Vitamin A by Normal and Selenium Poisoned Rats (Mean Values ± Stand. Error).

Exp.	No. rats	Days on selenium	Avg wt	Supplement	Time interval, hr*	Liver + kidney Carotene Total, μg	Carotene μg/g	Vit. A Total, μg	Vit. A μg/g
1	5	0	147 ± 3.6	100 μg carotene orally	65	0		26.4 ± 1.3	3.9 ± .3
	5	8	129 ± 3.0		65	0		13.6 ± 2.7	2.3 ± .4
2	6	0	100 ± 4.0	95 μg carotene inj.	6	28.2 ± 1.2	6.0 ± .2	9.0 ± 1.5	2.6 ± .4
	6	22	96.1 ± 3.0		6	26.3 ± .9	6.0 ± .3	5.8 ± .2	1.1 ± .1
3	3	0	84.7 ± 6.0	68 μg carotene inj.	6	20.8 ± .7	4.7 ± .4	7.7 ± .5	2.2 ± .2
	4	14	80.0 ± 3.5		6	20.5 ± .9	4.2 ± .2	2.6 ± .8	1.0 ± .3
4	5	0	97.6 ± 5.6	100 μg vit. A orally	24	0		31.6 ± 3.7	5.8 ± 1.0
	8	7	80.0 ± 2.5		24	0		13.8 ± 1.7	2.9 ± .3
5	5	0	83.0 ± 1.6	100 μg vit. A + 0.3 mg vit. E orally	24	0		32.9 ± 2.6	7.5 ± .5
	8	7	77.1 ± 2.1		24	0		12.9 ± 1.4	3.2 ± .4

* Interval animal was permitted to survive after giving the supplement.

ppm of selenium, as sodium selenite, in the diet. Due to differences in maternal diet, some groups became depleted of vit. A in 17-21 days while others required 30-35 days. Selenium did not affect rate of depletion. Food intake of control rats was restricted to the amount consumed by the selenium-fed group. Animals were caged singly in air-conditioned room at 75 ± 1°F. Supplements of crystalline carotene (85% β–, 15% α–) dispersed in water with 20% Tween 40 or of vit. A acetate dissolved in cottonseed oil,‡ were administered by tail vein or by stomach tube when vit. A deficiency was apparent. The rats were sacrificed 6 hours after injection, or 33-65 hours after stomach-tubing. In the latter groups, selenium was removed from diet 18 hours prior to giving carotene or vit. A supplement. All animals were fasted at least 8 hours prior to death. Vit. A in combined kidneys and livers was determined with antimony trichloride on an aliquot of the hexane extract of the unsaponifiable fraction, using correction for color due to carotene. Analyses of deficient rats revealed no detectable vit. A in livers and kidneys. Rats depleted of vit. A as described above were injected with trypan blue or carbon tetrachloride prior to administering carotene, and the tissues analyzed for vit. A. The conditions are given in Table II.

Results. In Table I it can be seen that utilization of carotene, as measured by tissue storage of vit. A, was impaired in rats fed selenium. Regardless of whether carotene was given orally (Exp. 1) or intravenously (Exp. 2 and 3), the amount of vit. A was only about one half that of control animals. To determine if selenium might diminish utilization of pre-formed vit. A, 2 groups were dosed orally with vit. A acetate (Exp. 4). Tissues of selenium-treated animals again contained less than one-half as much vit. A as normal animals. In Exp. 5, dl,α-tocopherol was incorporated into the vit. A supplement to protect the vitamin against possible oxidation in the digestive tract. This failed to prevent decrease in storage of vitamin in selenium-fed animals.

Discussion. Rosenfeld and Beath(4) found that in selenium fed sheep a marked drop in vit. A content of blood and liver occurred, even though the diet contained an adequate amount of carotene. Ascorbic acid in these tissues was also significantly depressed by selenium.

In the present study, it is possible that selenium impaired intestinal absorption of vit. A. In view of the relatively efficient utilization of orally administered carotene, however, it appears that this factor was not of great significance. The effect of selenium on vit. A may be explicable by two different mechanisms, first, that seleniferous tissues destroy vit. A, but not carotene, and secondly, that poisoned liver may have decreased capacity for holding the vitamin. It has been shown *in vitro* that ascorbic acid is oxidized by se-

TABLE II. Carotene Utilization by Rats Injected with Trypan Blue or Carbon Tetrachloride.*

Treatment	No. rats	Avg wt	Carotene supplement	Vit. A, μg/g Liver	Vit. A, μg/g Kidneys
None	4	113 ± 6.5	120 μg, I.V.	2.7 ± .2	1.9 ± .1
Trypan blue†	6	107 ± 2.5	*Idem*	2.1 ± .6	2.3 ± .4
CCl_4‡	6	113 ± 2.7	"	2.4 ± .1	2.1 ± .3

* All rats were killed 6 hr after inj. with carotene.
† Inj. I.P. with 1 ml of 2% trypan blue; inj. with carotene 24 hr later.
‡ Inj. subcut. with 0.2 ml CCl_4 in cottonseed oil (50% w/vol) for 3 consecutive days; inj. with carotene on fourth day.

lenium salts and this mechanism was proposed as the cause of decreased ascorbic acid in livers from selenium-poisoned rats(5). However, we have not observed increased destruction of vit. A incubated in rat blood containing added sodium selenite (unpublished observation).

In support of the second possibility, that selenium alters storage capacity of the liver, the recent study of Krishnamurthy and Ganguly(6) may be quoted. They found that rats injected with India ink decreased liver storage of vit. A ester, but not of vit. A alcohol. This would support the theory previously proposed by these workers(7) that different proteins may specifically bind various carotenoids in blood. The fact that storage of carotene was not affected in our study, whereas storage of vit. A was, would lend further support to this theory. However, it should be pointed out that the 2 types of poisons (selenium and India ink) damage different structures in livers. In contrast to the results of Krishnamurthy and Ganguly, impairment of reticulo-endothelial system by trypan blue had no effect on carotene utilization in our study (Table II).

The observation that rats with selenium-poisoned livers could still convert carotene injected intravenously supports our previous conclusion(1) that this organ is not of significance in conversion of pro-vitamin. Recently, McGillivray *et al.*(8) have shown that injected carotene can be transformed to vit. A in the rat even when the entire liver has been removed.

Summary. Rats fed sodium selenite had impaired utilization of carotene given orally or intravenously. Storage of pre-formed vit. A was reduced, but not storage of injected carotene. Poisoning with trypan blue or carbon tetrachloride did not affect carotene conversion.

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Studies on the Intestinal Absorption of Radioactive β-Carotene and Vitamin A in Man

Conversion of β-carotene into vitamin A

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Abstract: BLOMSTRAND, R. & WERNER, B. Studies on the Intestinal Absorption of Radioactive β-Carotene and Vitamin A in Man. *Scand. J. clin. Lab. Invest.* 19, 339-345, 1967. β-carotene-15, 15'-^{14}C, β-carotene-15, 15'-^{3}H, vitamin A alcohol-15-^{14}C, and vitamin A-15-^{3}H acetate were fed to eight patients in whom polyethylene cannulae had been inserted in the thoracic duct in the neck. The lymph lipids were extracted and chromatographed on columns and on thin-layer plates of alumina. Only 8.7-16.8 per cent of the fed labelled β-carotene is normally absorbed via the lymphatic pathway and mainly recovered as retinyl esters. Unchanged labelled β-carotene comprised only 1.7 to 27.9 per cent of the radioactivity after ingestion of labelled β-carotene.

The recovery of the radioactivity in the lymph lipids after feeding labelled vitamin A alcohol or acetate was in the range of 6.7 to 66.9 per cent. Absorption of radioactivity into the lymph followed the appearance of the chylomicron triglycerides.

Labelled retinyl esters, mainly retinyl palmitate, represented 75 to 90 per cent of the absorbed radioactivity after feeding labelled vitamin A, and 60 to 75 per cent after feeding labelled β-carotene. It is concluded that the reaction mechanism of vitamin A biosynthesis in the human intestinal mucosa consists of central cleavage of β-carotene into two molecules of vitamin A alcohol.

In one patient fed β-carotene-15, 15'-^{14}C, 46.9 per cent of the fed radioactivity was recovered as β-carotene, suggesting the presence of an enzymatic block in the conversion mechanism.

Index-words: Carotene; vitamin A; gastrointestinal system; carbon isotopes; hydrogen isotopes

Since the demonstration by von Euler, von Euler & Hellström (6) and Moore (12), that β-carotene relieved vitamin A deficiency in rats, the mechanism of its conversion into vitamin A has been the subject of intense interest. Two major mechanisms have been suggested. The first is the cleavage of β-carotene into two molecules of vitamin A as initially proposed by Karrer, Helfenstein, Wehrli & Wettstein (10). Secondly it was suggested by Glover & Redfearn (7), that one molecule of vitamin A might be formed from one molecule of β-carotene by a stepwise oxidation of β-carotene from one end of the conjugated central chain.

Thompson, Braude, Coates, Cowie, Ganguly & Kon (17) demonstrated the appearance of retinyl ester*, but not of β-carotene, in the mesenteric lymph of pigs and rats given test meals of β-carotene. Wagner, Wyler, Rindi & Bernhard (18) fed ^{14}C-labelled β-carotene to lymph fistula rats and observed the appearance of labelled retinyl

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* The terms retinol, retinal, retinoic acid, and retinyl ester refer to vitamin A alcohol, aldehyde, acid, and ester respectively.

ester, but not of labelled β-carotene in lymph. Using ^{14}C-labelled β-carotene in studies on rats, Olsson (13, 14) obtained results which supported the theory of a central cleavage mechanism. Furthermore he showed that β-carotene is absorbed from the intestine and is converted into vitamin A only in the presence of bile acids. Huang & Goodman (9) fed radioactive β-carotene and retinol dissolved in oil to rats and analysed the lymph lipids. Retinyl esters were the predominant labelled components in the lymph. There was no absorption of unchanged labelled β-carotene into the lymph. In a recent monograph on the absorption of lipids via the lymphatic pathway in man (4) we presented preliminary data on the absorption of labelled β-carotene and vitamin A suggesting a significant difference in the absorption of β-carotene and vitamin A in man. In the present report these experiments have been extended after feeding ^{14}C-labelled β-carotene, β-carotene-15, 15'-^{3}H, vitamin A alcohol-15-^{14}C and vitamin A-15-^{3}H acetate to patients in whom polyethylene cannulae had been inserted in the thoracic duct in the neck.

MATERIAL AND METHODS

Labelled compounds. We are grateful to Dr. U. Gloor of Hoffman-La Roche, Basel, Switzerland, for a generous gift of labelled β-carotene and retinol. Retinol-15-^{14}C (specific radioactivity 29.6 μCi/mg) and retinyl-15-^{3}H acetate (specific radioactivity 100 μCi/mg) were used without further purification. β-carotene-15, 15'-^{3}H (specific activity 1.09 mCi/mg) and β-carotene-15-15'-^{14}C (specific activity 15 μCi/mg) was purified on a column of deactivated alumina (Woelm II) just before use.

Table I. *Clinical data for the case series*

Patient	Age	Sex	Diagnosis
1	50		Mammary carcinoma (status post) Pulmonary metastases
2	52	♂	Lipoma in supraclavicular region + cholelithiasis
3	39		Mammary carcinoma
4	58		Mammary carcinoma
5	55	♂	Chronic lymphatic leukemia
6	52	♂	Gastric carcinoma
7	63	♂	Oesophageal carcinoma
8	72	♂	Biliary dyskinesia

Collection of samples. Thoracic duct lymph was collected from eight patients. Clinical data on those patients are given in Table I. Cannulation of the thoracic duct in the neck was carried out according to Werner (20). The patients fasted overnight before cannulation. Fasting lymph was collected for a few hours after cannulation. Blood samples for lipid analyses were collected from a cubital vein after the operation. On the day of operation the patients were given the labelled compounds in varying amounts of vegetable oil. All patients were in the fasting state when they received the formula. The composition of the test meals is given in Table II. During the investigation, the patients were allowed only water, tea, or coffee, except in case 5 who was allowed a free diet.

The lymph was collected every second hour by gravity drip in heparinized vessels, kept in a refrigerator at 4°C. The cells were removed by slow speed centrifugation and the samples were held at −20°C until analyzed.

In patient 8, who had a low lymph flow, the lymph was not collected in fractions. A cumulative absorption curve can therefore not be given in this case.

The clinical and experimental procedure is depicted in Fig. 1.

Lipid extraction and analyses. In each study, the time course of the absorption of the labelled compounds was determined by extracting 1.0 ml of each lymph sample with 20 ml $CHCl_3$: MeOH (2:1, v/v) followed by radioassay of the total lipid extract so obtained. The lymph samples containing the main peak of absorbed radioactivity were then pooled for subsequent analyses. A mixture of unlabelled carriers consisting of 250 μg of retinol plus 100 μg each of β-carotene, retinal, retinoic acid, and each of 4 retinyl esters (palmitate, stearate, oleate and linoleate) was added to

Table II. *The liquid formula given to the patients*

Patient	Type of fat	g	Glucose g	Protein (egg white) g	Water ml	Skim milk ml	Isotope fed	Amount (μCi)
1	Olive oil	22	41.5	37.5	160	–	β-carotene-15, 15'-^{14}C	20
2	"	2	–	–	–	50	β-carotene-15, 15'-^{3}H	50
3	Corn oil	5	–	–	–	50	β-carotene-15, 15'-^{14}C	6
4[a]	Soybean oil	2	–	–	–	50	β-carotene-15, 15'-^{3}H	50
4[b]	"	2	–	–	–	50	Retinol-15-^{14}C	20
5	Olive oil	22	41.5	–	160		Retinyl-acetate-15-^{3}H	97
6	"	50	–	–	–	–	Retinyl-acetate-15-^{3}H	192
7	"	2	–	–		50	Retinol-15-^{14}C	10
8	"	10	–	–		50	Retinol-15-^{14}C	10

[a] Formula given the first day of cannulation.
[b] Formula given the second day of cannulation

each sample at the time of extraction. The lower chloroform phase was collected and evaporated to dryness. The total lipid extract was weighed, dissolved in benzene and stored under argon at −15°C.

Portions of each lipid extract were chromatographed on columns of deactivated Al_2O_3 (Woelm, grade II) as described by Olsson (13, 14). A column of 5 g of deactivated alumina was eluted in the following way: Fraction 1, β-carotene (20 ml n-hexane); Fraction 2, retinyl esters (20 ml benzene-hexane, 3:17); Fraction 3, retinal (20 ml benzene: hexane, 1:1); Fraction 4, retinol (50 ml benzene); Fraction 5, more polar compounds (20 ml methanol). Portions of each column fraction were assayed for radioactivity.

The composition of the labelled retinyl esters was determined by a combination of two different chromatographic separations as described by Huang & Goodman (9). A carrier mixture of unlabelled retinyl esters, comprising 50 μg each of retinyl palmitate, stearate, oleate and linoleate was added to each sample just prior to chromatography. Separation of the retinyl esters on the basis of the degree of unsaturation of the fatty acid component was first accomplished by thin-layer chromatography on alumina gel C impregnated with silver nitrate. The saturated, and other, esters were then separated according to fatty acid chain length by reversed phase chromatography on silicone impregnated paper.

Other procedures. Triglycerides were determined by a modified procedure of Blankenhorn, Rouser & Weimer (2).

The fatty acid composition of the total lipid extract of each sample was determined by gas-liquid chromatography of the methyl esters of the fatty acids as described previously (8). Radioassay of ^{14}C and ^{3}H was carried out by dissolving a sample in 15 ml of 0.6 per cent 2,5-diphenyloxazole in toluene, followed by assay with a Packard Tri-Carb liquid scintillation counter.

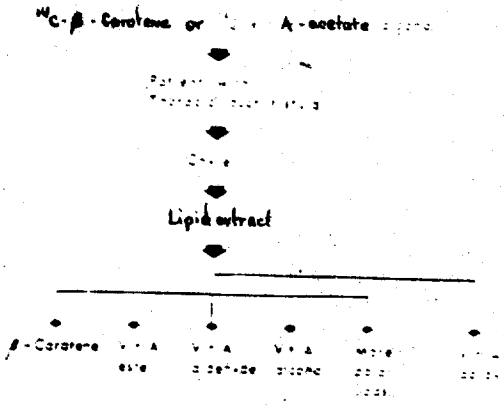

Fig. 1. Clinical and experimental procedures

Table III. *Recovery of the radioactivity in human thoracic duct lymph after oral administration of the test meals described in Table II*

Patient	Isotope fed	Collection time of lymph after test meals hours	Per cent of administered radioactivity recovered in lymph
1	β-carotene-15, 15[1-]^{14}C	18	14.6
2	β-carotene-15, 15[1-]^{3}H	22	8.7
3	β-carotene-15, 15[1-]^{14}C	22	52.3
4*a*	β-carotene-15, 15[1-]^{3}H	21	16.8
4*b*	Retinol-15-^{14}C	20	21.4
5	Retinyl-acetate-15-^{3}H	26	66.9
7	Retinol-15-^{14}C	18	41.4
8	Retinol-15-^{14}C	20	6.7

RESULTS

Table III shows the percentage of administered radioactivity recovered in the lymph lipids in the different patients, and Fig. 2 the cumulative absorption curves. It is apparent from these data that the labelled β-carotene is not absorbed as well as labelled retinol and retinyl acetate. In two patients (Nos. 1 and 2) only 14.6 and 8.7 per cent of the fed radioactivity is recovered in the thoracic duct lymph lipids, and in three studies (Nos. 4*b*, 5, and 7) where labelled vitamin A alcohol or acetate were fed 66.9–21.4 per cent of the fed radioactivity was recovered. On the other hand there seem to be individual differences. In one patient (No. 8) fed ^{14}C-retinol, only 6.7 per cent of the fed radioactivity was recovered in the lymph lipids. It should be emphasized that all of the patients showed a normal lymph flow during the investigation. One of the patients (No. 3), fed ^{14}C-labelled β-carotene, had a remarkably high recovery of 52.3 per cent of the fed radioactivity in the lymph lipids.

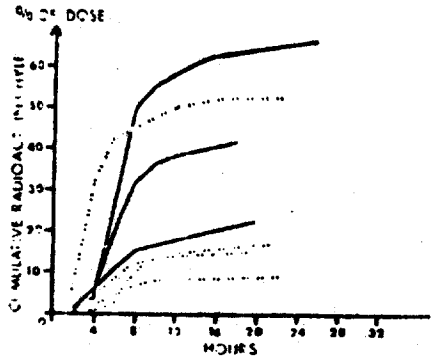

Fig. 2. Cumulative absorption curves of labelled β-carotene (- - -) and labelled vitamin A acetate and alcohol (——) in thoracic duct lymph of different patients.

Fig. 3 shows the time course curves after feeding labelled β-carotene. The appearance of the radioactivity in the lymph lipids coincided with the peak of lymph triglycerides. The main peak of absorption after feeding both β-carotene and retinol appeared between four and eight hours after feeding.

Table IV summarizes the distribution of radioactivity in the thoracic duct lymph in four subjects given labelled β-carotene (patients 1, 2, 3, and 4*a*). In these patients most of the radioactivity was found in the retinyl ester fraction (Fraction 2) which contained about 68 to 88 per cent of the total radioactivity in the lymph lipids. Analysis of a portion of the retinyl ester fraction from each lymph sample demonstrated that the radioactivity in this column fraction definitely resided in long-chain fatty acid esters of retinol. Smaller amounts of radioactivity were also recovered in the β-carotene fractions (Fraction 1), except in patient No. 3, who had significant amounts of activity in that fraction.

For four patients (Nos. 4*b*, 6, 7 and 8) fed labelled retinol or retinyl ester, the distribution

Table IV. *Percentage distribution of radioactivity in human lymph after ingestion of radioactive β-carotene*

Alumina column fraction	Patient 1	Patient 2	Patient 3	Patient 4a
β-carotene	1.7	11.3	89.6	27.9
Retinyl esters	87.9	73.2	2.1	68.2
Retinal	5.5	6.3	7.0	2.3
Retinol	4.9	9.2	1.3	1.6

Table V. *Percentage distribution of radioactivity in human lymph after ingestion of radioactive retinol or retinyl ester*

Alumina column fraction	Patient 4b	Patient 6	Patient 7	Patient 8
β-carotene	3.0	6.3	4.3	1.4
Retinyl esters	88.9	82.6	89.2	92.3
Retinal	5.8	7.0	2.9	1.8
Retinol	2.3	4.1	3.6	2.5

of radioactivity in lymph is presented in Table V. In all patients most of the radioactivity absorbed into the lymph was found in the retinyl ester fraction which contained 82.6 to 92.3 per cent of the absorbed radioactivity.

DISCUSSION

The studies reported here demonstrated that during the intestinal absorption of dietary β-carotene in man, the carotene is largely converted to vitamin A, and the newly synthesized vitamin A is then esterified and transported in the lymph. Dietary vitamin A is absorbed to a higher degree than dietary β-carotene. In one patient investigated the labelled β-carotene was as well absorbed as vitamin A and in this patient the β-carotene was not converted to vitamin A. Dietary retinol is almost completely esterified with long-chain fatty acids, and the retinyl esters are then transported via the lymphatic route, mainly in the form of lymph chylomicrons in the same way as the newly synthesized vitamin A. Apparently, in general, the human intestine possesses only a limited ability to absorb unchanged dietary carotene into the lymph.

The composition of the lymph retinyl ester was notably constant in all the patients investigated. Retinyl palmitate predominated in all samples, and saturated esters (retinyl palmitate stearate) consistently comprised approximately 80 per cent of the labelled esters. A detailed report on the analysis of the retinyl esters of the thoracic duct lymph and chylomicrons in some of these patients will be published elsewhere (8). The results of the present investigation indicate that there is one significant difference between man and rat: the finding that man is capable of absorbing small but significant amounts of unchanged dietary β-carotene into the lymph, whereas virtually no dietary β-carotene can be absorbed intact into rat lymph (9, 14).

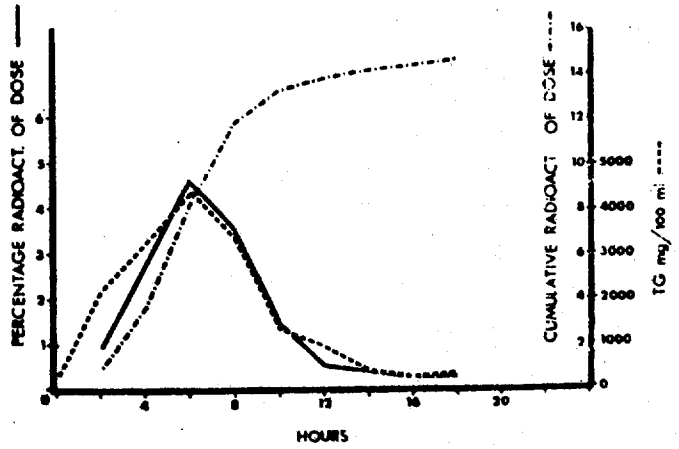

Fig. 3. The time course of the absorption of radioactivity into the thoracic duct of patient (No. 1) after ingestion of β-carotene-15, 15-^{14}C. TG= triglycerides

The labelled β-carotene employed in these studies contained ^{14}C at the central two carbon atoms (C-15 and 15') or was labelled with tritium at the central carbon atoms. In one patient fed β-carotene-15, 15'-^{14}C essentially the same results were obtained after analysis of the lymph lipids as after feeding β-carotene-15, 15'-^{3}H. The results with the ^{14}C-labelled β-carotene together with the experiments with tritium-labelled β-carotene indicate conclusively that the reaction mechanism of vitamin A biosynthesis consists of the central cleavage of β-carotene into two molecules of retinol in man. There is a possibility that some ^{3}H label might be lost during the metabolism of this β-carotene during its conversion to vitamin A. Studies with rat intestinal mucosa by Goodman et al. (8) have demonstrated that the hydrogen atoms attached to the central two carbon atoms of β-carotene are completely retained during this conversion process. β-carotene-15, 15'-^{14}C and β-carotene-15, 15'-^{3}H hence are both suitable compounds to use alone or in combination in studies of the metabolism of β-carotene.

The patient (No. 3) who after ingestion of ^{14}C-labelled β-carotene absorbed 52.3 per cent of the fed radioactivity without significant conversion into vitamin A might have a block in the enzymatic mechanisms involved in this conversion. This might represent an inborn error of metabolism, a primary defect in the intestinal mucosa. Unfortunately, due to malignancy, we have not been able to follow up these observations on this patient.

The presence of hypercarotenemia in various pathological conditions has been observed by many investigators (2, 5, 11, 15, 16, 19). The mechanism of hypercarotenemia is in many instances obscure. It has been suggested that the conversion of β-carotene to vitamin A is impaired in diabetes and hypothyroidism (5, 16). From the present investigation it is apparent that there are normally variations in the degree of intestinal absorption and conversion of β-carotene to vitamin A. Furthermore in extreme cases there might be a complete block in the enzymatic mechanisms involved in the conversion of β-carotene to vitamin A. Such a defect might then explain some cases of hypercarotenemia, where an extreme dietary intake of carotenoids can be excluded.

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Retinitis Pigmentosa

Vitamin A Serum Levels in Relation to Clinical Findings

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The majority of subjects affected with retinitis pigmentosa show a subnormal level of vitamin A and of carotenoids in the blood. This feature persists for many years both in hereditary and non-hereditary cases, and in a number of their unaffected relatives.

The absorption of vitamin A has been found to be normal, and the serum level of vitamin A can be raised by continuous therapy with vitamin A, vitamin E, vitamin A and E together, or by the administration of β-carotene. It falls again within a few weeks of the cessation of treatment.

The promotion of the blood vitamin A to a normal level over a considerable period has a beneficial effect on the retina in an appreciable number of cases. It is made evident by an increase of over 10% in the total area of both visual fields (binocular field area) and by an improvement in dark adaptation as shown by the measurements of the cone and rod threshold.

The degree of vitamin A deficiency which is found in retinitis pigmentosa is discussed in relation to the carotenoid/vitamin A ratio in the blood and to the mode of action of the vitamins selected for therapeutic trial.

1. Introduction

In discussing the levels of vitamin A in the blood of subjects affected with retinitis pigmentosa in relation to the clinical findings we must first draw attention to the essential nature of the disease, and recall some of our earlier data concerning vitamin A.

Human retinitis pigmentosa is a rare disease characterized by night blindness and by two primary features—a particular form of pigmentary degeneration of the retina and a progressive loss of the visual fields. Over 60% of the cases are hereditary, and two-thirds of these occur in male subjects.

The retinal changes are not often visible ophthalmoscopically until early adolescence, but are usually preceded by night blindness. The course of the disease can best be assessed by relating the degree of loss of visual field with the decline in dark adaptation. In the early cases both changes reflect a loss of rods rather than of cones, since the field loss begins in the equatorial part of the retina in which rods preponderate, and progresses until only a very small central area remains (Fig. 1).

The first indication of a decline in dark adaptation (Fig. 2) is a rise in the final rod threshold as measured after an interval of 30 min. (a) In severe cases cone function may be affected simultaneously (b) but in the majority the cone threshold taken after 7 min of dark adaptation does not decline until rod function has already been seriously affected.

The examination of a large number of cases has shown a correlation between the extent of dark adaptation and the residual area of retina (Campbell, 1962). There is in fact an overall linear relationship between the logarithmic value of the field area, whatever the shape of the field, and the logarithmic values of both the cone and rod thresholds, particularly the latter. This indicates a fundamental relationship between the number and function of live visual cells, which in the case of the rods may be concerned with their ability to accumulate rhodopsin.

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Owing to the rarity of the disease it has taken many years to collect a group of cases sufficiently large to enable us to carry out a reliable clinical analysis of this disease, which varies tremendously in its rate of progress in individual subjects. There is often a rapid deterioration during adolescence and the disease thereafter progresses much more slowly or may become stationary. This suggests that there may be some hormonal dysfunction at the time when the individual reaches maturity.

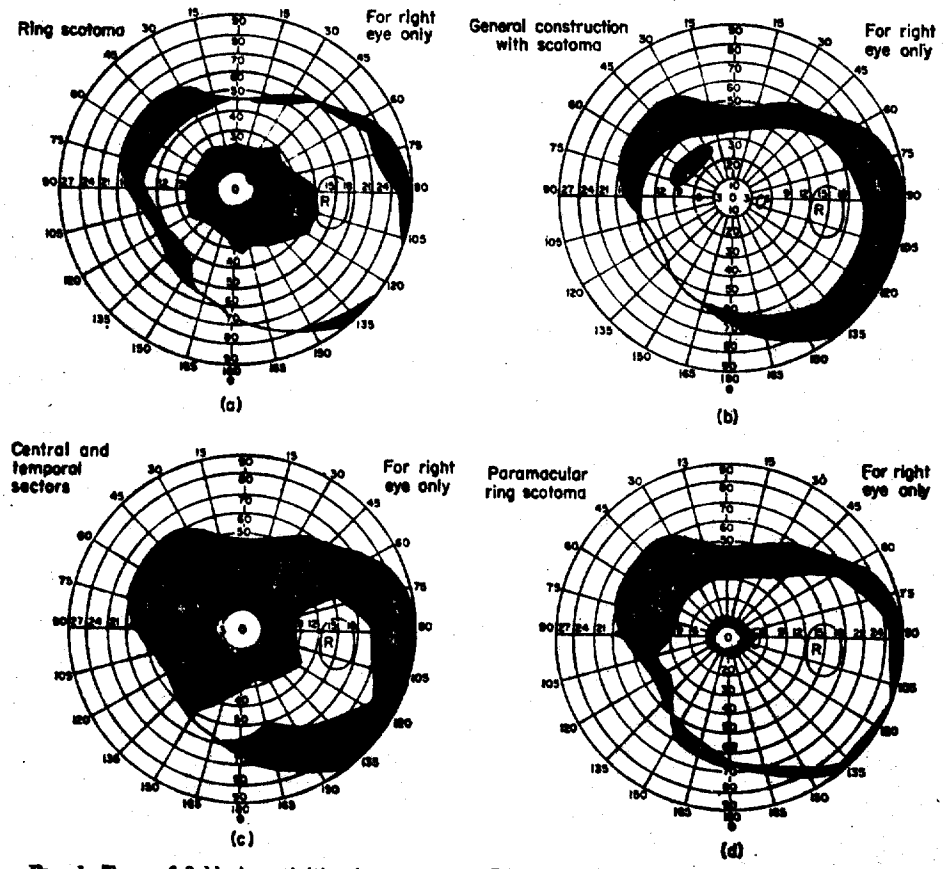

FIG. 1. Type of fields in retinitis pigmentosa. (a) Ring scotoma, (b) field reduced to central and temporal sector, (c) early localized scotoma, (d) paramacular ring scotoma.

In a group of 100 subjects studied at the Birmingham Eye Hospital no clinical difference could be found between hereditary and non-hereditary cases.

The early histological changes in the human disease, owing to the scarcity of pathological specimens, are not well known, but there is an undoubted resemblance between the early case described by Verhoeff (1931) and the corresponding stage of degeneration found in a strain of rats affected with hereditary retinitis pigmentosa as described by Bourne, Campbell and Tansley (1938).

Of even greater interest is the close resemblance of the disease in rats to the condition first described by Johnson (1939) and later investigated by Dowling (1960), which can be produced in the rat retina as the result of vitamin A deficiency.

The resemblance is so marked as to suggest that the human disease might exhibit a

similar deficiency. A biochemical survey, carried out in 1962 (Campbell and Tonks, 1962), proved that this was the case (Figs. 3 and 4). Whereas all the ordinary constituents of the blood were present in normal amounts, the levels of vitamin A and carotenoids were subnormal in 91% and 70% of the cases, respectively.

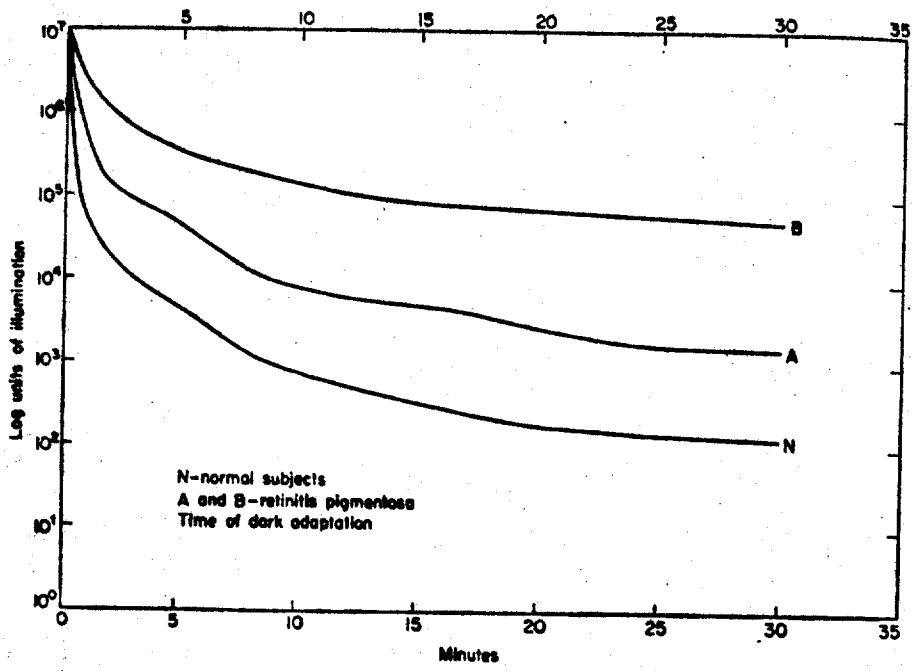

FIG. 2. Dark adaptation in retinitis pigmentosa in comparison with normal. A, early; B, advanced case.

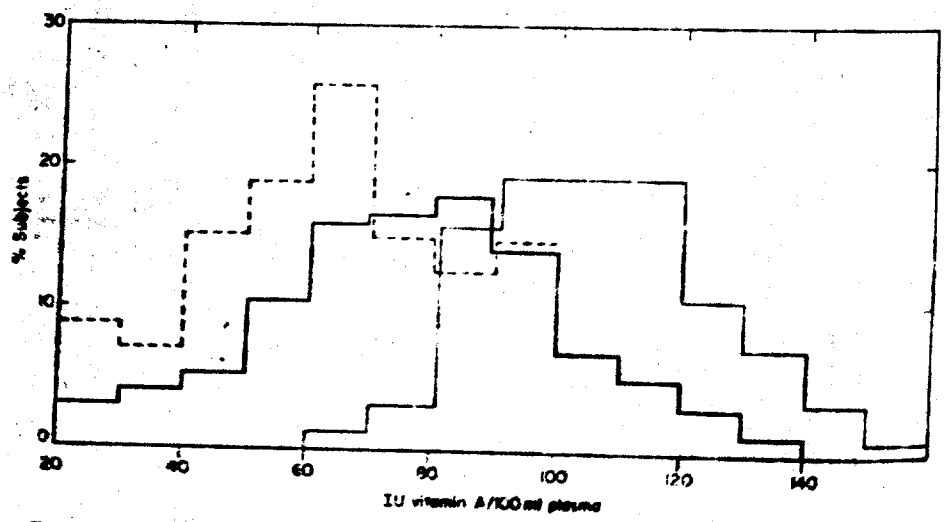

FIG. 3. Percentage distribution of blood vitamin A in retinitis pigmentosa in children and adults [illegible] pigmentosa children.

The mean value for vitamin A was only 82 IU and for carotenoids 96 μg/100 ml. This is well below the present-day normal average of 131 IU. A and 137 μg carotenoids quoted by Moore (1957, p. 362). The figure for vitamin A is even below the value which two of us (D. A. C. and E. L. T.) found for a Midland population when still on rations in 1949, viz. 108 IU with carotenoids at 85 μg/100 ml.

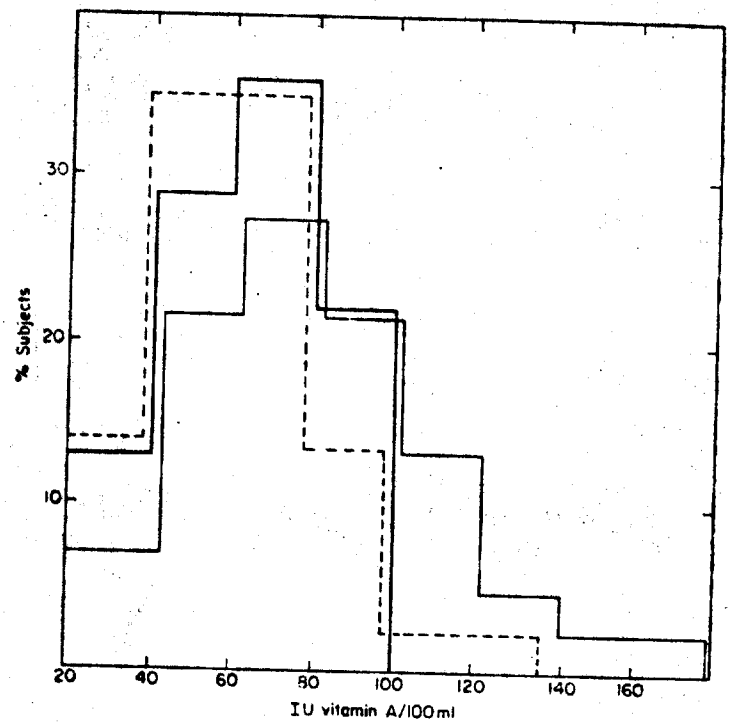

FIG. 4. Percentage distribution of blood vitamin A in children. (- - -) Normal, from relatively poor homes; (——) normal, on excellent diets according to data of Josephs, Baber and Conn (1941); (—) R. pigmentosa.

The subnormal values persist for many years in untreated subjects and have been found not only in hereditary and non-hereditary cases alike, but also in their unaffected relatives (Campbell and Tonks, 1962).

The values obtained both for vitamin and carotene were exceptionally low in affected children. There are a few published figures for normal children, but Josephs, Baber and Conn (1941) related their vitamin A figures to the state of nutrition and, in comparison, our retinitis pigmentosa children gave values which fell between two groups of normals, (a) those "from relatively poor homes but with evidence of good care and attention" and (b) "children from well-to-do homes with excellent diets" (Fig. 4).

These observers commented on the fact that it was "only in the presence of the worst conditions of diet that vitamin A in the serum gives evidence of deficiency, or that there was any difference in dark adaptation." This was again fully demonstrated in the case of normal adults by the "Sheffield" experiment on vitamin A deficiency carried out by Hume and Krebs in 1949.

The observations of Josephs et al. (1941) seem to indicate that a low level of vitamin A in children is a normal feature, but that in the untreated case of retinitis pigmentosa

it does not subsequently rise to the normal value in adult life. We say this advisedly because, when rationing came to an end, the blood vitamin A level in some of the affected subjects undoubtedly rose, and a series of absorption tests showed that the majority were able to absorb vitamin A quite well (Campbell and Tonks, 1962). It appeared, however, that the vitamin A in the blood could not be maintained at a normal level on an ordinary diet.

Although the level of carotenoids was subnormal, we at first regarded it as insignificant, having regard to the wide fluctuation which can occur in relation to the individual intake of carotene, but it proved to be unaffected by the general improvement in diet. Mr. Shearer has obtained evidence which points to the fact that carotene is being converted immediately to vitamin A to make up for the deficiency of the latter.

2. Therapeutic Trials of Vitamin A and Related Vitamins

From the foregoing evidence it would appear that the loss of retinal function in retinitis pigmentosa may arise not only from a lack of the appropriate enzymes which carry out the conversion of vitamin A to rhodopsin in the retina itself, but also from a systemic defect which results in an inadequate supply of vitamin A to the retina.

In order to explore this possibility a therapeutic trial has been carried out, over a considerable period, of the effect of certain vitamins upon retinal activity—viz. vitamin A; vitamin E which acts as a "sparer" of vitamin A; of vitamin A and E given together; and of β-carotene.

Methods

The results were assessed throughout the period of therapy by (1) serial measurements of the visual fields of both eyes, by (2) records of binocular dark adaptation, and by (3) estimations of the current fasting levels of vitamin A, and of the carotenoids in the blood (method as described by Campbell and Tonks, 1949). Parallel investigations were carried out on a control group receiving no treatment.

The serial measurements of visual fields were made by one of us (R. H.) using a Hamblin perimeter under a standard illumination of 65 lux, and were measured by a planimeter. Concurrent binocular dark adaptation was recorded by the "integral" method on the Goldmann adaptometer. The methods used have been described in detail elsewhere (Campbell, 1962).

It should be stated that since the field test may be regarded as a subjective test, an increase in the binocular field area was only regarded positive when it exceeded 10% of the original area.

In the integral method for testing dark adaptation the whole area of both retinae is illuminated by the test light—the head of the subject being inside a globe lined with white. As is evident from the figures, the dark adaptation curve obtained in this way does not always show a clear rod-cone break—as is the case when a point fixation light is used. We were, however, able to use the dark adaptation curve of a congenitally night-blind subject as the standard for a pure cone response. It was monophasic and reached its maximum after 7 min, at log intensity of illumination $10^{4\cdot6}$. The curves for affected subjects, as described by François (1961), were diphasic or monophasic according to the degree of loss of rod and cone function.

The results obtained by this test proved to be remarkably constant in the untreated subject and an improvement of 0·3 log units in the cone threshold at 7 min, or in the final rod threshold at 30 min, was regarded as positive.

Owing to the individual variation in size and shape of field and in the extent of the dark adaptation, the results have been summarized for each group on the criteria already stated according to the percentage of subjects showing improvement, no change or decline in the area of the binocular field, rod threshold and cone threshold.

The daily dose of vitamin was chosen as being that amount which raised the fasting

value of vitamin A in the blood to a normal level. No signs of hypervitaminosis have been observed, although some subjects have been on treatment for as long as 6 years with intervals of 3–6 months without therapy. The longest uninterrupted course on any one vitamin has been 31 months.

3. Vitamin A Therapy

In the therapeutic trial with vitamin A, 32 subjects who had been under observation for some time were given 24,000 IU daily in the form of Roavit, and the results compared with an untreated group of 29 subjects.

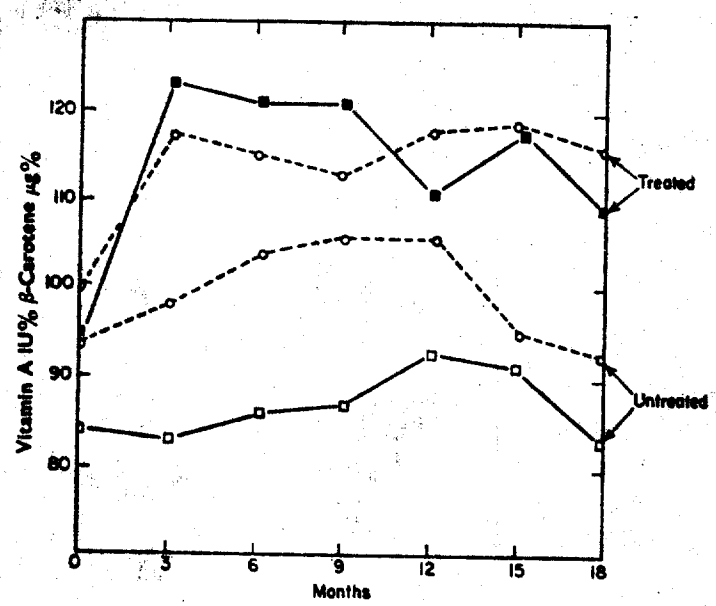

FIG. 5. Average blood levels of vitamin A IU% and carotenoids μg% in 32 subjects treated with vitamin A and in 29 untreated. Vitamin A: — ■—■, treated; □—□, untreated. Carotene: ●—●, treated; ○—○ untreated.

TABLE I

Treatment with vitamin A

	32 subjects treated		
	% Improved	Unchanged	Declined
Binocular field	47	28	25
Rod threshold	37	52	11
Cone threshold	33	63	4

	29 subjects untreated		
	% Improved	Unchanged	Declined
Binocular field	0	30	70
Rod threshold	3	90	7
Cone threshold	3	83	14

comparison with a rise of only 84–93 IU/100 ml in the untreated cases. The level fell again within 2–3 weeks of the cessation of treatment. No less than 47% of the cases showed an increase in visual fields—and there was a parallel decrease in the number showing a decline. An appreciable number also showed an improvement both of cone and of rod function.

Subject 12 is an individual example showing a surprising degree of improvement both in the visual field and in dark adaptation, although his initial levels of vitamin A and of carotenoids were both low (Fig. 6).

4. Treatment with Vitamin E

There are some interesting records of the protective action of vitamin E in pigmentary degeneration of the retina. For instance, Babel (1944) reported that vitamin E will give protection in rabbits against the experimental production of retinitis pigmentosa by injections of sodium iodate.

There are also several accounts of its therapeutic value in human retinitis pigmentosa. One of the earliest is that of Wibaut (1938) who reported a good response in 14 out of 45 cases; after treatment with Menformon in weekly doses of 50,000 IU vitamin E, given intramuscularly, there was an increase in visual fields and an improvement in dark adaptation. Meyer (1941) also found an enlargement of visual fields, with a decline on cessation of treatment with vitamin E, while Dominguez (1950) reported favourably on the use of L-tocopherol in cases of retinitis pigmentosa and in one case of retinitis punctata albescens.

Hegner in 1949 reported his close observation of a girl of 18 affected with retinitis pigmentosa whose visual fields and dark adaptation declined at the onset of menstruation. Several years ago Dr. Gebertt and one of us (D. A. C.) observed a similar phenomenon in several subjects (unpublished work).

Hegner's case improved on treatment with vitamin E and he suggested that the action of this vitamin might be connected with the increased production of gonadotrophins by virtue of its stimulating action on the anterior lobe of the pituitary. It is interesting to record in this respect that among our cases were two sisters who were pituitary dwarfs, with a low urinary excretion of gonadotrophic hormones and a low level of vitamin A and of carotene in the blood.

These records from previous observers, together with the more recent knowledge that vitamin E can act as a "sparer" of vitamin A by virtue of its antioxidant properties were the reason for including vitamin E in our therapeutic trials. It was given alone to a group of 25 patients, and in combination with vitamin A to a second group of 27 patients, in each case over a period of 12 months.

Dosage. Vitamin E was given in the form of Ephynal 50 mg twice daily, with or without 24,000 IU vitamin A daily.

5. Results

The results are shown best by a comparison of the two groups. In both instances there was an initial rise in the vitamin A in the blood, which was maintained through-

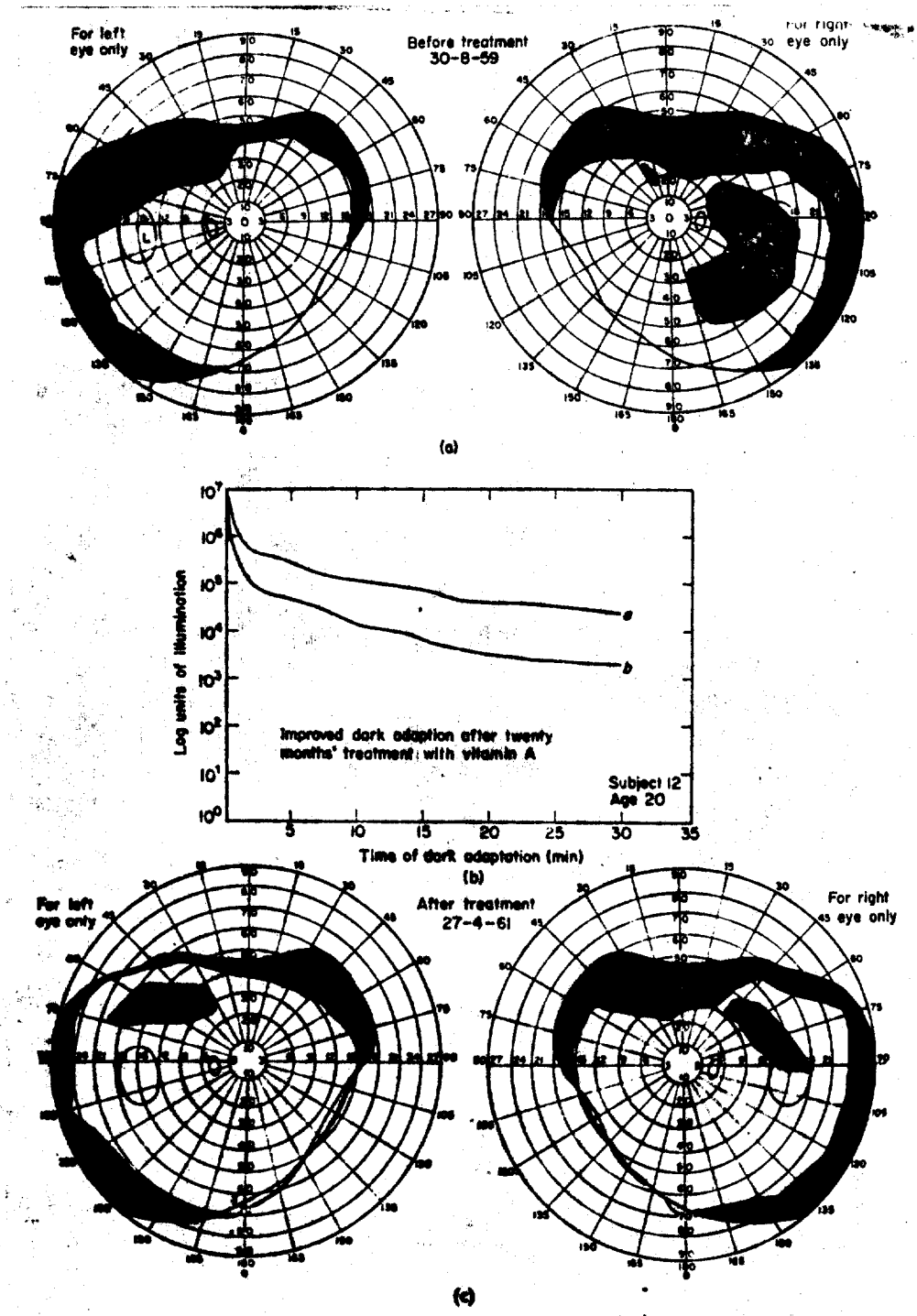

FIG. 6. Improvement in fields and in dark adaptation after vitamin A therapy in man aged 20 (subject 12).

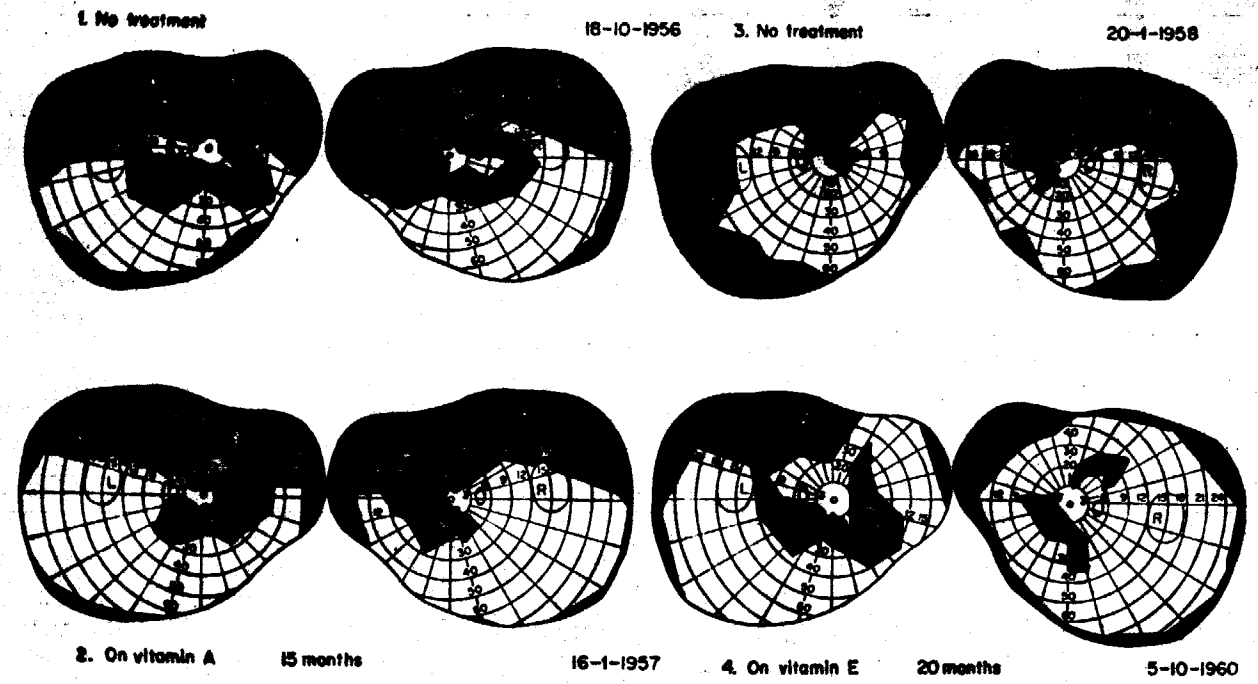

FIG. 8. Effect of vitamin A and vitamin E on visual fields (case 53).

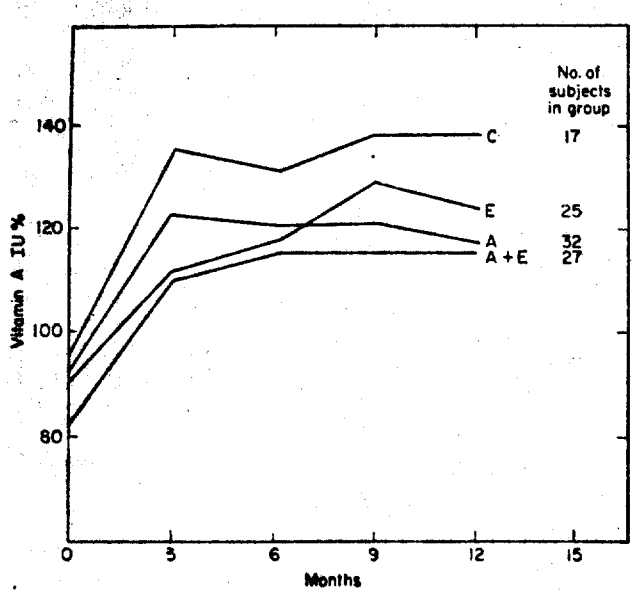

FIG. 7. Average rise in blood vitamin A on therapy. Daily dose (C) carotene 100 mg, (E) ephynal 100 mg, (A) vitamin A 24,000 IU, (A + E) 24,000 IU A + 100 mg vitamin E.

TABLE II

Response to therapy with vitamins A and E

	Response to vitamin E therapy 25 subjects		
	% Improved	Unchanged	Declined
Binocular field	60	20	20
Rod threshold	21	68	11
Cone threshold	42	42	16

	Response to vitamin A and E 27 subjects		
	% Improved	Unchanged	Declined
Binocular field	67	22	11
Rod threshold	24	71	5
Cone threshold	38	62	0

out the period to a maximum of 128 IU/100 ml after vitamin E alone, and 118 IU/100 ml after A and E (Fig. 7).

The number of subjects showing an expansion of visual field was notably higher on E, and on A and E, than on A alone.

A large number also showed improved cone function, particularly on vitamin E, while the number showing improvement in rod function was lowest on E, slightly higher on A and E, and highest on A alone.

Vitamin E proved to be most efficacious in young subjects, of whom case 53 is an

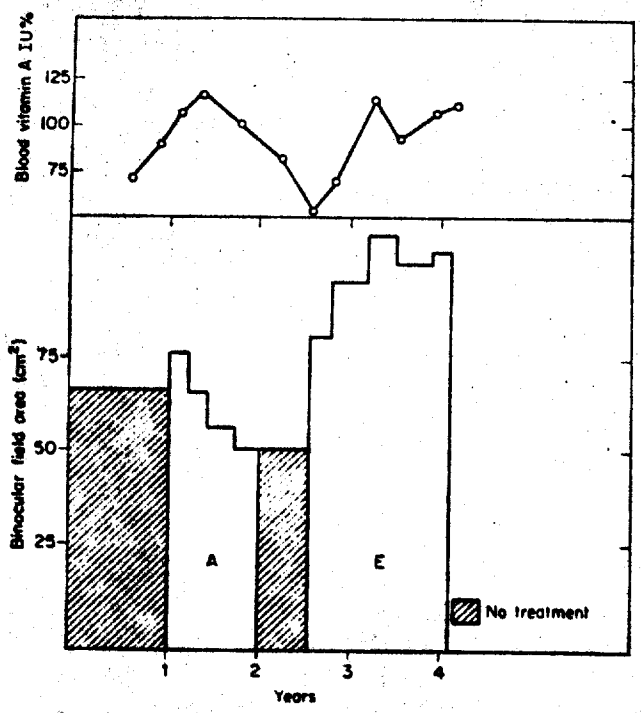

FIG. 9. Effect of vitamin A and vitamin E on serum vitamin A and on field area (case 53).

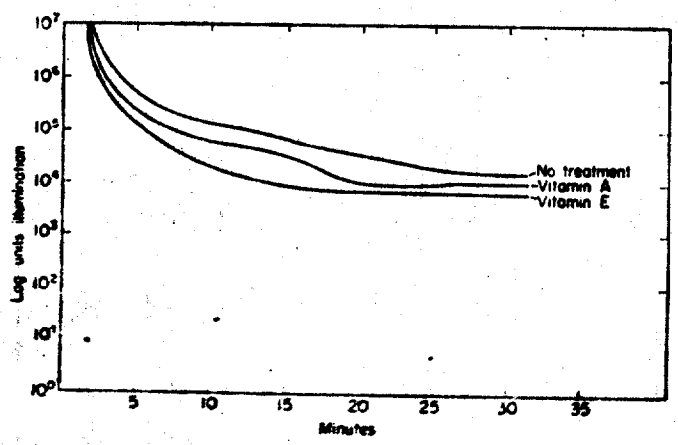

FIG. 10. Effect of vitamin A and vitamin E on dark adaptation (case 53).

example. He was a boy observed from the age of 12, and treated from the age of 15 over a period of 3 years, first with vitamin A for 12 months and then, after an interval of 6 months, with vitamin E for 20 months. On vitamin A his visual fields at first improved, but then declined. On vitamin E there was a much more marked improvement, particularly in the left eye (Figs. 8 and 9). His dark adaptation curve before treatment

showed a total loss of rod function and a considerable loss of cone function. Nevertheless, it improved considerably with both vitamin A and vitamin E, particularly in respect of cone function (Fig. 10).

6. Treatment with Carotene

There is only one reported trial of carotene as a remedy for retinitis pigmentosa. Josephson and Freiberger (1937) observed that large doses given orally were without effect, whereas injections of from 30,000 to 60,000 new USP units given one to three times a week produced a relief of night blindness within a week in all the early cases. Continuation of this treatment restored dark adaptation to "normal" in 1–4 months.

Method

A small group of 17 patients, all of whom had a low level of carotenoids in the blood, was available for trial and received a daily dose of 100 mg of β-carotene over a period of 12 months. It was given as gelatin-impregnated beadlets which will form an aqueous suspension.

Results

On this dose the vitamin A in the blood rose from 97 to a maximum of 138 IU/100 ml (Fig. 7), i.e. to a higher level than after the administration of either vitamin A or vitamin E, or of A and E in combination. The effect on the cone and rod thresholds was, however, only moderate.

TABLE III

Response to β-carotene

	17 subjects		
	% Improved	Unchanged	Declined
Binocular field	35	47	18
Rod threshold	29	57	14
Cone threshold	29	50	21

7. Comparison of Results

In comparing the various forms of therapy it is evident that the level of vitamin A in the blood can easily be raised and maintained for an appreciable period at a normal level

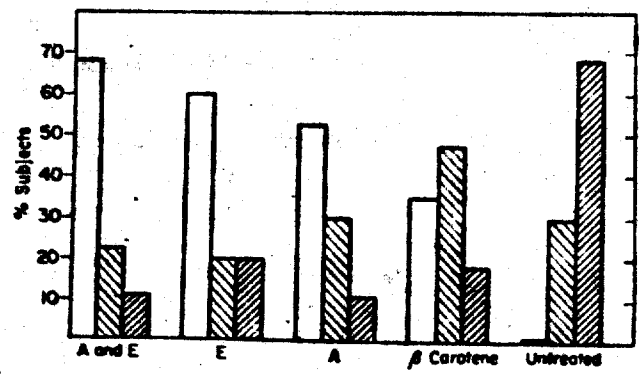

FIG. 11. Response obtained in visual fields by vitamin therapy. ☐ improved, ▨ unchanged, ■ declined.

by any one of the vitamins used, but it falls again in each case within a few weeks on the cessation of treatment.

The beneficial effect on the retina of each vitamin can be compared by the percentage of subjects showing an enlargement of the binocular visual field and an improvement in dark adaptation, as well as by those showing a decline.

In the case of vitamin A therapy the effect on the blood level of A can be attributed to the absorption of an increased amount of vitamin A, whereas vitamin E probably achieves the same effect by "saving" the vitamin A available in the body from the diet. When β-carotene is given the rise must be due to its active conversion into vitamin A.

In no instance was there any urinary excretion of vitamin A.

TABLE IV

Carotenoid/vitamin A index in blood

		Mean values		
		Untreated	During therapy	
Normal subjects	Walton		92·0	
	Campbell and Tonks		94·6	
R. pigmentosa subjects				
	Controls		115	
	on vitamin A		127	96
	Vitamin E		116	93
	Vitamin A and E		125	91
	β-Carotene		68	65

Dr. Walton (1964) has referred in his paper to the value of the carotene/vitamin A ratio in the blood as a useful index of the state of deficiency of vitamin A. His figure of 92·0 ± 25 for normal subjects compares favourably with the mean of 94·6 calculated from the figures of Campbell and Tonks (1962). It also agrees quite closely with

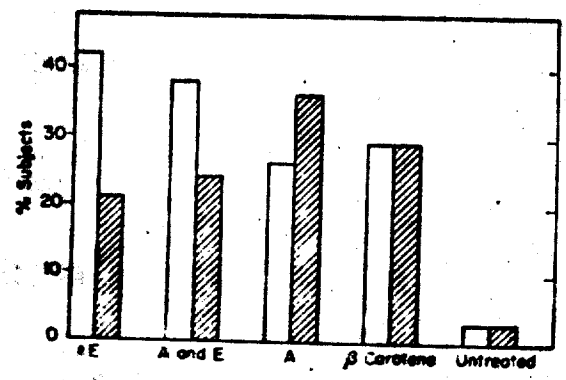

Fig. 12. Percentage of subjects showing improvement in dark adaptation on vitamin therapy. □ Cone threshold, ▨ rod threshold.

the average maximum index attained during these therapeutic trials, except in the case of those subjects treated with β-carotene. The very low initial index in this group before treatment was due to the fact that their average level of carotenoids was relatively much lower than that of vitamin A (61 μg as compared with 90 IU/100 ml).

In spite of the fact that their vitamin A rose to a higher level than with the other forms of vitamin therapy, the index remained uniformly low, and indicated a state of deficiency which was being remedied by excessive conversion of β-carotene.

β-Carotene also had the least effect on the visual fields, although it improved the dark adaptation, particularly rod function, in an appreciable number of subjects.

7. Discussion

The improvement in visual fields and dark adaptation that took place during the therapeutic trials may be attributed to an increase in the availability of vitamin A to the retina. In comparison, according to Fang, Hall and Hwang (1960), no improvement in dark adaptation occurs in normal subjects after giving large doses of vitamin A or of vitamin E, although the rod threshold can be lowered by a massive dose of A and E together (200,000 IUA + 400 mg of tocopherol).

Again the work of Hume and Krebs (1949) has shown that normal subjects have to be deprived of vitamin A for a very long period before they show a decline in dark adaptation. In the "Sheffield experiment" there were 3 subjects affected in this way and their mode of recovery, as summarized by Moore (1957, p. 379), is of particular interest in relation to the curative effect of vitamin A and of β-carotene.

The first subject, after 14 months' deprivation, was given 1,300 IU of preformed A daily with an improvement in dark adaptation which began after 2 weeks and continued slowly for 6 months. During this time the level of vitamin A in the blood rose from 22 IU to a maximum of 88 IU/100 ml. Increasing the dose to 2,600 IU/day had little effect.

The second subject, after 18 months' deprivation, received 1,250 IU of β-carotene daily for a month, with a rise in the level of the vitamin A in the blood from 28–63 IU, but a further decline in dark adaptation. On increasing the dose to 2,500 IU the dark adaptation was still subnormal after 6 months—the blood level of vitamin A was much the same and the carotenoids had only risen to 42 mg/100 ml.

The third subject, after 22 months' deprivation showed a remarkable improvement in dark adaptation in 7½ hr after a single dose of 2,500 IU carotene. After 3 weeks the blood vitamin A had risen from 19–96 IU, but the carotenoids had only increased from 17 to 31 μg/100 ml.

The close similarity between the response of these A-deficient subjects to treatment with vitamin A or with β-carotene, and that of retinitis pigmentosa subjects, lends further support to the suggestion that the latter have no ability to store vitamin A and that they depend largely on their carotene to make up for their deficiency of A.

The subnormal levels of vitamin A and of carotenoids in the serum of subjects affected with retinitis pigmentosa is an expression of their deficiency of available vitamin A. Although the absorption of A and its transport may be normal, the absorption of β-carotene is often defective, thus depriving the subject of an alternative source of supply of vitamin A.

Dr. Scott's work suggests that this type of retinal degeneration arises not solely from a lack of vitamin A, but possibly from some accompanying protein deficiency. Dr. Dowling has shown that, in the rat, there is a histological difference between the early stage of the degeneration in the dystrophic rat as compared with the vitamin A-deficient animal. The similarity between the two conditions is not quite so close as we at first thought.

The prospect of finding the additional factor or factors is, however, improved by the

ability to maintain an A-deficient animal in good health by giving vitamin A acid, as described by Dowling (1960) and by Thompson (Thompson and Howell, 1964).

Our results have shown that it is possible to alleviate the disease in man, and to delay the onset of blindness, by the therapeutic administration of vitamin A and/or of vitamin E in suitable doses, controlled by concurrent estimations of vitamin A in the blood. The therapeutic use of β-carotene does not appear to be so satisfactory.

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OBSERVATIONS ON CAROTENEMIA *

By THE LORD COHEN OF BIRKENHEAD, M.D., D.Sc., LL.D., F.R.C.P., F.A.C.P. (Hon.), *Liverpool, England*

YELLOWISH pigmentation of the skin (*xanthodermia*) is most commonly due to jaundice; it is then accompanied by characteristic changes in the mucous membranes, blood, urine and feces. It is, however, seen also when substances such as picric acid, saffron and mepacrine are ingested and stain the tissues. In Great Britain during World War II, when fats were in short supply, xanthodermia was met not infrequently, due to an excessive intake of carrots which followed an exhortation from the Ministry of Food on their virtues. This condition of carotenemia had been earlier described under the names *aurantiasis* and *carotenosis cutis*, but it was regarded as a rare phenomenon.

It was the occurrence of hypercarotenemia during this period, and the fact that my colleague, Professor R. A. Morton, was working in the biochemical field of carotenoids and vitamin A, which stimulated an investigation on the clinical types of carotenemia.

CAROTENOIDS

Carotenoids are pigments of plant origin, and there is no evidence that animals can synthesize them from simpler compounds. Karrer, Kuhn and their collaborators have established the structural formulae of the principal carotenoids. Their general formula is $C_{40}H_{56}O_n$, where n is 0–6. The hydrocarbons (here $n = 0$) are termed *carotenes*, and the hydroxyl derivatives, *xanthophylls*.

The carotenoids occur in all green vegetables and especially carrots, and are also found in butter, eggs and oranges. (Goodwin [4] gives complete

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analyses of the carotenoid content of various foods.) Beta-carotene is the most important precursor of vitamin A; it comprises 10 to 30% of the total pigment and is often accompanied by cryptoxanthin (3-hydroxy-beta-carotene), another vitamin A precursor. In animals vitamin A is stored chiefly in the liver but to a lesser extent in the kidney. It is mainly in the form of an ester but is liberated continuously in small amounts into the blood-

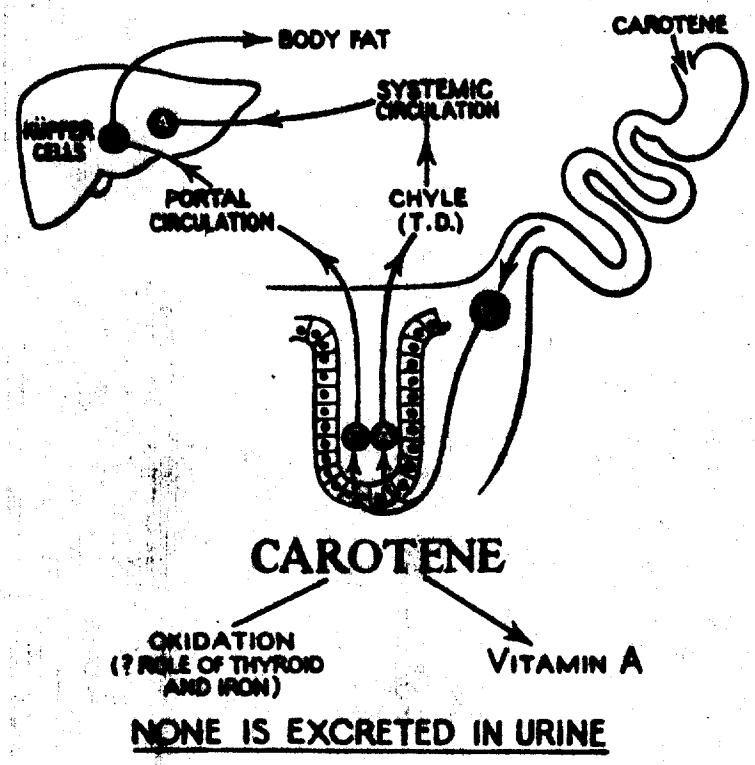

FIG. 1. Shows ingestion of carotene (C); followed by (a) absorption to storage in liver and body fat and (b) conversion into vitamin A (A) in intestinal mucosa, then absorption through thoracic duct and storage in liver.

stream as free vitamin A alcohol. Normally there are 100 μg. of carotenoids per 100 ml. of blood, and the xanthophyll : carotene ratio is 2 : 1. The earlier name of *lipochromes* points to their fat solubility.

Moore [7] was the first to demonstrate that carotene is a vitamin A precursor. He recorded an increase in the level of the vitamin A stores of rats receiving carotene, and it was assumed that the liver was the site of conversion, though there can be no doubt from recent work that the main conversion into vitamin A is extrahepatic and occurs in the intestinal mucosa (figure 1), though this is not the exclusive site. The amount of beta-carotene absorbed from different foodstuffs varies with their nature; for example, it is less from

non-fatty foods. Mineral oils such as liquid paraffin seriously reduce carotene absorption. (For a comprehensive discussion of this problem see Lowe and Morton [6] and Morton and Goodwin.[8])

There is evidence that absorption of beta-carotene varies directly with thyroid activity in some species, though it may well be that this is an indirect effect, for a rat with hyperthyroidism may accumulate more vitamin A than a normal rat, either because improved absorption of beta-carotene means that more is presented to the enzyme system responsible for the conversion, or, alternatively, that the thyroid hormone renders the enzyme system more effective.

The normal range of vitamin A in the blood is 120 to 150 i.u. per 100 ml., and deficiency symptoms, especially of dark adaptation, tend to appear when the level remains below 50 i.u. per 100 ml. for a year or longer.

Carotenemia Due to Excessive Ingestion

We have observed 50 cases of well marked hypercarotenemia due to a raw carrot intake of from four to eight pounds daily. Most of these were seen during World War II, but a few were observed both before and since. In recent years a raw carrot diet to induce slimming has been a prominent cause. Xanthodermia tended to appear after about six to eight months, but if carrots were then omitted from the diet the yellowish tinge faded in from two to six weeks. The main sites of the pigmentation were the face (especially the nasolabial folds) and the palms and soles. The mucous membranes and conjunctivae were clear, and no carotene was found in the urine. There were no subjective symptoms, and the patients were usually referred with "jaundice" or "anemia."

The excessive carotene in the blood can be demonstrated and differentiated from jaundice by the simple method of Greene and Blackford [5]: "Equal volumes of serum, absolute alcohol, and petroleum ether are shaken together in a test-tube, and left to stand. In carotenemia, the top layer of petroleum ether shows a yellowish discoloration. In jaundice, it is in the middle layer of alcohol that the bile pigments are dissolved and these often colour also the bottom layer of precipitated proteins." Carotene often rose to 500 μg. per 100 ml. in this group of cases; the xanthophyll : carotene ratio remained more than 1, and vitamin A was always present in excess, though the blood lipids were normal.

Carotenemia in Diabetes Mellitus

Before the introduction of insulin the most marked examples of carotenemia were seen in severe diabetics, especially in children who were on a severely restricted carbohydrate diet which consisted largely of "5%-carbohydrate" green vegetables. Here excessive intake was clearly the prime but not the only cause, for even without excessive intake a diabetic may show carotenemia. A possible explanation is the accompanying hyperlipemia, for

in many hyperlipemic xanthomatoses, and in nephrotic states, carotenemia is commonly found.

Case 1. This patient was a 27 year old female with *familial xanthomatosis palpebrarum* and carotenemia. Blood analysis showed:

Vitamin A	77	i.u./100 ml. plasma
Carotenoids. Total	243	μg./100 ml. plasma
Carotene	70	μg./100 ml. plasma
Xanthophyll	130	μg./100 ml. plasma
Total lipid (two hours after food)	1.19	gm./100 ml. plasma

Case 2. This patient was a 16 year old boy with *a nephrotic syndrome.* Blood analysis showed:

Vitamin A	86	i.u./100 ml. plasma
Carotenoids. Total	259	μg./100 ml. plasma
Carotene	76	μg./100 ml. plasma
Xanthophyll	160	μg./100 ml. plasma
Total lipids (fasting)	1.30	gm./100 ml. plasma
Cholesterol	340	mg./100 ml. plasma

It is, however, interesting to note that Rosenberg and Sobel[9] showed that alloxan-diabetic rats had an impaired capacity to convert beta-carotene into vitamin A in the isolated intestinal mucosa. If this were true of the human diabetic it might explain in part the carotenemia, but there is no evidence of associated vitamin A deficiency in the diabetic as there is in the patient to be described later, in whom carotenemia is demonstrably associated with vitamin A deficiency.

Carotenemia in Myxedema

It is more than 20 years since Escamilla et al.[8] described carotenemia in myxedema, and this has been amply confirmed since. For example, in two patients under the care of Cadman and myself the following changes were found:

Table 1

Carotenemia in Myxedema

Case	Date	Carotenoid μg./%	Carotene μg./%	Vitamin A μg./%	Cholesterol mg./%	Thyroid Condition	Note
S.	July '51	375	145	46	605	Myxedema	Untreated
	May '52	105	—	28	198	Almost euthyroid	Treated
	Nov. '52	76	—	23	147	Normal	
H.	Oct. '53	119	31	33	409	Myxedema	Untreated
	3 wks. later	62	18	29	250	Much improved	Treated

The mechanism whereby these changes occurred engaged our attention, and Cadman[1] undertook a study of the influence of dysthyroidism on the blood carotene and vitamin A to decide whether the thyroid hormone plays a significant part in the conversion of carotene into vitamin A.

Carotene and Thyroid Function

Reference was made earlier to the fact that in certain species the thyroid hormone aids the absorption of carotene from the intestine, though this has not been shown in man.

The factors which have been demonstrated to influence the plasma carotene are (1) the diet; (2) the stability of carotene in the intestinal tract; (3) the rate and amount of carotene absorption; (4) the rate of conversion of carotene into vitamin A; (5) the rate of removal of plasma carotene by liver or tissues; (6) the possible effects of other blood constituents, e.g., proteins, lipids.

Cadman's work was directed toward determining which of these factors might be influenced by the thyroid hormone. Using spectrophotometric methods, chromatography and the Carr-Price reaction, he established values for serum carotenoids, carotene and vitamin A in the normal state and in hyperthyroid and hypothyroid states. These are summarized in table 2.

Table 2

Clinical State	No. of Cases	Carotenoids μg.% plasma		Carotene μg.% plasma		Xanthophyll μg.% plasma		Vitamin A μg.% plasma	
		Means S.D.*	Ranges	Means S.D.*	Ranges	Means S.D.*	Ranges	Means S.D.*	Ranges
Normal	61	50±17	21–94	15±7	4–31	36±11	16–78	36±11	6–68
Hyperthyroidism	13	43±19	14–85	14±7	7–37	29±14	5–52	34±11	19–62
Hypothyroidism	16	105±81	33–375	33±31	10–145	72±52	22–230	36±8	24–48

* S.D. = standard deviation.

From this it can be seen (1) that there are wide ranges in the plasma carotenoids, carotene and vitamin A levels in both normal and dysthyroid states; (2) that the normal and hyperthyroid values for carotenoids and vitamin A cover approximately the same ranges, and (3) that the standard deviations of the carotenoids in the hypothyroid are considerable, and overlap the corresponding normal values, but reach much higher maximal figures. The plasma vitamin A rises but little in hypothyroidism, so that the ratio of carotene to vitamin A increases. Moreover, although high levels of blood cholesterol and lipids were found in this series of hypothyroid patients, no clear evidence of a constant correlation between carotene, vitamin A and cholesterol was found. The two cases of myxedema which have been quoted illustrate the return to normal of plasma levels of carotene during effective treatment.

Although in the hyperthyroid group there is no significant variation from the normal range of plasma carotene, when such patients are treated and rendered euthyroid there is a trend toward higher carotene levels as the clinical condition improves.

It is not yet possible to answer with certainty the question, Does the

thyroid hormone in man facilitate the absorption of carotene or promote its conversion into vitamin A, or both? But it is highly probable that the promotion of the conversion of carotene to vitamin A by thyroid is an important element, for the following reasons:

The level of plasma carotene is the difference between the amount absorbed and the conversion of carotene to vitamin A in the wall of the gut. In hyperthyroidism the blood carotene is lowered; the work of Cama and Goodwin [2] suggests that the carotene directly absorbed is increased, thus conversion of carotene into vitamin A must be increased. Conversely, in hypothyroidism blood carotene is increased but absorption is decreased; thus conversion of carotene into vitamin A must be decreased.

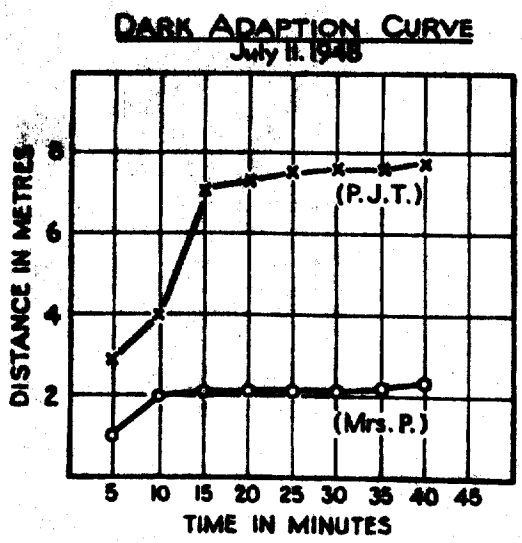

FIG. 2. Shows the dark adaptation curve of Mrs. D. P. when first seen, compared with a normal.

Cadman found also that the plasma xanthophyll levels undergo changes parallel to the plasma carotene levels, suggesting that although the xanthophylls are not provitamin A, thyroid plays a part in the destructive oxidation of the xanthophylls.

CAROTENEMIA AS AN INBORN ERROR OF METABOLISM

We have observed what is, in our experience, a unique case in which it would appear that hypercarotenemia resulting from a failure to convert carotene into vitamin A is due to an inborn error of metabolism.

Case 3. It is now nearly nine years since we first saw Mrs. D.P., then aged 38 years, who was referred as "hemolytic jaundice" or "anemia." She gave a history dating back several years of a yellow tinge of the skin and some loss of weight. There was no relevant past illness or family history, and she had taken a normal diet.

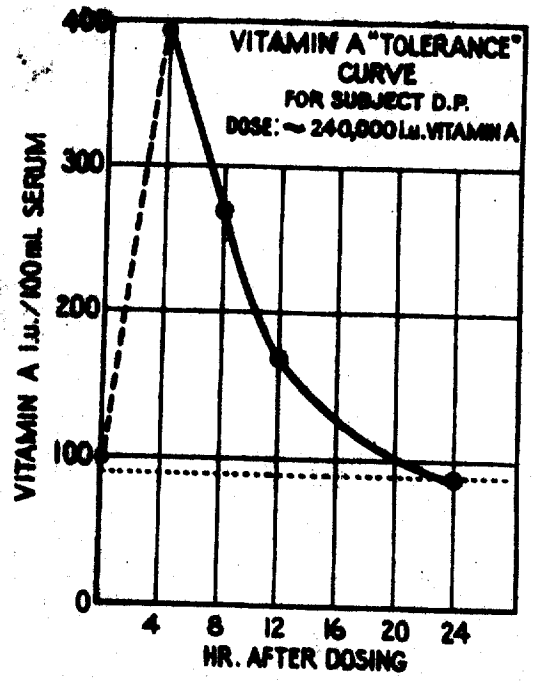

FIG. 3. Shows a normal vitamin A absorption by Mrs. D. P. after 240,000 i.u. vitamin A.

TABLE 3

Blood Analyses (Mrs. P.)

Date	Comments	Cholesterol mg./100 ml.	Vitamin A i.u./100 ml.	Carotenoids µg./100 ml.		
				Total	Carotene	Xanthophyll
	Normal values	120-220	120-150	100	30–60	50–70
					C<X	
21-5-48	Admission	156	15	282	162	120
8-7-48	After 6 weeks of adequate vitamin A		100	206	135	71
13-7-48	Four samples taken			175 218 178 181	136	38
19-7-48	Low carotene diet started: additional vit. A, B, C, D.		100	140		
21-9-48	Oxalated blood Clotted blood		142 139	257 218	207 169	50 49
19-5-49		180	110	276	180	90

On examination she showed general xanthodermia, most marked in the palms and soles but also slightly in the lips and buccal mucosa. Her sclerotics were clear and her hair was lustreless and rusty and her skin dry. The results of the investigations were as follows: *Urine:* no bile or urobilinogen. *Feces:* normal stercobilin. *Blood: moderate iron deficiency anemia* (red blood cells, 4.6 million per cubic millimeter; hemoglobin, 64%; white blood cells, 4,000 per cubic millimeter; neutrophils, 2,900; lymphocytes, 800; monocytes, 240), with *histamine-fast achlorhydria.* Reticulocytes, 0.1%. *Hypercarotenemia:* total carotenoids, 282 μg./100 ml. (carotene, 162; xanthophyll, 120). *Vitamin A deficiency,* 15 i.u./100 ml. Cholesterol, 156 mg./100 ml. Total protein, 7.75 gm./100 ml. (A.G. = 1.22:1.) Wassermann reaction, completely negative. Glucose tolerance not impaired. Sternal marrow, no significant change. *X-rays* of chest, gall-bladder and gastrointestinal tract, no abnormality. *Liver function tests:* alkaline phosphatase, thymol turbidity, bilirubin, colloidal gold, zinc turbidity, thymol flocculation, cephalin cholesterol, colloidal gold and hippuric acid, all normal. *Basal metabolic rate,* minus 15%. *Defective dark adaptation* (figure 2). *Normal vitamin A tolerance* (figure 3). Table 3 shows the detailed results of blood analysis when she was first seen in 1948, and the effects on her blood carotene and vitamin A of giving vitamin A and a low carotene diet. These findings have remained unchanged over the years following. She presented clinically, therefore, with hypercarotenemia, vitamin A deficiency, hypothyroidism, an iron deficiency anemia and achlorhydria.

The evidence that her hypercarotenemia was due to failure of conversion of carotene into vitamin A might be summarized as follows:

1. Absence of excess of carotene from the diet.
2. Persistent hypercarotenemia with low carotene intake over several years.
3. Normal blood lipids.
4. Vitamin A deficiency, though absorption of vitamin A is normal.
5. Carotene content of the serum constantly greater than its xanthophyll content.

It is of interest that what appears to be primarily a hypercarotenemia is accompanied by a moderate degree of hypothyroidism, another indication of a probable reciprocal relationship between carotene and vitamin A. Moreover, as in many cases

TABLE 4

Hypercarotenemia

Probable Mechanism	Plasma Carotene	Xanthophyll: Carotene Ratio	Plasma Vitamin A	Blood Lipids
1. Excessive intake	Increased	>1	Increased	Normal
2. Hyperlipemia a. Diabetes mellitus b. Nephrotic syndromes c. Hypercholesterolemic lipoidoses d. Hypothyroidism*	Increased	>1	Normal or increased	Increased
3. Failure of conversion of carotene into vitamin A				
a. Inborn error of metabolism	Increased	<1	Decreased	Normal
b. Hypothyroidism*	Increased	>1	Often increased	Increased
4. Failure of oxidation of carotene (? role of thyroid and iron)	Increased	?	Increased	Normal

* The two mechanisms brought into play in hypothyroidism are responsible for variable results.

of carotenemia, there are here an iron deficiency anemia and achlorhydria. There is some evidence to suggest that in an iron deficiency anemia there may be failure of oxidation of carotene, with resulting carotenemia.

Summary

Table 4 summarizes the conditions under which hypercarotenemia might be found clinically, and the suggested mechanisms and blood changes which help in their differentiation.

Summario in Interlingua

Le causas de xanthodermia include (1) jalnessa, (2) injection de colorantes jalne, per exemplo safran, e (3) carotinemia.

Carotinoides es de origine vegetal. Illos es incontrate in omne vegetales verde, multo concentrate in carotas, e etiam in butyro e ovos. Lor formula general es $C_{40}H_{56}O_n$ (ubi n = 0 a 6). Carotinas es hydrocarburos (con n = 0). Lor derivatos hydroxylic es xanthophyllas. Beta-carotina es le plus importante precursor de vitamina A. Normalmente, 100 ml de sanguine contine circa 100 mg de carotinoides e 120 a 150 unitates international de vitamina A. Le proportion de xanthophylla a carotina es approximativemente duo a un.

Le major recognoscite causas de carotinemia es: (1) Ingestion excessive de vegetales verde e, specialmente, de carotas—frequentemente in dietas adoptate pro reducer le peso corporee. In iste typo le nivello de carotina pote montar a supra 500 mg per 100 ml; etiam le contento de vitamina A cresce, sed le proportion de xanthophylla a carotina remane plus que 1. (2) Diabete mellite. On crede que duo factores es active in tal casos, i.e. le ingestion excessive de carotina e hyperlipemia. (3) Altere statos hyperlipemic. Istos include lipoidoses hypercholesterolemic, syndromes nephrotic, e hypothyroidismo. (4) Un error metabolic in que le conversion de carotina in vitamina A es defective. In tal casos, carentia de vitamina A accompania le presentia de excessos de carotina in le sanguine, durante que le absorption de vitamina A es normal.

Es discutite le relation inter carotina e le metabolismo thyroide.

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Council on Foods and Nutrition

THE COUNCIL HAS AUTHORIZED PUBLICATION OF THE FOLLOWING REPORT.

GEORGE K. ANDERSON, M.D., Secretary.

MINERAL OIL (LIQUID PETROLATUM) IN FOODS

Ever since the reports of Dutcher and his collaborators[1] and of Burrows and Farr[2] in 1927 it has been known that liquid petrolatum (mineral oil) interferes with the absorption of carotene. Several years ago the Council, as a result of considerations of a salad dressing containing mineral oil, which was designed for convenience in the formulation of therapeutic diets low in fat, called attention to the evidence then available about this possible deleterious effect and concluded that salad dressings containing mineral oil should be used only under the direction of a competent physician.[3] Since that time the situation has changed. The use of mineral oil in foods has increased, and much of the mineral oil so used is being taken without proper medical advice and often no doubt without the knowledge of the person consuming the food. The evidence obtained from newer studies of the effects of the ingestion of mineral oil shows conclusively that the harmful potentialities of mineral oil are far greater than had been supposed. Because of these developments the Council decided to prepare the present brief account of the evidence now available, with the view to reaching a decision about the acceptability of food products containing mineral oil and to informing physicians and others of its views.

As long ago as 1914 the Council on Pharmacy and Chemistry[4] published a report on liquid petrolatum in which it was mentioned that the introduction of this laxative on a fairly extensive scale in medical practice occurred in the period around 1905 to 1910. Today self medication with mineral oil and other laxative drugs regrettably is common. Although constipation usually is a symptom of little significance, Logan[5] has pointed out that it caused more than half the patients with gastric cancer to seek advice at the Roosevelt Hospital in New York.

Mineral oil has been recommended for the cooking of foods. Potato chips prepared in mineral oil have been placed on the market, and the use of mineral oil in the frying of doughnuts has been suggested.

From reports that have come to the attention of the Council it is evident that the use of mineral oil in foods has increased considerably, especially in the form of imitation mayonnaise or salad dressing in which mineral oil replaces the usual food oils that one would expect to find in such preparations. Even when these products are labeled to declare the presence of mineral oil, it is obvious that the consumer has no opportunity to read the labels when they are affixed on gallon size containers and sold to restaurants and institutions. Federal and state authorities having jurisdiction over foods can take suitable action against food products introduced into commerce without proper labeling. However, even labeling showing the presence of mineral oil in salad dressings does not afford adequate protection to the consumer who has no opportunity to read the label or who is possessed of insufficient information to comprehend the significance of statements required by regulations.

There are a number of reasons why there has been an increased use of mineral oil as an ingredient of certain foods. Physically these products can be made so that they cannot be differentiated, except in a laboratory, from ordinary products containing true fats or oils. The mineral oil products do not become rancid, and they may be kept for long periods of time without refrigeration. Mineral oil provides no food value, and products containing it are sometimes referred to, especially on restaurant menus, as "nonfattening" or "slenderizing." They usually can be produced much more cheaply than foods containing animal or vegetable oils and, unlike ordinary food oils and fats, mineral oil is plentiful and requires no ration points. Is it any wonder that the use of mineral oil in foods has tended to increase alarmingly?

THE ALIMENTARY BEHAVIOR OF MINERAL OIL

Absorption.—In 1884 Randolph[6] studied the absorption of a preparation of petrolatum and showed that, within the limits of analytical error, it could be recovered quantitatively in the feces. Other workers later showed that liquid petrolatum is relatively nonabsorbable from the alimentary tract, whether the oil is of the paraffin hydrocarbon type obtained from Pennsylvania crude oils or is of the squalene and naphthene type produced from Russian or California crude oils. It was not until 1932 that it was reported by Channon and Collinson[7] that small traces of mineral oil could be absorbed by animals and deposited in the liver. Stryker[8] in 1941 made a thorough microscopic and chemical study of the absorption of liquid petrolatum by rabbits, white rats and guinea pigs. The amounts of mineral oil administered daily were large; for example, 20 or 30 cc. to rabbits for several months. Gross examination of the bodies of the experimental animals after they had been killed showed white nodules in the intestine of the rabbits, involvement of the mesenteric lymph nodes of the rats with yellow flecks in the liver, and no demonstrable lesions in the guinea pigs. Microscopic examinations revealed the presence of oleophages in certain cells, usually in the superficial lamina propria near the tips of the villi, but occasionally deeper in the cells and, in all three species of animals, both intracellular and extracellular vacuoles in the mesenteric lymph nodes. Animals fed mineral oil for a considerable length of time showed pathologic changes in the liver consisting of vacuolated cells and extracellular vacuoles. Examinations of the mesenteric lymph nodes of selected human autopsy material revealed the same type of vacuoles, which did not reduce osmic acid and stained yellow by the Carminati method. From the mesenteric lymph nodes of both human beings and experimental animals an unsaponifiable oil could be obtained which did not absorb hydrogen and which had an index of refraction and specific gravity of liquid petrolatum. Frazer, Stewart and Schulman[9] in 1942 reported that considerable absorption of liquid petrolatum from the intestine can occur if the oil is emulsified. When the droplets of oil were approximately 0.5 micron in diameter they could be observed in the cells of the intestinal wall of the rat. By means of the chylomicrographic method it was possible to demonstrate oily particles in the blood. Absorption was as high as 60 per cent, which these authors point out compares favorably with the absorption of olive oil when similarly tested.

The evidence thus is conclusive that liquid petrolatum can be absorbed in small amounts from the intestine. Further study is needed of the extent and significance of lesions of the liver and other organs that may be produced by long continued ingestion of mineral oil. It appears, however, that there is no real basis for any belief that mineral oil has any carcinogenic effect.

Effect on Carotene and Vitamin A.—As a result of a great deal of investigation, especially by Curtis and his collaborators,[10] there now has been obtained a quantitative appreciation of the influence of mineral oil on the absorption of carotene. Curtis[11] has calculated that each ounce of mineral oil at body temperature is able to dissolve 140,000 international units of carotene and, at room temperature, 120,000 international units. The magnitude of these figures explains the profound effect exerted by mineral oil, and the difference in the solubility at

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body and room temperatures explains the experimental observation that mineral oil continues to exert its deleterious effect even when it has been saturated at room temperature with carotene. The diet ordinarily provides each day from 5,000 to 10,000 international units of vitamin A and, with the normal choice of foods in the United States, about two thirds of the total is contributed by the carotene of plant foods. Animal food sources of vitamin A are relatively few, being restricted to milk and other dairy products containing butterfat, egg yolk, beef fat, liver and fish liver oils. Helen S. Mitchell[12] reported about ten years ago that rats need from ten to twelve times more spinach to supply their vitamin A requirement when the spinach is fed with 0.5 cc. of mineral oil, and eight times as much when the mineral oil and the food are fed six hours apart. Observations such as these emphasize the undesirability of incorporating mineral oil in foods.[13]

Although liquid petrolatum has a profound adverse effect on the absorption of carotene, its effect on vitamin A itself is much less because vitamin A has a lower solubility in mineral oil than carotene. For this reason Curtis[11] has suggested that, whenever mineral oil is the cathartic of choice and is to be used for any period of time longer than a month, there should be administered with it 1 or 2 teaspoons of cod liver oil or its equivalent of other fish liver oil. "This is like borrowing from Peter to pay Paul," he has written, "but nevertheless it answers the problem of carotene loss to mineral oil." But vitamin A is not the only dietary essential whose assimilation may be adversely affected by the ingestion of mineral oil, and taking cod liver oil with mineral oil or saturating mineral oil with some form of vitamin A may actually lead to a false sense of security.

Constipation frequently occurs in pregnant women and in persons on restricted diets, and these are the very people who, because of the difficulty of meeting their dietary requirements with foods, should use mineral oil as a laxative understandingly or not at all. Hirst and Shoemaker[14] concluded as a result of their observations on 328 pregnant women under good antepartum dietetic management that "dietetic insufficiency in pregnancy should be assumed, and artificial vitamin A supplement offered in all cases." Some indication of the frequency with which mineral oil is used in conjunction with therapeutic diets is provided in the reports of cooperative investigations of the American Dietetic Association. Thus Johnson[15] has written that mineral oil was used in five of fifteen of the 1,500 calory diets reported, in eleven of twenty-six of the 1,200 calory diets and in six of twenty-one and six of thirty-eight of the 800 calory and the low fat diets, respectively. These reports of diets were obtained from thirty-six hospitals in widely separated sections of the United States. While the vitamin A values for all these diets were considered satisfactory, if mineral oil was not consumed, it was emphasized that nearly all of the vitamin A was due to carotene, and the actual vitamin A content of the diets was extremely low unless 3 ounces or more of liver was included in the diets at least once a week.

Vitamin D, Calcium and Phosphorus.—In 1940 Smith and Spector[16] reported their studies on the effect of the ingestion of mineral oil on the utilization of vitamin D. Even though the vitamin D was fed separately as cod liver oil, it was found that three times as much was needed to heal rickets in rats when the basal diet contained 5 per cent mineral oil. When the basal ration contained 10 per cent of mineral oil from five to ten times more cod liver oil was needed to heal rickets. These investigators also demonstrated with growing puppies that the ingestion of mineral oil interferes with the retention of calcium and phosphorus. It was found also that puppies reared on a diet which contained 10 per cent of mineral oil with adequate amounts of calcium and phosphorus did not show normal calcification of the bones even when the amount of cod liver oil administered was increased as much as fivefold.

Vitamin K.—The effect of mineral oil on vitamin K was studied by Elliott, Isaacs and Ivy.[17] A large number of rats were fed on a stock ration to which had been added 20 per cent of mineral oil by weight. Prothrombin deficiency, as measured by the Quick prothrombin time method, developed in the blood, and the condition was cured by the subcutaneous administration of a vitamin K preparation. It was the opinion of these investigators that the mineral oil interfered with the absorption of vitamin K. Hepatic injury was not ruled out but was considered to be unlikely.

The foregoing observations of Ivy and his collaborators received support and elaboration from the studies of Javert and Macri.[18] While investigating the prothrombin concentration during normal pregnancy they found in one woman a lowering of the prothrombin values below normal, and this condition was not improved by the administration orally of a vitamin K preparation. Intramuscular injections were effective. It was learned that this woman had been taking mineral oil during the course of her pregnancy. When the mineral oil was discontinued while the administration of vitamin K was continued, the prothrombin values increased to normal. However, at parturition the prothrombin of the cord blood was only one-third normal despite the administration of large amounts of vitamin K to the mother. Detailed studies then were made of 9 women who were found to be taking mineral oil intermittently during pregnancy. Of these women 3 had low prothrombin values. Of 10 other pregnant women taking mineral oil daily, 7 were found to have hypoprothrombinemia. The authors believed that the mineral oil produced its effect either through adsorption or the prevention of absorption of the vitamin K, or possibly through interference with bacterial synthesis. Their observation that the oral administration of vitamin K may be of little or no value if mineral oil also is being given is worthy of emphasis and serious thought.

MEDICAL OPINION REGARDING MINERAL OIL

Conflicting views are recorded regarding the effect of mineral oil per se on the alimentary tract. Many physicians consider mineral oil preparations the laxative of choice. It is probable that under medical supervision mineral oil can be properly used, but the ease of obtaining the preparations as well as other laxative drugs readily leads to abuse. Proctologists have experienced difficulty in visualizing the wall of the rectum because of an adhering film of oil in persons who take liquid petrolatum. The seepage of mineral oil is well known to be one of the discomforts that may attend the use of this substance. Morgan[19] has written forcefully about the need of caution in the use of liquid petrolatum. He has mentioned a syndrome to which he has ascribed the term "mineral oil poisoning" which may result from the continual oral administration of liquid petrolatum and which is relieved by discontinuance of the oil together with supportive measures to overcome the weakness which accompanies this syndrome. The most frequent signs and symptoms are anorexia, indigestion, flatulence, fatigue, nervousness, dyschesia and anal leakage, accompanied in many cases by considerable loss of weight. On the basis of reports such as these there can be no justification for the incorporation of liquid petrolatum in foods.

CONCLUSIONS

It has been shown that the ingestion of liquid petrolatum is capable of interfering seriously with the absorption of carotene, vitamin D, calcium and phosphorus and vitamin K. The effects

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of its prolonged use have not been thoroughly investigated, but there is sufficient evidence of possible harmful effects to justify the conclusion that its indiscriminate use in foods or in cooking is not in the interests of good nutrition, and any such use should be under careful supervision of a physician.

The Council previously has accepted, with a special requirement that the products be promoted for use only under the direction of a physician, salad dressing or imitation mayonnaise containing mineral oil, for use in therapeutic diets. In view of the abuses which have developed through the production and sale of food products containing mineral oil to the public, the impracticability of providing suitable and adequate warning of the possible harmfulness of such preparations, and the fact that physicians wishing patients to use such products readily can supply directions for their preparation on a small scale from liquid petrolatum and other ingredients, the Council has voted, on the basis of the evidence reviewed in the present report, to withdraw its acceptance of these products.

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Preliminary Communication

HYPERCAROTENÆMIA IN GHANAIANS

CAROTENE-RICH foods, such as palm oil, mangoes, *nkontomire* (a green leafy vegetable), tomatoes, and pawpaw, are extensively consumed in some parts of West Africa. Palm oil, a common ingredient of stews, may contain as much as 113,000 international units (I.U). of carotene per 100 g.,[1] and a medium-sized mango (150 g.) over 8000 I.U. During the season many mangoes may be eaten each day.

In these circumstances we suspected a hypercarotenæmia, and possibly also a hypervitaminosis A, in at least some of the inhabitants of Accra. Accordingly, we examined the concentrations of carotene and vitamin A in the serum and, wherever possible, also of the livers of men and women in Accra.

METHODS AND RESULTS

Vitamin A and carotene in the serum were determined by the method recommended by the Interdepartmental Committee on Nutrition for National Defence,[2] and vitamin A in the liver according to a modification of the method of Ames et al.[3] Calculations were made from a standard curve.

Carotene and vitamin A were determined in the sera of sixty-seven pregnant women (aged 15–44) and fifteen soldiers (aged 22–45), as well as in forty-nine livers of patients coming to necropsy at the Korle Bu Hospital.

The vitamin-A content of the serum in pregnant women ranged from very low values at the one extreme (6 μg. per 100 ml.) to very high values (292 μg.) at the other (see table). However, in two-thirds of the women the vitamin-A levels were 70 μg. per 100 ml. or less, giving a mean value of 66 μg. per 100 ml. The median value of 40 μg. in the pregnant women corresponds closely with that of 43 μg. reported from the U.S.A., Great Britain, and Norway,[1] although the mean value of 66 μg. is considerably higher.

In the fifteen soldiers the range in the vitamin-A content of the serum was not as wide as that observed in the larger group of pregnant women, and the overall average was less—56 μg. per 100 ml. (see table). Nevertheless, this concentration is higher than the average reported by Moore [1] from the Western countries.

CONCENTRATION OF VITAMIN A AND CAROTENE IN THE SERUM

Subject	Total cases	Vitamin A (μg. per 100 ml. serum)			Carotene (μg. per 100 ml. serum)		
		Range	Median	Mean	Range	Median	Mean
Pregnant women	67	6–292	40	66	268–1950	604	681
Soldiers	15	20–131	51	56	185–1881	758	850

By contrast, the carotene concentrations in the serum were exceptionally high both in the pregnant women and in the soldiers, the average levels being 681 and 850 μg. In only two of the fifteen men and seven of the sixty-seven women were the carotene levels less than 400 μg. per 100 ml.

Of especial interest was the observation that in the pregnant women very low levels of serum vitamin A, almost bordering on a vitamin-A deficiency (< 10 μg. per 100 ml.), were always associated with an excessive concentration of carotene (> 500 μg.). In the few cases where the concentration of carotene was less than 400 μg. the vitamin-A levels were variable—i.e., either within "normal" limits or raised (> 100 μg.). No direct correlation could be established between the concentrations in the serum of carotene and vitamin A: we found abnormally high serum concentrations of carotene in association with both very high and very low concentrations of vitamin A.

The vitamin-A content of the forty-nine livers varied considerably—from 32 μg. per g. to 3635 μg. Twenty-four of the livers showed vitamin-A levels above 300 μg. per g., while the average concentration for the whole group was 409 μg. The latter value is approximately four times as high as any reported in the livers of patients dying from various acute and chronic diseases in Britain [1] and Holland,[4] and twice as high as the vitamin-A reserves in cases of accidental death reported from Scotland.[5] Values approximating to those found in our series are reported from New Zealand where the highest value was 6867 I.U. per g. (2289 μg. per g.)[6]

The carotene concentrations in the livers of Ghanaians (range 10–603 μg. per g., median value 47 μg. per g., mean value 92 μg.) were low compared with those obtained for the serum, both in the pregnant women and in the soldiers.

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COMMENT

From these observations there seems little doubt that many of the Ghanaians in Accra show a distinct carotenæmia which could often be seen by naked-eye inspection of the serum. Abnormally high concentration of serum-carotene, exceeding 500 μg. per 100 ml. was found in more than 80% of the sixty-seven pregnant women and fifteen healthy adult men. The sera of one-third of the pregnant women and of one-fifth of the men contained vitamin-A concentrations above 70 μg. per 100 ml.

Less easy to understand was the association of very low levels of vitamin A in the serum with a hypercarotenæmia. Since half the livers contained more than 300 μg. of vitamin A per gramme, and since vitamin A as such is not readily available in the diet, it would appear that much of the carotene consumed in the diet was converted into vitamin A and stored, at least in the liver. We suspect, though we have still to prove this by analysis of serum and liver in the same healthy subjects, that in some circumstances a hypercarotenæmia and low concentration of vitamin A in the serum may be associated with a high liver content of vitamin A, suggesting, in the presence of excess carotene in the blood, some impairment in the mobilisation of vitamin A. The hypervitaminosis A may not be without consequence. This and other aspects of the consumption of excess carotene in Ghana are being investigated.

We wish to express our appreciation to the Director of Medical Services, Ghana Army, and to Dr. F. Boi-Doku, specialist pathologist, Korle Bu Hospital, for their helpful cooperation.

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XL. VITAMIN A AND CAROTENE.

XI. THE DISTRIBUTION OF VITAMIN A IN THE ORGANS OF THE NORMAL AND HYPERVITAMINOTIC RAT.

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In a previous paper Moore [1931] described the distribution of vitamin A and carotene, as determined by colorimetric methods, in the organs of rats which had received large amounts of carotene as red palm oil or carrot-fat. It was found on the one hand that unchanged carotene persisted throughout the alimentary tract, while on the other a high concentration of vitamin A accumulated in the liver. Outside the liver only small amounts of vitamin were detected. Thus the concentration in the storage fat was only about 1/100,000 of that found in the liver-fat, while in most other tissues, which of course contained only small percentages of fat, the amounts of vitamin present were too small to be detected by colorimetric means. In agreement with previous biological experiments by Sherman and Boynton [1925] however and with the subsequent work of Simmonet and Busson [1932] the kidney and lungs gave faintly positive reactions in some cases. It was concluded that the liver must not only be able to store vitamin A to a much greater extent than any other organ, but must also in a complementary sense play a large part in keeping the concentration of vitamin A throughout the rest of the body within prescribed limits. This question was discussed further in the case of the cow [Moore, 1932, 1].

The experiments described below were carried out as an extension of the previous work in order to keep pace with recent developments in our knowledge of the physiological behaviour and significance of vitamin A as studied from other angles. In the first place work on the vitamin A of human livers obtained at autopsy has shown that chronic nephritis is one of the most noteworthy of those diseases which are characterised by the occurrence of a high proportion of cases having subnormal vitamin A reserves [Wolff, 1932; Moore, 1932, 2], while the same tendency, although to a much less well marked extent, is noticed in bronchitis and pneumonia [Moore, 1932, 2; Fox, 1933]. It is therefore of interest to decide whether measurable amounts of vitamin A are normally present in the kidneys and lungs, and how far these organs are to be differentiated from others in this respect. Secondly it seemed desirable to extend the previous work to include rats which had received large amounts of vitamin A in place of carotene. Finally the ready availability of comparatively pure preparations of vitamin A has led to the confirmation by many workers of Takahashi's original finding that excess of vitamin A has a toxic action [Takahashi *et al.*, 1925; Harris and Moore, 1928; 1929; Drigalski, 1933; Moll *et al.*, 1933; Bomskov and Seemann, 1933;

Collazo and Rodriguez, 1933]. Knowledge of the distribution of vitamin A in the organs of rats suffering from hypervitaminosis must obviously be of interest as a step towards the elucidation of the cause of the toxic action.

Experimental

Vitamin A was assayed colorimetrically, using the alkali digestion technique [Davies, 1933], in the organs of albino rats which had been given varying amounts of carotene or vitamin A for varying periods. In the case of small organs, or organs containing small amounts of vitamin, the fat obtained after digesting the tissues was made up in 1 or 2 ml. of chloroform instead of in the 5 ml. usually employed in routine work.

The presence of vitamin A in the kidneys of rats having moderate liver reserves.

Five rats which had been used in vitamin A tests, and which presumably possessed negligible vitamin A reserves at the end of the test period, were given for several weeks a diet containing carotene as 15 % of red palm oil, with the addition of carrots in some cases. Vitamin A was assayed in the liver, kidneys, lungs and (in 3 cases) the intraperitoneal fat. The results are given in Table I.

Table I. *Rats having moderate liver reserves.*

	Rat 1 ♂		Rat 2 ♂		Rat 3 ♂		Rat 4 ♂		Rat 5 ♂	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	900	90	1200	150	1500	150	1000	100	500	50
Kidneys	10	5	6	3	4	3	2	1	8	3
Lungs	6	3	>3	>1·5	0	0	0	0	0	0
Intraperitoneal fat	—	—	—	—	—	0	—	0	—	2

It will be seen that the dietary treatment resulted in the accumulation of moderate vitamin A reserves in the liver[1]. Under these circumstances small amounts of the vitamin were detected in the kidneys in all cases. Only in two out of five was a positive reaction given by the lungs, and in one case out of three by the intraperitoneal fat.

The distribution of vitamin A in the organs of rats given large, but non-toxic, doses of vitamin A concentrate.

Three adult rats were given about 3·5 mg. of B.D.H. vitamin A distillate (3000 B.U. per mg.) each daily for 38 days. They were anaesthetised and killed by bleeding. In order to facilitate the detection of vitamin A in tissues expected to contain only small amounts, the various organs from all three rats were combined after dissection. Results are shown in Table II.

In these rats the liver reserve was extremely high, being 160 times greater than the normal human reserve. The concentration in the kidneys was about 10 times greater than in the previous experiment, but even greater concentrations were found in the lungs and suprarenals, in both of which values were observed

[1] For purposes of comparison it may be mentioned that the "normal" human liver reserve in this country, taken as the median value over a wide range of cases dying by accident, is about 250 B.U. per g. A similar value was obtained in the case of a single wild rat shot in the vicinity of this laboratory.

Table II. *Rats given large, but non-toxic, amounts of vitamin A concentrate.*

Combined tissues from 3 adult ♀ rats, Nos. 6, 7, 8.

	Total B.U. per rat	B.U. per g.
Liver	300,000	40,000
Kidneys	70	50
Lungs	500	450
Suprarenals	100	2,500
Heart	1	1
Spleen	1	2
Pancreas	15	25
Thymus	4	12
Brain	0·5	0·3
Muscles	—	0·5
Blood	—	2
Intraperitoneal fat	—	80

which would be considered quite high for the liver in normally fed rats. In the brain, heart, spleen, muscles and blood only small traces of vitamin were detected, but slightly larger amounts seemed to be present in the pancreas, thymus and intraperitoneal fat. The value in the case of the pancreas may have been made unduly high through the inadvertent inclusion of fat deposits.

To obtain further evidence on the points of interest raised in this experiment further assays were carried out on the liver, kidneys, lungs, pancreas and suprarenals of four adult rats which had received large amounts of vitamin A concentrate. These animals differed from those just described in so far that the feeding of concentrate was discontinued some weeks before killing, an ordinary stock diet of bread and milk, cereals and vegetables being given in the meantime. The organs from each rat were kept separate. Results are given in Table III.

Table III. *Rats given large, but non-toxic, doses of vitamin A concentrate. Concentrate feeding discontinued some time before killing.*

	Rat 9 ♀		Rat 10 ♂		Rat 11 ♀		Rat 12 ♂	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	75,000	8,000	150,000	15,000	125,000	15,000	125,000	10,000
Kidneys	10	5	10	5	15	10	5	2
Lungs	250	150	60	35	75	50	125	60
Pancreas	0	0	5	5	0	0	0	0
Suprarenals	0	0	2·5	25	5	70	1·5	15

In most cases the concentration of vitamin A in the liver was about half that observed in the preceding experiment. The concentration in the kidneys was remarkably low, being little in excess of that found in the case of the rats having moderate reserves. In the lungs the concentration was again greater than in the kidneys but was much lower than the value found in the previous experiment. The amounts of vitamin present in the suprarenals were much smaller than in the preceding experiment, while in the pancreas a negative result was obtained in three rats, a trace being found in the case of one animal.

The distribution of vitamin A in the organs of rats given toxic doses of vitamin A.

Two young albino rats were given the following diet. "Light white casein" 20 %; rice starch 35 %; coconut oil 40 %; salt mixture 5 %; dried yeast 10 % (additional); radiostol (vitamin D) 1 drop per week. Vitamin A concentrate was given dissolved in the coconut oil at such a concentration that 40 mg. were

included in every 10 g. of diet. To avoid undue loss of the vitamin before ingestion by the rat the solution of the vitamin in the oil was stored separately and admixed with the remaining components of the diet daily immediately before feeding. The history of the rats was as follows.

Rat 13 ♂. Initial weight 95 g. Declined in weight from commencement of the experiment with the development of a hunched attitude, loss of hair round the mouth[1] and haemorrhagic rhinitis. Died after 12 days, final weight 60 g. Average daily food consumption 3·1 g.; consumption of concentrate 12·5 mg. per day. Total concentrate ingested, 150 mg. *Post mortem* examination revealed extreme emaciation and severe lung disease. Parts of the lungs were red, wet and soft, other parts were filled with putty-like hard nodules which were present in all sizes up to about 7 mm. in diameter.

Rat 14 ♀. Initial weight 81 g. Declined in weight from commencement with superficial appearances similar to those observed in the previous rat. Died after 33 days, the final weight being 55 g. Average daily consumption of food 3·6 g., of concentrate 14·3 mg. Total concentrate ingested 471 mg. At autopsy the lungs appeared slightly reddened but did not exhibit the gross lesions observed in the preceding rat. The immediate cause of death appeared to be extreme emaciation. The most striking feature, however, was the extreme softness of the bones. X-ray examination revealed that several of the larger leg bones had sustained spontaneous fractures, the broken ends of the bones being healed together in irregular shapes with the formation of calluses.

Table IV. *Rats given toxic amounts of vitamin A concentrate.*

	Rat 13 ♂		Rat 14 ♀	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	50,000	15,000	200,000	60,000
Kidneys	40	60	500	600
Lungs	2,500	1,250	1,000	1,000
Suprarenals	0	0	2·5	50
Heart	60	100	15	25
Spleen	0	0	2·5	15
Pancreas	0	0	1	10
Brain	25	15	20	15
Eyes	0	0	0	0
Testicles	0	0	—	—
Muscles	—	0	—	25
Bone	—	0	—	2
Total B.U. ingested	450,000	—	1,400,000	—
Found in faeces	1,500	—	6,400	—
Found in urine	0	—	0	—

The results of vitamin A assays carried out on the organs of these rats are given in Table IV. In the case of the rat dying after 12 days the liver reserve was considerably below the values obtained in some other cases. The lungs contained a very high concentration of vitamin, which in view of the diseased and unduly weighty condition is best judged in this case from the total vitamin present rather than the value expressed per g. of tissue. Considerable amounts of vitamin were present in the kidney and heart. A small amount was found in the brain. Other organs gave negative results.

[1] The loss of hair round the mouth would appear to be due to the irritant action of traces of diet remaining on the skin after feeding. It is noteworthy, however, that the concentrate exerts no immediate irritant action. Our solution of the vitamin A concentrate in coconut oil could be eaten by ourselves in small amounts without irritation or discomfort.

In the case of the rat dying after 33 days the concentration of vitamin A in the liver was four times greater than in the preceding rat. The total amount of vitamin present in the lungs was less, but in this case a high value was found for the kidneys. Significant amounts of vitamin were also found in all organs except the eyes[1].

In order to determine the efficiency of the absorption of vitamin A in these rats the faeces and urine were collected daily and stored in 5 % KOH until the end of the experiment, when vitamin A assays were carried out on aliquot portions by the usual technique. In each case the urine gave a negative result. Positive results were found for the faeces, but the amounts present were insignificantly small when compared with the difference between the amount of vitamin ingested and that found in the body. In each case only about ¼ of the ingested vitamin could be accounted for, the remainder presumably being lost by oxidation at some stage as yet unknown.

The distribution of vitamin A in the organs of rats given large doses of carotene.

Two albino rats from the same litter as those used in the preceding experiment were given the same diet except for the substitution of carotene (B.D.H.) in place of vitamin A. Owing to the limited solubility of the pigment it was found impossible to incorporate more than 10 mg. per 10 g. of diet (*i.e.* ¼ of the concentration of vitamin A in the preceding experiment), but since the food consumption was much greater the actual daily consumption of carotene was about half that of vitamin A. The animals grew rapidly and when killed after 34 days were found at autopsy to be perfectly normal. The storage fat was plentiful and quite colourless. Particulars were as follows.

Rat 15 ♂. Initial weight 95 g. Final weight 156 g. Average daily food consumption 8·3 g.; carotene consumption 8·3 mg. daily; total carotene ingested 281 mg.

Rat 16 ♀. Initial weight 83 g. Final weight 129 g. Average daily food consumption 7·9 g.; carotene consumption 7·9 mg. daily; total carotene consumption 268 mg. The distribution of vitamin A in the organs of these rats is shown in Table V.

Table V. *Rats given large amounts of carotene.*

	Rat 15 ♂		Rat 16 ♀	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	12,500	1,250	12,500	1,700
Kidneys	5	4	5	3
Lungs	30	30	20	12
Suprarenals	0	0	0	0
Heart	0	0	0	0
Spleen	1·5	3	0	0
Pancreas	2·5	10	0	0
Brain	0	0	0	0
Eyes	0	0	0	0
Testicles	0	0	—	—
Muscles	—	0	—	0
Bone	—	0	—	0
Intraperitoneal fat	—	5	—	2
V.U. carotene ingested	560,000	—	530,000	—
Found in faeces	55,000	—	25,000	—

[1] In view of the fact that the retina has been reported to contain considerable amounts of vitamin A [Smith *et al.*, 1931; Wald, 1933] this negative result should not be taken to imply that the vitamin was entirely absent, but it is evident that the concentration of the vitamin, if present, was not sufficiently high to compensate for the small amounts of tissue available for assay.

The vitamin A reserves found in the liver would represent high values for ordinary conditions of nutrition but are some 30 times lower than the maximum value obtained by giving vitamin A. In the kidney the concentration of vitamin was no greater than in the rats having only moderate liver reserves, but in the lungs the amounts present were of a slightly higher order. In each case a faintly positive reaction was given by the intraperitoneal fat, while in rat 15 traces of chromogen were also detected in the spleen and pancreas. In the case of the spleen the yellow/blue ratio indicated that the reaction was due to carotene.

In order to determine the efficiency of absorption of the carotene the faeces and urine were examined as in the preceding experiments, the amounts found representing about 10 % and 5 % of the ingested pigment in rats 15 and 16 respectively. Since the amount of vitamin A present in the body must in each case have represented only some 3–6 mg. of carotene, or about 1–2 % of the ingested carotene, it is obvious that the utilisation of carotene at such high levels must be very inefficient, and that the greater part of the pigment has not been accounted for.

Discussion.

The distribution of vitamin A in the organs of the rat.

The above experiments indicate that small amounts of vitamin A (*ca.* 5 B.U. per g.) are consistently present in the kidneys even when the liver reserves are of a moderate order (*ca.* 100 B.U. per g.). Traces of the vitamin are sometimes present in the lungs under these conditions.

When the liver reserve is raised by the inclusion in the diet of large amounts of carotene or vitamin A the order of the kidneys and the lungs is reversed, since the lungs now invariably show a greater concentration of vitamin A. As far as can be judged both from the present experiments and from previous work [Moore, 1931] carotene feeding does not result in the accumulation of vitamin A in high concentration in any organ except the liver, the highest value observed being 30 B.U. per g. for the lungs of rats Nos. 15 and 16 in the present work. The feeding of vitamin A concentrate on the other hand may raise the concentration in the kidneys and lungs to values that would be high even for the liver in the case of animals receiving ordinary diets. At the same time appreciable amounts of vitamin may appear throughout the whole body. In the case of the suprarenal glands very inconsistent results have been obtained. For rats 6, 7 and 8 a very high average value (2500 B.U. per g.) was observed, but in other experiments the results were much lower and in some cases negative. It may be noted that in view of the small size of these organs the actual amount of vitamin involved was relatively small.

Hypervitaminosis A.

The production of toxic symptoms in the present work may be considered to provide further evidence as to the reality of hypervitaminosis A in so far as the preparation used was a distillate, as opposed to the non-distilled concentrates used, as far as we are aware, by other workers in this field. Drigalski [1933] has already shown that the toxic power of the concentrates is destroyed with the vitamin on ultra-violet irradiation, it is now evident that the vitamin potency and toxic power are stable to distillation and are not separated by this means. Although only two rats were used in the present work we were fortunate in observing almost all the symptoms described by previous workers. The lung lesions observed in rat 13 exactly resembled those described in several cases by Takahashi *et al.* [1925]. It is perhaps noteworthy that these lesions should occur

in the organ in which the greatest concentration of vitamin A outside the liver was located. In rat 14 the bone lesions were exactly similar to those described by Bomskov and Seemann [1933] and also Collazo and Rodriguez [1933], our own observations representing the third independent discovery of the phenomenon.

Speculation as to the mechanism of the production of hypervitaminosis A would be premature at the present stage, but two clearly established points may be worth mention. In the first place it is obvious that the liver possesses a very high toleration for the vitamin, since concentrations of the order observed in the hypervitaminotic rats (Nos. 13 and 14) were also observed in healthy animals (Nos. 6, 7 and 8). At the maximum level observed the vitamin must have constituted about 5 % of the dry weight of the liver and have represented a supply sufficient to last the rat for about 200 years if economically used. Secondly it is plain that in the case of rat 13 death through hypervitaminosis intervened at a point when the liver still contained less than half the maximum observed concentration of vitamin. This finding shows that death may occur while there is still room for a considerably greater accumulation of vitamin A in the liver and suggests that the production of hypervitaminosis does not depend on the absolute amount of vitamin A present in the organism but on the ingestion of the vitamin at a greater rate than that at which it can be stored by the liver or eliminated by the organism.

The absence of toxic symptoms after feeding excess of carotene.

The absence of any symptoms of hypervitaminosis in the rats (Nos. 15 and 16) given about 8 mg. of carotene daily suggests that it is virtually impossible to induce hypervitaminosis A by carotene feeding. This conclusion has already been reached by Kuhn and Brockmann [1933], but since these workers administered carotene at the rate of only about 1 mg. per day, which is little greater than the dosage of 0·75 mg. used by Moore [1930] in earlier experiments, it is obvious that further evidence on this point was desirable.

It is still possible, of course, that large masses of solid carotene, as opposed to solutions of carotene in oil, might possess toxic action. Even if this proved to be the case, however, the phenomenon observed would almost certainly be quite different from the hypervitaminosis induced by giving vitamin A concentrate, since a comparison of the vitamin A contents of the livers of rats 13 and 14, which were given large amounts of vitamin A concentrate, with those of rats 15 and 16, which were given large amounts of carotene, indicates that the rate at which the liver reserve can be raised by the conversion of carotene (50 B.U. per g. per day) is much slower than the rate of increase obtained by giving vitamin A (1200–1700 B.U. per g. per day). The superior rate and efficiency of absorption of vitamin A suggest that in intensive vitamin A therapy preformed vitamin A should be more efficacious than carotene. At levels approaching the minimum dose, as previously shown [Moore, 1933], vitamin A and carotene appear to be equally efficiently utilised.

Summary.

1. Vitamin A was assayed by the antimony trichloride method in the organs of rats which had received varying amounts of vitamin A or carotene.

2. Even when the liver reserves were quite low (100 B.U. per g.) small amounts of vitamin A (5 B.U. per g.) were found in the kidneys and sometimes in the lungs.

3. When large, but non-toxic, doses (3·5 mg. daily) of vitamin A distillate were given to adult rats (*a*) the concentration of vitamin in the liver rose to

extremely high levels (40,000 B.U. per g.), (*b*) the concentration in the lungs was of the order found in the liver under normal conditions of nutrition, (*c*) the concentration in the kidney remained fairly low (50 B.U. per g.), (*d*) the concentration in other organs was extremely low, except that surprisingly high values were observed in the suprarenals (up to 2500 B.U. per g.).

4. Even more massive doses of the distillate (12–14 mg. per day) given to young rats proved toxic, causing emaciation, haemorrhagic rhinitis, loss of hair round the mouth and (*a*) in one rat, dying after 12 days, gross lung lesions, (*b*) in another rat, dying after 33 days, a peculiar softening and hollowing of the bones which had resulted in spontaneous fractures of the legs. The distribution of vitamin A resembled that found in rats given non-toxic doses except that the concentration in the lungs was even higher (1250 B.U. per g.), while in the rat dying after 33 days a high kidney value (625 B.U. per g.) was observed.

5. Attempts to induce hypervitaminosis A by feeding carotene failed. On account of its limited solubility in fats only 8 mg. of the pigment could be given daily. Rats receiving this amount grew rapidly and remained in perfect health. On killing after 34 days the liver reserve was found to be only moderately high (1250 B.U. per g.) pointing to only about 1 % absorption, as opposed to 15 % in the case of vitamin A. Only very small amounts of vitamin were present in the lungs and kidneys, negative results being obtained in all other organs.

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Fishwick-'57
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The Metabolism of Uniformly ^{14}C-Labelled β-Carotene in the Rat. By M. J. Fishwick and J. Glover. (*Department of Biochemistry, University of Liverpool*)

Two vitamin A-deficient rats were given orally 7·8 and 4·3 mg. respectively of crystalline ^{14}C-β-carotene (0·4 μc/mg. and $E^{1\%}_{1cm.}$ 450 mμ, 2550) dissolved in 1·0 ml. arachis oil and their respired CO_2 separately collected at frequent intervals for 24 hr. The animals were then killed and the intestinal, liver and kidney lipids examined for radioactivity.

Approximately 50–70 % of the dose was not absorbed. Of the absorbed material, just over 2 % was eliminated as $^{14}CO_2$ in the 24 hr. It first appeared 0·5 hr. after dosing, attained a maximum 4–5 hr. later and then slowly declined. It appears, therefore, that the ^{14}C-β-carotene was rapidly degraded during its absorption across the intestinal wall. The percentage radioactivity in the total tissue lipids of the second animal were as follows: liver 30; intestine 13 and kidney 0·3. The liver and intestinal lipids were resolved and the radioactivity determined in the following fractions: β-carotene, vitamin A, sterols and fatty acids (ether-soluble). For the liver, the values were: 3·0, 14·4, 1·3 and 1·4 %. Thus the ^{14}C-β-carotene has been degraded to yield intermediary metabolites as well as vitamin A. The nature of the first fragment(s) formed has not yet been elucidated, but in view of the relative stability of orally administered vitamin A or retinene, which are well absorbed and stored in the liver of the dosed animals, the above results indicate that the ^{14}C-β-carotene was attacked at some other position(s) than the central double bond.

To obtain information regarding possible intermediary metabolites, the C_{16}–C_{18} fatty acids and cholesterol were isolated in pure crystalline form and their specific activities compared. The values for the fatty acids in the intestine in two experiments were: (I) 11–17 c.p.m./mg. and (II) 6–10 c.p.m./mg. and for the cholesterol (I) 31 c.p.m./mg. and (II) 84 c.p.m./mg. respectively. The corresponding figures for liver in experiment (II) were: fatty acids 12–14 c.p.m./mg. and cholesterol 43·4 c.p.m./mg. The cholesterol values are all

higher than the others in spite of the fact that the β-carotene was administered in arachis oil which would provide a ready available metabolic pool of C_2 units and so tend to trap any radioactive C_2 units formed. A similar result, however, was obtained in a third experiment where the ^{14}C-β-carotene was administered in aqueous Tween 80 solution. The higher specific activity of cholesterol would suggest that the carbon fragments removed from the β-carotene entered the C_3–C_6 branched unit system more readily than the C_2 unit system of the fatty acid helix.

This work, and the observations of Fazakerley & Glover (1957), indicate that the formation of vitamin A from the larger fragment, which would result from oxidative fission of a terminal double bond in β-carotene, involves at least in part some other enzyme system than β-oxidation previously thought capable of degrading the unsaturated branched carbon chain (Glover & Redfearn, 1954).

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The Metabolism of β-Carotene and Vitamin A*

A. Intestinal Absorption and Metabolism in the Rat and in Man
B. Biosynthesis of Vitamin A with Rat Intestinal Enzymes

D. S. Goodman**

Vitamin A and β-carotene are molecules that have interested both chemists and clinicians for a great many years. Historically, the existence of vitamin A has been indirectly recognized for literally thousands of years. Thus the Eber's Papyrus, an ancient Egyptian medical treatise of about 1500 BC, recommended roast ox-liver, or the liver of a black cock, as the therapy for hight blindness [1]. Hippocrates also prescribed ox-liver for night blindness, but suggested that it be eaten after being dipped in honey [1]. More recently, Roels found in 1956, less than 10 years ago, that the use of cock's liver was still prescribed by medicine men in Ruanda, in Central Africa, for the cure of night blindness [2]. All of these prescriptions of course depend upon the fact that liver usually contains a high concentration of vitamin A. It has been estimated that there are millions of cases of clinical vitamin A deficiency in the world today, mostly in south and southeast Asia, and this is obviously a problem of enormous medical importance.

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Intestinal Absorption and Metabolism

In 1933, DRUMMOND, BEE and PALMER demonstrated that vitamin A is absorbed via the lymphatic route, by carrying out studies in a patient with chylothorax [3]. Since then this finding has been confirmed and amplified by studies with other animal species. [For reviews of earlier work see ref. [1] p. 192 ff., and refs. (4) and (5)]. It has been established that retinol* is largely esterified during its intestinal absorption, and partial information about the processes of absorption and esterification has been available from the studies of MAHADEVAN and GANGULY [6], MURTHY *et al.* [7], MAHADEVAN *et al.* [8] and POLLARD and BIERI [9]. In contrast to the reasonably detailed information on retinol, however, much less detailed information has been available concerning the intestinal absorption and metabolism of the provitamin A, β-carotene.

Studies designed to define the events occurring during the intestinal absorption of β-carotene and retinol in the rat were recently reported by Dr. HELEN HUANG and myself [10]. Tritium or C^{14}-labeled β-carotene or retinol were dissolved in oil and fed by gastric tube to rats with cannulae implanted in the thoracic duct. The C^{14}-carotene was produced biosynthetically from C^{14}-acetate by the fungus *Phycomyces blakesleeanus*. The H^3-carotene and the labeled retinol were generous gifts of Hoffmann-La Roche of Basel (Switzerland). In addition experiments have also been carried out with patients in whom polyethylene cannulae had been implanted in the thoracic duct in the neck, at the time of neck surgery for other reasons. These experiments with human subjects were carried out in collaboration with Drs. ROLF BLOMSTRAND and BENGT WERNER of Stockholm [11]. After the feeding of labeled β-carotene or vitamin A, chyle was collected and the total lipid extracted. This was then chromatographed on columns of deactivated alumina as described elsewhere [10], and 6 fractions were collected, namely: Fraction 1, β-carotene; Fraction 2, retinyl esters; Fraction 3, retinal; Fraction 4, retinol; Fraction 5, more polar but non-acidic compounds; and Fraction 6, acids including retinoic acid.

Table I summarizes our results with lymph-fistula rats, and shows the distribution of radioactivity in lymph after feeding purified labeled β-carotene or labeled retinol. Regardless of which

* The terms retinol, retinal, retinoic acid and retinyl ester refer respectively, to vitamin A alcohol, aldehyde, acid and ester.

Table I. Distribution of radioactivity in rat lymph.

Fraction	% distribution of absorbed C^{14} after ether containing labeled β-carotene*	Retinol*
(1) β-carotene	2 ± 0	2 ± 1
(2) Retinyl ester	89 ± 2	89 ± 1
(3) Retinal	1 ± 1	3 ± 1
(4) Retinol	3 ± 1	1 ± 1
(5 + 6) Polar; acids	5 ± 2	2 ± 1

* n = 2 for β-carotene and 1 for retinol. The values listed are the mean ± standard error of the mean.

Table II. Distribution of radioactivity in human lymph after β-carotene 15,15'-H^3.

Fraction	Subject AW Chylomicrons	Subject AW Rest of lymph	Subject IC Chylomicrons	Subject IC Rest of lymph
(1) β-carotene	23	22	30	27
(2) Retinyl esters	67	71	61	65
(3) Retinal	3	2	3	2
(4) Retinol	3	3	3	3
(5) More polar	4	2	3	3

labeled compound was fed, retinyl esters accounted for 89% of the absorbed radioactivity. These results confirm the reported inability of the rat to absorb unchanged β-carotene into the lymph [12, 13]. Small amounts of radioactivity were also found in the other chromatographic fractions, but only the radioactivity found in fraction 4 could be chemically identified, and was shown to reside in retinol [10].

A similar distribution of radioactivity was found in human lymph, after feeding labeled preformed vitamin A to two subjects. In both cases, 80 to 90% of the absorbed radioactivity, in either the chylomicron or the non-chylomicron portion of the lymph, was found in retinyl esters.

In contrast to the rat, man is capable of absorbing a small amount of unchanged β-carotene into the lymph. Table II demonstrates that after feeding H^3-labeled β-carotene to two human subjects, 20 to 30% of the absorbed radioactivity was found in β-carotene, which was identified by crystallization with carrier pure β-carotene to constant specific radioactivity. The absorption of unchanged β-carotene was, however, extremely limited. As with the rat, most of the

absorbed radioactivity resided in retinyl ester, the identity of which was chemically established by saponification to form retinol, followed by oxidation with MnO_2 to the aldehyde, retinal.

Analyses were then carried out to determine the composition of the lymph retinyl esters in each of the various feeding experiments. As described elsewhere [10], this was accomplished by chromatography in two different kinds of systems. First, thin-layer chromatography was carried out on alumina gel impregnated with silver nitrate. This method separates retinyl esters on the basis of the degree of unsaturation of the fatty acid moiety. The saturated, and other, esters were then separated according to fatty acid chain length by reversed phase chromatography on silicone-impregnated paper. These two procedures suffice to define the retinyl ester composition of a mixture.

Fig. 1 shows the composition of the retinyl esters found in the lymph chylomicrons of the two human subjects who were fed H^3-labeled β-carotene. Identical results were also obtained with the non-chylomicron portions of lymph. The left hand side of the Figure shows the fatty acid composition of the total chylomicron lipid, as determined by gas-liquid chromatography of the fatty acid methyl esters. As expected, the relative proportions of the various fatty acids in the total lipid reflected closely the composition of the dietary fat. Thus, oleic acid was the major fatty acid in the lymph of subject AW, who was fed H^3-β-carotene dissolved in olive oil. Linoleic acid was the major fatty acid in subject IC, who was fed H^3-β-carotene dissolved in a Swedish polyunsaturated oil called Kronolja. In contrast to the composition of the total lipids, however, the composition of the retinyl esters (shown on the right), remained remarkably constant, regardless of the diet, and bore no resemblance to the diet. Retinyl palmitate predominated in all samples, and the saturated esters (retinyl palmitate plus stearate, in a ratio of approximately 2 to 1) comprised about 80% of the esters in both subjects.

Virtually identical results were obtained with the lymph samples from the two human subjects fed labeled preformed vitamin A. In addition, we have also found almost this very same retinyl ester composition in rat lymph, after a much wider range of diets [10]. As in man, retinyl palmitate predominates in rat lymph, and saturated esters (palmitate plus stearate in a ratio of about 2 to 1) consistently comprise approximately three-fourths of the lymph retinyl esters, after feeding either β-carotene or retinol.

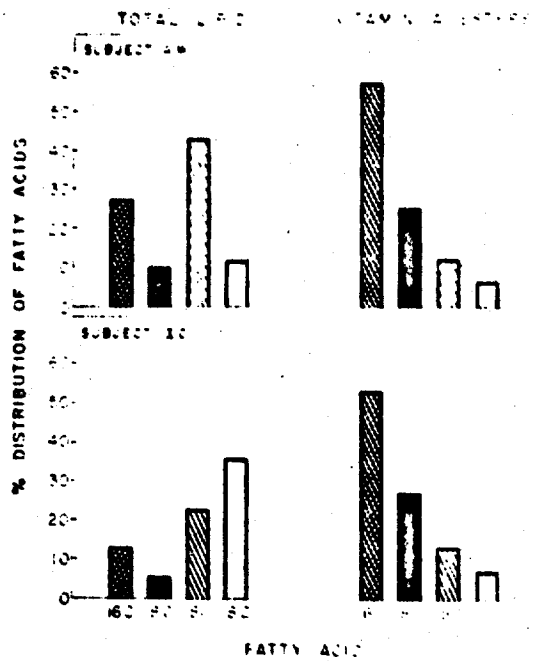

Fig. 1. The fatty acid composition of the total lipid (on the left) and the cholesterol esters (on the right) in lymph chylomicrons from two human subjects fed [illegible] H^3. Subject AW was fed a skim milk emulsion of H^3-β-carotene in olive oil, subject [illegible] an emulsion of H^3-β-carotene in a commercial polyunsaturated oil.

Biosynthesis of Vitamin A

The biosynthesis of vitamin A from β-carotene was first conclusively demonstrated by MOORE [11], by means of experiments in which β-carotene was fed to vitamin A-deficient rats. Subsequent examination of these rats showed that the feeding of β-carotene had produced a definite increase in the vitamin A content of their livers. Since then, the conversion of β-carotene to vitamin A has been demonstrated with a variety of preparations, including lymph-fistula animals [12, 13, 10, 11], rat intestinal loops [15] and slices [16], and isolated perfused rat livers [17]. Despite these studies, detailed information about the mechanism of the conversion of β-carotene to vitamin A has not been available, mainly because of the lack of a cell-free system capable of bringing about this conversion.

Table III. Identification of biosynthetic reaction product as retinal.

Sample		mg (of retinal or semicarbazone)	C^{14}, counts/min per μmole
Column fraction 3		4.3	19800*
Retinal semicarbazone:	Crystallization #1	2.3	17100
	Crystallization #2	1.2	16200
	Crystallization #3	0.6	16800
	Crystallization #4	**0.31	17100

* assuming all C^{14} in column fraction 3 resides in retinal
** m.p. 189 - 190 C

Dr. HELEN HUANG and I have recently reported the active conversion of β-carotene to vitamin A with cell-free homogenate fractions of rat intestinal mucosa [18]. The enzyme (or enzymes) responsible for this conversion is present in the soluble protein fraction (104,000 × *g* supernatant) of the mucosal homogenate, and has been partially purified by precipitation with ammonium sulfate between 25 and 45% saturation. There is an absolute requirement for the presence of molecular oxygen, and an almost absolute requirement for bile salts. The reaction also requires a heat-stable factor present in the particulate fraction of the mucosal homogenate. This factor can be extracted from the particulate fraction with 90% acetone; it has not yet been identified. The system is stimulated by the addition of glutathione, but is not affected by the addition of ethylene diamine tetraacetate, ascorbic acid, or reduced or oxidized pyridine nucleotides. The yield of retinal from β-carotene is usually in the range of 30 to 50%, after a 1 hour incubation.

The product of the reaction has been identified as retinal by addition of pure unlabeled retinal, followed by formation of the semicarbazone derivative [19], which was recrystallized 4 times without significant change in its specific radioactivity. The results shown in Table III demonstrate that 85% of the radioactivity in alumina column fraction 3 was established to reside in retinal by this procedure.

The product of the reaction has also been identified as retinal by addition of pure carrier retinal, followed by gas-liquid chromatography on a short column of SE-30 [20]. At the end of the column the effluent stream was split, so that part of it entered the mass detector, whereas most of it was collected at an exit port at one minute intervals for assay of radioactivity. Fig. 2 shows the results of this

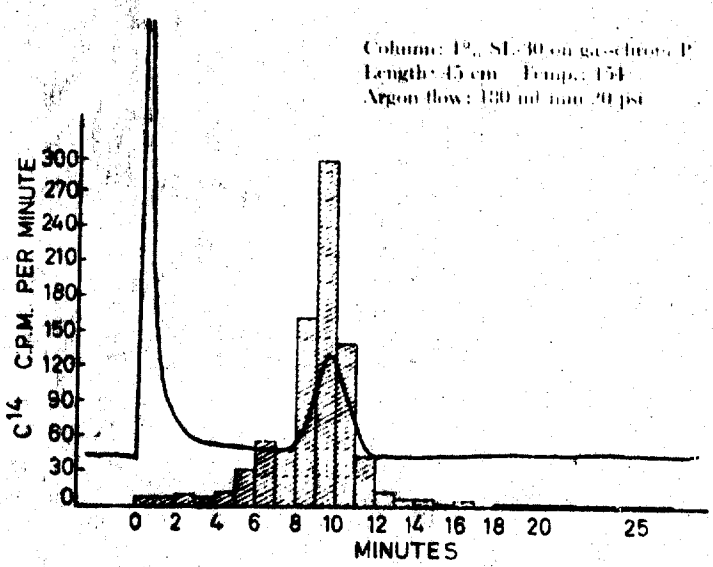

Fig. 2. Identification of the reaction product as retinal, after the *in vitro* biosynthesis of vitamin A from β-carotene. As described in the text, this Figure shows the gas-liquid chromatographic analysis of a mixture of newly-synthesized C^{14}-retinal (fraction 3) and carrier unlabeled retinal.

experiment. In Fig. 2 the solid curve shows the response of the mass detector, whereas the bar graph (referable to the scale on the left) shows the recovery of C^{14} from the column. Retinal emerged as a single symmetrical peak with a retention time of about 9.5 min. Most of the radioactivity also emerged as a single peak which was coincident with the peak of retinal. Since, in this experiment, there was a quantitative recovery of radioactivity from the column, this confirms the fact that most of the radioactivity in alumina column fraction 3 resides in retinal.

It has long been discussed whether β-carotene is centrally cleaved to form two molecules of vitamin A, or whether there is an attack at a site other than the central double bond of β-carotene, with the formation of only one molecule of vitamin A per molecule of carotene [21, 22].

Calculations based on the stoichiometry of our results indicate that most of the reaction product retinal must have arisen by central cleavage of the substrate β-carotene into two molecules of retinal. Thus, in one experiment, 7 incubations were conducted simultaneously with the same amount of substrate in each flask. Three control flasks did not contain enzyme and 4 flasks contained active enzyme.

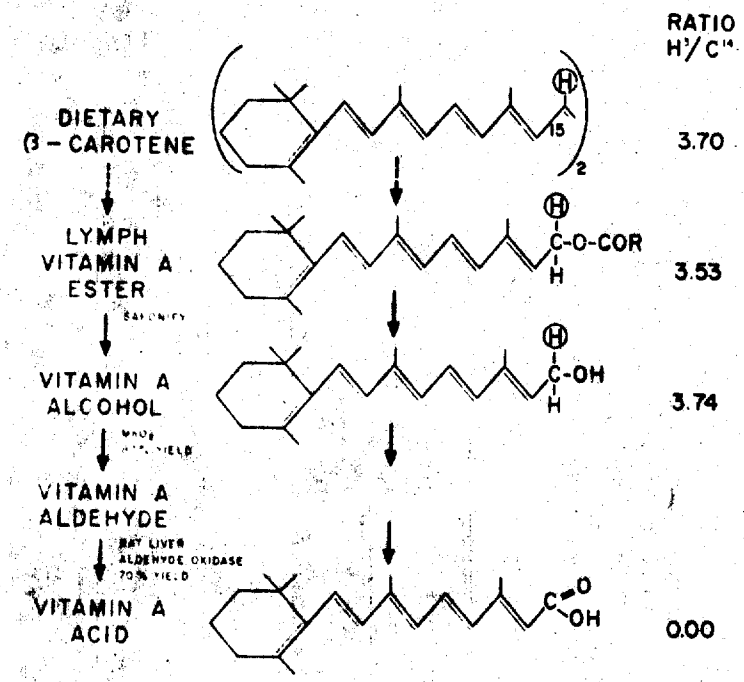

Fig. 3. Retention of the hydrogen atoms attached to the central carbon atoms (C-15 and 15′) of β-carotene, during the biosynthesis of vitamin A (see text for details).

After incubation without enzyme, 1029 ± 13 counts/min were recovered in the carotene fraction, and 55 ± 9 counts/min in fraction 3. After incubation with active enzyme, 535 ± 28 counts/min were recovered in fraction 1 and 460 ± 20 counts/min in fraction 3. These results are not consistent with the possibility that only one molecule of retinal is formed from one molecule of β-carotene.

Fig. 3 summarizes a study designed to find out whether the hydrogen atoms attached to the central carbon atoms of β-carotene are retained or are lost during the biosynthesis of vitamin A. β-carotene was prepared which was both uniformly labeled with C^{14}, and also specifically labeled with H^3 at the center of the molecule (at positions 15 and 15′). This double-labeled β-carotene was then fed to a lymph fistula rat. Lymph was collected and the retinyl esters isolated. As indicated in Figure 3, the ratio of H^3 to C^{14} in the lymph retinyl esters, and in the retinol obtained from these esters by saponification, was virtually identical with the ratio of tritium to C^{14} in the dietary carotene. Thus there was no loss of tritium, relative to C^{14}, during

the conversion of β-carotene to vitamin A, and hence the central hydrogen atoms of β-carotene must have been retained during the biosynthesis of vitamin A. Finally, in order to prove that the H^3 label was actually attached only to the central carbons of β-carotene, the retinol was oxidized to retinoic acid by a two-step procedure, involving first chemical oxidation to retinal, followed by enzyme oxidation of the retinal to retinoic acid. When this was done the H^3 completely disappeared, showing that all the H^3 had indeed been attached to the terminal carbon (carbon number 15) of retinol.

An experiment has also been carried out in which double-labeled β-carotene was converted to retinal during *in vitro* incubations. As before, the β-carotene was uniformly labeled with C^{14}, but was specifically labeled with H^3 at the central two carbons. In two incubations, in which there was a 56 and a 46% yield of retinal, respectively, there was no loss of H^3 relative to C^{14} during the conversion of β-carotene to retinal. This confirms the conclusion that the central hydrogen atoms of β-carotene are completely retained during the biosynthesis of vitamin A.

Our experiments have therefore demonstrated that the overall reaction of vitamin A biosynthesis consists of the central cleavage of β-carotene into two molecules of retinal, and that the hydrogen atoms attached to the central two carbon atoms of β-carotene are not removed during this reaction. Since molecular oxygen is required for the *in vitro* conversion of β-carotene to retinal, it seems highly likely that the mechanism of this reaction involves a direct reaction between molecular oxygen and the central two carbon atoms of β-carotene, followed by the cleavage of the central double bond to yield two molecules of retinal. The biosynthesis of vitamin A from β-carotene is hence most likely a dioxygenase reaction, as formulated in Fig. 4. The fine details of this dioxygenase reaction, such as the nature of the initial reaction and such as whether there is a cyclic peroxide intermediate as pictured in Fig. 4, cannot as yet be defined. The outline of the overall reaction seems, however, fairly secure.

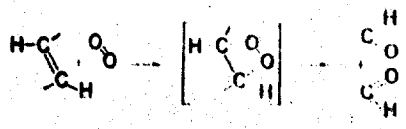

Fig. 4. Probable mechanism of the biosynthesis of vitamin A from β-carotene

Acknowledgement

The work reported in this paper was carried out as a collaborative effort between Dr. HELEN S. HUANG, Mr. TATSUJI SHIRATORI, and the author. Mrs. JOAN VORMBAUM provided expert technical assistance.

Summary

During the intestinal absorption of vitamin A (retinol), either in the rat or in man, the retinol is almost completely esterified and the esters are absorbed via the lymph mainly incorporated in lymph chylomicrons. During the absorption of β-carotene, the β-carotene is largely converted to retinal; the retinal is then reduced to retinol which in turn is esterified and transported in the lymph in a fashion similar to that of dietary preformed vitamin A. After ingestion of labeled β-carotene, unchanged labeled β-carotene comprised approximately one-fourth of the absorbed radioactivity in human lymph, but could not be demonstrated in rat lymph. The fatty acid composition of the retinyl esters was remarkably constant, regardless of the composition of the diet and regardless of whether the retinyl esters were derived from dietary retinol or from β-carotene. Retinyl palmitate predominated in all samples, and saturated esters (retinyl palmitate + stearate, in a ratio of about 2 to 1) consistently comprised about three-fourths of the labeled esters.

The active biosynthesis of vitamin A from β-carotene can be brought about with cell-free homogenate fractions of rat intestinal mucosa. Enzymatic activity resides in the soluble protein fraction of the mucosal homogenate. The reaction requires molecular oxygen and bile salts, and is stimulated by glutathione. A heat-stable factor in the particulate fraction of the homogenate is also required. The product, obtained in yields of up to 50 % has been identified as retinal via its semicarbazone derivative, and also by means of gas-liquid radiochromatography. The reaction mechanism involves the central cleavage of β-carotene into two molecules of retinal. During this reaction the hydrogen atoms attached to the central carbon atoms of β-carotene are completely retained. The conversion of β-carotene into retinal is most likely a dioxygenase reaction.

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The Intestinal Absorption and Metabolism of Vitamin A and β-Carotene in Man *

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The fact that vitamin A is absorbed via the lymphatic route was first demonstrated by Drummond, Hall, and Palmer in 1935, in studies carried out on a patient with chylothorax (2). Since then this finding has been confirmed and amplified by studies with other animal species. It has been well established that retinol § is largely esterified during its intestinal absorption, and partial information about the processes of absorption and esterification has been available from the studies of Ganguly and his associates (3–5) and Pollard and Biely (6). In contrast to retinol, however, much less detailed information has been available concerning the intestinal absorption and metabolism of the provitamin A, β-carotene.

Detailed studies of the events occurring during the intestinal absorption of β-carotene and retinol in the rat were recently reported by Huang and Goodman (7). Radioactive β-carotene or retinol was dissolved in oil and fed to rats containing cannulae implanted in the thoracic duct. Absorption of radioactivity occurred entirely via the lymphatic route, mainly in the form of lymph chylomicrons. Retinyl esters were the predominant labeled compounds in all samples of lymph and contained an average of 89% of the radioactivity absorbed after the administration of either labeled retinol or labeled purified β-carotene. There was virtually no absorption of unchanged labeled β-carotene into the lymph. The composition of the lymph retinyl esters was remarkably constant, regardless of the fatty acid composition of the diet and regardless of whether the retinyl esters were derived from dietary retinol or from β-carotene. Retinyl palmitate was the predominant ester in lymph, and saturated esters (retinyl palmitate plus stearate, in a ratio of approximately 2 to 1) consistently comprised two-thirds to three-fourths of the retinyl esters.

The studies reported here were designed to define the events occurring during the intestinal absorption of β-carotene and vitamin A in man. These studies represent both an extension, to man, of the studies carried out in the rat, and a continuation of earlier investigations on the transport of lipids via the thoracic duct in man (8).

Methods

Labeled compounds.[2] Retinol-15-^{14}C (specific radioactivity, 29.6 μc per mg) and retinyl-15-^{3}H acetate (specific radioactivity, 100 μc per mg) were stored under argon in sealed vials until used and were used without further purification. Previous assay of a different vial

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Parts of this work were presented at the Fifty-seventh Annual Meeting of the American Society for Clinical Investigation in Atlantic City, N. J., May 1965 (1), and at the Second International Symposium on Drugs Affecting Lipid Metabolism in Milan, Italy, September 1965. The preceding paper in this series is reference 9; this is paper no. 5 in the series.

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§ The terms retinol, retinal, retinoic acid, and retinyl ester refer, respectively, to vitamin A alcohol, aldehyde, acid, and ester.

[2] The labeled compounds used in these studies were the generous gift of Dr. U. Gloor of Hoffmann-La Roche, Basel, Switzerland.

from the same preparation of retinol-15-^{14}C had indicated that at least 98% of the radioactivity chromatographed with unlabeled retinol on alumina column chromatography (7). β-Carotene-15,15'-^{3}H (specific activity, 1.09 mc per mg) was chromatographed on a column of deactivated alumina (7) just before use. Further information about this preparation of labeled β-carotene has been provided elsewhere (9).

Subjects and collection of samples. Four studies were carried out in three subjects. In all subjects, polyethylene cannulae were implanted in the thoracic duct in the neck by the procedure described by Werner (10). Lymph was drained directly into containers kept in a small closed refrigerator, thus ensuring that the samples remained cold and in the dark during the time of collection.

Subject AW (study I) was a 61-year-old man who presented with a 2-month history of bilateral supraclavicular swellings. Past history included the presence of an esophageal diverticulum and of gallstones. Biopsy revealed the swellings in the neck to consist of lipomas. Lymph duct cannulation was performed at the time of surgical exploration of the swelling in the left supraclavicular region. At the time of cannulation the serum cholesterol concentration was 266 mg per 100 ml, and the lymph cholesterol in the fasting subject was 182 mg per 100 ml. On the day after cannulation the patient was fed a formula meal containing 52 μc β-carotene-^{3}H (47 μg) dissolved in 2 ml olive oil and emulsified with 50 ml skim milk. Lymph samples were collected at 1- to 2-hour intervals for 22 hours. The lymph flow was in the range of 15 to 25 ml per hour for most of the collection period. The patient was not fed during the collection period, but was allowed to drink water ad libitum.

Subject IC (studies II and III) was a 58-year-old housewife who presented with a lump in the left breast of 1 year's duration. Past history revealed a 1-year history of hypertension and mild congestive heart failure, treated with digitalis and chlorothiazide. Needle aspiration biopsy of the left breast showed the presence of malignant cells. Chest and skeletal X rays revealed no visible metastases. There was no history of gastrointestinal symptoms or abnormalities. Cannulation of the thoracic duct in the neck was performed at the time of scalene node biopsy, which was done to exclude the possibility of cervical node metastases. The serum cholesterol concentration was 310 mg per 100 ml, and the fasting lymph cholesterol was 158. One hour after cannulation the patient was given a formula meal containing 50 μc (46 μg) β-carotene-^{3}H, 91 μg unlabeled β-carotene, and 2 mg α-tocopherol, all dissolved in 2 ml of a polyunsaturated commercial vegetable oil [3] and emulsified with 50 ml skim milk. Lymph was collected at 1- to 2-hour intervals for the next 44 hours. A second formula feeding, consisting of 20 μc (0.68 mg) retinol-15-^{14}C plus 2 mg α-tocopherol dissolved in 2 ml of Kronolja and emulsified with 50 ml skim milk, was administered 23 hours after the first formula. The lymph flow was in the range of 15 to 30 ml per hour during most of the first collection period (study II) and in the range of 25 to 35 ml per hour during the second collection period (study III). The patient remained fasting for at least 10 hours after each of the two formula feedings, but was then allowed to drink milk (study II) or to eat a small meal (study III).

Subject HD (study IV) was a 52-year-old male engineer admitted for resection of a gastric carcinoma. A weight loss of 7 kg had occurred during the preceding year. The serum cholesterol concentration was 268 mg per 100 ml. At the time of total gastrectomy the liver appeared free of metastases, but regional lymph nodes contained metastatic tumor. The pathological diagnosis of the resected specimen was anaplastic carcinoma. Two hours before the start of gastrectomy, lymph duct cannulation was performed in the neck, in connection with a study of the appearance of malignant cells in lymph during and after gastric surgery. Four hours before lymph duct cannulation the patient was fed 50 ml of olive oil containing 194 μc (1.94 mg) of retinyl-^{3}H acetate. Lymph samples were collected at short time intervals (15 to 30 minutes) during the first 2 hours after cannulation, followed by infrequent collections thereafter.

Extraction and chromatography. In each study, the time course of the absorption of radioactivity was determined by extracting 0.5 ml of each lymph sample with 10 ml $CHCl_3$: MeOH (2:1, vol:vol), followed by radioassay of the total lipid extract so obtained. The lymph samples containing the main peak of absorbed radioactivity were then pooled for subsequent analyses. Portions of the pooled lymph were layered under an equal volume of isotonic saline and centrifuged for 45 to 60 minutes at 15,000 × *g* to 20,000 × *g*. The top, creamy zone of packed chylomicrons and the clear, chylomicron-free bottom fraction were separately collected and extracted with 20 vol of $CHCl_3$: MeOH (2:1, vol:vol), followed by the addition of 8 vol of 0.01 N H_2SO_4. A mixture of unlabeled carriers, consisting of 250 μg of retinol plus 100 μg each of β-carotene, retinal, retinoic acid, and of each of four retinyl esters (palmitate, stearate, oleate, and linoleate) was added to each sample at the time of extraction. The lower, chloroform phase was collected and evaporated to dryness. The total lipid extract was weighed, dissolved in benzene, and stored under argon at −15° C.

In study IV the lymph samples obtained during the first 3 hours of collection were all found to contain approximately 25,000 dpm of ^{3}H per ml. The lymph samples were pooled, and the total lipid of whole lymph was extracted as described.

Portions of each lipid extract were chromatographed on columns of deactivated alumina [4] as described previously (7). The order, identity and volume of eluent per 5 g of alumina were as follows: fraction 1, β-carotene (20 ml of n-hexane); fraction 2, retinyl esters (20 ml of benzene-hexane, 3:17); fraction 3, retinal (20 ml of

[3] The Swedish trade name of the vegetable oil is Kronolja. It consists mainly of soybean oil and contains a high proportion of linoleic acid.

[4] Woelm, grade III.

benzene-hexane (1:1); fraction 4, retinol (50 ml of benzene); fraction 5, more polar but nonacidic compounds (20 ml methanol); and fraction 6, acids including retinoic acid (20 ml methanol: 25% acetic acid, 3:1). Portions of each column fraction, and of each total lipid extract, were assayed for radioactivity.

Composition of retinyl esters. The composition of the labeled retinyl esters in each column fraction 2 was determined by a combination of two different chromatographic separations, as described in detail previously (7). A carrier mixture of unlabeled retinyl esters, comprising 50 μg each of retinyl palmitate, stearate, oleate, and linoleate, was added to each sample just before chromatography. Separation of the retinyl esters on the basis of the degree of unsaturation of the fatty acid component was first accomplished by thin-layer chromatography on alumina gel G impregnated with silver nitrate. The saturated, and other, esters were then separated according to fatty acid chain length by reversed phase chromatography on silicone-impregnated paper. The retinyl ester composition of each sample was calculated after expressing the results of each chromatographic separation as the percentage distribution of recovered radioactivity in the different esters.

Identification procedures. Radioactivity in column fraction 2 from the chylomicron samples of studies I and II (after feeding β-carotene-^{3}H) was established to reside in retinyl esters by the procedures described previously (7). These procedures included saponification to form labeled retinol, followed by acetylation to form labeled retinyl acetate (which was identified by column and thin layer chromatography). The material present in each column fraction 2 was also subjected to thin layer chromatography on alumina gel G, together with appropriate carriers, so as to separate long chain fatty acid esters of retinol from short chain esters (such as retinyl acetate) and from cholesterol esters (7). Elution and radioassay of the separated compounds indicated that in every sample, in all four studies, virtually all of the radioactivity in column fraction 2 resided in long chain retinyl esters.

Other procedures. The fatty acid composition of the total lipid extract of each sample was determined by gas-liquid chromatography of the fatty acid methyl esters, as described previously (7).

Pure lecithin was isolated from two of the samples and was subjected to hydrolysis with phospholipase A, as described elsewhere (11). The compositions of the fatty acids attached to the β and to the α′ positions of lecithin were then separately determined by gas-liquid chromatography.

Radioassay (for ^{3}H or ^{14}C or both) was carried out by dissolving samples in 15 ml of 0.5% diphenyloxazole in toluene, followed by assay with a Packard Tri-Carb liquid scintillation counter. Significant quenching was not observed.

The sources of all materials and compounds used in this study were described previously (7).

Results

The time course of the absorption of radioactivity into the lymph, in the two subjects fed β-carotene-^{3}H, is shown in Figure 1. In both subjects

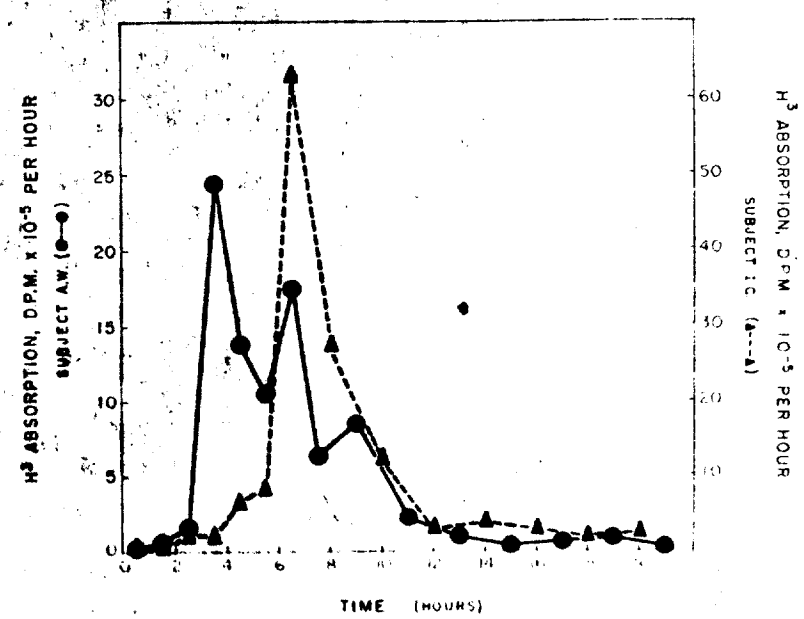

FIG. 1. THE TIME COURSE OF THE ABSORPTION OF RADIOACTIVITY INTO THORACIC DUCT LYMPH IN TWO HUMAN SUBJECTS FED β-CAROTENE-15,15′-^{3}H.

TABLE I

Distribution of radioactivity in human lymph after β-carotene-15,15'-³H

Alumina column fraction	Percentage distribution of absorbed ³H			
	Study I		Study II	
	Chylomicrons	Bottom fraction*	Chylomicrons	Bottom fraction*
1. β-Carotene	23	22	30	27
2. Retinyl esters	67	71	61	65
3. Retinal	3	2	3	2
4. Retinol	3	3	3	3
5+6. Polar; acids	4	2	3	3

* Lymph from which chylomicrons had been removed by centrifugation.

radioactivity was absorbed in a fairly sharp peak, which coincided with the peak of fat absorption into the lymph (as determined qualitatively by the degree of visible lactescence of the lymph samples). In study I (subject AW) the main peak of absorption occurred between 3 and 4 hours, and 88% of the absorbed ³H appeared in the lymph between 3 and 10 hours. The apparent second peak of absorption seen in this study immediately after the main peak probably reflects irregular gastric emptying of the formula meal containing the labeled β-carotene. In study II (subject IC) the main peak of absorption occurred between 6 and 7 hours, and 81% of the recovered ³H was absorbed between 5 and 11 hours. In both studies the absorption of radioactivity was virtually complete by 12 hours. The total amount of radioactivity recovered in thoracic duct lymph during the entire period shown in Figure 1 comprised 8.74% of the fed radioactivity in study I and 16.76% of the fed radioactivity in study II.

A similar time course of absorption of radioactivity was seen after feeding retinol-¹⁴C. Thus, in study III the main peak of absorption of ¹⁴C occurred between 3 and 5 hours, and 78% of the radioactivity absorbed into the lymph was absorbed between 2 and 8 hours. A total of 21.5% of the fed radioactivity was recovered in the lymph during a period of 20 hours.

Chylomicrons, isolated as described from the pooled samples representing the main peak of absorption of radioactivity, contained 70% of the absorbed radioactivity in study I and 80% of the absorbed radioactivity in study III. The relative amount of lymph radioactivity contained within lymph chylomicrons was not precisely determined in studies II or IV.

Table I summarizes the distribution of radioactivity in the chylomicron and the nonchylomicron ("bottom") portions of lymph in the two subjects given β-carotene-³H (studies I and II). In all samples, most of the radioactivity was found in the retinyl ester fraction (fraction 2), which contained 61 to 71% of the radioactivity absorbed into the lymph. Analysis of a portion of the retinyl ester fraction from each chylomicron sample demonstrated that the radioactivity in this column fraction definitely resided in long chain fatty acid esters of retinol (see above, under *Identification procedures*). Significant amounts of radioactivity were also recovered in the β-carotene fractions (fraction 1's), which contained 22 to 30% of the absorbed radioactivity. To determine whether this radioactivity resided in unchanged β-carotene, we added unlabeled pure β-carotene (250 μg) to a portion of each fraction 1 and serially crystallized the β-carotene two or three times, to constant specific radioactivity. Most (81 to 85%) of the ³H in each fraction 1 was established to reside in β-carotene by this procedure.

Comparable data for the distribution of radioactivity in lymph, in the two subjects fed labeled preformed vitamin A, are presented in Table II. In both subjects, most of the radioactivity absorbed into the lymph was found in the retinyl ester fraction, which contained 86 to 88% of the absorbed radioactivity in study III and 80% of the absorbed radioactivity in study IV. Further analysis showed that all of the radioactivity in these retinyl ester fractions resided in long chain fatty acid esters of retinol. This indicated that, in study

TABLE II

Distribution of radioactivity in human lymph after radioactive vitamin A

Alumina column fraction	Percentage distribution of absorbed label		
	Study III (retinol-¹⁴C)		Study IV (retinol-³H acetate)
	Chylomicrons	Bottom fraction	Whole lymph
1. β-Carotene	1	2	5
2. Retinyl esters	88	86	80
3. Retinal	6	5	6
4. Retinol	3	3	6
5+6. Polar; acids	2	4	3

TABLE III

Fatty acid compositions of the total lipid of human lymph

Study; oil in test meal	Sample analyzed	Percentage distribution of fatty acid mass					
		Palmitate (16:0)*	Palmitoleate (16:1)	Stearate (18:0)	Oleate (18:1)	Linoleate (18:2)	Arachidonate (20:4)
I. Olive oil	Chylomicrons	27	3	10	43	11	Trace
	Bottom	27	3	11	35	15	Trace
II. Kronolja	Chylomicrons	14	1	6	24	38	8
	Bottom	19	2	9	26	33	6
III. Kronolja	Chylomicrons	13	1	4	23	40	8
	Bottom	18	2	8	25	35	6
IV. Olive oil	Whole lymph	19	2	3	53	22	Trace

* Number of carbon atoms: number of double bonds. Only the major component fatty acids have been listed in this Table. A number of minor components, comprising together less than 10% of the total fatty acid mass, were observed in each sample.

IV, the dietary retinyl acetate had been hydrolyzed completely before or during absorption, followed by a re-esterification of the released retinol with long chain fatty acids. Small amounts of radioactivity were also recovered in all of the other column fractions. Most of this latter radioactivity probably represented artifactual chromatographic "smearing" secondary to oxidation or other alterations occurring during the processing of the samples. Similar slight smearing of radioactivity is commonly observed during the handling of pure labeled retinyl esters or retinol. Attempts to identify the radioactive material recovered in column fraction 4 [see (7)] demonstrated that only about one-third to one-half the radioactivity in the several fraction 4's definitely resided in retinol.

The fatty acid composition of the total lipid extract of each lymph sample studied is summarized in Table III. Since triglycerides constitute the major lipid in both the chylomicron and nonchylomicron portions of lymph, the compositions listed in Table III largely represent the triglyceride compositions of the several samples. As expected, the fatty acid composition reflected the composition of the dietary fat in all samples. Thus, linoleic acid predominated in lymph after test meals containing the polyunsaturated vegetable oil (Kronolja) (studies II and III), and oleic acid predominated after test meals containing olive oil. Furthermore, the degree of predominance of oleic acid was greater in study IV, in which 50 ml of olive oil had been fed, than in study I, in which only 2 ml of olive oil had been fed.

The composition of the labeled retinyl esters present in the retinyl ester fraction of each sample studied, for all four studies, is presented in Table IV. All samples contained a mixture of four retinyl esters: retinyl palmitate, stearate, oleate,

TABLE IV

Labeled retinyl ester compositions in human lymph

Study; labeled compound fed	Oil in test meal	Sample analyzed	Percentage distribution of labeled retinyl esters			
			Palmitate	Stearate	Oleate	Linoleate
I. β-Carotene-^{3}H	Olive oil	Chylomicrons	57	25	12	6
		Bottom	54	25	14	7
II. β-Carotene-^{3}H	Kronolja	Chylomicrons	53	27	13	7
		Bottom	57	26	12	5
III. Retinol-^{14}C	Kronolja	Chylomicrons	60	22	10	8
		Bottom	60	24	10	6
IV. Retinyl-^{3}H acetate	Olive oil	Whole lymph	56	19	16	9

TABLE V

Fatty acid compositions of chylomicron lecithin

Study, oil in test meal	Fatty acid position on lecithin	Percentage distribution of fatty acid mass					
		Palmitate	Palmitoleate	Stearate	Oleate	Linoleate	Arachidonate
I. Olive oil	α′	45.5	0.8	34.8	11.8	5.0	Trace
III. Kronolja	α′	42.8	1.2	31.2	9.9	9.4	3.8
I. Olive oil	β	3.7	1.4	1.2	18.3	58.5	11.2
III. Kronolja	β	3.2	1.6	2.0	14.4	64.5	13.6

and linoleate; there was no evidence for the presence of polyunsaturated retinyl esters containing more than two double bonds in the fatty acid moiety.[5] Retinyl palmitate was the predominant retinyl ester in all samples, and the two saturated esters, retinyl palmitate and stearate, together comprised 75 to 84% (average 79%) of the total retinyl esters in all samples. The ratio of retinyl palmitate to stearate was relatively constant ranging from 2.0 to 2.9 (average 2.4) in the seven samples listed in Table IV. The composition of the labeled retinyl esters bore no resemblance to the composition of the dietary fat or of the lymph total lipid (Table III). Furthermore, the retinyl ester composition was relatively constant regardless of whether the retinyl esters were derived from preformed vitamin A or from β-carotene and regardless of whether the chylomicron or the nonchylomicron portion of lymph was analyzed.

Analyses were also carried out to determine the fatty acid composition of the lecithin present in the chylomicron samples of studies I and III. It is known that lecithin comprises the major phospholipid in rat (12) and human (13) lymph and that the composition of lymph lecithin is relatively fixed, regardless of the composition of the diet (11–13). Furthermore, it has been pointed out (7) that, in the rat, the composition of the fatty acids attached to the α′ position of lymph lecithin strongly resembles the fatty acid composition of newly absorbed lymph retinyl esters.

Table V summarizes the composition of the fatty acids attached to both the α′ and the β positions of chylomicron lecithin for the two samples studied. Similar compositions were found in both lecithin samples. Saturated fatty acids predominated at the α′ position, comprising 74 to 80% of the α′ fatty acids in the two samples. Linoleic acid predominated at the β position. The ratio of palmitate to stearate, at the α′ position, was 1.31 and 1.37 in the two samples. These results are in accord with previously reported observations on the fatty acid distribution in human lymph lecithin (13).

Discussion

The studies reported here demonstrate that during the intestinal absorption of dietary preformed vitamin A, in man, the vitamin A is almost completely esterified with long chain fatty acids, and the retinyl esters are then transported via the lymphatic route mainly in association with lymph chylomicrons. During the absorption of dietary β-carotene, the carotene is largely converted to vitamin A, and the newly synthesized vitamin A is then esterified and transported in the lymph in the same fashion as dietary retinol. The composition of the lymph retinyl esters was remarkably constant in all the present studies, regardless of the fatty acid composition of the diet, regardless of whether the retinyl esters were derived from preformed vitamin A or from β-carotene, and regardless of whether the chylomicron or the nonchylomicron portion of lymph was analyzed. Retinyl palmitate predominated in all samples, and saturated esters (retinyl palmitate plus stearate, in an average ratio of 2.4 to 1) consistently comprised approximately 80% of the labeled esters.

These results indicate that the events occurring during the intestinal absorption of β-carotene and vitamin A in man are very similar to those occur-

[5] Although small amounts of radioactivity (5 to 11% of the total retinyl ester radioactivity) were consistently found at or just above the origin (below the linoleate zone) after thin layer chromatography, this radioactivity apparently resided in oxidation products formed during chromatography. Thus, after elution from the thin layer plate and rechromatography on an alumina column, less than 10% of this radioactivity was recovered in the retinyl ester fraction (column fraction 2).

ring in the rat. Thus, labeled retinyl esters predominated in rat lymph after the feeding of either labeled retinol or labeled β-carotene (7). Furthermore, the composition of the labeled retinyl esters found in rat lymph after a wide range of test meals was remarkably constant and was similar to the retinyl ester composition found here in human lymph. One difference was, however, observed between man and the rat: Man is capable of absorbing small but significant amounts of unchanged dietary β-carotene into the lymph, whereas virtually no dietary β-carotene can be absorbed intact into rat lymph. The quantitative difference in the ability of these two species to absorb intact β-carotene is, however, not very great, since most of the radioactivity absorbed into human lymph after feeding β-carotene-^{3}H was found in retinyl esters, and not in unchanged β-carotene. The human intestine therefore possesses only an extremely limited capacity for the absorption of unchanged dietary β-carotene.

Since the labeled β-carotene employed in these studies was labeled with tritium at the central two carbon atoms (C-15 and 15′), the question should be raised as to whether some ^{3}H label might not be lost during the metabolism of this β-carotene, particularly during its conversion to vitamin A. Recent studies with rat intestinal mucosa have demonstrated that the reaction mechanism of vitamin A biosynthesis consists of the central cleavage of β-carotene into two molecules of retinal, and that the hydrogen atoms attached to the central two carbon atoms of β-carotene are completely retained during this reaction (9, 14). β-Carotene-15,15′-^{3}H hence should be a suitable compound to use in studies of the metabolism of β-carotene. Because of its location the ^{3}H label would, of course, be lost during the conversion of newly formed retinal into retinoic acid, so that the present studies would not have detected the formation of retinoic acid had it occurred during absorption and metabolism of the β-carotene-^{3}H. Significant amounts of retinoic acid were not detected in rat lymph, however, in previous studies with ^{14}C-labeled β-carotene (7), and retinoic acid was not detected in human lymph, in the present study, after feeding retinol-^{14}C. The conversion of β-carotene into two molecules of retinal appears to be a dioxygenase reaction involving a direct reaction between molecular oxygen and the central carbon atoms of β-carotene (9). The newly formed retinal is then reduced to retinol (14), which in turn is esterified and transported via the lymph in the manner described herein.

The composition of the retinyl esters found in human and in rat lymph may be compared with the composition of the retinyl esters recently reported for a number of rat tissues (15). The composition of the labeled retinyl esters in each of six rat tissues was determined at several time intervals after the intravenous injection of chylomicrons containing newly absorbed labeled vitamin A (15). The retinyl ester compositions were remarkable in showing a consistent predominance of saturated esters in all tissues. Large differences were, however, seen in the relative amounts of labeled retinyl palmitate and stearate in different tissues. Thus, retinyl palmitate markedly predominated in liver, whereas similar amounts of retinyl palmitate and stearate were found in kidneys, and retinyl stearate predominated in adrenal glands. A marked predominance of retinyl palmitate in rat liver has also been reported by Futterman and Andrews (16). In addition, these workers determined the composition of the retinyl esters formed during incubation *in vitro* of homogenized retinas in the light (17); in four mammalian species the composition of the newly formed retinyl esters was similar to the retinyl ester composition observed here in lymph.

In a previous publication (7) it was pointed out that the fatty acid composition of retinyl esters in rat lymph is similar to the composition of the fatty acids present at the α′ position of lymph lecithin. It was also pointed out (15) that, since lecithin from practically all sources shows a striking predominance of saturated fatty acids at the α′ position, the predominance of saturated retinyl esters in all tissues extends the comparison between the fatty acid composition of retinyl esters and that of the α′ position of lecithin. In the present study, direct analysis of the fatty acid composition of lecithin from two of the chylomicron samples showed that, as with the retinyl esters, the composition of lecithin was relatively fixed and was hardly influenced by the composition of the test meal. The composition of the fatty acids attached to the α′ position of lecithin resembled the composition of the retinyl esters in being largely saturated; the ratio of palmitate to stearate at the

α' position of lecithin was, however, distinctly lower than that seen in the retinyl esters. Despite considerable similarities, therefore, the fatty acid compositions of retinyl esters and of the α' position of lecithin are certainly not identical.

The reason for the remarkably constant composition of the retinyl esters found in human and rat lymph is not known. It has been suggested (7) that retinol esterification during absorption may involve the direct reaction of retinol with a fatty acyl donor of composition similar to that seen in lymph retinyl esters. It has also been pointed out (7) that the similarity between the fatty acid composition of the α' position of lymph lecithin and that of retinyl esters suggests that retinyl ester fatty acids may derive either from the α' position of lecithin or from the precursor pool of the fatty acids of this position of lecithin. Some selectivity would, of course, have to exist to account for the different ratio of palmitate to stearate in retinyl esters and in lecithin. Further definition of the mechanism of retinol esterification during absorption will be required to decide between these and other, alternative possibilities.

Summary

β-Carotene-15,15'-^{3}H, vitamin A alcohol-15-^{14}C, and vitamin A-15-^{3}H acetate were fed to patients in whom polyethylene cannulae had been inserted in the thoracic duct in the neck. Serial samples of lymph were collected, and the lipid was extracted and chromatographed on columns and on thin layer plates of alumina. Absorption of radioactivity into the lymph mainly occurred between 3 and 10 hours. During this time washed chylomicrons contained 70 to 80% of the absorbed radioactivity. Labeled vitamin A esters predominated in all lymph samples, representing 80 to 90% of the absorbed radioactivity after the feeding of labeled preformed vitamin A, and 60 to 70% after β-carotene. The fatty acid composition of the vitamin A esters in lymph bore no resemblance to the composition of the diet and was remarkably constant, regardless of the fatty acid composition of the diet, regardless of whether the vitamin A esters were derived from dietary vitamin A or from β-carotene, and regardless of whether chylomicron or nonchylomicron lipid was analyzed. Vitamin A palmitate predominated in all samples, and saturated esters (vitamin A palmitate plus stearate, in an average ratio of 2.4 to 1) consistently comprised 75 to 85% of the labeled esters. Small amounts of vitamin A oleate and linoleate were also found in all samples. Unchanged labeled β-carotene comprised only 20 to 30% of the absorbed radioactivity, after ingestion of β-carotene-^{3}H. The human intestine possesses only an extremely limited ability to absorb unchanged dietary β-carotene into the lymph. The fatty acid composition of the lymph vitamin A esters was similar to but not identical with that of the α' position of lymph lecithin.

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Vitamin A and carotene in British and American diets

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1. The confusion sometimes found in discussions of dietary vitamin A and its precursors is illustrated. The relative potency of vitamin A and carotene is briefly discussed, and the procedure used in the National Food Survey of Great Britain explained. 2. Changes in the vitamin A and carotene contents of the British diet over the last two decades are illustrated. 3. The vitamin A and carotene contents of the American diet are estimated, and compared with those of the British diets; both estimates of intake are compared with recommended allowances. The difficulties of making such comparisons are discussed, in view of the different conventions used in the two countries. 4. An appendix, on the calculation of vitamin A in the diet, is included, in which interrelations between different methods of expressing vitamin A value are shown.

Discussion of dietary vitamin A and its precursors is sometimes confused because of the existence of three different ways in which quantities may be expressed: weight, international units (i.u.), and 'activity' or 'potency' (itself expressed in i.u.). Thus, the Committee on Nutrition of the British Medical Association (1950) state (p. 17) that a (British) 'mixed diet' contains one-third of its vitamin A as such and two-thirds as the provitamin, carotene, whereas Thompson (1965) and Plack (1965) estimate that a third 'of the total vitamin A intake' from the recent British diet is provided as carotene.

By internationally accepted definition (World Health Organization, 1949, 1950), 1 i.u. of vitamin A is equivalent to 0·3 μg of vitamin A alcohol and 1 i.u. of provitamin A is equivalent to 0·6 μg of β-carotene, these quantities having an equal effect at the low levels of intake used in a specific rat assay. At higher levels of intake, carotene is less well utilized both in rat and man, so that in human nutrition these two quantities have a different effect, and thus the notion of international units, which is useful for grouping unlike things with like properties, is by current definitions not very helpful.

Though there may be a more or less fixed 'biochemical conversion factor' between carotene and vitamin A, which has been assumed to be 2 in formulating the international definitions quoted above, there certainly is a highly variable 'nutritional conversion factor', which depends on species, the form in which the carotene is ingested, and the amount of carotene ingested. In the wartime Sheffield experiment reported by Hume & Krebs (1949) it was found that men were less efficient than rats in utilizing carotene, and further that the absorption of carotene from different sources varied greatly; a compromise 'nutritional conversion factor' of 3 was finally chosen, so making vitamin A six times more active (on a weight basis) in man than carotene as derived from an ordinary mixed diet. For cattle under natural conditions the sole source of vitamin A is carotene, and most of the evidence points to carotene

having, on a weight basis, one-fifth of the activity of vitamin A (S. Y. Thompson, personal communication).

On the evidence of the Sheffield experiment, the Medical Research Council (1945; and see McCance & Widdowson, 1960, p. 163) recommended that to obtain an approximate value for the total vitamin A potency of a mixed diet the total carotene in i.u. should be divided by 3 and added to the value for total preformed vitamin A. This is essentially the practice adopted in the National Food Survey, for which the divisor 3 is incorporated into the conversion factors for vitamin A for appropriate food groups, and thus all National Food Survey figures are in terms of vitamin A potency, as pointed out, for example, by Hollingsworth (1965). In the United States, however, a different practice is usually adopted (see below).

In considering requirements for vitamin A, the British Medical Association (1950), also following the results of the Sheffield experiment, considered that carotene is less well utilized than vitamin A to the extent of one-third (in terms of i.u.), but in the United States a factor of one-half is assumed: 'for practical purposes it is considered that 2 i.u. of β-carotene are equivalent to 1 i.u. of vitamin A in man' ((USA) National Research Council, 1964, p. 20).

When comparing one survey with another it is therefore important to know how the vitamin A values are expressed: the interrelations between different methods are expressed algebraically in the appendix to this paper. The resolution of the paradox posed in the first paragraph of the present paper is that the British Medical Association ratios were in terms of international units, whereas those of the later authors were in terms of potency (see Fig. 1).

Vitamin A and carotene in the British diet

Hollingsworth (1965) has discussed the trend, as revealed by the National Food Survey, in the sources of vitamin A in the British diet over the two decades 1942–62. Mean daily consumption has increased from about 3000 i.u. (vitamin A potency) per head for the years 1942–9 to about 4300–4400 i.u. for 1956 and subsequently; this increase was shown by Leitner, Moore & Sharman (1960) to be reflected in an increase in the vitamin A in the blood of both men and women. Over this period the percentage contribution from vegetables containing carotene dropped from more than one-third of the total in 1942 to about one-fifth, while the contribution from animal sources containing the preformed vitamin increased. There has thus been an obvious change in the relative proportions of preformed vitamin and precursor in the total vitamin A potency of the diet; these proportions are illustrated in Fig. 1. The proportions were calculated from the known contribution of different foods to the total vitamin A potency, allowance being made for the presence of carotene in milk, cream, cheese, butter and eggs. In terms of international units, preformed vitamin A accounted, during the war years, for about 30% of the total, and latterly rather nearer 40%; in terms of potency, or equivalent vitamin A activity, for about 56% during the war years, and latterly about 65%, carotene providing respectively about 44% and 35%.

A rather more sophisticated analysis, calculated from weights of vitamin A and carotene, is given in Table 1.

Vitamin A and carotene in the American diet

The hazards of comparing the results of one survey with those of another are many. Thompson (1965) compared an estimated intake of 4306 i.u. vitamin A per day, from the National Food Survey of Great Britain for 1962, with an estimate of 10155 i.u.

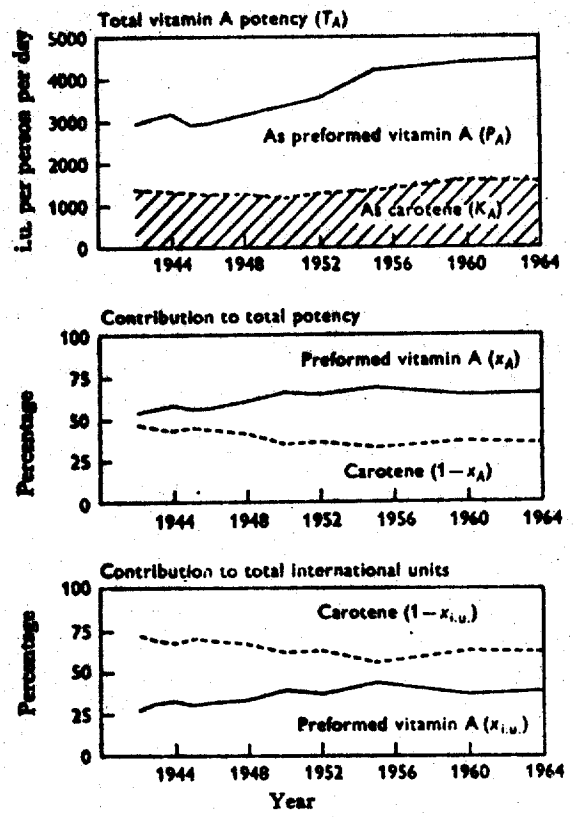

Fig. 1. Vitamin A and carotene in the British diet. Results of the National Food Survey: 1942–9, urban working class households; 1950–64, all households. Algebraic symbols are discussed in the appendix.

Table 1. *Results of the National Food Survey for all households for* 1964

Total vitamin A and carotene in terms of:	Percentage of total contributed by: Preformed vitamin A	Carotene
Weight	$26 = (x_{\mu g}).100$	$74 = (1-x_{\mu g}).100$
International units	$41 = (x_{i.u.}).100$	$59 = (1-x_{i.u.}).100$
Potency: UK assumption ($e = \frac{1}{3}$)	68	32
USA assumption ($e = \frac{1}{6}$)	58 $\Big\} = (x_A).100$	42 $\Big\} = (1-x_A).100$

from the United States Household Food Consumption Survey for 1955, concluding that, in the United States, 'the total vitamin A intake (10200 i.u. daily) is two and a half times that in Great Britain'. This conclusion is unwarranted for several reasons, with which Thompson, after consultation, fully agrees.

In the first place the American estimate quoted is not representative of the full

sample, in that the farm households have been excluded: the national figure is 9957 i.u. (about 9960 i.u.) (United States Department of Agriculture, 1957). In the second place these estimates are per 'nutrition unit' (or equivalent young adult male), whereas the British results are expressed on a per person basis; the appropriate American per person figure is 8540 i.u. (The complications arising from the fact that a 'person' is defined, for survey purposes, differently in the two surveys are not considered here.) Thirdly, and most importantly from a quantitative point of view, the American figures for 'vitamin A value' are in terms of total international units ('no allowance has been made . . . for differences in availability of the precursors as sources of vitamin A value from different types of food' (Watt & Merrill, 1963, p. 167), whereas the British figures, as has been stated above, are in terms of potency.

From a knowledge of the contribution of the different food groups to the total intake of 9957 i.u. per nutrition unit (United States Department of Agriculture, 1957, Table 5) it is possible to estimate that 4087 i.u. came from preformed vitamin A and 5870 i.u. came from carotene. If a divisor of 3 is applied to the carotene units—as in Britain—the vitamin A potency of the diet comes to 6044 i.u. per nutrition unit, or (from the values in Table 1 of the reference quoted immediately above) about 5180 i.u. per person. This is the figure which can be more properly compared with British figures, and is some 20%, markedly less than Thompson's estimate of nearly 150%, above the estimated British intake quoted by him.

Comparison of estimated intake with recommended allowances

Because the National Food Survey results are given as preformed vitamin A, or vitamin A potency, they are properly compared with the British Medical Association's (1950) recommended allowances in the same units; for example, the daily allowance for an adult is taken as 2500 i.u., and any assumption about the relative contributions of vitamin A and carotene to the total intake is obviated. The vitamin A potency of the average daily household food consumption in Great Britain in 1962 was about 4310 i.u. per person, estimated as 185% of the recommended allowance (Ministry of Agriculture, Fisheries and Food: National Food Survey Committee, 1964). In calculating this percentage a conventional deduction of 10% was made for non-ingestion of food, and allowances were made for meals served to visitors and for meals consumed outside the home (as described in Appendix G of the reference quoted immediately above, and in appropriate appendices of later annual reports of the National Food Survey Committee).

In the United States, the custom is to compare consumption in terms of nutrition units with the allowance for an adult male. Thus, in the example used above, the figure 9960 i.u. for vitamin A value is compared with an allowance of 5000 i.u. (United States Department of Agriculture, 1961, p. 9). However, the value of 5000 i.u., appropriate for a 'mixed diet', implies that one-third of the vitamin A activity is supplied by the preformed vitamin, since the equivalent allowance in terms of preformed vitamin A is 3000 i.u. ((USA) National Research Council, 1964 and earlier editions). However, if the estimates derived above for the amounts of preformed vitamin A and carotene in the American diet are correct, the proportion of vitamin A

activity supplied by the preformed vitamin is in fact $4087 \div (4087 + \frac{1}{2} \times 5870)$, or about 58%. If this proportion is accepted, rather than the assumed one-third, and if an allowance of 3000 i.u. in terms of preformed vitamin A is also accepted, then it can be shown that the allowance in terms of international units for a mixed diet of such proportions is 4260 i.u., rather than 5000 i.u. (see appendix, equation 8). The consumption figure of 9960 i.u.—less 10%—is 210% of the derived allowance of 4260 i.u., which is about 14% above the roughly equivalent British percentage of 185.

If the American data are considered in terms of preformed vitamin A a (necessarily) similar result is obtained. On the assumption that 2 i.u. carotene = 1 i.u. vitamin A, the total vitamin A potency of the diet is 7022 i.u. ($4087 + \frac{1}{2} \times 5870$ i.u.) per nutrition unit, to be compared with an allowance of 3000 i.u. Again with 10% allowed for wastage, the allowance is exceeded by 210%.

Finally it is hoped that the joint WHO/FAO Expert group which met towards the end of 1965 to consider requirements for vitamin A will have resolved some of these complications—perhaps by abandoning the use of international units—but in the meantime caution is advisable, and it is hoped that this paper, with its accompanying appendix, may assist in the manipulation of units, and explain the procedure used in the National Food Survey of Great Britain, as well as illustrating some changes that have occurred in the British diet over the last two decades.

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APPENDIX

On the calculation of vitamin A in the diet

(1) Let preformed vitamin A = P, carotene (assumed to be, or to be equivalent to, β) = K, total vitamin A plus carotene = T, overall efficiency of utilization of K = e, fraction of P in T = x. Quantities may be expressed in three different units:

weight, international units, or international units modified to represent 'equivalent vitamin A activity' or 'potency'.

Let quantities expressed by weight be denoted by suffix μg, quantities expressed in international units (unmodified) be denoted by suffix i.u., quantities expressed in terms of vitamin A potency, be denoted by suffix A.

By definition $P_A = P_{i.u.}$; $K_A = eK_{i.u.}$; $P_{i.u.} \times 0{\cdot}3 = P_{\mu g}$; $K_{i.u.} \times 0{\cdot}6 = K_{\mu g}$.

There are three possible definitions of x, depending on the units employed, namely

$$x_{\mu g} = \frac{P_{\mu g}}{T_{\mu g}}, \quad x_{i.u.} = \frac{P_{i.u.}}{T_{i.u.}}, \quad x_A = \frac{P_A}{T_A}.$$

Therefore

$$T_{\mu g} = P_{\mu g} + K_{\mu g}, \tag{1}$$

$$T_{i.u.} = P_{i.u.} + K_{i.u.}, \tag{2}$$

$$T_A = P_A + K_A, \tag{3}$$

$$= P_{i.u.} + eK_{i.u.}, \tag{4}$$

$$= \frac{P_{\mu g}}{0{\cdot}3} + \frac{eK_{\mu g}}{0{\cdot}6}. \tag{5}$$

(2) The British and American assumptions, as discussed in the text, can be expressed as follows: UK assumptions: $e = \frac{1}{3}$, $x_{i.u.} = \frac{1}{3}$; USA assumptions: $e = \frac{1}{2}$, $x_A = \frac{1}{3}$.

It should be noted: (*a*) x_A is related to e, as follows:

$$x_A = \frac{P_{i.u.}}{P_{i.u.} + eK_{i.u.}}; \tag{6}$$

$x_{i.u.}$ is independent of e, and is to this extent a better measure.

(*b*) The USA assumptions above are equivalent to an assumed value of $x_{i.u.} = \frac{1}{3}$, as may be seen by substituting into the equation

$$x_{i.u.} = \frac{ex_A}{1 - x_A(1-e)}. \tag{7}$$

(3) These relationships may be used to convert 'requirements' from one basis to another. For example, if requirements are given in terms of total international units of vitamin and provitamin in a mixed diet, what would requirements be in terms of preformed vitamin A only? In other words, given $T_{i.u.}$, what is T_A?

The solution depends on both e and x, and there are two expressions, to take account of the two ways in which x might be defined:

$$T_A = T_{i.u.}\left(\frac{1}{x_A + (1/e)(1-x_A)}\right) \tag{8}$$

$$\text{or} \quad T_{i.u.}(x_{i.u.} + e(1 - x_{i.u.})); \tag{9}$$

e.g. (*a*) if $x_A = \frac{1}{3}$, $e = \frac{1}{2}$ (USA assumptions) then from equation (8) $T_A = 0{\cdot}6\, T_{i.u.}$; therefore, if $T_{i.u.} = 5000$, $T_A = 3000$, etc.

Alternatively, equation (9) could be used to recalculate requirements on the USA scale, if the value of $x_{i.u.} = \frac{1}{3}$ (derived from equation (7)) is used.

(*b*) If $x_{i.u.} = \frac{1}{3}$, $e = \frac{1}{3}$ (UK assumptions) then from equation (9) $T_A = \frac{5}{9}T_{i.u.}$; therefore, if $T_{i.u.} = 5000$, $T_A = 2778$ i.u., etc.

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ACCUMULATION AND EXCRETION OF VITAMIN A-LIKE FLUORESCENT MATERIAL BY SEBACEOUS GLANDS AFTER THE ORAL FEEDING OF VARIOUS CAROTENOIDS*

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In our earlier work (1, 2), we injected various carotenoids intradermally and visualized them by their auto-fluorescence within the sebaceous complexes in the vicinity. Carotene and vitamin A were characterized operationally based upon their auto-fluorescence in the formalin fixed biopsy specimens: (a) Carotene was characterized by its yellow-gold, slow-fading fluorescence. (b) Vitamin A and/or a vitamin A-like material was characterized by a brilliant green fading fluorescence (3).

The vitamin A-like material was accumulated within the cells of the sebaceous glands, within the sebum in the hair follicles, and in the interstices of the stratum corneum. Based on these direct observations on normal individuals and on patients in whom the follicles were plugged, we hypothesized an external route in the skin for vitamin A.

Normally, neither vitamin A nor carotene is present in detectible amounts by the standard chemical or histologic methods (4, 5, 6). In this paper we will report the physiologic accumulation of the vitamin A-like material in the same external route via the sebum in man subsequent to the chronic ingestion of carotene; while failing subsequent to the chronic ingestion of equivalent or greater amounts of vitamin A. The serum carotene and vitamin A values were determined. It is noteworthy: (a) That the vitamin A-like fluorescence was present in the sebum and in the epidermis in proportion to the enhanced serum carotene values. (b) That a carotene tolerance developed as judged by chronic serum carotene values.

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METHODS

Serum carotene and vitamin A determinations (7) were made on the initial day of the carotenoid regimen and at monthly intervals thereafter.

The individuals tested were either out patient or hospital patients of the dermatology division at Cook County Hospital. They did not present absorption defects; they suffered from a variety of usually localized dermatoses not thought to be associated with vitamin A deficiency.

Punch biopsy specimens (8 mm.) from apparently normal skin were taken from the back usually at the time when the blood was drawn. The tissues were fixed (4 hours) in 10% formalin. Frozen sections (20 microns in thickness) were made and examined microscopically for their autofluorescence according to the method, somewhat modified (2), of Popper and Greenberg (3).

Results A. Group 1

The feeding experiments fell into three groups. Group 1 consisted of 2 patients: (a) One was fed small doses (equivalent to 10,000 U.S.P. units of vitamin A daily) of a Carotene Concentrate in Oil[1] preparation. (b) The other was fed carrot juice[2] containing carotene equivalent to 180,000 U.S.P. units daily. Within one to two months we observed: (a) The serum carotene concentration was increased 3 to 4 fold to values of 500-750 gammas per cent. (b) The green fading fluorescence was visualized in high intensity (4 plus) within the sebaceous gland cells, the sebum, the stratum corneum and the rete mucosum. (c) The serum vitamin A values remained essentially unchanged. (d) There were no clinical signs of vitamin A toxicity.

It is noteworthy that the vitamin A-like fluo-

[1] Carotene Concentrate in Oil. Wyeth Laboratories Inc., Phila. Pa. Derived from the non-saponifiable fraction of vegetables oils. 5000 U.S.P. (equivalent) Vitamin A per capsule.

[2] Everready Carrot Juice. Hawaiian Pineapple Co., Ltd., San Jose, Calif. Carotene content approximately 5,100 I.U. (equivalent) vitamin A per fluid ounce.

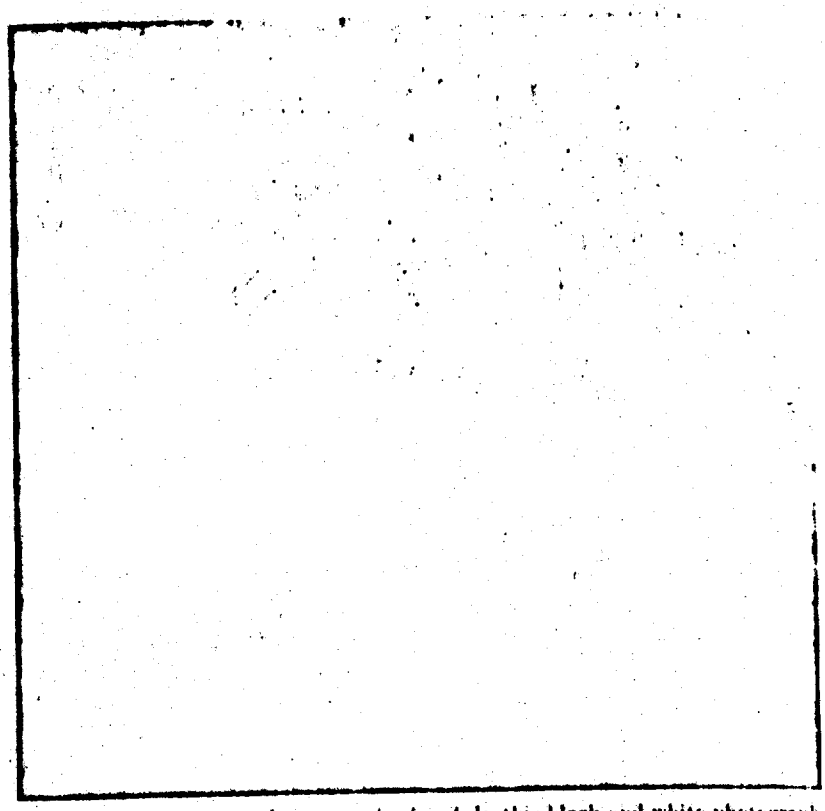

FIG. 1. *Subject in whom carotenoderma was developed.* In this black and white photograph, the bright green fluorescent hair follicles stand out as white dots. The photograph was made while the subject's back was being irradiated with an ultra-violet Wood's lamp.

resence seen histologically was readily observed at each of the hair follicles of the intact skin (See Figures 1 and 2). This macroscopic green fluorescence was observed in only these two subjects. Further there was a typical but slight carotenoderma in these subjects only. Histologically this was associated with yellow gold fluorescent flecks characteristic of carotene within the stratum corneum and within the dermis just beneath the tips of the papillae. In addition there was a salmon to brilliant red fluorescent material within the follicles and at the neck of the sebaceous glands. A red fluorescent material could be expressed from the follicles at this time and for months thereafter.

Results B. Group 2

The chemical data for the remaining subjects are summarized in Table 1. Carotene Concentrate in Oil,[1] the non-saponifiable fraction of vegetable oil; Roche Beta Carotene,[2] a crystalline carotene preparation; and Research Carrot Oil, a carrot oil extract, were fed. The serum carotene values remained essentially unchanged throughout the carotene ingestion period in the 9 subjects who received 5000-10,000 U.S.P. units daily of the Carotene Concentrate in Oil.[1] The serum carotene values were increased by 50% to 150% above the initial values within one month with all the carotene preparations within the dose range from 20,000 to 100,000 U.S.P. units daily. However, the cumulative rate of rise of the serum carotene values was either markedly diminished or ce-

[2] Roche Beta Carotene. Kindly supplied by Hoffman-La Roche, Inc. Nutley, N. Jersey. A crystalline preparation of beta carotene. (5 mgm. capsule)

[3] Research Carrot Oil. Kindly Supplied by Nutritional Research Associates, Inc. S. Whitley, Indiana. The fat soluble fraction of the carrot root. Standard potency: 25,000 U.S.P. (equivalent) vitamin A per gram. (0.8 gm. per capsule)

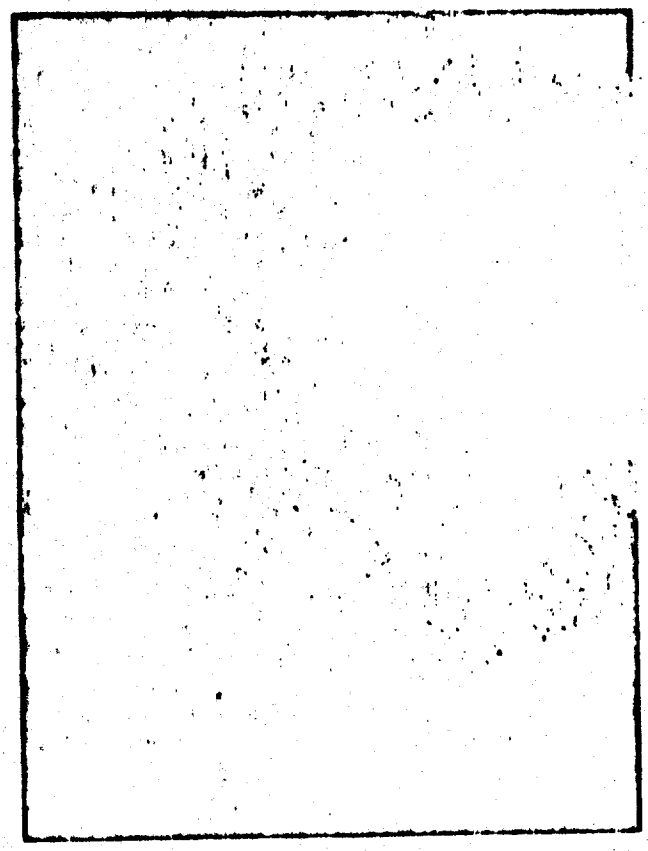

FIG. 2. *Normal subject for comparison.* This photograph was made as in Figure 1; the hair follicles were not fluorescent.

[illegible]ed in two thirds of the subjects in the second and third months.

Actually, a decreased serum carotene concentration was seen in the second month: (a) in 9 of [illegible] of the subjects receiving the various doses of Carotene Concentrate in Oil, (b) in 6 of 15 of the subjects receiving Roche Beta Carotene, and (c) in 2 of 5 of the subjects receiving the Research Carrot Oil. The serum carotene concentration showed a small percentage increase in the second month: (a) in 3 of 19 of the subjects receiving the Carotene Concentrate in Oil, (b) in 4 of 15 of the subjects receiving the Roche Beta Carotene, and (c) in 2 of 5 of the subjects receiving the Research Carrot Oil.

Essentially after one month, there were obtained three characteristic serum carotene curves: (a) a slowly rising curve in about ⅓ of the subjects, (b) a plateau curve in about ⅓ of the subjects, and (c) a slowly decreasing curve in about ⅓ of the subjects.

The serum carotene curves obtained were remarkably low and remarkably similar despite the wide range of doses administered.

The serum vitamin A values (see Table 1) remained essentially unchanged throughout the carotene ingestion period. There were no clinical signs of vitamin A toxicity (8).

Generally, the initial biopsy specimens were devoid of either of the autofluorescent carotenoids. In several instances trace intensities of the green fading fluorescence were visualized within the sebaceous glands. At the end of the first month of carotene feeding, the green fading fluorescence was visualized within the cells of the sebaceous glands in trace to 1 plus intensities in nearly every instance. At two months, the green fading fluorescence was in heightened intensity (1 plus to 2 plus) within the sebaceous glands. None of

TABLE 1

Serum carotene and vitamin A concentration in gamma % in 44 subjects during chronic carotenoid ingestion

Dose Day Equivalent to Vit. A in I.U.[1]	No. of Subjects		Initial	1 Month	2 Months	3 Mos
Roche Beta Carotene[2], 100,000.	15	Serum Carotene	128 (±68)	308 (±116)	313 (±141)	314 (± 184)
		Serum Vit. A	64 (±18)	71 (±9)	74 (±11)	82 (± 17)
R.C.O.[1] 40,000	5	Serum Carotene	113 (±47)	187 (±76)	194 (±101)	
		Serum Vit. A	69 (±16)	80 (±31)	74 (±21)	
Carotene concentrate in oil,[1] 40,000.	5	Serum Carotene	118 (±31)	211 (±95)	283 (±78)	
		Serum Vit. A	72 (±9)	76 (±38)	72 (±11)	
Carotene concentrate in oil,[1] 20,000.	5	Serum Carotene	159 (±54)	262 (±69)	—	288 (± 119)
		Serum Vit. A	65 (±19)	103 (±17)	—	88 (± 44)
Carotene concentrate in oil,[1] 5000–10,000.	9	Serum Carotene	139 (±95)	139 (±67)	—	104 (± 28)
		Serum Vit. A	72 (±17)	65 (±9)	—	60 (± 7)
Roche Vit. A Acetate,[3] 100,000.	5	Serum Carotene	87 (±39)	87 (±42)	—	
		Serum Vit. A	83 (±28)	153 (±108)	—	

the Group 2 subjects demonstrated the green fading fluorescence within the sebum, the interstices of the stratum corneum, or the rete mucosum as did the two subjects of Group 1. Neither did we observe the yellow gold fluorescence of carotene within any of these sites. However, small amounts of the unidentified salmon to red fluorescent material, as described for Group 1, were frequently observed within the follicles in the biopsy material after the second month of carotene ingestion.

Results C. Group 3

Similarly, a group of 5 subjects were fed vitamin A acetate[3] (100,000 U.S.P. daily) for one month. At one month the serum vitamin A values were increased from 50% to 200% while the serum carotene values remained essentially unchanged. Further, the sebaceous glands contained zero to trace intensities of the green fading fluorescent material except in one subject in whom we observed the fluorescence in moderately increased intensity. In no instance was the green fading fluorescence observed in the sebum or in the stratum corneum; nor did we observe the red fluorescent material within the hair follicles as with the subjects of Groups 1 and 2. The above histologic findings were equally true for several additional patients not listed in Table 1 who were fed three times to ten times the above dosage of vitamin A acetate for 2–3 months and longer.

[3] Vitamin A Acetate. Kindly supplied by Hoffmann-La Roche Inc., Nutley, N. Jersey. (50,000 I.U./capsule).

DISCUSSION

1. Carotene Hypothesis

Carotene is universally considered to be present in normal skin in the stratum corneum although the experimental evidence is meager and questionable (9, 10, 11). Several authors have alluded to its presence in sweat glands (12, 13, 15, 16, 17) or in sebaceous glands (13, 14, 15, 16, 17); to our knowledge neither carotene nor vitamin A have been demonstrated histologically or chemically either in these structures or their secretions (4, 5, 6, 10).

In our data, in the two subjects (Group 1) in whom the serum carotene values were raised 3–4 fold (500–750 gammas%) we observed the vitamin A-like fluorescence in high intensity within the sebum and within the entire epidermis and none within the sweat glands or ducts. In the other 39 subjects (Group 2) in whom the serum carotene values were raised 1 to 2 fold or less (200–300 gammas%), the vitamin A-like fluorescence was visualized in decreased intensity within the sebaceous glands only. The vitamin A-like fluorescence was not seen within the sebum when equivalent or greater amounts of vitamin A were ingested. We therefore hypothesize that the carotene rather than the vitamin A of the serum serves as the principal carotenoid for the sebaceous complex.

Vitamin A supplementation has been much used in therapy in dermatology; there are almost no reports (18) of the use of carotene supplements. Recently, in a symposium on nutritional disease,

Simshaw (19) reported remarkably low serum carotene values (44–85 gammas %) associated with serum vitamin A values (20–29 gammas %) but were within normal limits in school children in 3 Central American countries (except Guatemala). Follicular hyperkeratosis was present in 45–55% of these children, and it remained unchanged despite vitamin A supplements (40,000 I.U. daily for 14 weeks) or fat supplements (48 grams of lard, etc., 5 days a week for 56 weeks). In view of our hypothesis we would predict that carotene supplementation would benefit these school children; although it is conceivable that the hypocarotenemia in this population is the result of a generally enhanced carotene tolerance as discussed below.

2. *Carotene Tolerance*

A carotene tolerance, as judged by the serum carotene values, was developed with the chronic ingestion of carotene in 39 of 41 subjects. This phenomenon, though surprising to us, was described previously, although sketchily and on few patients, by Kaufman and Drigalski (20) and Wendt (21). And recently in an excellent work, Urbach *et al* (24) obtained a two fold increased serum carotene concentration which remained unchanged during a 6 month period of massive carotene ingestion in 4 subjects. Urbach's conclusion that the body does not have control mechanisms to regulate the level of serum carotene seems unjustified, even though vitamins A, C and E are regulated more efficiently.

Other authors have commented on the great variability amongst individuals in the development of carotenemia (13, 14, 15, 22). Where case histories have been obtained in patients with carotenoderma, it usually required from 4–12 months or longer of supplementary carotene (12, 14, 15, 17); however among the patients there were described several individuals in whom 1–2 months or less sufficed (14, 17, 22).

Significantly, we observed a several fold increase in the peak carotene values associated with carotenoderma in only 2 of 41 subjects. Probably, there is a previous natural selection for the clinical studies so that only the carotenoderma susceptible individuals present themselves to the physician.

Further, in our study and in Urbach's (24), the serum vitamin A values remained essentially unchanged throughout the carotene ingestion period. Thus there is no direct evidence for the concept that the low peak carotene values are related to an accelerated rate of conversion of carotene to vitamin A. Neither is this mechanism ruled out for vitamin A blood levels are known to be closely regulated (24). Significantly, despite the enormous carotene intake for several months there were none of the classical signs of vitamin A toxicity in our subjects (8, 23).

SUMMARY

1. A vitamin A-like fluorescent material was visualized within the sebaceous glands and in the sebum and throughout the epidermis in the 2 of 41 subjects in whom the serum carotene values were increased 3 to 4 fold by long term carotene supplementation.

2. In the remaining 39 of 41 subjects the vitamin A-like fluorescence was visualized in the sebaceous glands only and the serum carotene values were increased only 1 to 2 fold or less.

3. Vitamin A acetate in 3 to 10 times equivalent doses did not induce the vitamin A-like fluorescence within the sebum and the epidermis.

4. Based on these findings we hypothesized that the vitamin A-like material visualized in the sebum is derived principally from the serum carotene and not from the serum vitamin A.

5. Carotene tolerance as judged by the serum carotene values was developed in almost all of the subjects. This was discussed in relation to the variability in susceptibility to carotenoderma.

6. The above carotene tolerance may in part be the mechanism to explain the freedom from toxicity observed even with massive carotene alimentation.

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DISCUSSION

Dr. Victor R. Wheatley (Chicago, Ill.): The one point I should like to make here is that there is still no real evidence that the material is vitamin A. Giving the best judgment possible on your work, we can say that the carotenoids are converted into a green fluorescent substance, but I think it is premature to assume that it is vitamin A. Biologically the conversion of β-carotene to vitamin A is a complex process and not the simple piece of scissors biochemistry it appears on paper. I can't understand why when you feed large amounts of vitamin A you do not get it in the sebaceous gland. If it was vitamin A I would expect the reverse to be true.

Dr. Ruven Greenberg: I agree that we do not have positive proof that the green fading material is vitamin A. I was very careful to call it a vitamin-like material. I, too, agree that the increase of the fluorescence in the sebum does not come about by feeding vitamin A, rather it was produced by feeding carotene, and that's the whole point of this paper.

Originally, with the intradermal injection of a solubilized carotene, we demonstrated that this vitamin A-like material travels within the sebum via an external route to the stratum corneum. In this paper we were able to reproduce this route physiologically, in 2 of 41 patients in whom we raised the serum carotene level 3 to 4 fold while at the same time the serum vitamin A values remained essentially unchanged. This makes me feel that the serum carotene is the precursor of vitamin A; or at least it is the precursor of a vitamin A-like material within the sebum.

Dr. Theodore Cornbleet (in closing): We were rather disappointed when we could not duplicate the high levels of blood carotene attained in the earlier experiments.

Three patients were given vitamin A first, and then given carotenes afterward; in those three we attained much higher levels of serum-carotene and also a larger amount of carotene and vitamin A in the sebaceous glands. In the present experiments we did not administer vitamin A either before or during the feeding of carotene. Perhaps this may explain the modest rises in serum-carotene levels we are reporting.

One thing stands out in the clinical work—there are no ill effects, no untoward results from the giving of the large doses of any of the preparations of carotene we employed. This is in contrast to what could happen from the use of comparable doses of vitamin A. If there is a place for pharmacological doses of vitamin A in dermatology, then perhaps carotene could replace the vitamin, at least for greater safety.

CAROTINOID PIGMENTATION OF THE SKIN RESULTING FROM A VEGETARIAN DIET *

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Hess and Myers [1] described the occurrence of a yellow discoloration of the skin of the body, with the presence of carotin in the blood of children fed on a diet containing carrots. Head and Johnson [2] reported a similar occurrence in an adult who had eaten considerable quantities of carrots. The latter authors referred exhaustively to the articles concerning the carotin pigmentation of the skin that had appeared in English and German literature.

In Japanese literature we have no description of a yellow pigmentation of the skin resulting from a diet containing carrots; but the fact that a vegetarian diet may cause a yellow discoloration of the skin has long been known among physicians in Japan. Baelz [3] (1896) was the first to call attention to the golden-yellow discoloration of the skin ("aurantiasis cutis") developing as the result of eating large quantities of "mikan" (*Citrus nobilis* Lour., var. *microcarpa* Hassk., a kind of orange produced in Japan). He noted that the palms of the hands and the soles of the feet are tinted usually more heavily than other portions of the body, and he supposed that the horny layer of the epidermis, which is thicker in the above-mentioned portions of the body than others, may have a specific affinity for the yellow pigment of mikan present in the circulating blood.

A number of physicians have reported similar cases (Arima, 1896; Sano, 1900; Watanabe, Sato, Morita, 1903; Nagahama, 1904; Shishido, 1905; Oishi, 1906; Ono, 1906; Dohi, 1910). Nagahama and Shishido stated that an intense pigmentation of the palms of the hands and the soles of the feet is dependent partly on intense skin secretion occurring in these sites. Dohi and Ono examined microscopically a section of the skin showing aurantiasis, and they found only the horny layer of the epidermis to be tinted yellowish. Ishikawa, Okada and Yamada (1915) reported that orange-yellow pigmentation of the skin similar to that resulting from eating mikan had been observed in some people in Hokkaido who had been eating heavily of squashes. Kashida (1917) reported a case of aurantiasis cutis resulting from eating much laver. Prof. K. Miura [4] has stated that orange-yellow pigmentation of the skin, which had been described under the term aurantiasis cutis of Baelz, is due to coloring of the subcutaneous adipose tissue with carotin present in mikan, squash, laver, etc., owing to the affinity of fats for carotinoids, and he noted that the term "carotinosis" is more nearly exact than "aurantiasis" to express the true nature of this pigmentation. Oi (1918) reported three cases of aurantiasis cutis resulting from the liberal use of cucumbers produced in Formosa.

I have recently observed more than thirty-five cases showing a yellow discoloration of the skin after the eating of unusual amounts of squash. Here in Hokkaido, the northern island of Japan, where the yield of rice is not so great as in the mainland, farmers are in the habit of eating large amounts of bald barley, potatoes, legumes, squashes, etc., as substitutes for rice.[5] In the fall they usually eat squash, which is produced on their own farms, as freely as if it were the principal food of the season. Since the beginning of October and most frequently toward the end of the month I have noticed marked yellow discoloration of the skin in some of the patients who came up from the country to visit me. I have never encountered any similar cases in the city inhabitants. The majority of those who showed the squash pigmentation of the skin were farmers, aged from 20 to 40, working daily so hard on the farm that large amounts of carbohydrates were needed as a source of energy. The occurrence of the squash pigmentation of the skin, however, is not confined to farmers of these ages. Any one who eats heartily of squash may show a yellow discoloration of the skin.[6] An idiot, aged 3 years, and a man, aged 50, following a sedentary occupation and suffering from general paralysis, have shown the skin pigmentation; both of them had been eating large amounts of squash because of an abnormal appetite for it.

Having observed carefully all these cases of squash pigmentation of the skin, I have ascertained that this pigmentation is the same as that described in English and German literature as being caused by the liberal use of carrots in the diet. Some years ago at Professor Miura's medical clinic in Tokyo, I observed some cases of mikan pigmentation of the skin. And now I affirm that there is no difference in any respect between the skin pigmentation due to squash and that due to mikan. The palms of the hands, the soles of the feet, the forearms, and the nasolabial folds were more heavily tinted yellow than other parts of the body, whereas the sclerae were not affected. The color varied from a canary yellow to a deep orange gold. The condition may be easily differentiated from jaundice by the difference in the localization of the discoloration: The pigments of squash or mikan color most intensely such portions of the body as would be least stained in the case of jaundice.[7] Furthermore, in the case of squash or mikan pigmentation there is no itching of the skin, general malaise, fever, etc. When squash is removed from the daily diet, the yellow pigmentation of the skin disappears slowly. It is of interest here to note that the palate resumes its normal color most slowly, the yellow discoloration being observed here even several weeks after the disappearance of the pigmentation of the palms of the hands or the soles of the feet.

Squashes (*Cucurbita maxima*), as usually eaten by the people in Hokkaido, contain a large quantity of carotinoids. These are probably absorbed easily from the small intestine. I was able to demonstrate in vitro that these pigments dissolve readily in human duodenal fluid kept at the temperature of 37 C., though not in the least in gastric juice. The pigments thus absorbed from the intestine circulate in the blood. In some

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4. Miura, K.: Carotin als Ursache von Aurantiasis Cutis (Baelz), Festschrift gewidmet Keiso Dohi, Tokyo, January, 1917, p. 203.
5. The details are given by Morimoto, K.: The Standard of Living in Japan, Johns Hopkins University Studies in Historical and Political Science, Baltimore, 1918.
6. Dr. Morimoto, professor of economics in the Hokkaido Imperial University, told me that, when, in October, he visited several primary schools in the farming districts of Hokkaido to investigate school lunches eaten by children, he found that the majority of the children had an intense yellow pigmentation of the skin due to the large amount of squash consumed daily.
7. Only the palate, however, is tinted, though not intensely, in both cases.

cases of mikan or squash pigmentation I could ascertain the presence of carotin in the blood drawn by venipuncture. The blood, when allowed to coagulate, gave the serum a gold yellow—more intense in color by far than that of normal blood—which, when examined spectroscopically, showed absorption bands identical with those of carotin. The carotinoids have not been found in the urine excreted by man showing mikan or squash pigmentation of the skin—in such quantity as can be determined by any usual methods. Whether or not these pigments would be excreted into the intestines we do not know. The largest quantity of carotinoids present in the blood seems to be excreted by the skin secretions. The secretion of the sebaceous gland contains much fat. Sweat, even that of the palms of the hands or the soles of the feet, the skin of which portions of the body lacks the sebaceous gland, contains some fat. And, as generally known, fats have a great affinity for the carotinoids. The intense pigmentation of the nasolabial folds, frequently observed, may be due to intense secretion of the sebaceous gland in these sites.

As to the histologic question with respect to what part of the skin is pigmented to cause the yellow discoloration of the skin, as clinically observed, Dohi and Ono, who had examined microscopically a section of the yellow-pigmented human skin in a case of aurantiasis cutis, stated that only the horny layer of the epidermis was found to be tinted diffusely yellowish. The subcutaneous adipose tissue would be actually colored by carotinoids,[8] but, so far as I am aware, the yellow discoloration of the skin, as is observed clinically from the outside of the body, is chiefly due to the coloring of the horny layer of the epidermis, and not that of the layers lying deeper. It is of interest here to refer to the findings of Palmer and Kempster.[9] In the section of the highly pigmented shank skin of fowls, they found microscopically the granules of carotinoids in the corium, along blood capillaries, and most intensely accumulated in the malpighian layer. In a case presenting a marked squash pigmentation I could remove a part of the skin of the highly pigmented palm of the hand—the epidermis that had been separated from the corium as the result of combustio bullosa. After the epidermis had been removed, the corium appeared by no means yellowish, while the epidermis removed showed the yellow coloring of its horny layer. Microscopically examined, the horny layer of the epidermis was seen to be tinted diffusely yellowish, no yellow pigment granules being found. The malpighian layer was free from pigmentation. The fact that the carotinoids color the horny layer of the epidermis, in the case of the carotinoid pigmentation of the skin in human subjects, may account for the intense discoloration of the palms of the hands and the soles of the feet—more intense than in other portions of the body—where the horny layer is thickest.

In regard to the affinity for the fat-staining substances of the horny layer of the human epidermis, Stoehr stated in his textbook that paraeleidin is colored black as well as ordinary fats, but only after a long-continued action of osmic acid. Martinotti demonstrated by the aid of the chromchrysoidin reaction the presence of certain special kinds of fat in the human epidermis, keratohyalin, eleidin, etc., and noted the significance of fats in the biologic process of producing horny substances. It is evident that in the cells of the horny layer of the human epidermis there exist certain kinds of fat that are colored by fat-staining dyes slowly but steadily, and, once colored, maintain the color for a long while. In the case of carotinoid pigmentation of the skin it is highly probable that such kinds of fat present in the epidermis are colored by the carotinoids which are excreted by the skin secretions. An obscure point, however, is left yet undecided: whether the carotinoids coloring the human epidermis are carried out solely by the skin secretions, or further directly through the tissue of the corium from its blood capillaries.

Kaupe stated that there is some variation in individuals with respect to the ease with which the carrot pigmentation may develop. Klose noted that well nourished children were prone to the same affection. Having observed many cases of squash pigmentation, I can scarcely confirm Klose's findings. Individual variation in the intensity of skin secretion or in the thickness of the epidermis rather than the nutritional condition of the body seemed to determine the tendency for the skin pigmentation of the subjects following a squash diet.

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8. The discoloration of the palate might presumably be due to the yellow coloring of the adipose tissue underlying a thin mucous membrane.

9. Palmer, L. S., and Kempster, H. L.: The Influence of Specific Feeds and Certain Pigments on the Color of the Egg Yolk and Body Fat of Fowls, J. Biol. Chem. 39: 331 (Sept.) 1919.

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C. HENSCHEN

HYPERVITAMINOSIS A IN MAN

(Atoxic carotenoses and genuine A-toxicosis)

From the Surgical University Clinic, Basel.

The prominent carotenoid expert L. ZECHMEISTER writes: "No natural hypervitaminosis A exists; none died as a result of carotene intoxication so far". Only nutritional factors related to geography, nutritional emergencies, psychopathic pica or wilful misuse can actually break through the multiple defense mechanism which prevents vitamin A damage. Pharmacologist K. SCHUBEL is unable to report any example of hypervitaminosis A in man in his work entitled "Toxicology of the Vitamins" (1937), despite [illegible] evaluated evidence and despite the fact that the sequelae of A-toxicosis had by then been verified and confirmed in animal tests. The above shortcoming of the findings was compensated by a case of pathologic hypervitaminosis A in man, which I was accidentally able to follow. Therefore carotenoses in man are now observable, starting from their harmless, non-pathologic sub-levels, to the occurence of genuine toxic A-lesions. The following forms are distinguishable on the basis of clinical symptom classification:

1. Simple xanthemiae ("invisible xanthomas"), without chronic xanthodermatoses, merely with the sporadic occurence of a rapidly fading yellow discoloration on the palms and foot soles, reveal to the expert's scrutiny that excess supply is involved, or that an inter-vitamin equilibrium malfunction is present,

which was not fully compensated by the organism. Example: The long-known yellow discoloration of palms and soles in babies and infants after the consumption of carrots. The subsequent fading of the xanthic pigmentation despite continued ample carotene supply (HEYMANN) demonstrates the efficiency of the body's adjustment capacity.

2. Manifest xanthemias with chronic xanthodermia without pathologic disorders (compensated atoxic carotenoses). Examples: "aurantiasis cutis" (orange-yellow discoloration of the foot soles and palms after excessive tangerine consumption); "ochro-dermatoses" of Japanese farmers after excessive consumption of giant squash ; xantho-dermatoses after consumption of baked squash with meatless diet which is however rich in spinach, salad and butter (Hungary, England).- Food rich in carotene content (milk, eggs, butter, margarine, oranges, lemons, carrots, rose-hips, paprika, salad) increases the serum carotene level considerably already after 1 to 2 weeks when the A storage of the liver is overloaded (MIAKE: after daily consumption of 25 to 30 oranges, carotenemia; on the 16th day, carotenuria as well). Prolonged increase of the serum lipochromia in healthy humans to yellow discoloration of foot soles, palms, naso-labial folds and finally of the entire body as well, with the exception of the sclera. However, individual-- possibly merely temporary--inclination is involved as well , since only certain members of a family group on the same diet are affected. Said pigmentation is the sequela of equilibrium upset between the vitamin formation capacity of the liver, vitamin A-decomposition, the carotene storage capacity at storage sites, deficient excretion of polyene in the stool

and through the skin and reduced oxidation processes. When carotene is excreted in perspiration (maximal discoloration in the main perspiration zones) and (with higher fatty acids) when it is excreted with the cutaneous fats, the pigment accumulates in organic form within lipid drops, maximally in the corneous layer; it likewise accumulates in small spots within the subcutis. The first prominent fact, i.e. that persons on a raw vegetarian diet do not always become "canary-people" is ascribable to the fine adjustment capacity of our internal laboratory; it is likewise attributable to the circumstance that those on a raw vegetarian diet do not consume only tangerines and carrots; instead, they are on a mixed vegetable-fruit diet which, thanks to its favorable vitamin mixture, offers an opportunity for the organism to make the needed adjustments over the inter-vitamin correlations.

3. Decompensated atoxic or pretoxic xanthomias in patients with deblilitated liver. In cases of liver- and gall bladder diseases, manifest(" hemeralopia of liver patients") or concealed hypovitaminosis A and hypercarotenemia occur, with or without xanthodermatosis, as sequela of deficient vitamin A-formation. Depending on the variable functional condition of such livers, too little and soon again an excess of vitamin A, in the course of compensating overproduction, is formed; therefore an "oscillating" xanthosis can occur. This implies oscillation beteeen A-deficient carotenemia and regression of the carotene level following A-enrichment.

This oscillation between two antipolar conditions recalls the interplay of Graves' disease - myxedema, hyperglycemia - hypoglycemia, etc. It remains questionable whether these carotenemias of liver patients lead to the progressive slagging of the liver and spleen with carotenoids which remain unprocessed (excess storage of hepatolienal R.E.S.?). Similar fluctuations in the carotene- vitamin A - metabolism occur also in cases of liver cirrhosis and cancer (abnormal A-excretion through liver and kidneys ; therapeutic hypervitaminosis due to "Vogan" administration in cancer patients with debilitated liver); they likewise occur in pre-senile and senile liver insufficiency due to advanced age, in which cases unbalanced and excessive carrot consumption is especially apt to cause carotene retention and yellowish-brown - orange xanthodermatosis (SCHUSSLER, GROTT).

4. Decompensated xanthoses (vitamin-A toxicoses).

First, the clinical aspect of hypervitaminosis A:

Comparatively small A-doses (2000 to 10,000 international units) inhibit the metabolism of rats and guinea pigs on standard diet up to 10%. Massive A-supply accumulates in the rat liver in volumes which "would correspond to the requirement for a century" (SANTOS, RAITZ, DAVIS and MOORE). Vitamin A supply below the damage limit (1500 i.u.) in sound young rats promotes growth and fatty deposits.

Overdosage results in a whole series of disorders already within 5 y to 8 days: roughening of the coat; trophic skin disorders with seborrhoea; apathy; rhinitis hemorrhagica; conjunctivitis and exophthalmos; lack of appetite and diarrhea; strong growth inhibition; drop of basic metabolic rate; weight loss; high-grade anemia with drop of thrombocyte count; rachitis-type stiff walk with spastic leg contractions; deformation of the rib cartilage; spontaneous fractures (as during osteogenesis imperfecta and general osteo-dystrophia fibrosa), with insignificant modification of the blood phosphate and blood calcium, but with decreased bone calcium and bone phosphorus (mineral deficiency due to blocking? osteogenetic cell damage?); inhibition of bone thickening jointly with cortical thinning; after 2 to 4 weeks, rapid death. Anatomic findings: necrotizing glomerular necrosis with sclerosis, enormous fatty deposits (cholesterol impragnation of the entire R.E.S.), in particular of the KUPFFER star cells, of the liver; minor spleen parenchyma lesion, high-grade necrotizing and toxic damage of the testicle endothelials with semen formation disorders. 8-weeks-old animals show a behavior which is does not differ substantially from the above. Thyroxin and application of Vitamin B and C prevent death and the entire series of disorders. -

the characteristics of A-hypervitaminosis in guinea pigs are: severe shifting of the fat metabolism, i.e. hyperlipemia and hyperlipoidemia, lipoid storage with fatty infiltration of the KUPFFER cells during fat deficiency, nucleus fragmentation and enrichment of the liver cells, lipoid storage in the spleen pulpa, fatty infiltartion of capillaries, of kidney tubuli and of glomerula endothelials.

In a nutshell, my findings in a case of human hypervitaminosis A are:

Mrs. A. W. B. Admission diagnosis (November 1935): "lithogenetic jaundice". Clinical diagnosis: "Pseudo-icterus caused by alimentary xanthodermatisis as a result of excessive carrot consumption". In late spring 1935, after strenuous nursing (work?), the woman, 49 years old at that time, developed physical debility; her face was sallow, and she was affected by insomnia. When iron tincture, prescribed "for anemia" proved to be ineffective, spontaneous excessive consumption of vegetables, which resulted in an insurmountable pica-type desire for carrots; for months, she consumed 2 to 3 bunches of carrots daily (up to 1 kg), mostly raw, often untrimmed (she surreptitiously recovered carrots from the garbage can which had been discarded by concerned relatives); this went hand in hand with a strong craving for head lettuce (2 to 3 heads per day); no aversion to meat, but aversion to sweets and fresh fruit. In September 1935: yellow pigmentation of the face, hands and feet, finally of the entire body, with paresthesiae of the

fingers and feet; itching of the left hand back; intensified fatigue, dull pain in the liver area, "dyspnée d'effort", ankle edema.

Diagnosis on November 6, 1935: short-statured, slender woman; littl subcutaneous fat; mucous membranes pale; nervous system, hearing and eyes normal; sclera clear. Pale orange discoloration of the face, especially in the naso-labial folds; the rest of the body shows similar discoloration; brick-red discoloration of palms and foot soles. Skin dry, somewhat flaccid; no evidence of scratching. Tongue slightly coated. Conjunctival and mucous membranes of the mouth show yellowish tint. Neck and chest organs normal. Pronounced liver enlargement without presence of gall bladder tumor; lack of cholelithiatic pressure points; enlarged spleen definitely palpable; no abnormal lymph gland enlargements; for ½ year, hypermenorrhea (uterus myoma). Motility, sensitivity, reflexes and circulation of the extremities without evident symptoms. Occasionally, subfebrile temperature. Blood pressure 140/70. - Sedimentation rate tests, October 28, 1935: 32/67½/127; November 7, 1935 : 19/50/124; November 13, 1935: 14/32/110; January 1936,1936: 32/60/118. - Blood counts: October 28, 1935: hemoglobin (hgl) 57%; red blood corpuscles 3,440,000; "F.J." 0,85; "St.L." 4%; neutrophyls (N) 62,4%; lymphocytes (Ly) 28,7%; monocytes (Mono.) 4,6%; eosinophils (Eos.)0,3%; macrophages (Ma.) 0. - November 6,1935: hemoglobin 57%; red blood corpuscles 3,760,000; "F.J." 0,77; total leucocytes (L) 3%; "St.L." 1,3%; neutrophyls (N)51%; lymphocytes (Ly.) 34,3%; monocytes (Mono.) 12%;

eosinophils (Eos.) 0,7%; macrophages (Ma.) 0,4% ; anisocytosis; poly-chromasia . - January 24,1936 : hemoglobin (Hgl)64%, red blood corpuscles 3,952,000; "F.J." 0,81; total leucocytes (L) 4750 ; "St. L." 5,3%; neutrophils (N) 53,4%; lymphocytes (Ly.) 28,0%; monocytes (Mono.) 10,7%; macrophages (Ma.) 1,3%; minor aniso- and poikilocytosis, polychr. -

Bilirubin (October 29, 1935): indirect trace only (0,24 mg%).-

Blood urea (November 7,1935): 29 mg%.-

Serum examination:

Extinction wave length 430 (light source) = E 430/10 cm

Carotene	Thinning = V 1 : 10	0,3497
	(normal: 0,07 - 0,19)	
Free xantophylls	E 430/10 cm	
	V 1 : 10	0,15
	(normal: 0,06 - 0,12)	

Serum diastase at 32. - Urine, October 28,1935: normal; subsequently: variable urobilin (+) and phosphates.

Thrombus formation during thrombometric examination: poor.-

Vitamin C metabolism, October 14,11935: excretion of 31,4 mg in urine, from 300 mg Redoxon, within 10 hrs (urine concentration prior to loading 0,5%); hypovitaminosis C.

Professor KARRER obligingly undertook the processing of the serum: "Regrettably, the obtained chromatogram was extremely indistinct, and the single fractions likewise yielded extremely indistinct absorption spectrums (partial modifications and oxidation respectively, of the carotene). It is therefore impossible to determine reliably whether the serum originally contained non-modified α-carotene or β-carotene, and whether these pigments had in part undergone additional modifications".

Pathogenesis: after the prohibition of vegetables with high carotene content on December 2, 1935, considerable regression of the yellow skin pigmentation, except on the palms and foot soles (where it varied a great deal, even without dietetic errors), while the condition improved subjectively; venisection 400 cc; replacement transfusion 350 cc. January 31, 1936: serum control (Physiology-Chemistry Institute); the result can be regarded as normal 0,2 to 0,3). - June 9, 1936 : mild sub-anemia, minor leukocytopenia without counterclockwise displacement with mono-, aniso- and poikilocytosis and basophilic red blood corpuscles.

January 26,1937: except for remnants on hands and foot soles, the yellow pigmentation vanished; serum redoxy (S.R.) 20/15/123. Blood urea 21 mg%; serum bilirubin 0,36 mg%; serum diastase 32; hemoglobin 64%; red blood corpuscles 4,848,000; "F.J." 0,66; total leukocytes (L) 6,7%; neutrophyls (N) 56,7%; lymphocytes (Ly.) 22,7%; monocytes (Mono.) 9,6%; eosinophils (Eos.) 2,0%; marcrophages (Ma.) 2,3%. Mild anisocytosis. Thrombometry: good but soft thrombus, tensile (test) weight 175 g; high coagulation valence 1,0, low valence 2,5; fibrinogen 0,19; coagulation time (G.Z.) 45 minutes. Retractability 8 mm - all counts accordingly show near-normal values. -

January 12, 1940. Serum redoxy (S.R.) 10/26/101. Blood sugar 0,103/0,101. Blood urea 20 mg%; hemoglobin 72 %;

red blood corpuscles 4,576,000; "F.J." 0,80; thrombocytes 228,800; leukocytes (L) 3700; "St.L." 8 %; neutrophils (N) 47%; lymphocytes (Ly.) 35 %; monocytes (Mono.) 7%; macrophages (Ma.) 2%. Moderately strong aniso- and poikilocytosis.- Early summer of 1940: myomectomy. - For 1 year, bilateral cataract formation. - July 23, 1940: blood count normal. - Since September 1940, Benevain-injections; since December, Menformon-therapy.

The principal clinical characteristics of this pathography were: debility, weight loss, painful liver enlargement with questionable hypervitaminogenic lithiasis, spleen enlargement, urobilinuria, increased serum redoxy (S.R.), simple hypochromic anemia as sequela of liver, spleen and bone marrow lesion, intermittent leukopenia and monocytosis, hypobilirubinemia, secondary C-deficiency, skin paresthesia (irritating effect of the pigment grains?), cataract formation. Since crystallized carotene failed to generate these pathologic symptoms in the experiment, it must be assumed that they are the sequelae of A-surplus production, or the sequelae of the joint effect of vitamin A + excess storage of carotene.[1] The liver enlargement presumably is compounded by "hepatosis chymatosa" and lipoid storage in the KUPFFER cells. Watery solutions of acid bile salts make the absorption of substances possible which are otherwise not water-soluble ;by pairing of the liver- and bladder-cholates with carotene and xantophylls and possibly with vitamin A as well . Therefore excess polyene in the bile with reduced production of protective colloids necessarily results in the formation of gall-sand and gall-stones. Specifically, it will result in

the fromation of cholesterol sand and cholesterol stones (as a sequela of hypercholesteremia, generated by A-toxicosis), in which crystallized carotene can be stored.

Carotenoids (mainly β-carotene) are stored in large quantities in the corpus luteum. The ovaries are thereby hardly "interceptor organs" only; instead, they need the substances for the processing of the burst follicle, as well as indirectly, for their hormone production. In fact, a certain correlation exists between the sex hormones and vitamin A and its precursor, i.e. carotene (GUGGISBERG). Since our patient was already at the climacteric stage, and since her genital chemistry (involving an uterus myomatosus) was probably unbalanced, the obstruction of this bypass-mechanism could have contributed to excessive carotene retention.

It is highly probable that the cataract which developed in our patient is a delayed sequela of the hypervitaminosis. The close connection existing between vitamin A and the external cotyledon (accelerated renewal of the epithelials and of the nerve substance) makes it hardly surprising that the crystalline lens, as an epithelium-derivative, likewise becomes the victim of vitamin-related disorders within the A-cycle.

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1) The results of vitamin experiments in animals are admittedly not readily applicable to man. For example: beriberi in man causes tachycardia; beriberi in the rat causes bradycardia. A. ZELLER. (see p. 10 of translation)

In the course of experimental avitaminosis A in young rats the dams of which had been kept on a slightly modified McCOLLUM diet, numerous cases of crystalline lens clouding occur which terminate in the fissuring of the nuclear zone and in characteristic layered cataract formation, whereby only the first stages of the crystalline lens clouding are reversible. PILLAT observed two human pre-xeroses due to A-deficiency with clouding of the subepithelials and of the deep frontal lens cortex layers. The crystalline lens needs three factors to maintain its internal respiration, its nutrient supply, i.e. for the maintenance of its anatomic structure: a H-ion concentration of approximately p_d - 7,5, a certain amount of glutathione and it needs the reduction system (GOLDSCHMIDT). It is not coincidental that in the redoxy systems, glutathione, vitamin C and vitamin B_2 (v. SZILY) are specifically contained in the crystalline lens, in high concentrations. Vitamin C present in the lens of healthy humans undergoes a reduction in the lens affected by cataract to such an extent, that the vitamin in question is no longer determinable at all. Since xerophthalmia can occur when the A-content is high or even excessive, it appears likely that the C-deficiency of the organism resulting from excessive A-enrichment, the shift of the "favorable" vitamin mixture optimum, interfered with the redoxy system, needed to prevent crystalline lens injury.

Since our patient's vitamin C intake was certainly sufficient during carotene hyperconsumption (approximately 100 mg from carrots and salad alone), no primary, i.e. nutrition-related hypovitaminosis C can be at issue here. The complete upheaval of the carotene-vitamin A metabolism presumably shifted the inter-vitamin balance to the disadvantage of vitamin C.

Multiple safeguards exclude the risk of hypervitaminosis in case of normal state of health and well-regulated nutritional conditions. Such safeguards are: self-control of the body by means of a "concealed regulating chemism" (ZECHMEISTER); chemical corrections on the part of the healthy liver and by means of correlations between vitamins which are utilized for self-defense by the organism. According to EULER carotene and vitamin C form part of a coupled redoxy system. Much vitamin C is also found in lipochrome- (carotenoid)-rich tissues (suprarenal gland, corpus luteum, retina); simultaneous C-administration protects rats and guinea pigs from hypervitaminosis A (WENDT and SCHROEDER). Until recently, in necessarily unilateral animal tests the significance of the inter-vitamin compensation mechanism was overlooked. Our patient was C-deficient; therefore an important link of this balance safeguard system was missing in her case. It remains problematic whether the excessive provitamin A supply and ballast accumulation of the finished vitamin could finally have resulted in the elimination of C-reserves needed for body function until deficiency occurred, or whether adequate enrichment of the C-reserves was inhibited.

Last but not least, hypervitaminosis A is therefore the result of inter-vitamin correlation upsets, involving disordered carotene- or A-distribution in the tissues, disrupted excretion, [illegible] specific individual resorption conditions in the gastro-intestinal tract, excessive R.E.S. storage and other factors.

The experimental hypervitaminosis A as well as the involuntary hypervitaminosis A in man show, in some respects, a curious coincidence with the corresponding avitaminosis, also to some extent with hypervitaminosis D and hypovitaminosis D (rachitic bone changes in case of hypervitaminosis A). [illegible] were experimentally and clinically determined:

Growth inhibition (modified longitudinal growth) due to the lack of a growth vitamin.- Drop of body weight caused by wasteful utilization of the so-called "sebaceous glands" which represent A-storage deposits (intensification of the neutrophyl (N) metabolism, "dystrophia alipogenica" in A-deficient children). - Drying, flaking, inflammation, callousing and decay of the skin- and mucous membrane epithelium, (KOLPO-keratosis of the vagina; transformation of the multi-layered urethra epithelium into callous platelet epithelium, as a result of missing "epithelium protection vitamin". Sequence of organs affected by epithelium damage: vagina; respiratory and urinary tract (bronchial ectasiae, chronic cystitis); salivary glands (dried up saliva secretion; secretion accumulation); eyes with [illegible] glands (tear glands, [illegible] glands); liver (degenerative hepatosis;

intestine (gland villus atrophy); other organs (suprarenal glands, ductus deferens, mamma); finally and rarely, the pancreas; therefore proneness to catarrhs of the respiratory tract and to infections (prone to infections in the skin-mucuous membrane and urinary tract-areas; increased paratyphoid disposition), and prone to cancer development[2]. - Keratomalacia, night blindness (no visual purpur renewal as sequela of "purpur-vitamin" deficiency) and xeropathalmia as sequela of missing anto-xerophthalmic A-protection (pregnant women are especially susceptible, as a result of the fetal vitamin-requirement). - Impairment of bone vitality .- - Spermatogenesis malfunctions; proneness to premature birth.- Formation of kidney-, gall bladder- and urinary bladder stones. Post-hepatotic and post-hepatitic entero-hepatic disorders with A deficiency symptoms following poor resorption of fat-soluble vitamin A. - Diseases of the pancreas.- Disorders of the nervous system (deafness).- Deficient blood formation with disappearance of the blood platelets.

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2) A ZELLER (Effect of vitamins A and B on the growth of the Jensen-sarcoma. Discussion of the Swiss Association of Physiologists, 1938): " Vitamin A overdosages (100 to 600 international units) and in particular, vitamin A deficiency definitely inhibit the growth of the sarcoma and in a large number of cases (provided that the specific diet was started as early as 4 weeks before inoculation), controls always indicate continued growth in 80 to 100% of the cases. Growth inhibition due to vitamin deficiency is in part explainable as follows: (. . illegible) vitamin represents the tumor growth factor, while inhibition by means of hypervitaminosis can be attributed to the fact that tumor development is lagging , in an environment with unbalanced vitamin correlations. In case of a spontaneous tumor, the organism and its defensive reactions are damaged by the vitamin ratio upset; the tumor growth will therefore accelerate under such conditions.

A minor example related to our findings is the carotenase caused by the carotenoid capsanthine ($C_{35}H_{50}O_3$) of the paprika red, after excessive consumption of "sweet Szeged paprika", reported by v. TOKAY("capsicism").

Female psychopath, 20 years old: within two weeks, consumption of 2 kg paprika; even larger quantities were consumed later. Lack of appetite, variable undefined pain in the entire body, irritation, somnolence, red pigmentation of the hands, foot soles, ears, naso-labial folds, neck folds, armpits, knee bends, the mucous membrane of the palate and edges of the tongue, while the sclera remained white. Achlorhydria and gastritis mucosa; urine and blood count normal; serum dark yellow; no diabetes. The presence of these numerous general symptoms proves that the disorder was not merely "cosmetic" as v. TOKAY assumed; instead, it was the start of a general hypervitaminotic toxicosis; capsanthine differs from all other carotenoids insofar as its adsorption affinity is high and it has a powerful storage capacity.

Daily doses of 0,6 y of the pure vitamin A-preparations, calibrated in growth tests, are sufficient to assure the normal development of the young rat kept on a standard diet. The daily vitamin A need of man amounts to 2500 international units; minimum value 2000 i.u.; serum level of the healthy individual 100 i.u., with a daily vitamin A load of 2500 i.u. = value of the vitamin A equilibrium; standard average A-value per 100 cc blood = 70 i.u.- The organism needs the double volume of the principal provitamin: β-carotene, i.e. 5000 i.u. As a result of the latter's poor digestive utilization, maximally 46% is utilized for vitamin A formation; 54% and more, however--depending on individual factors and intestinal resorption--is not utilized and is excreted with the feces; carotene need of man: 30 to 60 y per kg of body weight; optimal daily dose: 3,5 mg (TSPP), minimum 1 mg; average values of the β- carotene at the serum level per 100 cc blood: about 40 i.u. Carotene is contained in butter, egg yolk, milk, cheese, in all green vegetables (jointly with chlorophyll a and b and xantophyll), in dates, apricots, plums, figs and especially in the carrot (nearly pure β-carotene); in the carrot, it is coupled with xantophyll, the pigment betanine and with the aglycon betanidine. Fresh carrots contain 0,135 % carotene, (garden carrots) up to 0,023% and (field carrots) contain approximately 0,1 g carotene per 1 kg. When we determined the daily carotene need of man according to TSPP as 3,5 mg , our patient's consumption would be nearly the 30-fold amount of the optimal daily dose.

The carotinoids absorbed from the food and the vitamin A generated by the liver enter the blood stream where they are determinable in the serum only, but not in the erythrocytes; the carotinoids released by the neutral lipids of the food are detectable in the lymph stream 2 to 3 hours after intake. The following diagram shows the distribution key and the destinations of the nutritional carotenoids in the body.

Carotenoid content of the food

1) remains unresorbed
2) excreted with the feces
3) is resorbed
4) in circulation
5) in the liver
6) in other organs
7) deposited in fat
8) is decomposed and oxidized
9) partially decomposed
10) partially converted into vitamin A
11) additional transformations (unknown)

The liver, acting as storage-and regulating organ of the carotene-vitamin A metabolism, additionally stores xantophyll and--from tomatoes, rose hips and water-melons--the polyen-hydrogen lycopine which has a purely aliphatic structure, with the overall formula $C_{40}H_{56}$; the vitamin A reserve of the organ is thereby mostly in excess of its carotene content.

Further carotene incorporation sites are: the so-called deposit fat, the carotenoids of which can be mobilized again in case of A-deficiency; the lipoid cells of the corpora lutea of the ovaries (crystallizing of small red crystals), the testicles, the cortex and marrow of the suprarenal glands, the bone marrow, the hypophysis and thymus, the retina and presumably the crystalline lens as well. The carotene which is not converted into vitamin A in the liver probably enters the circulation in regular waves or in suddenly subsiding streams; another part is excreted with the bile or is presumably utilized for further chemical conversions. However, in case of maximal nutritional supply, unprocessed carotene is likewise stored as ballast in the liver (excess R.L.S. storage). I do not know whether a re-conversion of surplus vitamin A into carotene exists.

Effective antidotes against impending hypervitaminosis A are: scanty doses of the A-supply, jointly with adequate vitamin B, C and D administration, with strong restriction of fat in the diet, so as to reduce carotenoid resorption in the intestine; irradiation with ultraviolet light or soft X-rays which decolorize and deactivate carotene solutions; thyroid gland preparations; venesection-transfusion; administration of testicle- and ovary-preparations or of suitable synthetic hormones; bile diuretics; protective liver diet rich in carbohydrates; liver- and spleen diathermy.

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A-Hypervitaminose des Menschen (atoxische Karotinosen und eigentliche A-Toxikose)

Von C. Henschen

„Eine natürliche A-Hypervitaminose gibt es nicht; es ist noch niemand an Karotinvergiftung gestorben", schreibt der ausgezeichnete Kenner der Karotinoide, *L. Zechmeister*. Tatsächlich können nur ernährungsgeographische Gegebenheiten, Ernährungszwangslagen, eine psychopathische Pica oder Mutwille die einem Vitamin A-Schaden entgegenstehenden vielfachen Schutzsicherungen durchbrechen. Der Pharmakologe *K. Schübel* konnte seiner Arbeit „Toxikologie der Vitamine" (1937) kein Beispiel einer menschlichen A-Hypervitaminose beigeben, obschon die wertmäßigen Unterlagen und die Folgen der A-Toxikose im Tierversuch bereits sichergestellt und erkannt waren. Eine mir vom Zufall gebotene Beobachtung einer *pathologischen A-Hypervitaminose beim Menschen* füllt diese Erfahrungslücke, so daß damit die *Karotinosen des Menschen* von ihren harmloseren nicht pathologischen Unterstufen bis zum eigentlichen toxischen A-Schaden verfolgbar sind. *Es lassen sich in der Aufstufung der klinischen Erscheinungen folgende Formen auseinanderhalten:*

1. *Die einfachen Xanthämien („unsichtbare Xanthosen") ohne dauernde Xanthodermie, wo nur das gelegentliche Auftauchen einer rasch wieder abblassenden Gelbfärbung der Hand- und Fußteller kundigem Auge Luxuszufuhr oder vom Körper nicht völlig ausgeglichene Bilanzstörungen des intervitaminären Gleichgewichtes verrät.* Beispiel: Die den Kinderärzten schon lange bekannte Vergelbung der Handteller und Fußsohlen bei Säuglingen und Kleinkindern nach Genuß von Mohrrüben. Wie gut sich der Körper einzuregulieren vermag, zeigt die spätere spontane Aufhellung der xanthotischen Färbung trotz weiterer starker Karotinzufuhr (*Heymann*).

2. *Manifeste Xanthämien mit dauernder Xanthodermie ohne pathologische Störungen (kompensierte atoxische Karotinosen).* Beispiele: „*Aurantiasis cutis*" (orangegelbe Verfärbung der Fußsohlen und Handflächen nach übermäßigem *Mandarinengenuß*); „*Okrodermatosen*" japanischer Bauern bei übermäßiger *Ernährung mit Riesenkürbis;* Xanthodermien nach Genuß von *gebackenem Kürbis* bei fleischfreier, jedoch spinat-, salat- und butterreicher Nahrung (Ungarn, England). — Sehr karotinreiche Nahrung (Milch, Eier, Butter, Mandarinen, Orangen, Zitronen, Karotten, Hagebutten, Paprika, Salat) läßt nach Ueberfüllung der A-Speicher der Leber schon nach 1—2 Wochen das Serumkarotin erheblich ansteigen (*Wiyake:* Nach täglich 25—30 Orangen Karotinämie, am 16. Tage Karotinurie!). *Längerdauernde Vermehrung des Serumlipochroms durch polyenreiche Ernährung führt bei gesunden Menschen zu Gelbfärbung der Sohlen, Handteller, der Nasolabialfalten, schließlich auch des übrigen Körpers mit Ausnahme der Skleren; doch spielt eine individuelle, vielleicht auch bloß zeitweilige Disposition mit,* da in der Speichermöglichkeit der Karotin-Stapelungsorte, mangelnder Polyen-Ausschüttung durch Stuhl und Haut und Senkung der Oxydationsvorgänge. Bei der Ausscheidung des Karotins mit dem Schweiß (Stärkstverfärbung in den Hauptschweißdrüsen!) und (über höhere Fettsäuren) mit den Hautfetten sammelt sich der Farbstoff in Körnerform innerhalb von Lipoidtropfen am massenhaftesten in der Hornschicht, in kleinen Kleksen auch in der Subkutis. Die zunächst auffällige Tatsache, daß passionierte Rohköstler nicht regelmäßig zu „Kanarienmenschen" werden, erklärt sich aus dem feinen Regulierungsvermögen unseres Innenlandschaftes und daraus, daß der Rohköstler ja nicht einseitiger Mandarinen- oder Karottenvertilger ist, sondern eine gemischte Gemüse-Obst-Nahrung genießt, welche dank ihrer glücklichen Vitaminmischung dem Körper über intervitaminäre Korrelationen die richtigen Korrekturen ermöglicht.

3. *Dekompensierte atoxische oder prätoxische Xanthosen bei Leberschwächlingen.* Bei Leber- und Gallenblasenerkrankungen kommt es infolge mangelnder A-Bildung zu einer manifesten („Hemeralopie der Leberkranken") oder verschleierten *A-Hypo*vitaminose und zu Hyperkarotinämie mit oder ohne Xanthodermie. Indem je nach dem wechselnden Funktionszustand solcher Lebern bald zu wenig, bald wieder in nachholendem Ueberbetrieb ein Zuviel an A gebildet wird, kann eine *„oszillierende" Xanthose* auftreten, ein Hin- und Herschwanken zwischen A-defizitärer Karotinämie und Wiederherabgehen des Karotinspiegels nach A-Anreicherung; dieses Oszillieren zwischen zwei gegenpoligen Zuständen erinnert an die Wechselspiele Basedow-Myxödem, Hyperglykämie-Hypoglykämie usw. *Ob diese Karotinämien der Leberkranken zu einer fortschreitenden Verschlackung der Leber und Milz mit unverarbeitet bleibenden Karotinoiden führen, bleibe dahingestellt* (Ueberspeicherung des hepato-lienalen R.E.S. ?). Gleiche Schwankungen im Karotin-Vitamin-A-Haushalt treten auf bei Leberzirrhosen, Krebskranken (abnorme A-Ausschüttung durch Leber und Nieren durch Voganzufuhr bedingte therapeutische Hypervitaminose leberschwacher Krebskranker!) und bei präseniler und seniler Altersinsuffizienz der Leber, bei welcher einseitiger übermäßiger Mohrrübengenuß besonders gerne zu Karotinstauungen und gelblichbräunlicher orangefarbener Xanthodermie führt (*Schüßler; Grott*).

4. *Dekompensierte Xanthosen (Vitamin A-Toxikosen). Zunächst das Bild der experimentellen A-Hypervitaminose:*

Relativ kleine A-Gaben (2000–10,000 Internat.-Einheiten) hemmen den Stoffwechsel normal ernährter Ratten und Meerschweinchen bis zu 16%. Massive A-Zufuhr speichert in der Rattenleber Mengen, die „dem Bedarf für ein Jahrhundert entsprechen würden" (*Santos, Ruitz, Davis* und *Moore*). *Unter der Schadengrenze* (15,000 I. E.) bleibendes A-Angebot befördert bei gesunden *Jungratten* Wachstum und Fettansatz. Ueberdosierung führt schon nach 5—8 Tagen zu einer ganzen Skala von Störungen: Struppigwerden des Felles; trophische Hautstörungen mit Seborrhoe; Apathie; Rhinitis haemorrhagica; Konjunktivitis und Exophthalmie; Freßunlust und Durchfälle; starke Behinderung des Wachstums; Senkung des Grundumsatzes; Gewichtssturz; hochgradige Blutarmut mit Thrombozytensturz; rhachitisartiger Steifgang mit krampfartigen Kontrakturen der Beine; Rosenkranz-Deformierung der Rippenknorpel; Spontanfrakturen (ähnlich der Osteogenesis imperfecta und der generalisierten Osteodystrophia fibrosa) bei unwesentlicher Veränderung des Blutphosphates und -kalziums, aber Abnahme des Knochenkalkes und -phosphors (Mineralverarmung durch Blockierung? Schädigung der osteogenetischen Zellen?); Hemmung des Dickenwachstums des Knochens unter Kortikalisverdünnung; nach 2—4 Wochen rascher Tod. *Anatomisch finden sich nekrotisierende Glomerulonephrose mit Verkalkung, enorme Fettspeicherung (Cholesterinimprägnation des ganzen R.E.S.) besonders der Kupffer'schen Sternzellen der Leber, geringer Parenchymschaden der Milz, hochgradige nekrotisierende und toxische Schädigung der Endothelien der Hoden mit Störung der Samenbildung.* 8 Wochen alte Tiere verhalten sich nicht wesentlich anders. *Thyroxin und Zufuhr von Vitamin B und C verhüten den Tod und die ganze Störungsreihe.* — Das Bild der *A-Hypervitaminose des Meerschweinchens* ist gekennzeichnet durch die *schwere Verschiebung des Fetthaushaltes:* Hyperlipämie und Hyperlipoidämie. Lipoidspeicherung mit Fetteinlagerung der Kupffer'schen Zellen bei Fettarmut. Kernzerstäubung und Mitosenreichtum der Leberzellen, Lipoidspeicherung der Milzpulpa, Verfettung der Kapillaren, der Nierentubuli und des Glomerulusendothels.

In nuce meine Beobachtung einer A-Hypervitaminose beim Menschen:

faules Aussehen, Schlaflosigkeit. Nach Erfolglosigkeit einer „wegen Anämie" verordneten Eisentinktur spontan überreichlicher Genuß von Gemüsen, wobei es zu einem unüberwindlichen, Pica-mäßigen Verlangen nach Karotten kam, von denen monatelang täglich 2—3 Büschel (bis 1 kg), meist roh, oft ungereinigt, verzehrt wurden (heimliches Herausholen von durch die sorgenden Angehörigen weggeworfenen Karotten aus dem Abfalleimer!); daneben gleich starkes Verlangen nach Kopfsalat (3—4 Stück pro Tag!), keine Abneigung gegen Fleisch, wohl aber gegen Süßigkeiten und frisches Obst. September 1935 Gelbfärbung des Gesichts, der Hände, Füße und schließlich des ganzen Körpers mit Parästhesien der Finger und Füße, Juckreiz über dem linken Handrücken, zunehmender Müdigkeit, dumpfem Schmerz in der Lebergegend, dyspnée d'effort, Knöchelödem.

Befund am 6. November 1935: Kleingewachsene grazile Frau; wenig Subkutanfett; blasse Schleimhäute. Nervensystem, Gehör und Augen normal; *Skleren rein.* Gesicht, besonders in den Nasolabialfalten, blaßorange verfärbt, desgleichen der übrige Körper; tiefgelbrote Verfärbung der Handflächen und Fußsohlen. Haut trocken, etwas schlaff, ohne Kratzeffekte. Zunge leicht belegt. Konjunktival- und Mundschleimhaut gelblich angefärbt. Hals- und Brustorgane gesund. Deutliche Schwellung der Leber bei fehlendem Gallenblasentumor und fehlenden cholelithiatischen Druckpunkten; Vergrößerung der deutlich tastbaren Milz; keine abnormen Lymphdrüsenschwellungen; seit ½ Jahr Hypermenorrhoe (Uterusmyome!). Motilität, Sensibilität, Reflexe und Zirkulation der Extremitäten ohne Auffälligkeiten. Temperatur ab und zu subfebril. Blutdruck 140/70. — *Senkungsreaktionen* 28. Oktober 1935: 32/67½/127; 7. November 1935: 19/50/124; 13. November 1935: 14/32/110; 24. Januar 1936: 32/60/118. — *Blutbilder:* 28. Oktober 1935: Hgl. 57%, R. Bk. 3,440,000, F.J. 0,85; L. 5300: St.L. 4%, N. 62,4%, Ly. 28,7%, Mono. 4,6%, Eos. 0,3%, Ma. 0. — *6. November 1935:* Hgl. 57%, R.Bk. 3,760,000, F.J. 0,77; L. 5600: St.L. 1,3%, N. 51%, Ly. 34,3%, Mono. 12%, Eos. 0,7%, Ma. 0,4%; Anisozytose; Polychromasie. — *24. Januar 1936:* Hgl. 64%, R. Bk. 3,952,000, F.J. 0,81; L. 4750: St.L. 5,3%, N. 53,4%, Ly. 28,0%, Mono. 10,7%, Ma. 1,3%; schwache Aniso- und Poikilozytose, Polychr. — *Bilirubin* (29. Oktober 1935): nur indirekt Spur (0,24 mg%). — *Blutharnstoff* (7. November 1935): 29 mg%. — *Serumuntersuchung:*

Extinktion bei Wellenlänge 430 (Lichtquelle)	= E 430/10 cm	
Karotin	Verdünnung = V 1 : 10	0,3497
		(normal: 0,07—0,19)
Freie *Xanthophylle:*	E 430/10 cm	
	V 1 : 10	0,15
		(normal: 0,06—0,12)

Serumdiastase bei 32. — *Urin* 28. Oktober 1935 normal; später verschiedentlich Urobilin (+) und Phosphate. — *Thrombusbildung* bei thrombometrischer Untersuchung schlecht. — *Vitamin C-Stoffwechsel 14. Oktober 1935:* Ausscheidung von 31,4 mg im Urin von 300 mg Redoxon innert 10 Std. (Urinkonzentration vor der Belastung 0,5 mg%): *C-Hypovitaminose.*

Prof. *Karrer* war so freundlich, das Serum aufzuarbeiten: „Leider fiel das Chromogramm sehr unscharf aus und auch die einzelnen Fraktionen gaben sehr unscharfe Absorptionsspektren (teilweise Veränderungen bzw. Oxydierung des Karotins!). Es ist darum nicht mit Sicherheit zu sagen, ob das Serum ursprünglich unverändertes α-Karotin oder β-Karotin enthielt oder ob diese Pigmente bereits teilweise weiter verändert waren."

Verlauf: Auf Verbot stark karotinhaltiger Gemüse am 2. Dezember 1935 bereits starker Rückgang der Gelbfärbung bis auf Handteller und Fußsohlen (hier auch ohne Diätfehler stark wechselnd) bei subjektivem Besserbefinden; Aderlaß 400 ccm, Auffüllungstransfusion 350 ccm. *31. Januar 1936* Serumnachkontrolle (Physiologisch-chemische Anstalt): E 430/10 ccm, Verdünnung 1 : 10 ergibt 0,306; da Pat. nicht nüchtern war, kann das Ergebnis als normal betrachtet werden (0,2—0,3). — *9. Juni 1936:* Leichte Subanämie, geringe Leukozytopenie ohne Linksverschiebung bei Mono-, Aniso- und Poikilozytose und Basophilie der R. Bk. — *26. Januar 1937:* Gelbfärbung bis auf Reste an Hand- und Fußtellern verschwunden. S.R. 20/45/123. Blutharnstoff 21 mg%. Serumbilirubin 0,36 mg%. Serumdiastase 32. Hgl. 64%, R. Bk. 4,848,000, F.J. 0,66; L. 4700: St. L. 6,7%, N. 56,7%, Ly. 22,7%, Mono. 9,6%, Eos. 2,0%, Ma. 2,3%. Leichte Anisozytose. Thrombometrie. Guter, aber weicher Thrombus, Zerreißungsgewicht 175 g; große Gerinnungsvalenz 1,0, kleine Val. 2,5; Fibrinogen 0,19; G.Z. 45 Min.; Retraktilität 8 mm = in allen Ziffern demnach nahezu normale Werte. — *12. Januar 1940* S.R. 10/26/101. Blutzucker 0,103/0,101. Blutharnstoff 20 mg%. Hgl. 72%, R. Bk. 4,576,000, F.J. 0,80. Thrombozyten 228,800. L. 3700: St.L. 8%, N. 47%, Ly. 35%, Mono. 7%, Eos. 0,3%, Ma. 2%. Ziemlich Aniso- und Poikilozytose. — *Frühsommer 1940* Myomektomie. — Seit 1 Jahr doppelseitige Kataraktbildung. — *23. Juli 1940* normales Blutbild. — Seit *September 1940* Benervainjektionen, seit Dezember Menformonbehandlung.

Die klinischen Hauptzüge dieses Krankheitsbildes waren: *Abmattung, Abmagerung, schmerzhafte Leberschwellung mit fraglicher hypovitaminogener Lithiasis, Milzschwellung, Urobilinurie, gesteigerte S.R., einfache hypochrome Anämie als Folge der Leber-Milz-Knochenmarkschädigung, zeitweilige Leukopenie und Monozytose, Hypobilirubinämie, sekundäres C-Defizit, Hautparästhesien (Reizwirkung der Farbstoffkörner?), Kataraktbildung.* Da *kristallisiertes Leberschwellung* dürfte sich summieren aus einer *„Hepatosis parenchymatosa"* und einer *Lipoidspeicherung der Kupffer'schen Zellen.* Da wässerige Lösungen gallensaurer Salze durch Paarung der Leber- und Blasencholate mit dem Karotin, dem Xanthophyll und wohl auch mit dem Vitamin A die Aufnahme dieser sonst wasserunlöslichen Stoffe ermöglichen, so führt Polyenüberreicherung der Galle bei verminderter Bildung von Schutzkolloiden notwendigerweise zur *Bildung von Gallengrieß und -Steinen* und zwar — wegen der durch die A-Toxikose erzeugten Hypercholesterinämie — von *Cholesteringrieß* und *Cholesterinsteinen,* in die kristallisiertes Karotin eingelagert sein kann.

Karotinoide (vorwiegend β-Karotin) sind reichlich im *Corpus luteum* einmagaziniert. Die Eierstöcke sind dabei kaum bloße „Abfangorgane", vielmehr benötigen sie die Stoffe zur Verarbeitung des gesprungenen Follikels und damit indirekt zu ihrer Hormonproduktion; besteht doch eine gewisse Verwandtschaft der Sexualhormone zum Vitamin A und dessen Vorstufe, dem Karotin (*Guggisberg*). Da unsere Kranke bereits in der Klimax war und bei ihrem Uterus myomatosus einen wohl entgleisten Genitalchemismus hatte, kann die Sperrung dieses Ausweichgeleises seinerseits zur Karotinüberstauung beigetragen haben.

Es ist mehr denn wahrscheinlich, daß der bei unserer Kranken aufgetretene Katarakt eine Spätfolge der Hypervitaminose ist. Bei den engen Beziehungen, welche das Vitamin A zum äußeren Keimblatt hat (beschleunigte Erneuerung der Epithelien und der Nervensubstanz!), ist es nicht verwunderlich, wenn auch die *Linse als Epithelabkömmling Opfer vitaminotischer Störungen des A-Kreises* wird. Bei der experimentellen A-Avitaminose von Jungratten, deren Muttertiere auf etwas abgeänderte McCollum-Kost gesetzt waren, entstehen in großer Zahl Linsentrübungen, schließlich Zerklüftungen des Kerngebietes und typische Schichtstarbildung, wobei nur die ersten Anfänge der Linsentrübung noch rückbildungsfähig sind. *Pillat* sah bei zwei menschlichen Präxerosen infolge A-Mangels Trübungen der subepithelialen und der tiefen vorderen Linsenrindenschichten. Nun bedarf die Linse zur Aufrechterhaltung ihrer inneren Atmung, ihrer Versorgung mit Nährstoffen und somit zur Wahrung ihrer anatomischen Struktur dreier Faktoren: einer H-Ionenkonzentration von etwa $p_H = 7.5$, einer gewissen Menge Glutathion und des Reduktionssystems (*Goldschmidt*). Es ist kein Zufall, daß von den Oxydoreduktionssystemen das Glutathion, das Vitamin C und das Vitamin B_2 (*v. Szily*) gerade in der Linse in hohen Konzentrationen enthalten sind. Das in der gesunden Menschenlinse vorhandene C-Vitamin nimmt in kataraktösen Linsen so stark ab, daß es überhaupt nicht mehr nachweisbar ist. Da Xerophthalmie auch bei hohem, ja überhohem A-Gehalt auftreten kann, ist es wahrscheinlich, daß *das durch die A-Ueberreicherung eintretende C-Defizit des Organismus, die Verschiebung des „glücklichen" Vitamin-Mischung-Optimums* das für die Unversehrtheit der Linse notwendige Redoxsystem gestört hat. Da unsere Kranke während der Karotin-Hyperkonsumption sicher genügend Vitamin C aufnahm (durch die Karotten und den Salat allein ungefähr 100 mg[1]), kann es sich *nicht um eine primäre,* d. h. durch die Ernährung bedingte C-Hypovitaminose handeln; wahrscheinlich hat der voll: außer Rand und Band gebrachte Karotin-Vitamin-A-Stoffwechsel die intervitaminäre Gleichgewichtsbilanz zu Ungunsten des C verschoben.

Die Gefahr einer Uebervitaminisierung ist bei normalem Gesundheitszustand und geordneter Ernährungslage über mehrfache Sicherungen ausgeschaltet: Durch die Selbststeuerung des Körpers mittels eines „verborgenen Regulierchemismus" (*Zechmeister*), durch chemische Korrekturen seitens einer gesunden Leber und durch intervitaminäre Wechselbeziehungen, welche der Körper zu seinem Selbstschutz ausnützt. Nach *Euler* gehören *Karotin und C-Vitamin zu einem gekoppelten Redox-System:* Findet sich doch in lipochrom- (karotinoid-) reichen Geweben (Nebenniere, Corpus luteum, Retina) auch viel C-Vitamin; *Ratte und Meerschweinchen sind bei*

[1]) Die Ergebnisse des Vitamin-Experimentes am Tier sind freilich nicht ohne weiteres für den Menschen auswertbar; Beispiel: Beriberi des Menschen erzeugt Tachykardie, die der Ratte Bradykardie. A. Z. Red.

Tier mit Reinvitaminen hat bis in die neueste Zeit die Bedeutung dieses *intervitaminären Ausbalanzierungsmechanismus* übersehen lassen. Da unsere Kranke C-defizitär war, fehlte ihr ein wichtiges Glied dieser Gleichgewichtssicherung; es bleibe dahingestellt, ob nicht übermäßige Zufuhr der A-Provitamine und Ballaststapelung des fertigen Vitamins schließlich betriebsnotwendige C-Reserven zur defizitären Bilanz austrieb oder nicht genügend anreichern ließ. *Die A-Hypervitaminose ist darum nicht zuletzt Ergebnis einer intervitaminären Korrelationsstörung*, an deren Entstehung ungeordnete Karotin- oder A-Verteilung in den Geweben, gestörte Ausscheidung, die besonderen individuellen Resorptionsbedingungen im Magen-Darmkanal, Ueberspeicherung des R.-E.-S. u. a. m. eine Rolle spielen.

Die A-Hypervitaminose des Experiments wie die unfreiwillige des Menschen zeigen in manchen Zügen eine seltsame Uebereinstimmung mit der zugehörigen Avitaminose und in gewissem Grade auch mit der D-Hypervitaminose und D-Hypovitaminose (rhachitisartige Knochenveränderungen bei A-Hypervitaminose!). Als Folgen des A-Mangels sind durch Versuch und Klinik festgestellt:

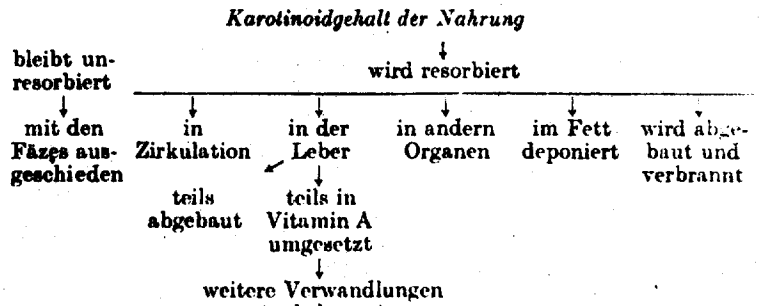
Wachstumsstillstand (verändertes Längenwachstum) infolge Wegfalles eines der Wachstumsvitamine. — *Verfall des Körpergewichtes* durch Raubverbrauch der sog. „Fettdrüsen", welche A-Reservedepots darstellen (Erhöhung des N-Stoffwechsels! „*Dystrophia alipogenica*" A-armer Kinder!). — Austrocknung, Abschilferung, Entzündung, Verhornung und Verfall des Haut- und Schleimhautepithels (Kolpo-Keratose der Vagina; Umwandlung des mehrschichtigen Harnröhrenepithels in verhornendes Plattenepithel) infolge *Ausfalls des „Epithelschutz-Vitamins"*; Reihenfolge der von der Epithelschädigung betroffenen Organe: Scheide, Luft- und Harnwege (Bronchiektasien! Chronische Zystitis!), Speicheldrüsen (Versiegen der Speichelsekretion; Sekretstauung), Auge mit Drüsenannexen (Tränen-, Lidrüsen), Leber (degenerative Hepatose), Darm (Atrophie der Drüsenzotten), übrige Organe (Nebenhoden; Ductus deferens; Mamma), zuletzt und selten das Pankreas; darum Neigung zu Katarrhen der Luftwege und Infektionen (Neigung zu Infekten im Haut-, Schleimhaut- und Harnwegebiet; erhöhte Paratyphusdisposition) und zu Krebsbildung[2]). — *Keratomalazie, Nachtblindheit* (mangelnde Erneuerung des Sehpurpurs infolge Defizit des „Purpurvitamins") und *Xerophthalmie infolge Ausfalles des antixerophthalmischen A-Schutzes* (besondere Anfälligkeit schwangerer Frauen als Folge des Vitamin-Bedarfes des Fötus). — *Beeinträchtigung der Knochenvitalität.* — *Störungen der Spermatogenese; Neigung zu Frühgeburten.* — *Steinbildung* in Niere, Harn- und Gallenblase. — Posthepatotische und posthepatitische *entero-hepatische Störungen* mit A-Mangelerscheinungen infolge schlechter Resorption des fettlöslichen Vitamin A. — *Erkrankungen des Pankreas.* — *Störungen am Nervensystem* (Taubheit). — *Mangelhafte Blutbildung mit Verschwinden der Blutplättchen.*

Unsere Beobachtung hat ein abgeschwächtes Beispiel an der von *v. Tokay* mitgeteilten *Karotinose durch das Karotinoid Kapsanthin* ($C_{35}H_{50}O_3$) *des Paprikarots* nach übermäßigem Genuß des „süßen edlen Szegediner Paprikas" („*Kapsizismus*"):

26jährige Psychopathika; in 2 Wochen Genuß von 2 kg Paprika, später weiterhin Verzehren noch größerer Mengen. *Appetitlosigkeit, wechselnde unbestimmte Schmerzen im ganzen Körper, Verstimmung, Schläfrigkeit, Rotfärbung der Hände, der Fußsohlen, der Ohren, der Nasolabialfalten, der Falten des Halses, der Achselhöhle und Kniekehle, der Gaumenschleimhaut und der Zungenränder bei Weißbleiben der Skleren; Achlorhydrie* und *Gastritis mucosa;* Urin und Blutbild normal; *Serum dunkelgelb;* kein Diabetes. Das Auftreten einer Reihe von Allgemeinerscheinungen beweist, daß nicht bloß eine „kosmetische Störung" vorlag, wie *v. Tokay* annimmt, sondern eine in Gang kommende *allgemeine hypervitaminotische Toxikose.* Zeichnet sich doch gerade das Kapsanthin vor allen andern Karotinoiden durch hohe Adsorptionsaffinität und starkes Einspeicherungsvermögen aus.

Von den reinen, am Wachstumstest geeichten Vitamin A-Präparaten reichen Tagesdosen von 0,6 γ hin, die normale Entwicklung einer normal genährten Jungratte zu sichern. *Der A-Tagesbedarf* 2500 I.E. = Wert des Vitamin A-Gleichgewichtes, normaler durchschnittlicher A-Wert pro 100 ccm Blut = 70 I.E. — *Vom Hauptprovitamin β-Karotin benötigt der Körper die doppelte Menge* = 5000 I.E., da wegen dessen schlechter Verdauungsausnützung höchstens 46% der A-Herstellung dienen, 54% und mehr Prozent aber — je nach den individuellen Bedingungen der Darmresorption — unverwertet mit dem Stuhl abgehen: Karotintagesbedarf des Menschen 30—60 γ pro kg Körpergewicht; optimale Tagesdosis 3,5 mg (*Stepp*), Minimum 1 mg; Durchschnittswerte des β-Karotins im Serumspiegel pro 100 ccm Blut um 40 I.E. Karotin ist enthalten in Butter, Eigelb, Milch, Käse, in allen Grüngemüsen (hier zusammen mit Chlorophyll a und b und Xanthophyll), in Datteln, Aprikosen, Pflaumen, Zwetschgen, Feigen und namentlich in der Mohrrübe (fast reines β-Karotin!), in dieser verkoppelt mit Xanthophyll und den Farbstoffen Betanin und dem Aglykon Betanidin. Frische Karotten enthalten 0,135% (Gartenrüben!) bis 0,023% (Feldkarotten!), in 1 kg ungefähr 0,1 g Karotin. *Wenn wir den täglichen Karotinbedarf des Menschen mit Stepp auf 3,5 mg ansetzen, so hätte unsere Kranke täglich fast das 30fache der optimalen Tagesdosis konsumiert* und zwar allein aus den Karotten! Die Ausscheidung des Fertigvitamins erfolgt zeitlich sehr viel später als die des β-Provitamins.

Die aus der Nahrung aufgesaugten Karotinoide und das von der Leber fabrizierte Vitamin A gelangen in den Blutstrom, wo sie nur im Serum, nicht aber in den Erythrozyten auffindbar sind; die in den Neutralfetten der Nahrung aufgelösten Karotinoide finden sich 2—3 Stunden nach der Einnahme im Lymphstrom. Folgendes Schema von *Zechmeister* gibt den *Verteilungsschlüssel und die Schicksalswege der Nahrungskarotinoide im Körper:*



Die *Leber*, Umformungs-, Stapel- und Regulierorgan des Karotin-Vitamin-A-Haushaltes, stapelt außerdem *Xanthophyll* und — aus Tomaten, Hagebutten und Wassermelonen — den rein aliphatisch gebauten Polyen-Wasserstoff *Lycopin* von der Bruttoformel $C_{40}H_{56}$; die Vitamin A-Reserve des Organs ist dabei meist größer als der Karotingehalt. *Weitere Einlagerungsstellen* des Karotins sind das sog. *Depotfett*, dessen Karotinoide bei A-Mangel wieder mobilisiert werden können, die Lipoidzellen der Corpora lutea der *Eierstöcke* (Auskristallisierung von kleinen roten Kristallen), *Hoden, Rinde und Mark der Nebennieren, Knochenmark, Hypophyse und Thymus, Retina* und wahrscheinlich auch die *Linse*. Das in der Leber nicht zu Vitamin A umgesetzte Karotin dürfte in regelmäßigen Wellen oder in plötzlich abflutenden Strömen in den Kreislauf gelangen, zum andern Teil durch die Galle ausgeschieden oder für weitere chemische Verwandlungen gebraucht werden; bei stärkster alimentärer Anflutung wird es jedoch auch unverarbeitet in der Leber ballastmäßig gestapelt (Ueberspeicherung des R.E.S.!). Ob es eine Rückverwandlung von überschüssigem Vitamin A in Karotin gibt, ist mir nicht bekannt.

Wirksame *Gegenmittel gegen eine drohende A-Hypervitaminose* sind: Knappe Dosierung der A-Zufuhr bei genügender Darreichung der Vitamine B, C und D unter starker Einschränkung der Fettkost, um die Resorption der Karotinoide im Darm zu vermindern; Bestrahlung mit ultraviolettem Licht oder weichen Röntgenstrahlen, welche Karotinlösungen entfärben und entaktivieren; Schilddrüsenpräparate; Aderlaß-Transfusion; Darreichung von Hoden- und Eierstockspräparaten oder den entsprechenden Kunsthormonen; gallentreibende Mittel; kohlehydratreiche Leberschutzkost; Leber- und Milzdiathermie.

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[2]) *A. Zeller* (Die Beeinflussung des Wachstums des Jensen-Sarkoms durch die Vitamine A und B_1. Verhandl. fr. Vereinig. schweiz. Physiologen 1938): „Vitamin A-Ueberdosierung (100—600 I.E. A) und besonders Vitamin A-Mangel *hemmen* sehr deutlich das Wachstum des Sarkoms und heilen es in einer großen Zahl der Fälle (falls die besondere Kostform schon 4 Wochen vor dem Impfen begonnen wird!), während die Kontrollen stets zu 80—100% der Fälle progredientes Wachstum aufweisen. Die Wachstumshemmung durch Vitaminmangel ist teilweise so zu erklären, daß das Vitamin den limitierenden Faktor für das Tumorwachstum darstellt, während die Hemmung durch Uebervitaminisierung auf mangelndes Gedeihen des Tumors in einem Milieu mit unausgeglichenem Vitamingleichgewicht zurückzuführen ist. Beim *Spontantumor* wird der Organismus und seine Abwehrreaktion durch ein *gestörtes Vitaminverhältnis* geschädigt, so daß der Tumor unter diesen Bedingungen erst recht wachsen kann."

Bomskov, Ch., und *G. Seemann:* Z. exper. Med. 89, 771 (1933). — *J. A. Collazo* und *J. Sanchez Rodriguez:* Klin. Wschr. 12, 1732, 1768 (1933). — *W. v. Drigalski* und *W. Laubmann:* Klin. Wschr. 12, 1171 (1933). — *Guggisberg, H.:* Biol. u. Pathol. des Weibes, Bd. VIII/2, Lfg. 45, 1928. — *Hary, Margarete:* Frankf. Z. Path. 48, 283 (1935). — *Rothmann* und *Schaaf:* Chemie der Haut. Hdb. Haut- u. Geschlechtskrankh. Bd. 1, 2, 161, bes. 267. Berlin, J. Springer 1929. — *Schübel, K.:* Med. Welt 1937, 21, 705. — *Solmssen, U.:* Untersuch. über Karotinoide. Diss., Berlin 1936. — *V. Szily, A.:* Hdb. d. spez. pathol. Anat. u. Histol. v. Henke u. Lubarsch, Bd. III/3. Berlin, J. Springer 1937. — *v. Tokay, L.:* Mschr. Psychiatr. 82, 346 (1932) (Lit.). — *Wagner, H.-H.:* Z. Klin. Med. 137, 653, 659 und 664 (1940). — *Zechmeister, L.:* Karotinoide. Berlin, Springer 1934 (Lit.); Erg. Physiol. 39, 117 (1937) (Lit.).

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HYPERVITAMINOSIS A AND CAROTENEMIA

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The effect on man of the ingestion of large amounts of carotene has been the subject of many articles in the past twenty years. On the other hand, knowledge of the results of overdosage with vitamin A is almost entirely confined to the effects on rats and mice. The lack of experience with hypervitaminosis in man is easy to understand, since only artificial concentrates contain sufficient vitamin A to be dangerous, and the ability to obtain these is limited. Early recognition of the possible danger of hypervitaminosis has limited the employment of excessive amounts in therapy. Moreover, the zone of safety between an adequate prophylactic dose and a dangerous dose is very large. One safeguard is lacking, that against the zeal of a person who is "sold on" vitamins. We have recently observed a case in which enthusiasm for vitamin therapy was present in a mother and eventually in her child.

REPORT OF A CASE

History.—B. B., a 3 year old boy of Jewish parentage, had appeared well up to the fall of 1938, when he was about 18 months old. At that time his appetite diminished and he seemed to be disinclined to play. Six months later (May 1939) he had a severe attack of tonsillitis, for which he was given sulfanilamide for two days. Two months after this, July 26, 1939, he had a tonsillectomy followed by hemorrhage, after which he received three transfusions. A blood-count done after the hemorrhage showed severe anemia and 2,900 white cells per cubic millimeter, of which 44 per cent were neutrophils. The patient's liver and spleen were found to be enlarged, and clubbing of the fingers was noted. At this time he lost much of his hair, and that which grew back was sparse, dry and coarse.

During the next eight months, the child failed to improve, and because of the enlarging spleen and the continuing anemia he was admitted to the Harriet Lane Home for study with the tentative diagnosis of Gaucher's disease (March 1940).

Physical Examination.—When the patient entered the hospital in March 1940, his hair was sparse, dry and rather coarse, and eyebrows and fine hair over the body were lacking. The fingers and toes were slightly clubbed. He was pale and sallow, but there was no increase in pigmentation and no jaundice and the scleras were clear. The heart and lungs showed no abnormality. The liver was felt 2 cm. below the costal margin, the edge being sharp and firm. The spleen was enlarged and firm and was palpable 3 cm. below the costal margin. The child's height was 89 cm. (about normal for his age).

Laboratory Data.—Most of the laboratory data are summarized in table 1. In addition, a test of dextrose tolerance gave the following results: fasting level, 81 mg. per hundred cubic centimeters of blood; levels after ingestion of dextrose, one-half hour 152 mg., one hour 168 mg., two hours 140 mg. and three hours 111 mg. The results (in milligrams per hundred cubic centimeters of blood) in the levulose tolerance test were: fasting, 91; half-hour, 132; one hour, 131; two hours, 105, and three hours, 102. The erythrocyte sedimentation rate (corrected) was 6 mm. in one hour on one occasion and zero on two others. The bromsulphalein test of hepatic function showed no retention of the drug. Excretion of urobilin was not increased in either the stools or the urine. The Wassermann and tuberculin tests gave negative reactions.

Roentgenograms of the chest and skull showed no abnormalities, but those of the limbs revealed considerable irregularity in cortical structure. In the phalanges and metacarpals the cortex was extremely thin and the medullary cavities appeared to be "vacuolated." The epiphyses of the upper ends of the humeri and tibias were mottled in appearance, and the distances between epiphyses and diaphyses were greater than normal. There were five carpal centers of ossification, indicating that skeletal development was in advance of the child's age in years.

A biopsy was done on a piece of bone, but unfortunately the tissue used was from the tibia, in which no change was disclosed by roentgenographic examination; as a result we are unable to say what was the nature of the abnormal process which occurred predominantly in the upper extremities. It is, however, possible to state definitely that the marrow showed no evidence of hypoplasia, nor was there any accumulation of reticuloendothelial cells. The only abnormality was a predominance of the more immature cells of the myeloid series. It was noted that the bones became decalcified in the fixative more rapidly than usual, but there was no evidence from the biopsy of any process that might cause decalcification of the bone.

Course.—The one outstanding feature of the examination that appeared to point to a possible etiologic factor was the enormously high content of vitamin A in the serum such as would not ordinarily be encountered except four to six hours after ingestion of an excessive dose of vitamin A. On questioning the mother, it was learned that beginning at 2 to 3 months of age the child had received a teaspoonful of halibut liver oil a day (about 240,000 U. S. P. units of vitamin A) up to the present time. Moreover, he had on occasion drunk the oil directly from the bottle in undetermined amounts.

As soon as it was appreciated that the case might be one of hypervitaminosis, the child was discharged with instructions that he should not be given any extra vitamin A. Improvement was immediate. Within two months, the appetite had improved, the patient had gained weight and his hair had begun to grow back normally. By the end of six months his appearance and the composition of his blood were normal and the vitamin A content of the serum was within the upper limit of normality. Certain features remained, however; the phosphatase was still elevated and the serum proteins were still depressed. For a time after he was discharged from the hospital the child showed a desire for

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butter and carrots, which led to a considerable though temporary increase in serum carotene and a temporary increase in serum lipids.

Two and a half years after leaving the hospital, the child was to all external appearances normal and of average height and development. He had had no illness except measles, and there was nothing in the mother's account to indicate that any pathologic change was still present. However, his liver was palpable as a firm sharp edge about 2 cm. below the costal margin; the spleen was felt as a large, firm mass occupying the upper left quadrant of the abdomen, and his fingers were still slightly clubbed. The laboratory data were essentially normal except for a slight elevation of the phosphatase content of the blood. The bones appeared normal in structure, but skeletal development was slightly in advance of the chronologic age (7 carpal centers of ossification at 5½ years of age).

In order to understand the significance of the conditions observed in this case, it will be necessary to review in considerable detail what is known concerning overdosage with vitamin A and its precursor, carotene. It has seemed best to carry out the review in the following order: (*a*) the known results of overdosage with vitamin A and carotene as determined by experiments on animals; (*b*) carotenemia as it is observed clinically, including the factors influencing the accumulation of carotene in the blood and the clinical effects of carotenemia, (*c*) hypervitaminosis, (1) the factors leading to accumulation of vitamin A in the blood and (2) the possible mechanisms the body employs to protect itself against the harmful effects of overdosage with vitamin A.

TABLE 1.—*Laboratory Data for Patient with Hypervitaminosis A*

Date	7/26/39	1/29/40	3/7/10	3/19/40	4/6/10	4/19/40	4/15/10	6/21/10	10/14/10	10/31/[illegible]
Erythrocytes, number/cu. mm.	2,100,000	3,800,000	4,300,000	3,000,000	...	3,400,000	...	4,400,000	4,600,000	5,000,[illegible]
Hemoglobin, Gm./100 cc.	7.5	8.0	10.1	11.0	...	10.1	...	11.3	12.0	[illegible]
Leukocytes, number/cu. mm.	2,900	6,100	6,200	4,600	...	4,800	...	5,700	8,400	7,[illegible]
Neutrophils, %	44	40	31	38	...	40	...	63	67	[illegible]
Lymphocytes, %	51	54	58	62	...	59	...	30	31	[illegible]
Monocytes, %	5	6	2	0	...	1	...	1	1	[illegible]
Eosinophils, %	0	1	4	0	...	0	...	1	1	[illegible]
Reticulocytes, %	..	..	..	2	...	..	...	..	..	[illegible]
Platelets, number/cu. mm.	..	..	..	68,000	...	70,000	...	34,000	42,000	120,[illegible]
Fasting blood sugar, mg./100 cc.	..	..	..	81	...	..	...	..	..	[illegible]
Serum nonprotein nitrogen, mg./100 cc.	..	..	..	21	...	..	...	..	..	[illegible]
Serum protein, %	..	..	..	4.3	...	5.0	...	3.8	5.6	[illegible]
Albumin, %	..	..	..	2.9	...	3.6	...	2.6	3.5	[illegible]
Globulin, %	..	..	..	1.4	...	1.4	...	1.2	2.3	[illegible]
Fibrinogen, %	..	..	..	0.58	...	...	...	...	...	[illegible]
Serum bilirubin (van den Bergh), mg./100 cc.	..	..	..	1.6	...	1.4	...	1.4	1.6	[illegible]
Serum calcium, mg./100 cc.	..	..	..	10.6	...	10.0	...	9.7	9.6	[illegible]
Inorganic phosphorus, mg./100 cc.	..	..	..	5.3	...	3.1	...	5.0	4.7	[illegible]
Phosphatase, Bodansky units	..	..	..	13.7	...	15.5	...	[illegible]	[illegible]	[illegible]
Serum cholesterol, mg./100 cc.	..	..	..	251	...	...	...	243	242	[illegible]
Serum total lipid, mg./100 cc.	..	..	..	1,110	800	...	770	9.0	[illegible]	630
Serum vitamin A, U. S. P. units/100 cc.	..	..	..	910	650	...	450	4.0	2.0	470
Serum carotene, micrograms/100 cc.	..	..	..	110	100	...	100	2.0	150	60

RESULTS OF OVERDOSAGE WITH VITAMIN A AND CAROTENE IN ANIMALS

As has been said above, knowledge of hypervitaminosis A is confined to the effects in animals, particularly in rats and mice, which are especially sensitive to the toxic effects of overdosage with vitamin A. Since different species vary greatly in degree of sensitivity to vitamin A and also possibly in the type of change produced by excessive amounts, one must be cautious in applying the knowledge gained by experimentation with animals to the interpretation of occurrences in the human being. There are, however, certain changes that have occurred in all the species tested, and these should form the basis for an understanding of the effects of intoxication with vitamin A. The following account will be based on the effects of excessive doses of vitamin A on the rat, since that animal has been most extensively studied.

When rats are given toxic doses of vitamin A (over 15,000 units a day), they lose weight almost immediately and may die in a week or ten days. There are certain rather constant symptoms; the hair, especially about the head becomes unkempt and falls out and the skin becomes greasy and has a peculiar odor suggestive of hydrous wool fat. Later the eyes become sore, there is a bloody secretion from the nose and diarrhea develops.[1] These symptoms along with the extreme loss of weight and frequent loss of use of the posterior extremities provide a superficial resemblance to vitamin A deficiency. An almost constant feature is the development of multiple spontaneous fracture.

1. (*a*) Domagk, G., and von Debeneck, P.: [illegible] chows Arch. f. path. Anat. 290:385, 1933. (*b*) M[illegible] T.; Dalmer, O.; von Debeneck, P.; Domagk, G.; Laquer, F.: Arch. f. exper. Path. u. Pharmakol. [illegible] 176, 1933. (*c*) von Drigalski, W.: Klin. Wchn. 12:308, 1933. (*d*) Bomskov, C., and Scott[illegible] Ztschr. f. d. ges. exper. Med. 89:771, 1933. (*e*) D[illegible] A. W., and Moore, T.: Biochem. J. 28:[illegible], 1934. [illegible] Baumann, C. A., and Moore, T.: ibid. 33:1[illegible], 1[illegible] (*g*) Noetzel, H.: Ztschr. f. d. ges. exper. Med. 1[illegible] 83, 1939.

of the bones of the extremities.[2] Some investigators reported exophthalmos,[3] the cause of which is unknown; others stated specifically that they had never seen this result.[2f]

Histologically two features stand out, rarefaction of the bones with extreme thinning of the cortex[4] and increased deposition of fat in the reticuloendothelial cells, especially in the Kupffer cells of the liver and in the spleen.[5] There has been considerable disagreement over the degree of accumulation of fat and the extent of the hyperplasia of the reticuloendothelial cells; some investigators reported almost none,[5e] while one author likened the histologic picture to that observed in cases of Schüller-Christian disease.[2d]

Other histologic changes were noted by various authors, some of which may be of considerable significance if confirmed. Some authors, for instance, found evidence of increased activity of the thyroid gland[6]; one stressed increase in the basophilic cells of the anterior lobe of the hypophysis,[7] and hypertrophy of the island tissue of the pancreas was reported by another.[8]

Although the anatomic studies have usually been made on severely intoxicated animals, certain of the changes, especially those in the reticuloendothelial cells, are known to occur also in animals less severely poisoned. This fact indicates that such changes are the result not of a general intoxication but of a process more specifically associated with excess of vitamin A. Investigations of the relationship of overdosage with vitamin A to fat metabolism tend to confirm this suggestion. Since this subject has recently been reviewed,[9] it need only be touched on here.

When vitamin A in large but nontoxic daily doses was given to rats, guinea pigs or rabbits, a rise in cholesterol and in total lipids of the serum occurred.[10] This rise was only temporary, however, and tended to disappear after a time, even while the large doses of vitamin A were being given.[11] The same effect was observed in human beings[12] but not in dogs.[10c] In rats previous deficiency intensified and prolonged the response,[9] and a similar reaction was seen after pneumonia in infants receiving vitamin A.[13] Apart from this lipid reaction, the effect of vitamin A on physiologic processes has been little studied. No effect was observed on the formation of blood in animals.[14] The serum calcium and phosphorus were not affected, though there was an increase in excretion of calcium, which was attributed to "decalcification" of the bones.[14] The basal metabolic rate was decreased.[15] An observation of considerable significance, if confirmed, was that function of the liver was at first improved and then depressed by excessive vitamin A.[16] All authors are agreed on the fact that recovery is prompt when the excessive intake of vitamin A is discontinued.

In contrast to the harmful results of overdosage with vitamin A, toxic effects have not been seen in animals after ingestion of excessive amounts of carotene; the animals have not lost weight or died and have not shown any of the outward manifestations of hypervitaminosis.[17] The changes in the metabolism of fat and the histologic changes in the reticuloendothelial "system," especially the increase in the storage of fat, are the same after the ingestion of carotene

2. (a) Bomskov and Seemann.[1d] (b) Davies and Moore.[1e] (c) Baumann and Moore.[1f] (d) Collazo, J. A., and Rodriguez, J. S.: Klin. Wchnschr. **12**:1768, 1933. (e) Strauss, K., and Ziegl, B.: Beitr. z. path. Anat. u. z. allg. Path. **94**:345, 1934. (f) Weslaw, W.; Wronski, B.: Wroblewski, A., and Wroblewski, B.: Klin. Wchnschr. **17**:777, 1938.

3. (a) Noetzel.[1g] (b) Strauss and Ziegl.[2e] (c) Collazo, J. A., and Rodriguez, J. S.: Klin. Wchnschr. **12**:1732, 1933. (d) Josephs, H. W.: Personal observation.

4. (a) Bomskov and Seemann.[1d] (b) Strauss and Ziegl.[2e] (c) Wolbach, S. B., and Bessey, O. A.: Physiol. Rev. **22**:233, 1943.

5. (a) Domagk and von Dobeneck.[1a] (b) Noetzel.[1g] (c) Collazo and Rodriguez.[2d] (d) von Drigalski, W., and Laubmann, W.: Klin. Wchnschr. **12**:1171, 1933. (e) Uotilla, U., and Simola, P. E.: Virchows Arch. f. path. Anat. **301**:523, 1938.

6. Uotilla, U.: Virchows Arch. f. path. Anat. **301**: 535, 1938.

7. Cornil, L.; Chevallier, A.; Paillas, J. E., and Chouquet, J.: Ann. d'anat. path. **16**:83, 1939.

8. Cornil, L.; Chevallier, A., and Paillas, J. E.: Ann. d'anat. path. **16**:74, 1939.

9. Josephs, H. W.: Bull. Johns Hopkins Hosp. **71**: 265, 1942.

10. (a) Collazo, J. A.; Torres, I., and Rodriguez, J. S.: Klin. Wchnschr. **13**:1678, 1934. (b) Jusatz, H. J.: ibid. **13**:95, 1934. (c) Wendt, H.: ibid. **14**:9, 1935. (d) Chalier, J.; Jeune, M.; Simon, F., and Alocoque, P.: Compt. rend. Soc. de biol. **129**:605, 1940.

11. (a) Josephs.[9] (b) Wendt.[10c]

12. (a) Wendt.[10c] (b) Jusatz, H.: Deutsche med. Wchnschr. **60**:573, 1934. (c) Lasch, F.: Klin. Wchnschr. **13**:1534, 1934.

13. Josephs, H. W.: Studies in Vitamin A: Vitamin A and Total Lipid of Serum in Pneumonia, Am. J. Dis. Child. **65**:712 (May) 1943.

14. Bomskov, C., and Sievers, G.: Ztschr. f. d. ges. exper. Med. **89**:780, 1933.

15. Chevallier, A., and Baert, H.: Compt. rend. Soc. de biol. **116**:1037, 1934.

16. Ikogaki, I.: Ztschr. f. Vitaminforsch. **9**:1, 1940.

17. (a) Moll and others.[1b] (b) Davies and Moore.[1e] (c) Chevallier, A.; Cornil, L., and Combe, R.: Compt. rend. Soc. de biol. **118**:574, 1935. (d) Lewis, J. T., and Reti, L.: ibid. **118**:577, 1935.

as after large but nontoxic doses of vitamin A.[18] The most obvious reasons for the less pronounced effects with carotene are: (1) carotene is less well absorbed than vitamin A; (2) the effect of carotene is seen only after conversion to vitamin A, and the time taken for this conversion would prevent the flooding of the body with the active vitamin which is necessary for the production of toxic effects. This subject will be more fully discussed in the section devoted to the body's defense against hypervitaminosis.

CLINICAL CAROTENEMIA

Factors Influencing the Carotene Level of the Blood.—1. Diet: Recognition of the primary importance of diet in carotenemia came as the result of the work of Palmer[19] and van den Bergh[20] and of a large number of clinical observations, most of them on children and persons with diabetes. These studies revealed correlations of xanthosis of the skin with increased content of lipochrome[21] in the blood and with diets rich in lipochromes (carotenoids).[22] Carrots are the primary food associated with carotenemia; also important are squash,[22a] orange[23] and egg yolk[24] (egg yolk supplies xanthophyll rather than carotene). Xanthosis of dietary origin is most common in the late summer[22] and a number of observers have noted that the carotene of the blood is normally at a higher level at this time of the year.[26] In the cases reported in pediatric clinics, young infants appeared to be spared; the condition is observed only in later infancy and early childhood. It is generally recognized that carotenemia and xanthosis are slow to develop, and relatively slow to recede after the high intake of carotene is discontinued.[27] Not every one reacts in the same way to the same diet, and it has been observed that some persons who acquire xanthosis easily do so on a surprisingly small extra amount of carotene.[22j] Moreover, the reactions of the same person may vary; it has been noted that a carotene-rich diet may cause xanthosis at first but that the yellow color may later disappear not to return during continuance of the diet.[28]

2. Level of Serum Lipids: The suggestion was made early that the serum lipids might have something to do with the development of carotenemia. The conditions other than ingestion of diets high in carotenoids under which carotenemia occurs in human beings are in general those associated with hyperlipemia—diabetes, nephrosis[29] and hypothyroidism.[28b] In cases of diabetes of the type in which the serum lipids are increased there is also a tendency for carotenemia to develop.[30] Rabinowitch[30e] reported

18. (*a*) Wendt.[10c] (*b*) Ralli, E. P.; Pariente, A. C.; Brandaleone, H., and Davidson, S.: Effect of Carotene and Vitamin A on Patients with Diabetes Mellitus, J. A. M. A. **106**:1975 (June 6) 1936. (*c*) Ocana, T.: Compt. rend. Soc. de biol. **120**: 267, 1935. (*d*) Stern, K., and Willheim, R.: Ztschr. f. d. ges. exper. Med. **97**:354, 1935.

19. Palmer, L. S.: Carotenoids and Related Pigments, New York, Chemical Catalogue Company, 1922.

20. van den Bergh, H.; Muller, P., and Broekmeyer, J.: Nederl. tijdschr. v. geneesk. **2**:127, 1920.

21. The terminology in this field is confusing. "Lipochrome" refers only to a colored substance associated with fat and is the older term. The early recognition and the importance of carotene among the lipochromes has led to the gradual use of the term carotenoids to designate this class of substances, all the members of which have similar chemical structures. In most clinical work "carotene" is used to designate anything that gives a yellow color in the purified benzine (petroleum ether) layer above an alcohol-water mixture. This is an incorrect use of the term but does no harm as long as the fact is recognized. "Carotenemia" is a term introduced by Hess and Meyers to include the entire clinical picture of yellow skin and increase of carotene in the blood; as such it has been widely accepted. It must be recognized, however, that the yellow color of the skin is not due to the carotene content of the blood, and, although there is a relationship, it is not always a close one. There is now a tendency to distinguish between "carotenemia" (excessive carotene in the blood) and "xanthosis cutis" (yellowness of the skin). As in the case of the faulty use of "carotene," the use of "carotenemia" to cover the entire syndrome does no harm, but it is well to recognize the possibilities for confusion.

22. (*a*) Hess, A. F., and Myers, V. C.: Carotinemia: New Clinical Picture, J. A. M. A. **73**:1743 (Dec. 6) 1919. (*b*) Kaupe, W.: München. med. Wchnschr. **66**: 330, 1919. (*c*) Klose, E.: ibid. **66**:419, 1919. (*d*) Stoeltzner, W.: ibid. **66**:419, 1919. (*e*) Ryhiner, P.: Jahrb. f. Kinderh. **94**:225, 1921. (*f*) Kohn, B.: Ztschr. f. d. ges. exper. Med. **36**:447, 1923. (*g*) Hashimoto, H.: Carotinoid Pigmentation of Skin Resulting from Vegetarian Diet, J. A. M. A. **78**:1111 (April 15) 1922. (*h*) Miyake, I.: Arch. f. Dermat. u. Syph. **147**:184, 192[illegible] (*i*) Greene, C. H., and Blackford, L. M.: M. Clin. North America **10**:733, 1926. (*j*) von Drigalski, W.: Ztschr. f. Vitaminforsch. **3**:37, 1934. (*k*) Clausen, S. W., and McCoord, A. B.: J. Pediat. **13**:635, 1938.

23. (*a*) Miyake.[22h] (*b*) Josephs, H. W.: Personal observation.

24. (*a*) Stoeltzner.[22d] (*b*) Clausen and McCoord.[22k]

25. (*a*) Clausen and McCoord.[22k] (*b*) Catel, W.: Monatschr. f. Kinderh. **73**:316, 1938. (*c*) Kimble, J. S.: J. Lab. & Clin. Med. **24**:1055, 1939.

26. (*a*) Kaupe.[22b] (*b*) Stoeltzner.[22d] (*c*) Kohn.[22f]

27. (*a*) Clausen and McCoord.[22k] (*b*) Kauffmann, F., and von Drigalski, W.: Klin. Wchnschr. **12**:[illegible] 1933. (*c*) Heymann, W.: Carotinemia in Diabetes, J. A. M. A. **106**:2050 (June 13) 1936.

28. (*a*) Klose.[22e] (*b*) Umber, F.: Klin. Wchnschr. **53**:829, 1916.

29. (*a*) Wendt.[10c] (*b*) Clausen and McCoord.[22k] (*c*) Josephs, H. W.: Personal observation.

30. (*a*) Wendt.[10c] (*b*) Umber.[28b] (*c*) Labbé, M.: Presse méd. **33**:17, 1925. (*d*) Rabinowitch, I. M.: Canad. M. A. J. **18**:527, 1928; (*e*) Carotinemia [illegible]

that those patients with the highest levels of serum carotene tended in general to have the highest levels of lipid. If a large enough number of cases is studied to obtain the extreme variations in values which are encountered only under pathologic conditions, it can be shown that a correlation does exist.[31] However, the lack of demonstrable correlation in small series and under normal conditions has led several observers to deny that the lipids have any significance in carotenemia.[32]

In a recent study, it was found that during and after pneumonia, the levels of carotene and of lipid could be correlated when account was taken of the ingestion of carotenoids and the time required after ingestion for accumulation of the carotenoids in the blood. When carotene was given by mouth the rate of accumulation in the blood stream depended in general on the level of lipid during the period of ingestion.[13] In a recent study of hypothyroidism the values of serum carotene were found to be dependent on those of the serum lipid.[33] A close relationship between carotene and cholesterol has been stressed, and it has been claimed that the yellow color of the nodules present in xanthoma is due to their content of carotene.[34]

3. Other Factors: The fact that when carotenoids are removed from the diet the carotene of the serum falls to an extremely low level suggests that the stores of carotene in the fat depots are only to a minimal extent available for maintenance of the level in the blood. Moreover, it has been found that thin people have a higher concentration of carotene in the fatty tissue than well nourished persons,[35a] which indicates that even when fat is mobilized from depots in the tissues, carotene remains behind. The fate of the carotene which disappears from the blood is not entirely known. Some of it is evidently deposited in the fatty tissues. A large part is removed from the circulation in the liver, where it undergoes conversion to vitamin A. Some of it is undoubtedly oxidized. Whether or not it has any function other than as a precursor of vitamin A is unknown.

The increase in the activity of the reticuloendothelial cells after the ingestion of excess carotene may explain why the high initial carotenemia tends to disappear later; the rate at which removal is accomplished is at first exceeded by the rate of intake but later increases to take care of the excess carotene. It has been suggested that hypothyroidism[33] and diabetes[36] interfere specifically with the conversion of carotene to vitamin A, but it is hard to see how this would lead to accumulation of carotene in the blood unless the mechanism of removal were also impaired. Several investigators have advanced the hypothesis that failure of oxidation of carotene is the determining factor, especially in diabetes and hypothyroidism.[37] Until more is known concerning the part that oxidative processes play in the removal of carotene from the blood in the living organism, and not merely in vitro, this hypothesis can hardly be proved or disproved.

Carotenemia has been noted among Europeans living in the tropics, and Castellani and Chalmers[38] mentioned a condition which they called ochrodermatosis, which occurred only at sea level and disappeared when the patients were removed to higher altitudes. The diet was not mentioned. Sequeira[39] saw what he considered a similar case in Africa apparently not dependent on diet and without hypercholesteremia. Improvement occurred only after the patient was given small amounts of thyroid. Bounin and Lévinson[37c] reported carotenemia in a group of patients with chronic malaria who had been inadequately treated.

Effects of Carotenemia.—Xanthosis Cutis: Carotenemia has been considered completely harmless, its only symptom being the development of the characteristic yellow color of the skin which generally disappears as soon as the responsible conditions have been corrected. Usually the yellow color varies with the level of carotene in the blood, but a close parallelism is not always present. A striking exception to the general rule is the absence of xanthosis cutis in persons with carotenemia with nephrosis

Diabetes: Relationship Between Sugar, Cholesterol and Carotin Contents of Blood Plasma, Arch. Int. Med. **45**:586 (April) 1930. (*f*) Barbudo, N., and von Querner, E.: Wien. Arch. f. inn. Med. **30**:279, 1937.

31. Josephs, H. W.: Bull. Johns Hopkins Hosp. **65**: 112, 1939.

32. (*a*) Ralli, Pariente, Brandaleone and Davidson.[18b] (*b*) Burger, M., and Reinhardt, A.: Deutsche med. Wchnschr. **45**:430, 1919. (*c*) Ralli, E. P.; Brandaleone, H., and Mandelbaum, T.: J. Lab. & Clin. Med. **20**:1266, 1935.

33. Josephs, H. W.: To be published.

34. (*a*) Thomson, J. G.: Ztschr. f. d. ges. exper. Med. **92**:692, 1934. (*b*) Bennhold, H.: Klin. Wchnschr. **13**:36, 1934.

35. Fasold, H., and Heidemann, L. R.: Ztschr. f. d. ges. exper. Med. **92**:53, 1933.

36. (*a*) Ralli, Pariente, Brandaleone and Davidson.[18b] (*b*) Ralli, Brandaleone and Mandelbaum.[32c]

37. (*a*) Heymann.[27c] (*b*) Stannus, H. S.: Internat. Clin. **1**:146, 1929. (*c*) Bounin, E., and Lévinson, M.: Ztschr. f. Vitaminforsch. **5**:12, 1936.

38. Castellani, A., and Chalmers, A. J.: Manual of Tropical Medicine, ed. 3, London, Baillière, Tindall & Cox, 1919.

39. Sequeira, J. H.: Brit. J. Dermat. **49**:69, 1937.

(Clausen and McCoord[22k] and personal observations). Histologic examination shows that the carotene is found mostly in the superficial horny layer of the skin and that its concentration becomes less the deeper one goes.[40] The fact that it is present in sweat and sebaceous material has led to the hypothesis that its presence in the skin is dependent on this excretion and the subsequent rubbing of the carotene into the horny layer. Its predominant occurrence in those areas in which the horny layer is particularly thick or in which sweating is most marked (palms and soles, forehead, nasolabial folds, axillas and groins) would tend to bear out this theory.

2. Increased Serum Lipids: Anderson and Soley[41] noted an increase in serum lipids and pronounced fall in basal metabolic rate in several California farmers who had eaten vegetables in large amounts. The liver was enlarged in 4 of the 9 patients. An increase in serum lipid was noted by two other observers[42] but, in general, little attention has been paid to the blood lipids in carotenemia.

3. Harmful Effects: Some recent work throws doubt on the generally accepted harmlessness of carotenemia. Sequeira[39] reported weakness, loss of weight and low blood pressure. He was speaking, however, of cases observed in the tropics in which diet was apparently not a factor; hence it is possible that the carotenemia was secondary to some underlying factor responsible for the whole condition. Anderson and Soley reported that the liver was enlarged in about half of their patients and that the removal of rose bengal from the blood was slower than normal.

A more certain example of harmfulness is furnished by a case recently reported by Henschen.[43] A woman of 49 acquired a pica-like craving for carrots during an attempt to cure "anemia" with a vegetable diet. She ate 2 to 3 bushels a month (about 2 pounds [0.9 Kg.] a day), mostly raw, for six months, and a generalized xanthosis cutis developed. She also complained of paresthesias of the hands and feet. She was thin, pale, rather easily fatigued and subject to dyspnea. The liver and spleen were enlarged. The yellow color was general over the body, but the scleras were clear. Examination of the blood gave the following data: The hemoglobin content was 57 per cent. Erythrocytes numbered 3,[illegible]00,000 per cubic millimeter and leukocytes 5,300, with neutrophils 62 per cent, lymphocytes 28 per cent and monocytes 4 per cent. There was poor clot formation, but no estimation of the number of platelets was made. There was 0.347 mg. of carotene per hundred cubic centimeters of serum (normal 0.07 to 0.19). Serum xanthophyll was not increased. When carrots were removed from her diet, the patient slowly improved, though the number of white cells remained low. Later cataracts developed.

Reports of Earlier Cases of Dietary Carotenemia.—In the records of the Johns Hopkins Hospital there are 6 cases of carotenemia in which the diet alone either was or might have been responsible. This series is exclusive of cases of carotenemia occurring in the course of diabetes or nephrosis or of hypothyroidism, in which the low thyroid function is definitely the primary factor. They are included at this point because they have a bearing on the discussion of hypervitaminosis and because they confirm the hypothesis that carotenemia may not be as harmless as heretofore pictured.

CASE 1.—The patient was a child of 16 months, who had recently come from California, where she had been indulged in oranges, the only source of carotene that she could be ascertained to have received in significant amount. She recovered promptly, and at no time did untoward symptoms develop.

CASE 2.—The serum of this patient, also a child, was received for diagnosis. All that I can ascertain is that no harm resulted from the carotenemia and that the clinical diagnosis was made from the yellow color of the skin. It is unusual to see xanthosis cutis with the serum carotene as low as it was in this case.

CASE 3.—Carotenemia was diagnosed in a well-to-do asthmatic man who enjoyed vegetables in large quantities. No special studies were made, and the case is included only as one in which the patient was not harmed by the carotenemia.

CASE 4.—A 38 year old clergyman had as his only complaints yellowness of the skin and loss of weight. He had been supplementing his diet with carrots and carrot juice for about seven years. When carrots were removed from his diet, he recovered rapidly.

CASE 5.—This patient, a colored farmer who had lived largely on vegetables, especially spinach, was extremely undernourished. Low blood pressure, emaciation and extreme muscular weakness led to a first impression of hypopituitary cachexia (Simmond's disease). Later hypothyroidism was suggested on the bases of the high serum cholesterol and low basal metabolic rate. On a diet of high caloric value he improved extraordinarily rapidly.

CASE 6.—A well-to-do woman of 45, because of imagined idiosyncrasies to meat, eggs, milk and starchy foods, had lived largely on vegetables and fruits for a number of years. In addition to having carotenemia she was markedly underweight, with low blood pressure

40. (a) Hashimoto.[22g] (b) Miyaki.[22h] (c) Ansai, cited by Zechmeister, L.: Ergebn. d. Physiol. **39**:117, 1937.

41. Anderson, H. H., and Soley, M. H.: Am. J. M. Sc. **195**:313, 1938.

42. (a) Kohn.[22f] (b) Lian, C.; Abaza, A., and Sassier, R.: Bull. et mém. Soc. méd. d. hôp. de Paris **51**: 801, 1935.

43. Henschen, C.: Schweiz. med. Wchnschr. **71**: 331, 1941.

and asthenia. Hypothyroidism was diagnosed on two occasions when she was in the hospital. She was greatly improved by a diet high in calories, given in the hospital.

The patients in the first 3 of these cases were symptomless and entirely unharmed by the carotenemia. In case 1, however, the serum lipids were well above the normal. The last 2 cases considered by themselves would be hard to evaluate; in case 5 the extremely inadequate diet might have caused the entire clinical picture, and in case 6 the impression of hypothyroidism was particularly strong and in addition the diet was inadequate. Certainly the cases in which there is evidence of harm were those in which the patient had taken the carotene-containing foods for a long time or had lived largely on them. Case 4 is the link connecting the cases of innocuous carotenemia with those in which harm has resulted; in this case there is no doubt of the primary position of the carotenemia, and at the same time the patient showed loss of weight, asthenia, high blood lipids and cholesterol and a low basal metabolic rate.

From these cases plus those described by Anderson and Soley, of carotenemia in California farmers,[11] one may form a picture of the effects of carotenemia. Loss of weight is usual, but it is not possible to be certain to what extent this is the direct result of carotenemia and to what extent the result of the concurrent inadequacy of the diet. The same is true of muscular weakness. Hypotension is even more difficult to evaluate. It was not mentioned by Anderson and Soley. In case 6 of the series just described the low blood pressure antedated the abnormal diet; in case 5 the extreme inadequacy of the diet may have caused the low blood pressure, and in case 4 the blood pressure was not excessively low. The high values for blood lipid and cholesterol and the low basal metabolic rate are definitely part of the picture of carotenemia, occurring as they did in Anderson and Soley's cases as well as in 3 of the 4 cases reported here in which the lipids were determined. A tendency to leukopenia also appears to be a characteristic, but one that may be absent; in the series I have reported it occurred in 2 of the 3 cases with symptoms (4 and 6), and it was present in Henschen's case. Anderson and Soley merely stated that the blood was normal, without reporting the actual counts.

Perhaps the most confusing aspect is the relationship of carotenemia to hypothyroidism. Every symptom that has been mentioned as occurring with carotenemia except the loss of weight could occur in hypothyroidism. Only consideration of the fact that the diet came first and the symptoms followed will allow a correct evaluation to be made. A possible explanation of this similarity lies in the reported antagonism between carotene or vitamin A and thyroxin. According to this theory the symptoms of hypothyroidism may exist not only as the result of diminished activity of the thyroid gland, but as the result of "neutralization" of thyroxin by carotene or vitamin A.

Another point that must be emphasized is that it is the abnormal intake of carotene rather than the amount in the blood that is the cause of trouble. In conditions such as diabetes and nephrosis, in which carotenemia may occur without excessive intake, none of the symptoms cited are present. Apparently under ordinary condi-

TABLE 2.—*Laboratory Data for Patients with Carotenemia*

Case No.	Age, Sex	Source of Carotene	Date	Serum Carotene, Micrograms/100 Cc.	Serum Vitamin A, U. S. P. Units/100 Cc.	Serum Lipids, Mg./100 Cc.	Serum Cholesterol, Mg./100 Cc.	Basal Metabolic Rate	Loss of Weight	Weakness	Hypotension	Leukocytes, No./Cu.mm.	Comments
1	16 mo. F	Oranges	2/8/38	610	90	1,100	...	..	0	0	...	.....	
2	Child ?	Unknown	Jan. 1939	220	55	650	...	..	0	0	...	.....	
3	37 yr. M	Carrots	Oct. 1936	...	...	.....	...	..	0	0	...	8,500	Diagnosis made on appearance of skin
4	36 yr. M	Carrots and carrot juice for 7 years	9/12 9/28 12/10	600 420 150	60 100 120	1,410 970 720	441	−19	+	+	Slight	4,100	Symptomatic improvement on discontinuance of carrots and carrot juice
5	18 yr. M	Vegetables, especially spinach, principal source of food for 2 years	12/22	550	...	1,250	420	−45	++	++	++	6,800	Rapid improvement on high calory diet
6	47 yr. F	Vegetables and fruit principal source of food for 4 years	....	...	...	.....	Elevated	−45	++	++	+	4,100	Considered to be hypothyroid on 2 occasions, but improved rapidly on high calory diet

tions carotene must be ingested in excessive amount for a long time or must form the major part of the diet to the exclusion of other constituents in order to be harmful.

CLINICAL HYPERVITAMINOSIS

Factors Influencing Vitamin A of the Blood.—Little is known of the factors leading to the increase of vitamin A in the serum, and that little has come principally from experimentation on animals.

1. Diet: Increasing the dietary intake of vitamin A beyond a certain optimum will increase the amount stored, but it is usually not possible to push the level of vitamin A in the blood above what might be called a normal maximum, except for a few hours after administration of a large dose. A child of 4 years who was given percomorph liver oil in large doses for resistant rickets received about 120,000 U. S. P. units of vitamin A every day for a month with no more than a minimal effect on her serum vitamin A, determined twenty-four hours after the last dose. Two infants were given 200,000 U. S. P. units of vitamin A a day for several weeks. There was a slight temporary rise in vitamin A in the blood, lasting about a week. Likewise, in rats ingestion of 2,500 U. S. P. units a day raised the level of vitamin A from 1 unit per cubic centimeter of serum to about 2 units, and, although a large amount was stored in the liver, the serum vitamin A fell to nearly its former level within two or three days after the high ingestion of the vitamin was discontinued.[44]

2. Level of Serum Lipids: The conditions in which hyperlipemia occurs tend to be associated with increases in vitamin A as well as with carotenemia. Also, as in the case of carotenemia, the relationship is of a general nature and one must utilize pathologic extremes to be able to demonstrate it.[31] An important difference lies in the far more rapid removal of excessive vitamin A than of carotene from the blood. The post-absorptive curve of vitamin A is influenced by the level of serum lipid just as is that of carotene[13] but the specific removal of vitamin A in the liver effectively prevents prolonged accumulation in the blood, while the slower removal of carotene permits an accumulation which is also aided by the relatively higher solubility of the latter substance in the blood lipids. It seems likely also that the solubility in lipids is a less important factor in the case of vitamin A than of carotene. If vitamin A and lipids tend to vary together, it is because they are subject to similar influences.[45]

The highest values for vitamin A seen in over 700 cases did not exceed 2.5 units per cubic centimeter except immediately after ingestion, even though the series included some cases with high levels of lipid (cases of nephrosis and 1 of myxedema). Following pneumonia there was a tendency for hyperlipemia to develop in some of the children observed; under these circumstances the vitamin A might temporarily reach a value of 3 units per cubic centimeter of serum (patients not receiving supplementary vitamin A). These levels were the highest seen with the exception of those in the case of vitaminosis reported here.

Protection Against Hypervitaminosis.—A factor of safety for the animals that rely on carotene for their source of vitamin A is the relative harmlessness of carotene. Also any damage which it does cause is apparently not permanent and rapid and complete recovery is to be expected as soon as the excessive intake of carotene is stopped.

The facts just reviewed point to the existence of a mechanism for the maintenance of the level of vitamin A in the blood within certain limits. It seems highly significant that the mechanism exists in the case of the vitamin which can produce toxic effects, and not in the case of its precursor, carotene, which appears to be relatively harmless. It is interesting that the dog, which apparently lacks this mechanism for the limitation of the level of serum vitamin A (personal observation), is the one animal thus far studied in which none of the toxic effect from vitamin A have been elicited.[10c]

Another protective mechanism is the storage of vitamin A in the liver, where it appears to be harmless. It is not the total amount of vitamins in the body that determines the presence of toxic symptoms but the ingestion of amounts large enough to overwhelm the ability of the liver to remove it from the circulation.[46] Seemingly it is possible to push the storage in the liver without limit, and no symptoms of intoxication are produced unless the daily dose is raised above the "toxic" limit. When vitamin A or carotene is given intravenously the excess is removed with great rapidity.[47] In this removal the cells of the reticuloendothelial system play a primary role particularly the Kupffer cells of the liver. This

44. Josephs, H. W.: Bull. Johns Hopkins Hosp. **71**: 253, 1942.

45. Josephs.[9, 13]

46. (*a*) Davies and Moore.[10] (*b*) Ikegaki, I.: Ztschr. f. Vitaminforsch. **7**:113, 1938.

47. (*a*) Drummond, J. C.: Gilding, H. P., and McWalter, R. J.: J. Physiol. **82**:75, 1934. (*b*) Gr[illegible] H., and Skurnik, L.: Acta med. Scandinav. **101**:[illegible] 1939.

has been shown by fluorescence microscopy [48] as well as by "blockade" experiments.[49] The increase in activity of these cells under the influence of overdosage with vitamin A may therefore be looked on as a factor in the defense against hypervitaminosis.

When excessive vitamin A is given to rats over a period of time, a rise in the level of serum lipid occurs, which, however, is temporary. This has been taken as evidence that one of the fundamental effects of vitamin A is on the metabolism of lipids, but that ordinarily the effect is adequately and promptly compensated for. When the vitamin is suddenly introduced in large amounts the compensatory process is temporarily overcome and a rise in blood lipid occurs. It has been suggested that this compensatory process involves increased activity of the thyroid gland.[50] Support for this idea comes from the demonstration of antagonism between vitamin A and thyroxin.[33] The continuance of the high content of lipid in the serum in the cases of carotenemia cited in this paper suggests that this compensatory factor may be more effective in the rat than in man.

COMMENT ON THE CASE

From this review of reports of experimental and clinical carotenemia and hypervitaminosis A, one obtains some insight into the significance of certain pathologic changes in the case which is reported. However, since final proof of the mechanisms involved is lacking, the interpretations which follow must be looked on as suggestions rather than as demonstrations of the manner in which excessive vitamin A acts.

Abnormal Appetite.—Whether or not it is justifiable to label as "abnormal" a liking for an article of food which most people would not eat by choice, there is no doubt that the child's liking for halibut liver oil was a factor in the case which is reported. It is interesting that this desire for the oil was replaced by a desire for butter and carrots after the administration of the oil was discontinued, and that as the patient improved clinically he lost all such desires. Henschen's patient with carotenemia had developed such an appetite for carrots that she was impelled to search garbage cans to satisfy her craving. Two of Hashimoto's patients among Japanese peasants had an abnormal appetite for squash, the source of their carotene. Clausen and McCoord mentioned a case in which the patient had an abnormal craving for carrots; the serum carotene in this case was the highest in their series. These examples are cited to show that an abnormal appetite for carotene-containing foods may occur.

It is possible that the appetite existed first and was primarily responsible for the large intake, but it seems more probable that the increased intake was begun for economic or other reasons and that the abnormal appetite developed subsequently. Desire for some particular dietary constituent not infrequently results from the necessity of correcting a deficiency or some other condition, for example, the craving for salt in persons with adrenal insufficiency. It is conceivable that excessive vitamin A or carotene taken at first through economic necessity or zeal might give rise to changes that would create or increase the desire for the material that caused the changes. The change in desire from halibut liver oil to carrots and butter in our patient when use of the oil was stopped suggests that this was the case. It may be noted from table 1 that after the patient was discharged from the hospital the content of carotene in the serum rose for a short time to a definitely abnormal level, which confirms the history of increased intake of carotene.

Hepatomegaly and Splenomegaly.—The original impression of Gaucher's disease was suggested by the presence of a large liver and spleen together with anemia of the hypoplastic type. The roentgenograms of the bones were not indicative of this condition except that the changes in the metacarpals and phalanges suggested the presence of a process replacing normal marrow with pathologic tissue. The increased storage of fat in animals subjected to intoxication with vitamin A suggests the possibility that such storage may have occurred in our patient; however, we have no direct evidence of this or of any reticuloendothelial proliferation, at least in the bone marrow, though such a proliferation may account for the enlargement of the liver and spleen. The improvement of the patient when ingestion of excessive vitamin A was stopped is evidence that vitamin A intoxication was primarily responsible for the clinical picture. It may be pointed out in confirmation that Henschen's patient also had hepatomegaly and splenomegaly with a hypoplastic type of anemia, and that in the cases of carotenemia which I summarized the patients who had any symptoms tended to have leukopenia and a slight reduction in the number of red cells.

The idea that vitamin A and carotene are capable, under conditions that lead to accumulation in the blood, of causing a disturbance in the

48. Popper, H., and Brenner, S.: J. Nutrition **23:** 431, 1942.

49. Lasch, F., and Roller, D.: Klin. Wchnschr. **15:** 1636, 1936.

50. Wendt, H.: Deutsche med. Wchnschr. **62:** 1213, 1936.

storage of lipids with certain features in common with the changes which occur in xanthomatosis, Gaucher's disease or Niemann-Pick disease is an attractive one. However, the reported effect of vitamin A on reticuloendothelial proliferation in animals has not yet been thoroughly confirmed, nor has it been shown that the splenomegaly or hepatomegaly in clinical cases is actually due to increase in the storage of lipids.

Increased Vitamin A in Serum.—The vitamin A of the blood of our patient when first seen was at a level which has otherwise been encountered only at the height of the postabsorptive curve, four to six hours after a relatively large dose. Not only that, but it remained at a high level after all excess ingestion of vitamin A had been stopped and only slowly returned to normal over a period of six months. As stated previously, attempts to cause a rise in serum vitamin A in other patients to more than a limited extent or over more than a limited time have proved unsuccessful. While this paper was in preparation a child of 8 months was brought to the outpatient department after receiving 3 teaspoons of percomorph liver oil a day during a period of four months (corresponding to about 500,000 U. S. P. units of vitamin A per day). The vitamin A of the serum was 110 U. S. P. units per hundred cubic centimeters and the total lipid 730 mg. per hundred cubic centimeters, both normal values. This case exemplifies the efficiency of the mechanism by which the constancy of the blood vitamin A is maintained and at the same time indicates that this mechanism in the patient whose case is reported here was seriously impaired.

In the discussion of the body's protection against hypervitaminosis the role played by the reticuloendothelial cells in removing vitamin A and carotene from the blood was cited. If, as has been suggested, the cells of the reticuloendothelial system are made more active by an overdose of vitamin A the vitamin would be more, not less, effectively removed from the blood. The fact that in the case under discussion the vitamin was less effectively removed indicates either that the child had some defect in his reticuloendothelial response or else that intense, prolonged overdosage is capable of injuring this response. The only work that I could find bearing on this point is that of Ikegaki,[46b] who found that in early stages of overdosage with vitamin A liver function increased, whereas later it decreased; the functions dealing with removal of dye from the blood stream and with detoxification were particularly affected. In our patient, bromsulphalein was removed normally but the serum bilirubin was consistently somewhat raised (1.6 mg. per hundred cubic centimeters). In Andersón and Soley's cases of carotenemia, 5 of the 8 patients tested showed impairment of the ability of the liver to remove rose bengal from the blood.

A second possibility in this case is that the limit of the liver's ability to store vitamin A was exceeded. While little is known of the capacity of the human liver, work on rats shows that when storage of vitamin A has been pushed to a high point the vitamin disappears with great rapidity when excessive intake is discontinued, supposedly by oxidation or some other form of destruction. Whether or not prolonged administration of vitamin A could impair this mechanism for getting rid of amounts in excess of the storage capacity is not known. As I have pointed out however, excessive storage has little influence on the level in the blood, so that it would be difficult to explain the continuance in this patient of a high level over a prolonged period after the intake of vitamin A was discontinued, especially since the total amount in circulation is only a minute fraction of the amount in storage.

Increased Level of Serum Lipids.—The increase in lipid in the blood in the patient under discussion was definite though not excessive (The normal range is approximately 550 to 750 mg. per hundred cubic centimeters, though higher may be encountered in otherwise normal persons.) The gradual fall after excessive intake of vitamin A was stopped leaves no doubt regarding the causation. The level of carotene in the patient's serum was not excessively high except on one occasion, as previously mentioned, just after his return home, when he had a craving for carrots and butter. It may be noted that on this occasion the content of lipids also increased slightly. A rise in blood lipids appears to be a significant part of the picture of hypervitaminosis A as well as of carotenemia. The cholesterol also takes part in the rise of lipids but in some cases, as in those reported by Anderson and Soley, the increase is not correspondingly great. The basal metabolic rate was not determined in the patient discussed with hypervitaminosis A, but it was decreased in most of my patients with carotenemia. In fact, the picture of carotenemia resembles that of hypothyroidism to such an extent that a distinction may be difficult. Only the definite association of the condition with a vegetable diet and its disappearance as soon as the source of carotene was removed lead to a correct diagnosis of carotenemia. The explanation for the similarity between the two conditions may lie in the vitamin A-thyroid antagonism to which reference was made previously in which case the symptoms of hypothyroidism in carotenemia would be due to the presence of

an excess of thyroid antagonist and not to an actual diminution in the activity of the thyroid gland.

It was pointed out previously that the rise in lipids which occurs in animals subjected to continued excessive doses of vitamin A or carotene was a temporary phenomenon and that the return to normal levels was evidence of the development of a compensatory process. Wendt suggested that the compensation might involve an increase in thyroid activity.[10c] I have suggested that the continuance of the high level of lipids in the patient reported on here might also indicate that the "compensatory" process is less effective in man than in rats. The fact that the dose required to elicit the lipid response in man is relatively far smaller than that for rats [12] points in the same direction. If, as suggested, increased activity of the thyroid supplies the "compensatory" process, its relative failure in man might explain both the persistence of the high levels of serum lipid and the symptoms of hypothyroidism.

Skeletal Abnormalities.—The skeletal changes which occurred are difficult to evaluate, and the biopsy was of no aid. The speed of decalcification was in line with the observation that in animals decalcification of bones is one of the most constant effects of hypervitaminosis. The most outstanding features in the roentgenograms of the patient were the evidences of a more rapid epiphysial development, the irregularities, the apparent stippled effect of the epiphysial centers, the increased distance between epiphysis and diaphysis and the advanced development of carpal centers of ossification. Even two and a half years after the removal of excessive vitamin A the carpal centers were in advance of the patient's chronologic age. The child, however, has not grown unduly and at present is no more than at the upper limit of normal stature for his age. It may be mentioned that Wolbach and Bessey [4c] in a recent review stated the opinion that vitamin A acts to increase the rate of development of bone; however, they gave none of the evidence on which the opinion was based.

Associated Abnormalities.—Four other abnormal conditions present in this case are worth noting. (*a*) The rise in serum phosphatase depends on the activity of processes which also give rise to changes in the bones, and it is to be considered in relation to these. Phosphatase is also increased in diseases of the liver, and, as has been pointed out, there is evidence of hepatic damage in this case. (*b*) The low level of serum protein, especially of the globulin fraction, is probably of considerable significance, but it cannot be evaluated in the present state of knowledge. (*c*) Sparse, coarse hair was a prominent characteristic of the clinical picture. Loss of hair by rats subjected to vitamin A intoxication has been a prominent feature of some descriptions. In the patient, the return of hair of normal texture and distribution was almost the first sign of improvement. (*d*) Clubbing of the fingers was a striking feature of the clinical picture when the child was first seen. It was still present though less prominent after two and a half years. Its significance is not known, but it may possibly indicate damage to the liver, since clubbing of the fingers and toes is not infrequently observed in cirrhosis in children.

The question of complete recovery of the patient in the case reported is still open. Clinically the child is well and has remained so during the past two years. The only causes for anxiety are the continued presence of splenomegaly and to a less degree of hepatomegaly and the persistent advance in skeletal development. In Henschen's case splenomegaly was slow to disappear and leukopenia persisted for at least a year. In view of the magnitude and the duration (three years) of the intake of vitamin A it would be surprising if some permanent change had not resulted. My fear is not so much that there may be a progressive process still active as that there may have been some scarring of the liver that will later give rise to trouble.

SUMMARY

Severe hypervitaminosis A occurred in a boy of 3 years who had received about 240,000 U. S. P. units of vitamin A daily since he was about 3 months of age. The condition was characterized by hepatomegaly, splenomegaly, hypoplastic anemia, leukopenia, increased serum vitamin A, increased serum lipids, advanced skeletal development, clubbing of the fingers and sparse, coarse hair; it was accompanied with an abnormal appetite for halibut liver oil, the source of the vitamin. Most of the symptoms cleared promptly when the excess vitamin A was removed from the diet.

Experimental and clinical observations indicate the existence of a mechanism for the maintenance of a constant level of vitamin A in the blood, possibly related to the activities of the reticuloendothelial cells. No such mechanism seems to exist for carotene. The occurrence of hypervitaminosis A in this child indicates either that some defect existed in the system responsible for the protective reaction or else that intense, prolonged overdosage is capable of injuring the regulatory mechanism.

Johns Hopkins Hospital.

Komatsu, S.: TERATOGENIC EFFECTS OF VITAMIN A. I. EFFECTS OF BETA-CAROTENE. Shika Gakuho, Vol. 7, pp. 2067074, 1971. Department of Orthodontics, Dental Research Institute, Tokyo College of Dentistry (Director: Prof. Y. Yamamoto). Faculty of Pharmaceutical Science, Tokyo College of Dentistry (Instructor: Prof. S. Tamura)

INTRODUCTION

Vitamin A is a nutritionally important vitamin. TThe deficiency of this vitamin is said to cause growth retardation, opthalmological, dermatological, reproductive, and epidemic disturbances (14,22,24). There have been a great number of detailed studies on the relationship between vitamin A and the dark adaptation of the eye. With respect to communicable diseases, particularly measles, vitamin A is excessively consumed, which leads to internal deficiency of the vitamin.. During the postwar food shortage, infants were raised on defat milk, which gave a rise to keratitis sicca, which occasionally led to loss of sight. As a result, there was a general tendency of giving large doses of vitamin A to infants.

About that time, Josephs published a paper on hypervitaminosis A and carotenemia in 1944 (6), in which he reported that infants and toddlers, rather than adults, suffered from the condition characterized by anorexia, growth retardation, hypererethism, dermatological disturbance, baldness, pain and swelling of bones, hypertrophy of the liver and pancreas, and increased hemorrhagic tendency (19), and cited increased vitamin A level in the blood as the decisive diagnostic indicator.

The most characteristic symptoms in animals suffering from this condition are the spontaneous fracture of long bones and internal hemorrhage. The fact that the excess intake of this vitamin during pregnancy causes unique deformation was reported by Cohlan (1953) (2), and a similar observation was made by Giroud and Martinet (1959) (7), Millen and Woollam (1957) (15), Inaba (1958) (9), Baba et al. (1959) (1), Deuschle et al. (1959) (5), Kalter and Warkany (1959) (12), (1961) (13), Takekoshi (1961) (23), Murakami et al. (1965) (18), Ezaki (1964) (6), Ibara et al. (1966) (8), and Kajiwara (1965) (11).

Plant foods contain carotene and carotenoid, which are also ingested in the form of food coloring (21). These substances change into vitamin A in human or animal body. Since there has been no report made on the teratogenic effect of hypercarotenemia due to a large intake of carotene, the author specifically studied the teratogenic action of beta-carotene, a vitamin A precursor.

A part of this paper was published at the 176th meeting of Tokyo College of Dentistry, the 39th Kanto Regional Meeting of the Japanese Society of Pharmacology, and the 9th meeting of the Japanese Society of Congenital Deformity.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL ANIMALS:

Healthy Wistar albino rats inbred since 1950 at Tamura Laboratory of Experimental Medicine, with minimal individual variation and equal hereditary factor were used. The experimental conditions are shown in Table 1.

Table 1. Experimental methods

Group	Drug	Doses	Total number of female rats treated	Route of administration	Stage
Control	Physiological saline	1ml/100g	17	Oral	From 9th to 12th day of pregnancy
A	Vitamin A	30,000I.U./100g	15		
CAR—1	β-carotene	(15mg/100g, 30,000I.U./100g)	10		
CAR—2	β-carotene	(36mg/100g, 60,000I.U./100g)	12		
CAR—3	β-carotene	(180mg/100g, 300,000I.U./100g)	10		

ENVIRONMENT:

Since the incidence of experimental deformities is said to vary according to the season, amount of noise, and condition of the cage, the animals were kept in a clean metal-mesh cage with a sufficient floor space per rat with constant temperature and humidity in a quiet chamber.

EXPERIMENTAL FEED:

The basal feed used at this faculty is composed of 80% crushed wheat, 15% casein, 4% salts, and 1% composite vitamin. Containing about 20% protein and a necessary amount of salts, the feed is nutritionally complete. Mature rats were given 15 g of this feed daily, the amount of vitamin A ingested being 375 I.U. per day. Water was given upon demand.

CONFIRMATION OF PREGNANCY

The vaginal wax of female rats was tested for the confirmation of the estrus, and those found to be in the estrus were placed in the same cage with a healthy male rat, 10 - 11 weeks old, of the same strain from 6:00 p.m. to the following morning. Pregnancy was determined by the presence of sperms in the vagina, and the duration of pregnancy was determined with 0:00 a.m. on the same day regarded as the 0 day of pregnancy.

OBSERVATION PERIOD

The mother rats were killed on the 21st day of pregnancy, precisely in 20 days and 12 hours of pregnancy, and the condition of the fetuses was examined.

The abdomen of the mother rats was cut open immediately, and the fetuses were removed. After weighing them, their external appearance was observed macroscopically and in the stereomicroscope of low magnification. When the fetuses were dead the uterus was examined with respect to the

number of implantations, number of placental remnants, and macerated fetuses.

After the examination of external abnormality, skeletal specimens of the fetuses were prepared according to the modified version of Noback and Downson's method (15,4).

PREPARATION OF SKELETAL SPECIMENS

1. The abdomen of the fetus is opened, and the organs are removed.
2. The remainder is fixed with 95% ethanol for 2 days.
3. The skin and muscles are removed.
4. The remainder is immersed in a solution prepared by dissolving 20 mg of alizarin red S in 1 liter of 1% KOH for 1.5 - 2 days.
5. The skeleton is placed in a 20% glycerin solution for 1 day.
6. The skeleton is immersed in a 40% glycerin solution for 1 day.
7. The skeleton is immersed in a 60% glycerin solution for 1 day.
8. The skeleton is placed in an 80% glycerin solution.

The skeletal specimen thus obtained was examined macroscopically or in a stereomicroscope with regard to the presence and degree of abnormality.

EXPERIMENTAL RESULTS

1. BODY WEIGHT OF MOTHER RATS (TABLE 2)

The weight gain of mother rats given beta-carotene (CAR) showed no difference from that of the control group, but was arithmetically in excess of that of the vitamin A group (A).

Table 2. Mother's condition

		Control	A	CAR-1	CAR-2	CAR-3
Body weight of pregnant mothers(g)	The first day of pregnancy	209.5	203.9	187.8	194.0	191.5
	The first day of administration	229.5	235.6	213.3	217.5	221.4
	The last day of administration	239.5	234.5	229.8	234.0	234.4
	The day of autopsy	309.4	297.3	287.8	292.5	291.6
	Gained body weight	99.9 ±28.6	93.4 ±25.5	100.0 ±8.8	98.5 ±17.3	100.1 ±22.5

2. IMPLANTATION (Table 3)

The CAR-2 group, just as the control group, showed no fetal death. There were incidences of resorptions in CAR-1 and CAR-3 groups, 4.7% for the former and 12.2% for the latter, which is close totthe 11.2% of the A group, and the incidences of placental remnants were 5.6% for the A group, 1.9% for the CAR-1 group, and 1.6% for the CAR-3 group. Macerated fetuses occurred to 1.7% of the A group but none of the CAR groups.

The rate of surviving fetuses was 100% for the control, 81.5% for the A group, 100% for the CAR-2 group, 93.5% for the CAR-1 group, and 86.2% for the CAR-3 group.

Table 3. Pregnancy status

		Control	A	CAR-1	CAR-2	CAR-3
Total number of implantations		184	178	107	130	123
Mean number of per a mother		10.8	11.9	10.7	10.8	12.3
Number of fetal deaths	Resorptions	0	20	5	0	15
	Placental remnants	0	10	2	0	2
	Macerated fetuses	0	3	0	0	0
Percentage of fetal deaths (%)	Resorptions	0	11.2	4.7	0	12.2
	Placental remnants	0	5.6	1.9	0	1.6
	Macerated fetuses	0	1.7	0	0	0
Number of survived fetuses		184	145	100	130	106
Percentage of survived fetuses(%)		100	81.5	93.5	100	86.2
Sex ratio female/male		5.5/5.3	4.7/5.0	5.8/4.3	6.0/4.8	5.8/4.2
Mean weight of survivors(g)		4.07 ±0.14	3.79 ±0.36	3.95 ±0.41	3.89 ±0.29	3.83 ±0.27
Number of fetuses with growth retardation		4	1	0	2	1
Percentage of fetuses with growth retardation(%)		2.2	0.7	0	1.5	0.9
Weight of placenta(g)		4.20	3.99	3.98	3.99	4.50

The body weight of surviving fetuses was the same in all group including the control group. Fetal growth retardation showed no variation between the groups, nor was there any appreciable difference in placental weight between the groups.

3. EXTERNAL FETAL ABNORMALITY (Table 4)

No external abnormality had occurred to the fetuses of Wistar albino rats inbred for many years at Tamura Laboratory for Experimental Medicine.

The incidence of deformities for the A group, however, was 82.6% as compared with 0% for any of the CAR groups as shown in Table 4.

4. DEGREE OF OSSIFICATION IN THE FETUSES

a. OCCIPITAL OSSIFICATION

No incidence of incomplete occipital ossification occurred to the Wistar albino rats subjected to this experiment as shown in Table 5. Incomplete ossification occurred to 72.7% of the A group, to none of the CAR-1 groups or the control group. The incidence among the CAR-2 and 3 groups was considerably lower.

b. STERNEBRAL OSSIFICATION

Incomplete sternebral ossification occurred to 16.7% of the control and 75.5% of A group. Severe forms of this deformity were noted in some of the fetuses of the CAR-groups, the incidence being 80.0% for CAR-1, 83.1% for CAR-2, and 55.8% for CAR-3. These results suggest that the administration of CAR causes incomplete sternebral ossification of considerable severity.

Table 4. Incidence and types of malformations

		Control	A	CAR-1	CAR-2	CAR-3
Total number of survived fetuses		184	145	100	130	106
Percentage of abnormal fetuses	(%)	0	82.6	0	0	0
Microcephalia	(%)	0	5.5	0	0	0
Exencephalia	(%)	0	0	0	0	0
Auricular malformation	(%)	0	40.4	0	0	0
Open eyelid	(%)	0	14.7	0	0	0
Stomatic malformation	(%)	0	1.8	0	0	0
Pseudomacroglossia	(%)	0	20.2	0	0	0
Macrognathia	(%)	0	17.4	0	0	0
Cleft palate	(%)	0	61.5	0	0	0
Low jaw cleft	(%)	0	10.1	0	0	0
Cleft tongue	(%)	0	1.8	0	0	0
Micromelia	(%)	0	0.9	0	0	0
Anal atresia and ecaudate	(%)	0	0	0	0	0

Table 5. Ossification patterns

	Control	A	CAR-1	CAR-2	CAR-3
Fetuses with retardation of ossification of the occipital bone(%)	0	72.7	0	9.2	1.1
Fetuses with retardation of ossification of the sternebrae(%)	16.7	75.5	80.0	83.1	55.8
Mean number of ossified metacarpal bones per fetus	7.89 ±0.24	6.30 ±0.84	7.28 ±0.81	7.57 ±0.40	6.97 ±0.80
Mean number of ossified metatarsal bones per fetus	8.00 ±0.00	7.87 ±0.25	8.00 ±0.00	8.00 ±0.00	8.00 ±0.00
Mean number of ossified coccyges per fetus	4.95 ±0.46	5.00 ±0.64	4.40 ±0.39	4.76 ±0.37	3.97 ±0.96

Table 6. Deformities of the bone structures in fetuses

		Control	A	CAR-1	CAR-2	CAR-3
Formation of the 14th rib	left (%)	0	27.9	0	0	0
	right (%)	0	24.5	0	0	0
Brachygnathia	(%)	0	3.4	0	0	0
Abnormality of the humerus	(%)	0	8.2	0	0	0
Shortening of the ulna	(%)	0	8.8	0	0	0
Shortening of the radius	(%)	0	7.5	0	0	0
Shortening of the femur	(%)	0	4.1	0	0	0
Shortening of the tibia	(%)	0	1.4	0	0	0
Shortening of the fibula	(%)	0	8.2	0	0	0

c. OSSIFICATION OF THE METACARPAL AND METATARSAL BONES AND THE COCCYX

The number of ossified bones in the metacarpal and metatarsal regions of the control group was rated 7 - 8% and that in the coccyx, approximately 4 - 5%. The number of ossified metacarpal bones of group A was somewhat less, but that in the metatarsal and coccygeal regions of the same group showed no appreciable difference.

The numbers of ossification centers in the metacarpal and metatarsal bones and the coccyx of CAR groups were same as those of the control group.

d. ABNORMALITIES OF FETAL SKELETON

No skeletal deformities were shown by the fetuses of the control group. Group A showed a high incidence of the deformity of the 14th rib, and incidences of the shortening of the mandible, radius, ulna, peroneal bone, tibia, etc. None of these deformities occurred to the CAR groups.

DISCUSSION

Since beta-carotene is converted into vitamin A in vivo, it was given to the rats at doses equivalent to 30,000, 60,000, and 300,000 I.U. of vitamin A. At these doses, the vitamin exerts a teratogenic action. In this experiment, a considerably high incidence of resorption was noted in the massive beta-carotene group which received the compound at a dose equivalent to 300,000 I.U. of vitamin A. Severe forms of incomplete ossification of the sternebrae were noted in all of the beta-carotene groups, but the skeletal deformities in fetuses as produced by excessive vitamin A ingestion did not occur to these groups.

The above results indicate that not all of the beta-carotene ingested was converted into vitamin A. The organ where the conversion takes place is the liver, and hepatic dysfunction may reduce the amount converted into vitamin A, but since the present experiment included only healthy rats, such disturbance is not likely. Nieman and Obbink (17) stated that since animals acquire a large amount of carotene from different food stuffs, they tend to develop hypercarotenemia, subsequently xanthosis, but such condition should be distinguished from hypervitaminosis A. Ostwalt and Briggs (20) observed 4 generations of rats which were given 0.1% beta-carotene, but failed to witness any toxic action of the compound. In other words, hypervitaminosis A did not occur despite the life-long intake of the compound at a daily dose equivalent to 1600 I.U. of vitamin A. Josephs et al. (10) failed to produce hypervitaminosis A even with massive doses of carotene. Dagadu and Gillman (3) published results of their experiment to the effect that a large intake of carotene elevated the serum carotene level, but not the serum vitamin A level. He interpreted the result as that the vitamin A stored in the liver inhibited the conversion of carotene into vitamin A.

The author experimentally produced hypercarotenemia in pregnant rats by giving them large amounts of beta-carotene, but the fetuses developed none of the deformities or skeletal abnormalities caused by vitamin A at

equivalent doses. This demonstrates that hypercarotenemia does not immediately induce hypervitaminosis A.

Different foods, with a wide distribution of carotene and carotenoid, are ingested by animals, but, by an ingenuity of nature, hypercarotenemia does not induce hypervitaminosis A.

CONCLUSION

1. Deformities were produced in Wistar albino rats inbred for more than 20 years at Tamura Laboratory of Experimental Medicine, with 30,000 I.U. of vitamin A. Beta-carotene at 18 mg, which is equivalent to 30,000 I.U. of vitamin A, 36 mg, equivalent to 60,000 I.U., and 180 mg, equivalent to 300,000 I.U., were also given to rats of the same strain and their effects were investigated.

2. The beta-carotene 180 mg group showed resorptions as induced by other drugs at massive doses.

3. Severely retarded sternebral ossification occurred to all carotene groups.

4. There was no incidence of skeletal abnormality or deformity among CAR groups, unlike the vitamin A group.

The authors wish to express their deep gratitude to Prof. Yamamoto, Prof. Tamura of the Faculty of Pharmaceutical Science, and Prof. Tsutsumi for their instruction and encouragement. The cooperation from the staff of the Faculties of Pharmaceutical Science and Orthopaedics is gratefully acknowledged.

FIGURES

Fig. 1. The abnormal fetus with low jaw cleft, auricular malformation, open eyelid and malformation of the mouth.(Vitamin A treated)

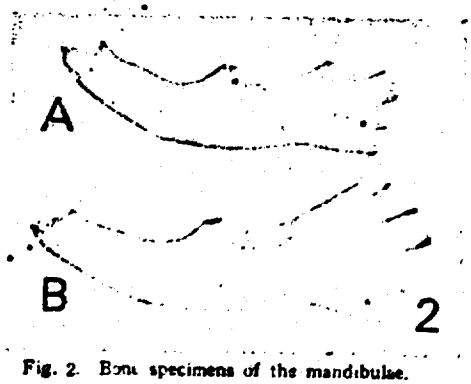
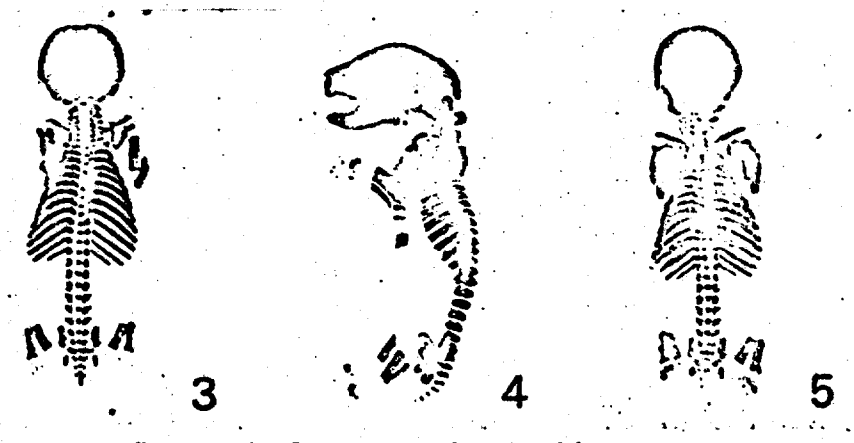
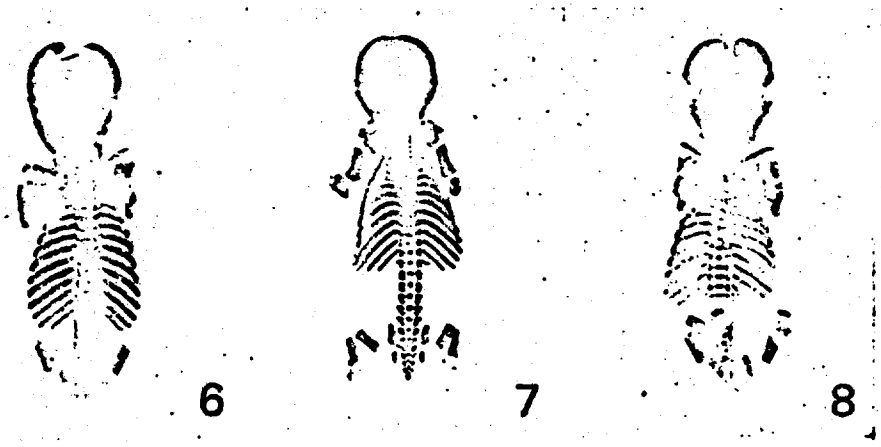


Fig. 2. Bone specimens of the mandibulae.
A. Vitamin A-treated.
B. Control.

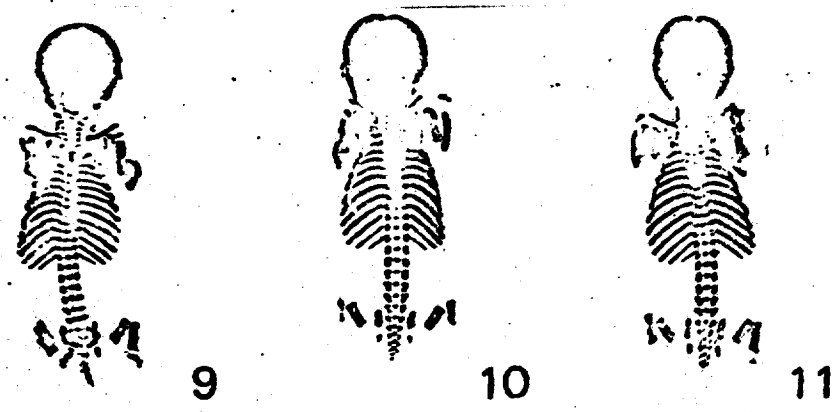


Figs. 3, 4 and 5. Bone specimens of the normal fetuses.



Figs. 6, 7 and 8. Bone specimens of the fetuses with abnormal skeleton.

6. Retardation of ossification of the occipital bone.(Vitamin A treated)
7. Incomplete formation of the 14th rib.(Vitamin A treated)
8. Lack and retardation of ossification of the sterneorae.(Vitamin A treated)



Figs. 9, 10 and 11. Bone specimens of the fetuses with abnormal skeleton

9. Lack of the 5th sternebra.(β-carotene treated)
10. Retardation of ossification of the occipital bone.(β-carotene treated)
11. Incomplete formation of the 13th rib.(β-carotene treated)

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—— 原　　著 ——

Vitamin A による催奇形作用に関する研究

1. β carotene の影響について

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緒　　言

vitamin A は栄養学的にきわめて重要な vitamin である。この vitamin の欠乏は成長，眼，皮膚，繁殖，伝染性疾患の発生に関係を及ぼすといわれている[14,22,24]。特に眼の暗調反応についてはきわめて詳細な研究が多数発表されている。伝染性疾患，特に麻疹では，vitamin A が消費され，これの体内の欠乏を誘起し，また戦後食糧事情の不良状態下で脱脂粉乳で育成された乳児には，角膜乾燥症を発生し，次いで失明するものもあり，小児科領域では第二次世界大戦を前後して，かなり大量の vitamin A を飲用させる傾向であった。

あたかもこの時を同じくして，Josephs が1944年[10]，hypervitaminosis A と carotenemia という論文を発表し，ヒトでは成人よりも乳児，幼児に多く，食欲不振，成長停止，興奮性の増加，皮膚障害，脱毛症，疼痛と骨膜腫を伴う骨障害を訴え，肝腫，脾腫の増大，出血傾向の促進などを認めることを報告した[19]。しかし，その診断を決定する最終の根拠は，血液中の vitamin A 量の上昇であるという。

動物実験で最も特有な症状は，長骨の自然骨折と内出血である。また妊娠中にこの vitamin の過剰摂取は，特有の奇形の発生に関係することが Cohlan(1953)[2]によって指摘され，Giroud および Martinet(1959)[7]，Millen および Woollam(1957)[15]，稲葉(1958)[8]，Baba ら(1959)[1]，Deuschle ら(1959)[3]，Kalter および Warkany(1959)[12]，(1961)[13]，竹越(1961)[23]，Murakami ら(1965)[16]，山崎(1964)[6]，伊原ら(1966)[5]，梶原(1965)[11]によっても同じような結果が報告された。

植物性食品には広く carotene および carotenoid が含まれ，また食用品の色素[21]として広く摂取されているが，ヒトや動物体内でこれらの物質は vitamin A に変化する。しかし carotene を多量に与えた hypercarotenemia が発生した際の催奇形作用の有無については報告されていない。そこで著者は，vitamin A precursor としての β-carotene を選び，これについて催奇形作用の有無を調べた。

実 験 方 法

実験動物：

田村実験医学研究所で1950年から封鎖的に飼育し，遺伝因子を比較的等しくした，個体差の少ない健康な Wistar 系 albino rat を用いた。実験条件は（第1表）のごとくである。

飼育環境：

実験期に誘起された奇形の発生の難度は，季節によって異なるともいわれ，騒音，群居なども影響があるという。この実験は，静かな環境の中温，恒湿の室内で，しかも清潔で1個体当りの面積の十分にある金網籠の中で飼養した。

実験飼料：

ラットを飼養した当教室の基礎飼料の組成は，砕小麦80%，casein 15%，塩類4%，総合 vitamin 1%からなっており，この飼料には蛋白質が20%内外含有され

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Table 1. Experimental methods

Group	Drug	Doses	Total number of female rats treated	Route of administration	Stage
Control	Physiological saline	1ml/100g	17	Oral	From 9th to 13th day of pregnancy
A	Vitamin A	30,000I.U./100g	15		
CAR-1	β-carotene	(18mg/100g) (30,000I.U./100g)	10		
CAR-2	β-carotene	(36mg/100g) (60,000I.U./100g)	12		
CAR-3	β-carotene	(180mg/100g) (300,000I.U./100g)	10		

て，必要な塩類，vitamin を含む栄養学的に完全な飼料である。この飼料を成熟ラットでは1日15g 与えたので，vitamin A の摂取量は1日375 I. U. であった。なお飲料水は自由に与えた。

妊娠判定：

雌ラットの膣脂膏から発情期を確かめ，夕方6時頃から翌朝まで，前に述べた系統の生後10～11週の健康な雄ラットとともに1匹づつ同居させ，雌ラットの膣内精子の存在の確認によって妊娠と判定し，その日の午前0時を妊娠第0日と定め，妊娠期間を起算した。

観察時期：

妊娠第21日目，正確には妊娠20日と12時間で母ラットを殺し，その母，仔ラットの種々の状態を観察した。

すなわち，母ラットを直ちに開腹し，生存胎仔を取り出し，体重を測定後，弱拡大実体顕微鏡下および肉眼的に外形の観察をおこなった。また母ラット子宮については，胎仔死亡の場合には着床痕跡数，遺残胎盤数，浸軟胎仔数を数えた。

外形異常の有無の観察を終えた仔ラットは，骨格異常を調べるため骨格標本を作った。その製作法は，Noback 法[10]，Dawson法[4] を修正した方法で次の通りである。

骨格標本作製法：

1. 仔ラットの腹部を切開し，臓器を除去する
2. 95% ethanol で2日間固定する。
3. 皮膚，筋肉を可及的除去する。
4. 1% KOH 1l 中に 20mgの alizaline red S を溶解した溶液に1.5～2日間入れる。
5. 20% glycerine 溶液中に1日入れる。
6. 40% glycerine 溶液中に1日入れる。
7. 60% glycerine 溶液中に1日入れる。
8. 80% glycerine 溶液中に保存する。

以上の操作を経て完成した骨格標本について，異常の有無，その程度を肉眼的または実体顕微鏡を用いて観察，記録した。

実験成績

1. 母ラットの体重(第2表)

β-carotene投与群(以下CARと略す)の母ラットの体重増加は，対照群に比べて，ほとんど差異は認められなかった。vitamin A投与群(以下Aと略す)よりも算術的にはまさった。

Table 2. Mother's condition

		Control	A	CAR-1	CAR-2	CAR-3
Body weight of pregnant mothers(g)	The first day of pregnancy	209.5	203.9	187.8	194.0	191.5
	The first day of administration	229.5	235.6	213.3	217.5	221.4
	The last day of administration	239.5	234.5	229.8	234.0	234.4
	The day of autopsy	309.4	297.3	287.8	292.5	291.6
	Gained body weight	99.9 ±28.6	93.4 ±25.5	100.0 ±8.8	98.5 ±17.3	100.1 ±22.5

2. 胎仔着床状態(第3表)

胎仔の死亡数は，CAR－2群が対照群と同じように皆無であった．CAR－1群，CAR－3群では吸収胚が認められ，CAR－1群では4.7%認められ軽度であったが，CAR－3群ではA群(11.2%)と同じように12.2%認められかなり高度であった。遺残胎盤はA群では5.6%認められたが，CAR－1群では1.9%，CAR－3群では1.6%認められた．浸軟胎仔はA群では1.7%認められたが，いずれのCAR群にも発生しなかった。

生存胎仔は対照群では100%であるのにたいして，A群では81.5%であった。CAR群では，CAR－2群は100%，CAR－1群は93.5%，CAR－3群は86.2%でA群よりは生存胎仔が多かった。

生存胎仔の体重は，対照群に比べて，A，CAR各群いずれも大差なく，成長不良な胎仔の出現も差異はなかった。また，胎盤重量も特に著しい変化は認められなかった。

3. 胎仔外形異常(第4表)

胎仔の外形異常は，田村実験医学研究所で長年にわたり飼養した Wistar 系の albino rat では，実験した例数には全く認められなかった。

しかし，A群では82.6%出現したが，CAR群のいずれの群も第4表に示したように出現しなかった。

4. 胎仔化骨進行度(第5，6表)

a．後頭骨化骨進行度

第5表のように実験に供した田村実験医学研究所の Wistar 系 albino rat では，後頭骨の化骨不全は出現しなかった。しかるに，A群では72.7%に化骨不全は出現したが，CAR群では，CAR－1群には対照群と同じように化骨不全は発生せず，CAR－2，3群において発生したが，その出現率は，はるかに少なかった。

b．胸骨分節化骨進行度

胸骨分節化骨不全は対象群では16.7%発生したが，A群では，はるかに多く75.5%出現した。CAR群でもかなり高度の化骨不全が認められ，1群で80.0%，2群で83.1%，3群で55.8%であった。この成績からCARの投与は，かなり高度の胸骨分節化骨不全を誘起するようである。

c．中手骨，中足骨および尾椎の化骨進行度

対照群でも中手骨，中足骨の化骨核数は7～8%，尾椎のそれは4～5%内外であった。A群では中手骨の化骨核数は，やや少なかったが，中足骨，尾椎の化骨核数には，ほとんど差異を認めなかった。

CAR群でも中手骨，中足骨，尾椎の化骨核数は対照群とほとんど差異を認めなかった。

d．胎仔骨格異常

Table 3. Pregnancy status

		Control	A	CAR-1	CAR-2	CAR-3
Total number of implantations		184	178	107	120	123
Mean number of per a mother		10.8	11.9	10.7	10.8	12.3
Number of fetal deaths	Resorptions	0	20	5	0	15
	Placental remnants	0	10	2	0	2
	Macerated fetuses	0	3	0	0	0
Percentage of fetal deaths (%)	Resorptions	0	11.2	4.7	0	12.2
	Placental remnants	0	5.6	1.9	0	1.6
	Macerated fetuses	0	1.7	0	0	0
Number of survived fetuses		184	145	100	130	106
Percentage of survived fetuses(%)		100	81.5	93.5	100	86.2
Sex ratio female/male		5.5/5.3	4.7/5.0	5.8/4.3	6.0/4.8	5.8/4.2
Mean weight of survivors(g)		4.07 ±0.14	3.79 ±0.36	3.95 ±0.41	3.89 ±0.29	3.83 ±0.27
Number of fetuses with growth retardation		4	1	0	2	1
Percentage of fetuses with growth retardation(%)		2.2	0.7	0	1.5	0.9
Weight of placenta(g)		4.20	3.99	3.98	3.99	4.50

Table 4. Incidence and types of malformations

		Control	A	CAR-1	CAR-2	CAR-3
Total number of survived fetuses		184	145	100	130	106
Percentage of abnormal fetuses	(%)	0	82.6	0	0	0
Microcephalia	(%)	0	5.5	0	0	0
Exencephalia	(%)	0	0	0	0	0
Auricular malformation	(%)	0	40.4	0	0	0
Open eyelid	(%)	0	14.7	0	0	0
Stomatic malformation	(%)	0	1.8	0	0	0
Pseudomacroglossia	(%)	0	20.2	0	0	0
Macrognathia	(%)	0	17.4	0	0	0
Cleft palate	(%)	0	61.5	0	0	0
Low jaw cleft	(%)	0	10.1	0	0	0
Cleft tongue	(%)	0	1.8	0	0	0
Micromelia	(%)	0	0.9	0	0	0
Anal atresia and ecaudate	(%)	0	0	0	0	0

Table 5. Ossification patterns

	Control	A	CAR-1	CAR-2	CAR-3
Fetuses with retardation of ossification of the occipital bone(%)	0	72.7	0	9.2	1.1
Fetuses with retardation of ossification of the sternebrae(%)	16.7	75.5	80.0	83.1	55.8
Mean number of ossified metacarpal bones per fetus	7.89 ±0.24	6.30 ±0.84	7.28 ±0.81	7.57 ±0.40	6.97 ±0.80
Mean number of ossified metatarsal bones per fetus	8.00 ±0.00	7.87 ±0.25	8.00 ±0.00	8.00 ±0.00	8.00 ±0.00
Mean number of ossified coccyges per fetus	4.95 ±0.46	5.00 ±0.64	4.40 ±0.39	4.76 ±0.37	3.97 ±0.96

Table 6. Deformities of the bone structures in fetuses

		Control	A	CAR-1	CAR-2	CAR-3
Formation of the 14th rib	left (%)	0	27.9	0	0	0
	right (%)	0	24.5	0	0	0
Brachygnathia	(%)	0	3.4	0	0	0
Abnormality of the humerus	(%)	0	8.2	0	0	0
Shortening of the ulna	(%)	0	8.8	0	0	0
Shortening of the radius	(%)	0	7.5	0	0	0
Shortening of the femur	(%)	0	4.1	0	0	0
Shortening of the tibia	(%)	0	1.4	0	0	0
Shortening of the fibula	(%)	0	8.2	0	0	0

胎仔の骨格異常は，対照群には現れなかった。A群では第11肋骨がかなりの頻度で出現した。また下顎骨短小，上腕骨彎曲異常，橈骨短小，尺骨短小，[illegible]骨短小，脛骨短小などが現われた。しかし，[illegible]群でもそのような骨格異常は発生しなかった。

考　　察

β-carotene は生体内で vitamin A に変化することから，vitamin A の催奇形作用を発現する30,000 I. U.，60,000 I. U.，300,000 I. U. に相当する β-carotene を与えた。この実験成績では vitamin A 300,000 I. U. に相当する β-carotene 大量群で，かなりの頻度に胚吸収が認められた。また，β-carotene 投与のいずれの群にも胸骨の化骨不全が高度に認められたが，vitamin A 投与に対して発生した胎仔の骨格異常は，全く認められなかった。

このような結果は，投与した β-carotene が生体内で直ちに全量，vitamin A に変化しなかったことを意味するようである。β-carotene が vitamin A に変化する器官は肝臓であり，これの機能障害は vitamin A への変化を減少させることも考えられるが，著者の実験は健康なラットであり，また β-carotene も大量与えたので vitamin A への変化が障害されたとは考えられない。Nieman および Obbink[17] は，動物はいろいろの食料から大量の carotene を摂取するので hypercarotenemia をおこし xanthosis が発生することがあるが，これは hypervitaminosis A と区別すべきであるという。Ostwald や Briggs[20] は，0.1% の β-carotene でラットを4代にわたり飼育しても毒作用を認めなかった。すなわち，1600 I.U. を毎日ラットの生涯を通じて与えても hypervitaminosis A の症状は全く認められなかった。また，Josephs ら[10] は，いくら大量の carotene を摂取しても hypervitaminosis A の症状は見られなかったと述べている。Dagadu および Gillman[3] は Ghanaian で，carotene を大量摂取すると血清 carotene 量は著しく増量するが，血清の vitamin A 量は低いことを発表し，肝臓の vitamin A の貯臓が carotene の vitamin A に変化するのを抑制していると考察した。

著者も妊娠ラットに多量の β-carotene を与えて hypercarotenemia を惹起させたが，生まれる胎仔には，それに相当する vitamin A で発生する奇形，胎仔の骨格異常などは全く認められなかった。このことは，ある一つの観点から hypercarotenemia は直ちに hypervitaminosis A を誘起しないということを証明する一つの実験とも考えられる。

食品には，carotene および carotenoid は広く分布されているが，それを動物は摂取している。しかし，hypercarotenemia が hypervitaminosis A の発生を惹起しないことは，自然の妙用ともいえよう。

結　　論

1. 田村実験医学研究所の約20年間封鎖的に飼育した Wistar 系 albino rat に 30,000 I.U. の vitamin A を与え奇形を発生させ，これに相当量の β-carotene [illegible]を，また60,000I.U. に相当する36mgを，また30[illegible] I.U. に相当する180mgを与えて，その影響を観[illegible]

2. その結果は，β-carotene 180mg を与えた[illegible]他の薬剤でも大量群に惹起されるような胚吸収が認められた。

3. carotene 投与のいずれの群にも胸骨の化骨不全が，かなり高度に認められた。

4. vitamin A 投与にたいして発生した奇形および胎仔の骨格異常は，全く認められなかった。

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Fig. 1. The abnormal fetus with low jaw cleft, auricular malformation, open eyelid and malformation of the mouth.(Vitamin A treated)

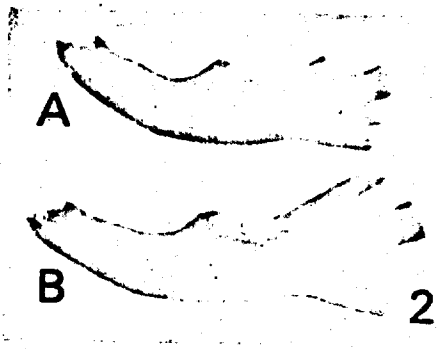
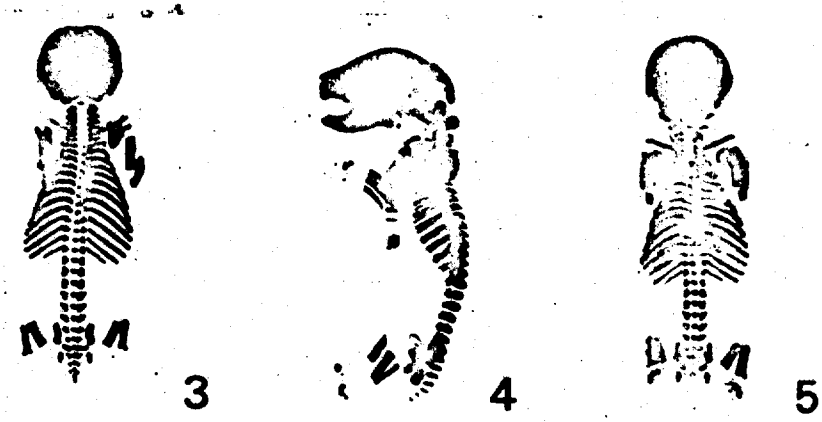
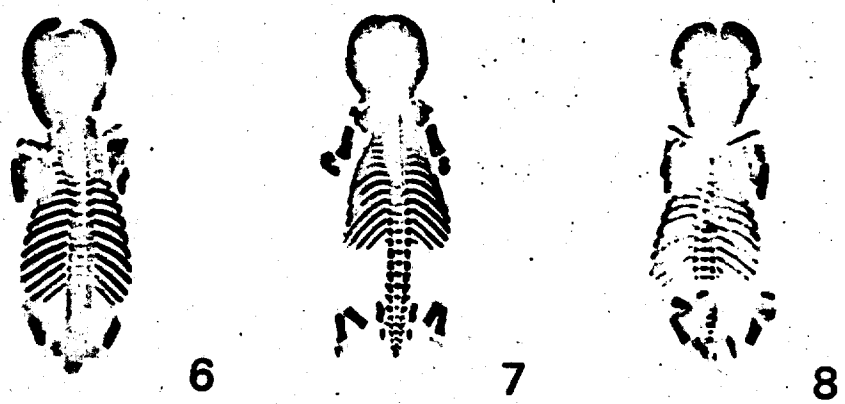


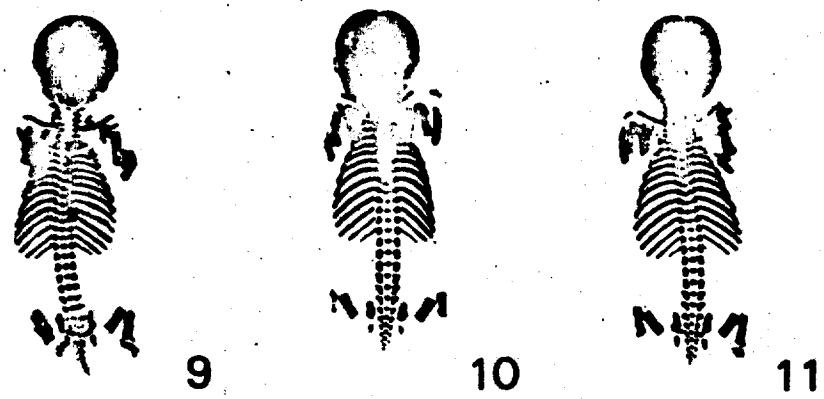
Fig. 2. Bone specimens of the mandibulae.
A. Vitamin A treated.
B. Control.



Figs. 3, 4 and 5. Bone specimens of the normal fetuses.



Figs. 6, 7 and 8. Bone specimens of the fetuses with abnormal skeleton.
6. Retardation of ossification of the occipital bone.(Vitamin A treated)
7. Incomplete formation of the 14th rib.(Vitamin A treated)
8. Lack and retardation of ossification of the sterneorae.(Vitamin A treated)



Figs. 9, 10 and 11. Bone specimens of the fetuses with abnormal skeleton.
9. Lack of the 5th sternebra.(β-carotene treated)
10. Retardation of ossification of the occipital bone.(β-carotene treated)
11. Incomplete formation of the 13th rib.(β-carotene treated)

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Metabolism of C^{14} Labelled Beta-Carotene in the Rat.* (23285)

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Our previous studies on the metabolism of C^{14} labelled beta-carotene have indicated that in the rat it is largely converted to a non-saponifiable substance or substances(1). At that time it was not possible to determine if all radioactivity of the non-saponifiable material resided in vit. A because no method was available for the isolation and purification of vit. A from this fraction. Since then, a method has been developed for isolation and purification of vit. A from the non-saponifiable extracts of liver tissue(2). With this technic it appeared worthwhile to continue our investigation on the metabolism of beta-carotene.

Method. Our previous commercial source of radioactive carotene was not available so it was necessary to develop a technic to produce it. Such carotene was produced by growing *Phycomyces blakesleeanus* in a suitable C^{14} labelled acetate medium.† Two samples of labelled C^{14} carotene were prepared. Sample No. 1 contained 1010 μg of beta-carotene with an $E^{1\%}_{1\,cm}$ of 2430‡ and a total radioactivity of 65,000 cpm. Sample No. 2 contained 1120 μg of beta-carotene with an $E^{1\%}_{1\,cm}$ of 2470 and a total radioactivity of 35,000 cpm. Each sample was prepared and fed to rats numbered 1 and 2 respectively, as previously described(1). Immediately after feeding, rats were placed in a metabolism chamber without food and water. The CO_2 was collected according to procedure of Skipper *et al.*(3). Both rats were sacrificed 24 hours after feeding the carotene. The non-saponifiable material was extracted from the liver and from the total extrahepatic tissue according to the method of Hjarde(4). The tissue extracts were freed from carotene by chromatographic procedures of Glover *et al.*(5). The carotene-free non-saponifiable extracts are referred to as CFNS fractions. After non-saponifiable material was extracted, the fatty acids were removed from the saponified extracts by acidifying them with 2N HCl, extracting with petroleum ether, and washing the latter extracts with distilled water. These washings were combined with the remainder of the saponified extracts and labelled "water soluble" fraction. The digitonin-precipitable sterols were isolated from the CFNS fraction according to the procedure of Kelsey(6). Vit. A was isolated from liver CFNS material by the procedure of Powell *et al.*(2). The water soluble fraction was oxidized and the CO_2 collected as described by Skipper *et al.* (3). Radioactivity of the various fractions was measured in a Tracerlab windowless gas flow counter and corrected for background. All CO_2 collected was counted in the form of $BaCO_3$ and corrected for self absorption. Vit. A content of various CFNS fractions was determined according to technic of Glover *et al.*(7). Due to technical difficulties, it was not possible to purify and count the vit. A in total extrahepatic tissues. Extinction of petroleum ether solutions was measured at 450 mμ and, accepting the $E^{1\%}_{1\,cm}$ for beta-carotene as 2500, the amount of carotene was calculated.

Results. From Table I it may be noted that the material in feces, urine, and intestinal contents contained approximately 50% of the total radioactive carbon fed. The total non-saponifiable material from these sources amounted to an average of 30% of the dose fed. Therefore, about 70% of the carotene was assumed to be absorbed. The carotene content of the non-saponifiable fraction from feces of rat No. 1 was 340 μg and from rat No. 2 was 360 μg. The fatty acids from feces, urine, and intestinal contents accounted for about 20% of the dose fed.

Table I summarizes the distribution of C^{14} from the labelled carotene in the tissue and

* This work was supported in part by Atomic Energy Commission contract.

† Unpublished.

‡ These "E values" were measured in a light petroleum ether (b.p. 30-40°C) solution at 450 mμ.

TABLE I. Distribution of C^{14} in Rats after Oral Administration of C^{14} Labelled β-Carotene.

	Total c.p.m. in sample		% of dose absorbed*	
	Rat 1	Rat 2	Rat 1	Rat 2
Fed	65,000	35,000		
Feces, urine, and intestinal contents	35,000	16,000		
Non-saponifiable	21,000	10,000		
Fatty acids	14,000	6,000		
H_2O soluble	—	—		
Total extrahepatic tissue	13,200	10,850	44.0	57.1
CFNS	9,700	7,050	32.3	37.1
Fatty	3,500	3,800	11.7	20.0
H_2O soluble	—	—	—	—
Total liver	1,425	872	4.8	4.6
CFNS	765	599	2.6	3.1
Vit. A	425	310	1.4	1.6
Sterols	60	39	.2	.2
Rest	280	250	.9	1.3
Fatty acids	510	173	1.7	.9
H_2O soluble	150	100	.5	.5
Expired CO_2	1,420	1,020	4.7	5.4
Recovered	51,045	28,742		
Non-saponifiable	31,465	17,649		
Fatty acids	18,010	9,973		
H_2O soluble	150	100		
CO_2	1,420	1,020		
Unaccounted for	13,955	6,258		

* Absorbed dose = Administered dose − (amt in feces, urine, and intestinal contents).

fractions examined. In both rats the highest amount of radioactivity resided in the CFNS fraction of extrahepatic tissue. Some activity was present also in the fatty acid fractions. The only "water soluble' fraction found to contain radioactivity was from the liver. About 1.5% of the absorbed C^{14} was found in liver vit. A. The combined activity in the CFNS and fatty acid fraction from extrahepatic and liver tissue accounted for an average of about 55% of the absorbed C^{14}. Approximately 5% of the absorbed C^{14} was recovered in the expired CO_2. Thus, about 60% of the absorbed C^{14} was accounted for. The average recovery for the total dose fed was about 80%.

The distribution of C^{14} in the 3 different fractions of the CFNS material from liver is such that vit. A composes 50% of the total activity of the fraction, the digitonin-precipitable sterols are only slightly active, the remaining non-saponifiable fraction is about as active as the vit. A fraction. The 2 latter fractions were examined and found to contain no vit. A according to accepted colorimetric or spectrophotometric technics.

Each rat liver contained approximately 3000 μg of vit. A, and the extrahepatic tissue approximately 150 μg of the vitamin.

Discussion. It is evident that C^{14} from rats fed labelled beta-carotene enters into compounds other than vit. A. These findings support the suggestion of Glover and Redfearn (7) and others(8) that carotene is not entirely converted into vit. A and that it might be directed into other metabolic pathways.

It is interesting to note that considerable radioactivity existed in the fatty acid fraction from feces, urine, and intestinal contents, but no detectable activity was found in the water-soluble fraction from this material. Also, it is of interest that of all the animal tissue, the only water soluble fraction that contained radioactivity was from liver tissue.

The presence of only about 5% of the absorbed C^{14} in expired CO_2 is much lower than Laughland(9) reported for the chick and rat. Failure to account for a large amount of C^{14} from absorbed carotene is in disagreement with our earlier findings where almost 100% of absorbed C^{14} was accounted for in the CFNS fraction. Two explanations seem plausible. One is that the position of labelling of the C^{14} in the carotene molecule may have been different in the 2 sources of carotene. The source of carotene in our earlier investigation was from algae which was supposedly uniformly labelled, and in the present study, from fungi where the type of labelling is unknown. Secondly, it is possible that since the animals used contained larger amounts of vit. A than the previous ones, carotene was not needed to supply the animal's need for vit. A, and hence it was directed into secondary metabolic pathways. Studies seeking support for both of these suggestions are now under investigation.

Summary. Feeding of C^{14} labelled carotene resulted in the deposition of approximately 1.5% of absorbed C^{14} into liver vit. A. In the total animal an average of about 15% of the absorbed C^{14} went into fatty acids, and 40% into non-saponifiable material. An av-

erage of about 5% of the absorbed C^{14} was found in the expired CO_2. Approximately 40% of the absorbed C^{14} remains to be accounted for.

The authors wish to thank Drs. V. G. Lilly, H. L. Barnett, and Mrs. Ruthann Folmer, West Virginia University, for assistance in production of labelled beta-carotene.

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Lewis, Jit; Reti, L.; On the Hyperavitaminosis A and the Harmlessness of Strong Doses of Provitamin A (crystallized carotene). (C.R. Soc. Biol., 1935, 118, 577-580).

Strong doses of various preparations which contain high concentrations of vitamin A are toxic for the rat (Takahashi (1), Harris and Moore (2), Drigalski (3), Collazo, and Sanchex-Rodriguez (4).) We have confirmed these observations with a commercial preparation (Vogan). Young rats (weighing 49 to 58 gr.) received (by mouth) 40,000 units each day. Growth was stopped, weight decreased, and death occurred after 10 to 14 days of treatment, in a state of pronounced cachexia. In the latter days we observed a paresis of the front limbs. The cutaneous lesions, limited to the head and to the front limbs were typical: the skin thickened, sometimes there was an obvious peeling, then small bloody ulcerations appeared and the fur fell off. Also noted was an imflammation of the conjunctiva and the eye lids. These cutaneous symptoms were provoked by local application of one drop of the preparation per day on the skin of the head. A similar application on the skin of the dorsal region did not produce any deterioration; only the covering skin tissue is sensitive.

The carrot lipids obtained by total extraction to benzene, as well as their non-saponfifiable fraction given (by mouth) the dosage of 1.5 cc per day, containing 0.04 gr. of carotene determined by colorimetrig, also produced a poisioning of the white rats. Death due to marasmus occurred after 3 to 6 days of treatment. We did not observe cutaneous symptoms. The same dose of crystallized carotene (168 degrees C.), dissolved in 1 cc. of peanut oil and given for 10 days, did not produce any changes. Growth was normal, the average weight increase was 25.4 gr. for the animals who received the carotene (average 20.5 gr.) and 35.5 gr. (average 31 gr.) for those control animals who received 1 c.c. of oil without carotene. Different terpenic derivitives (oil of sandalwood, citral, citronellol, terpinol, linalol) given at a 2% dosage of 1 c.c. of oily solution for 14 days did not produce any general symptoms nor any modification in growth. Local application on the skin of the head did not give any more cutaneous symptoms. Carrot oil at the dosage of 1 c.c. 10% solution (corresponding almost to the quantity contained in 1.5 c.c. of the unsaponifiable of all the lipids), was not toxic.

The fact that the concentrated preparations of vitamin A produced some toxic symptoms, does not allow us to maintain that this intoxication was a hypervitaminosos A. Other concentrated extracts with a nonsaponifiable part of the oils not containing vitamin A, such as carrot oil, are also toxic, although the symptoms are not identical. The toxicity could not be attributed to a non-specific effect of vitamin A, common to terpenes, when the different terpenic derivitatives that were tested were not toxic at the dosage used. It could not have been attributed to a specific action of hypervitaminosis, when experiments were carried out with the vitamin. Clearly, such work has been obtained by Karrer (5); otherwise, it would still be thought to be due to another substance contained in the non-saponifiable part, especially since Drummond and Magliano have worked with the concentrated products of vitamin A which are not toxic.

The harmlessness of high doses of carotene is well demonstrated by our experiments. It is true that a great part of the dosage given (40%) was found in the feces the last days of the experiment, but a certain proportion is stabilized by the tissues. The extract of the liver lipids contained 0.08 gr. per kgr., and that of the suparenals nearly 0.26 gr. per kgr. These glands could then stabilize the carotene when it is found in large doses in the system. Although this is not usually found in the suprarenal glands of the white rat. The white rat is also able to tolerate high doses of carotene administered intravenously or intraperitoneally, as we have been in the experiments which were shared later. The excess of carotene given digestively is, in part, eliminated by the feces and, in part, stabilized by the itssues, but probably also partially transformed into vitamin A (>). In the case where an excess of the latter would be toxic, it was necessary to acknowledge that the organism only forms it in a limited quantity, or stabilites it in such a way that it is not harmful. There was a standardization of the formation or discharge of vitamin A beginning with the carotene hypothesis supported by the facts described by Moore (8) on the transormation of carotene into vitamin A in the rat, and by Dann (9) on the transmission of vitamin A from the mother to the nursing infant in the same species (10).

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(2) Harris et Moore. Biochem. Journ.. Biochem Journ., 1928, 1. 22, p. 1461; ibid 1929, 1. 23, p. 1114.

(3) W. Drigalski. Klin. Wochenschr., 1933, 1. 12, p. 308. W. Drigalski et W. Laubmann. Klin. Wochenschr., 1933, 1. 12, 1. 1171.

(4) J. A. Collazo et J. Sanchex-Rodriguex. Klin. Wochenschr., 1933, 1. 12, pp. 1732 ct 1768.

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C. R. Soc. Biol. 118:577-580. 1935.

SOCIÉTÉ DE BIOLOGIE DE BUENOS AIRES

SÉANCE DU 29 NOVEMBRE 1934

Séance extraordinaire en l'honneur du Président, le Pr. B.-A. Houssay, qui vient d'accomplir ses 25 années de professorat universitaire.

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SUR L'HYPERVITAMINOSE A ET L'INNOCUITÉ DES FORTES DOSES DE PROVITAMINE A (CAROTÈNE CRISTALLISÉ),

par J.-T. LEWIS et L. RETI.

Les fortes doses de diverses préparations qui contiennent la vitamine A à des concentrations élevées sont toxiques pour le

Rat [Takahashi (1), Harris et Moore (2), Drigalski (3), Collazo et Sanchez-Rodriguez (4)]. Nous avons confirmé ces observations avec une préparation commerciale (Vogan). De jeunes rats (pesant de 49 à 58 gr.) recevaient *per os* 40.000 unités par jour. La croissance fut arrêtée, le poids diminua, la mort survenant après 10 à 14 jours de traitement, dans un état de cachexie prononcée. Les derniers jours, nous avons observé une parésie des membres antérieurs. Les lésions cutanées, limitées à la tête et aux membres antérieurs sont typiques : la peau s'épaissit, parfois il y a une desquamation marquée, puis de petites ulcérations sanguinolentes apparaissent et le poil tombe. On note aussi une inflammation de la conjonctive et des paupières. Ces symptômes cutanés peuvent être provoqués par l'application locale d'une goutte par jour de la préparation sur la peau de la tête. Une application semblable sur la peau de la région dorsale ne fut pas suivie d'altérations, seule la peau du capuchon est sensible.

Les lipides de carotte obtenus par extraction totale au benzène, ainsi que leur fraction non saponifiable donnés *per os* à la dose de 1,5 c.c. par jour, contenant 0,04 gr. de carotène déterminé par colorimétrie, produisent aussi une intoxication du rat blanc, la mort dans le marasme survenant après 3 - 6 jours de traitement. Nous n'avons pas observé de symptômes cutanés. La même dose de carotène cristallisé (p. f. = 168° C), dissous dans 1 c.c. d'huile d'arachide, donnée pendant 10 jours, n'a produit aucune modification. La croissance fut normale, l'augmentation moyenne de poids fut de 25,4 gr. pour les animaux qui reçurent le carotène (moyenne 20,5 gr.) et de 35,3 gr. (moyenne 31 gr.) pour les témoins qui reçurent 1 c.c. d'huile sans carotène. Divers dérivés terpéniques (essence de santal, citral, citronellol, terpinol, linalol, α et β - ionone) donnés à la dose de 1 c.c. d'une solution huileuse à 2 p. 100, pendant 14 jours n'ont pas produit de symptômes généraux ni une modification de la croissance. L'application locale sur la peau de la tête ne donne pas non plus des symptômes cutanés. L'huile essentielle de carotte, à la dose de 1 c.c. d'une solution à 10 p. 100 (ce qui correspond à peu près à la quantité contenue dans 1,5 c.c. de l'insaponifiable des lipides totaux), administrée pendant 6 jours, ne fut pas toxique.

(1) Takahashi, cité par J.-C. Drummond et H.-J. Channon. *Biochem. Journ.*, 1925, t. *19*, p. 1047.

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(4) J.-A. Collazo et J. Sanchez-Rodriguez. *Klin. Wochenschr.*, 1935, t. *12*, pp. 1730 et 1768.

Le fait que les Préparations concentrées de vitamine A produisent des symptômes toxiques, ne permet pas de soutenir que cette intoxication soit une hypervitaminose A. D'autres extraits concentrés de la fraction non saponifiable des graisses qui ne contiennent pas de vitamine A, telle l'huile de carotte, sont aussi toxiques, quoique les symptômes ne soient pas identiques. La toxicité ne peut pas être attribuée à une action non spécifique de la vitamine A, commune aux terpènes, puisque les divers dérivés terpéniques essayés ne furent pas toxiques à la dose employée. Elle ne pourra être attribuée à une action spécifique d'hypervitaminose que lorsqu'on aura effectué des expériences avec la vitamine A pure, telle qu'elle a été obtenue par Karrer (5); autrement, on pourra toujours penser qu'elle peut être due à une autre substance contenue dans la fraction non saponifiable, surtout depuis que Drummond et Magliano ont travaillé avec des produits concentrés de vitamine A, qui ne sont pas toxiques.

L'innocuité des hautes doses de carotène est bien démontrée par nos expériences. Il est vrai qu'une grande partie de la dose donnée (40 p. 100) fut trouvée dans les fèces les derniers jours de l'expérience, mais une certaine proportion est fixée par les tissus. L'extrait des lipides de foie contenait 0,08 gr. par kgr. et celui des surrénales à peu près 0,26 gr. par kgr. Ces dernières glandes peuvent donc fixer le carotène, lorsque celui-ci se trouve en grandes doses dans le régime ; quoique on ne le trouve pas habituellement dans les surrénales du rat blanc (4). Le rat blanc peut aussi tolérer de hautes doses de carotène administrées par voie intraveineuse ou intrapéritonéale, ainsi que nous l'avons vu dans les expériences qui seront communiquées plus tard. L'excès de carotène donné par voie digestive est en partie éliminé par les fèces et en partie fixé par les tissus, mais probablement aussi partiellement transformé en vitamine A (7). Dans le cas où un excès de cette dernière serait toxique, il faudrait admettre que l'organisme ne la forme qu'en quantité limitée, ou la fixe de telle façon qu'elle ne soit pas nuisible. Il y aurait une régulation de la formation ou de la libération de la vitamine A à partir du caretène, hypothèse appuyée par les faits décrits par Moore (8) sur la transformation du carotène en vitamine A chez le Rat et par Dann (9)

(5) Karrer. *Helv. Chim. Acta*, 1933, t. *16*, p. 625.

(6) S. Lévy, Palmer et C. Wennedy. *Journ. of biol. Chem.*, 1921, t. *46*, p. 559. L. Randoin et R. Netter. *Bull. de la Soc. de chim. biol.*, 1933, t. *15*, p. 944.

(7) T. Moore. *Biochem. Journ.*, 1930, t. *24*, p. 692.

(8) T. Moore. *Biochem. Journ.*, 1931, t. *25*, p. 375.

(9) W.-J. Dann. *Biochem. Journ.*, 1932, t. *26*, p. 1072.

sur la transmission de la vitamine A de la mère aux nourrissons, dans la même espèce (10).

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La méthode de Rosenthal pour le traitement de l'intoxication mercurielle aigue,

par E. Hug, A.-J. Llacer et F. Ruiz.

Rosenthal a trouvé que le formaldéhydesulfoxylate de soude est efficace pour traiter l'intoxication mercurielle aiguë chez l'Homme ou les animaux d'expérience.

Nous avons constaté que l'administration gastrique (par sonde) de 25 mgr. de bichlorure de Hg par kgr. de poids, en solution à 1 p. 100, tue les chiens en 2, 4, 11 jours. Pour éviter les vomissements, on administra 15 mgr. de chlorhydrate de morphine 40 à 80 minutes avant. On trouva à l'autopsie des lésions glomérulaires et tubulaires très intenses, avec de la néphrose nécrotique dans quelques cas.

Des chiens ainsi intoxiqués furent traités 30 à 45 minutes après par du formaldéhyde-sulfoxylate de sodium par voie gastrique (1 gr. par kgr.) ou intraveineuse (0,5 gr. par kgr.). Les chiens survécurent en bon état. Après 11 jours, on préleva les reins, qui présentèrent des lésions de glomérulo-néphrite légère, mais dans peu de glomérules et dans quelques tubes urinifères, le reste était normal.

Tandis que, chez les sujets non traités, l'azote non protéique du sang monte de 40-50 mgr. pour 100 c.c. à 102-133 (2 jours), 122-278 mgr. (4 jours), chez les traités il ne varie pas (examens faits 2, 4, 7 et 11 jours après l'intoxication).

A trois Chiens intoxiqués, nous avons administré par voie gastrique 50 c.c. de blanc d'œuf dilué dans 100 c.c. d'eau, sans lavage de l'estomac. Nous avons aussi injecté à ces animaux, par voie intraveineuse, 1 gr. par kgr. d'hyposulfite de sodium. Ils moururent entre 3 et 6 jours après, avec augmentation constante de l'azote non protéique (166 à 312 mgr. pour 100 c.c. de sang) ;

(10) Ces travaux ont été faits en partie grâce à une donation de la Fondation Rockefeller.

(1*) *Publ. Health Rep.*, 1933, t. *48*, p. 1543. *Journ. Amer. Med. Assoc.*, 1934, t. *100*, p. 1273.

TOXIC SYMPTOMS DUE TO CAROTENE-CONTAINING FOODS

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As known, vitamins taken in excessive quantities can cause toxic symptoms, not only in the form of drugs but also when foods contain them in concentrated form. Besides the known vitamin D overdosage symptoms, toxic vitamin A effects are also reported from the Artic after consumption of fish liver in large amounts (1).

However, not much information is available on the question whether the provitamins A--i.e. the carotenes--can likewise be held responsible for toxic symptoms when supplied in excessive amounts to the human organism.

By the end of the last century (1898) and at the beginning of this century only a single harmless property of the carotene was discovered namely: it causes a yellow pigmentation of the skin and mucous membranes under certain conditions, such as ample vegetarian food consumption or in cases of diabetes mellitus (2,3,4).
The yellow pigmentation was generally believed to be harmless. No reports on toxic symptoms were made. L. ZECHMEISTER (1934) has not witnessed any death up to that date due to carotene intoxication (5). Up to 1941(5) the view that carotene is harmless was generally accepted. Even Th. MOORE (6), in his book on vitamin A, makes no reference to toxic carotene effects.

Neverthless, the study of case histories reported in earlier works leads to the suspicion that carotene might have still other toxic effects. HEAD and JOHNSON (7) mention secondary anemia in a diabetic with carotene icterus; HASHIMOTO (8) reports on abnormal craving for squash in a feebleminded three-year-old and in a 50 years old man. Carotene was verified in the blood. V. TOKAY (9) observed a married woman, 20 years old,, who initially ate 2 kg. of sweet Szeged paprika daily for 2 weeks; during the next 3 weeks, she continued to consume ample amounts of paprika. The pica for paprika which caused brick-red skin discoloration due to capsaicin, a carotene, should, however--according to TOKAY--not be interpreted as a carotene effect. As the study indicates, said effect should instead be regarded as a neurotic symptom in a patient who descends from a family with psychopatic history. Finally, SEQUEIRA and others (10) mention a young man who lived in the tropics for 5 years and developed carotene icterus symptoms whenever he stayed in the interior of the country in question, and not in the vicinity of the coast. But the authors are unable to supply reliable information on the question whether the patient had consumed ample amounts of vegetables and fruits. Pronounced arterial hypotonicity and weight loss occurred. CLAUSEN and McCOORD (11) found low blood pressure and asthenia in two cases of hyper-carotenemia, but they cannot exclude morbus Addison.

Only HENSCHEN (5) undertook an in-depth investigation of possible toxic carotene effects in 1941. He classified the symptoms following excessive carotene consumption in 4 categories:

1. simple xanthemia without chronic xanthodermia, as in cases of carotene-babies and infants who received carotene-rich food. The skin will later clear spontaneously, when the excess carotene supply is compensated by the body;

2. manifest xathemias with chronic xanthodermia or other disorders, eventually in case of excessive tangerine consumption (aurantiasis cutis);

3. decompensated atoxic xanthoses in patients with liver ailments. So-called oscillating xanthoses occur allegedly thereby, depending on the condition of the liver, i.e. the yellow discoloration of the skin due to excess carotene consumption will be proportionate to the capacity of the liver to convert carotene (provitamin A)into vitamin A.Accordingly, HENSCHEN assumes that the liver is the site where carotene is converted into vitamin A.;

4. decompensated xanthoses as defined by HENSCHEN, i.e. the xanthoses specifically at issue here. He reports on a 49-years old woman whose case he diagnosed as "pseudo-icterus due to alimentary xanthodermia as a sequela of excessive carrot consumption". The details of the pathography are:

"Unsuccessful therapy with iron tincture during mild anemia", while the general condition of the patient was poor; she consumed spontaneously excessive amounts of carrots. It was found that she had a craving for carrots.

For months, the patient consumed up to 1 kg of carrots daily, mostly in raw condition, and untrimmed. Carrots thrown by concerned members of her family into the trashcan were secretely recovered by the patient. In addition , she ate 3 to 4 heads of lettuce daily ; she disliked sweets and fresh fruit. After several months xanthodermia, increasing lassitude, dull pain in the liver area knuckle edema, motor dyspnea developed. Even the the mucous membrane of the mouth showed yellow pigmentation, but the sclera were white. Enlargement of the liver and spleen. Blood count: secondary anemia with 57% hemoglobin. Bilirubin for s. not increased. Carotene in s. 349 y %. Bile pigments in the urine not increased. Furthermore, an uterus myoma and cataract were diagnosed. The therapy consisted in carotene-free diet. The xanthodermia disappeared. 4 months later the hemoglobin was 64% and carotene in s. 306 y % (the patient was not fasting).After blood-letting and blood transfusion ("Blutauffüllung" 4 years later: 72% hemoglobin. After myomectomy: normal blood count. On the basis of the carotene pica, the anemia, the poor general condition in conjunction with the increased carotene content of the blood, HENSCHEN concludes that a toxic carotene effect is involved. He believes that the patient has a debilitated liver and that the conversion of carotene into vitamin A is therefore obstructed; as a result, carotene accumulates in the body.

McCONAGHEY (12) observed two cases which clarify the significance of excessive carotene consumption. The patients were again female.

The first case concerns a 42-year-old woman who worked with foods in a restaurant. She reported a liver ailment and stated that she cannot eat much. She was affected by a catarrh and since she had read that carrots are good for a catarrh, she consumed enormous quantities of carrots; they were her almost exclusive food in summer. A few weeks prior to her visit the doctor's office: yellow discoloration of the palm surfaces and foot soles; sclera white; depression, lassitude; color of feces normal. Sligh epigastrium sensitivity. Complains of flatulence. Blood count: 78% hemoglobin and 4,9 mill.erythrocytes. Carotene in s. increased by 500 mg% (y%? author). The jaundice subsided after 6 weeks of carotene-free diet.

The other case involved a 40-year-old overweight woman. During her first pregnancy she ate large amounts of carrots. 3 years later, during her second pregnancy, she had a constant craving for carrots. After the menopause--6 months before medical observation--she consumed 450 g carrots daily. Yellow pigmentation of the skin, general weakness and "weak nerves". Blood count: 70% hemoglobin, with 4,4 mill erythrocytes. Carotene level in the serum: 300 mg% (y% ? the author). The patient ramained yellow since she was unable to give up carrots.

Another case, observed by me, can be addedd to these few cases of toxic carotene effect; they are in part not unequivocal:

Gerda R.,45 years old, a housewife, widowed, with 4 children. In 1923 jaundice for ¼ year following gastro-intestinal catarrh. Gall- bladder inflammation (after ample food consumption) with pain persisting for 10 weeks. No gall stones. Pain in the gall bladder area felt at this time, as well during faulty diet. From June to August 1956, after heart complain. treatment in "Waerland Home" for recovery. Anemia with 50% hemoglobin was diagnosed there. During said therapy Mrs. R. changed her nutritional method. The diet supplied at the Waerland Home was as follows:
7 a.m., ¼ l vegetable broth with soaked linseed, carrots and soup greens. 9 a.m.: ¼ l buttermilk containing wheat bread, a spoonful of ground linseed as well as dried fruit.
11 a.m.: herb tea. 12 noon: vegetables (salad., radishes) plus 1 carrot, radishes and red beet . A small amount of parsley root. This was followed by potatoes boiled in their skins, cottage cheese and linseed oil with buttermilk and 1 to 2 slices of Waerland-bread. 4 p.m.: a cup of herb tea or whey. In the evening: "Kruska", rice with raisins or stewed fruit and millet, raw grated potatoes, linseed oil or "Vitaquell" without butter.

At that time, Mrs. R. weighed 65 kg; she is 169 cm high. She lost weight during the treatment. She took additional iron while at the sanitarium; subsequently, the hemoglobin content rose over 60%. She adhered to the above dietary method later at home until spring 1957, but not as strictly as at the "Waerland-home".

As a consequence of domestic difficulties, her eating habits became irregular. In the winter of 1957, while attending to the heating installation in the basement, she began to eat occasionally some of the carrots stored there. Since the fall of 1957 she increased her consumption of raw carrots to 1 kg daily. In addition, she ate large amounts of head lettuce and parsley roots. Since the fall of 1957 she noticed a yellow skin discoloration and allegedly a yellow discoloration of the sclera as well. The hemoglobin was allegedly 50%. A pica for vetetables, in particular for carrots and parsley roots, developed. She felt listless and miserable and complained about smarting of the tongue tip. No appetite for sweets. She was admitted to the hospital on March 24, 1959--not on account of the yellow skin discoloration, but for taenia therapy (taeniasis could not be confirmed).

Diagnosis: reduced "E.Z." and "A.Z."; height: 169 cm.; weight: 58 kg. Skin discoloration, specifically on the palms and foot soles. Sclera white. No mucous membrane discoloration. No specifically strong urine pigmentation. No skin itch. Liver palpable 2 finger-widths below the costal arch, not painful when pressed. No spleen tumor. Bile pigments in the urine not intensified. Total bilirubin in the yellowish serum according to JENDRASSIK: 0,33 mg%; direct bilirubin not determinable. MEULENGRACHT count: 15. Total lipids: 1,42 g . . . Carotene in s. = 286 y% (determined in the Institute for Food Chemistry, Berlin).

Total cholesterol: 143 mg%; cholesterol ester:95 mg%; thymol ++; mod. TAKATA-ARA-reaction according to MINCKE-SOMMER = 60 mg%. Electrophoresis : 52.5%; albumans : 3,4% , α1,9,1% α2-, 13,5% β-; 21.5% y-globulin with 7,16% total albumen. Bromthalein-test result pathological. Osmotic erythrocyte resistance without pathologic finding. Blood count: hemoglobin 54%; erythrocytes: 2.51 mill.; leukocytes: 6900. Moderate anisocytosis. Diff. blood count normal. PRICE-JONES curve: regular ery-diameter. Gastric juice: refractory histamine anemia. Gall bladder probe: positive reflex, no path.-cytologic and bactereologic finding. X-ray gall bladder examination: no pathologic finding. Thorax X-ray: ∅ basal metabolism: + 5%.

The admission diagnosis of "hyper-carotenemia" was established on the basis of the yellow skin discoloration while the sclera were not affected, and on the basis of questions concerning the patient's eating habits. Therapy: all foods containing carotene were eliminated from the diet. Drug therapy included vitamin B, vitamin C and iron preparations.

The elimination of carotene from the food intitially failed to reduce the yellow skin coloration. The urgent, almost manic demands for carrots , made by the patient who continued to visit the floor kitchen, were disregarded. 15 days later the MEULENGRACHT count decreased to 8 and the serum color appeared lighter. Carotene level on the 23. day: 170 y%; pronounced fading of the skin pigmentation, but not on the skin of the palms and foot soles.

The completely carotene-free diet was unbalanced and could therefore not be maintained; a less strict diet formula was ordered. Only carrots and parsley roots were forbidden. After dieting for 2 months, yellow skin discoloration was no longer evident on the palms and foot soles either. The carotene pica subsided. Hemoglobin rose over 70%, with 3.51 mill. erythrocytes. The carotene level control test, performed 1 month after her discharge from in-patient therapy (May 5, 1958) showed that the carotene level had returned to normal in the serum (170 y%). The results of the liver function test were still pathologic: positive thymol-test; MANCKE-SOMMER reaction: 50 mg%, and slightly higher β- and y-globulin values. Minor weight gain and improvement of her general condition could be achieved.

When surveying the cases reported here, the conclusion can be reached that ample carotene consumption leads to yellow skin discoloration and eventually, yellow discoloration of the mucous membranes as well; significantly, the sclera always remain free of symptoms. The carotene requirement of man is estimated to 30 - 60 y per kg of body weight (5, 13, 14, 15, 16); therefore Mrs. R. , with a weight of 58 kg and a daily consumption of 1 kg carrots (18 yo 72 mg carotene content), consumed 310 to 1240 y carotene per day, i.e. the ten- to twentyfold of the physiologic value . The situation for the cases described in literature is similar.

Therefore , excess carotene consumption is certainly present. When the consumption of head lettuce (approx. 0,32 mg carotene/100g) and of parsley roots is taken into account as well, the consumed carotene volumes are certainly larger. The skin discoloration with a reddish hue is identical concerning its location in all cases; it persists longest on the skin of the palms and foot soles, obviously because metabolims and elimination respectively, are slower in these regions.

Excessive carotene consumption is caused by various factors. They might be accidental, as in case of Mrs. R.; or neurotic patients are involved (TORAY's case); a pregnancy might be the causative factor, or a diet might lead to ample carotene consumption (especially in case of children).

The carotene level in the serum is always elevated. When the bilirubin level is normal or low (5, case of Mrs. R.) the serum shows an intensively yellow pigmentation. The MEULENGRACHT count is higher (17), i.e. up to the chromatic identity with potassium bichromate solution (1 : 10 000) the serum has to be thinned over 1 : 6, whereby the ratio of the required thinning liquid volume indicates the grade for the yellow pigmentation. The conclusive evidence for the increased carotene level when serum bilirubin is normal and the MEULENGRACHT count is higher, is photometrically determined. In Mrs. R.'s case the serum was agitated with alcohol and petroleum ether.

The carotenes accumulate in the top layer of the petroleum ether while the other (alcohol) phase contains the bile pigments and xantophylls.

The petroleum ether layer, to be pipetted, is photometrically measured and the determined value is compared with a calibration curve, prepared with petroleum ether solutions of an oily carotene solution (18). 0,6y β-carotene = 1 I.E." and corresponds regarding its biological activity to approx. 0,3 y vitamin A- alcohol, or respectively, approx. 0,344 y vitamin A-acetate. The standard values in the s. are about 60 to 180 y% carotene (19).

In cases of hyperthyrosis and goitre (20,21, 22) a higher carotene level is found in the serum, since carotene and thyroxine are antagonists; furthermore, in case of diabetes mellitus increased carotene supply during vegetable-rich sugar diet, liver damage or pancreas insufficiency with deficient carotene conversion into vitamin A are held responsible (23).

A higher carotene level in the serum was also found in case of hyperlipemia (23, 24). The above-mentioned changes were not verified in the reported cases, except for the liver lesion and hyperlipemia diagnosed in the case of Mrs. R.

According to HENSCHEN, liver lesions in case of increased carotene supply in the food can result in failure to adjust the balance between carotene and vitamin A. However, more recent investigations indicate that carotene is converted

into vitamin A in the intestinal wall (25,26,27,28). It should therefore probably be assumed that no liver damage is involved during the development of hypercarotenemia (incidentally, HENSCHEN failed to specify such damages adequately in his earlier works). Even in the case of Mrs. R., where liver lesion is certainly at issue (increased bromthalein test, thymol ++,etc), the above factor presumably played no part.

Besides reference to xanthodermia and hypercarotenemia, the frequent reference made to (hyperchromatic) anemia is significant. But the anemia can be attributed to other causes than specifically to the toxic effect of carotene. In HENSCHEN's case a myoma was involved; the anemia subsided after its extirpation. In Mrs. R's case, anemia was present prior to excess carotene consumption and could have been conditioned by inadequate nourishment or resportion disorders respectively. In both of McCONAGHEY's cases (12) only one of the female patients was anemic; but data are missing for other test results on possible causative factors. The deteriorated physical condition and lassitude which are mentioned in most cases are probably due to concealed malnutrition and unbalanced vegetarian diet.

The pica for carotene, or respectively, for carotene-containing foods occurs in all cases, in part on a neurotic basis; in other cases, it indicates carotene intoxication (cases: HENSCHEN (5), MCCONAGHEY(12), Mrs.R.).

The reason why said pica develops has not been clarified. It seems questionable whether the sweetish taste of the carrots is responsible, since in case R. a craving for parsley root was present as well, while the taste of these roots is quite different.

Summary

In addition to findings in literature on toxic symptoms following excess consumption of foods containing carotene, the author reports on his own case of carotene intoxication. The conclusion is reached that besides a harmless yellow discoloration of the skin and muxuous membranes, merely pica for carotene-containing foods--in particular for carrots--develops, which cannot always be controlled.

Pica remains the only constantly recurrent symptom of the intoxication, additional pathologic changes observed to date, such as lassitude and anemia, could be due to other causes.

Toxische Erscheinungen durch karotinhaltige Nahrungsmittel

Aus dem Städt. Krankenhaus Berlin-Tegel-Süd (Ärztlicher Direktor: Chefarzt Dr. Wauer)

HANS LOECKELL

Es ist bekannt, daß Vitamine, in allzu großen Mengen eingenommen, toxische Erscheinungen hervorrufen können, und zwar nicht nur in Arzneiform, sondern auch wenn sie angereichert in Nahrungsmitteln enthalten sind. Neben den bekannten Vitamin-D-Überdosierungserscheinungen wird auch von toxischen Vitamin-A-Wirkungen nach reichlichem Genuß von Fischleber in der Arktis berichtet (1).

Aber nur spärlich sind die Mitteilungen darüber, daß auch die Provitamine A — die Karotine also — im Übermaß dem menschlichen Körper zugeführt, für toxische Erscheinungen verantwortlich zu machen sind.

Ende des vorigen Jahrhunderts (1898) und am Anfang dieses Jahrhunderts entdeckte man nur eine und zwar harmlose Eigenschaft des Karotins, nämlich unter bestimmten Bedingungen wie reichliche vegetabilische Ernährung oder bei Diabetes mellitus eine Gelbfärbung der Haut und der Schleimhäute hervorzurufen (2, 3, 4). Die Gelbfärbung wurde allgemein als harmlos angesehen. Über toxische Erscheinungen wird nicht berichtet. L. Zechmeister sah bisher (1934) niemand, der an einer Karotinvergiftung gestorben wäre (5). Bis 1941 (5) wurde die Meinung von der Harmlosigkeit des Karotins allgemein vertreten. Und selbst Th. Moore (6) in seinem Buch über das Vitamin A bemerkt nichts über toxische Karotinwirkungen.

Doch läßt das Studium der in den älteren Arbeiten mitgeteilten Krankengeschichten den Verdacht aufkommen, daß das Karotin noch andere, toxische Wirkungen zu entfalten vermag. Head und Johnson (7) erwähnen sekundäre Anämie bei einem Diabetiker mit Karotinikterus; Hashimoto (8) berichtet von anormalem Appetit auf Kürbis bei einem dreijährigen Idioten und bei einem fünfzigjährigen Mann. Karotin wurde im Blut nachgewiesen. V. Tokay (9) sah eine zwanzigjährige Ehefrau, die zunächst 2 Wochen lang täglich 2 kg Ia süßen edlen Szegediner Paprika aß und dann noch etwa 3 Wochen reichlich dem Paprika zusprach. Diese Pica auf Paprika, der infolge des Kapsaicins, eines Karotinoids, zur ziegelroten Verfärbung der Haut führte, muß aber nach v. Tokay nicht als Karotinwirkung gedeutet werden, sondern kann auch, wie aus der Arbeit hervorgeht, als Symptom einer Neurose der im übrigen aus einer psychopathisch belasteten Familie stammenden Patientin aufgefaßt werden. Schließlich erwähnen Sequeira et al. (10) einen jungen Mann, der 5 Jahre in den Tropen lebte und nur die Erscheinungen eines Karotinikterus bot, wenn er sich im Innern des betreffenden Landes und nicht in Küstennähe aufhielt. Verfasser wissen aber nicht sicher anzugeben, ob der Patient reichlich Vegetabilien und Früchte zu sich nahm. Auffällig war eine arterielle Hypotonie und Gewichtsabnahme. Clausen und Mc Coord (11) sahen in zwei Fällen bei Hyper-Karotinämie niedrigen Blutdruck und Asthenie, können aber einen Morbus Addison nicht ausschließen.

Erst Henschen (5) beschäftigte sich 1941 eingehend mit den möglichen toxischen Wirkungen des Karotins. Er teilte die Erscheinungen nach übermäßigem Karotingenuß in 4 Kategorien ein.

1. einfache Xanthämien ohne andauernde Xanthodermie, wie es z. B. bei den Karotinsäuglingen und Kleinkindern, die karotinreiche Ernährung erhalten, der Fall ist. Die Aufhellung der Haut erfolgt später spontan, nachdem der Körper die Luxuszufuhr von Karotin kompensiert hat.

2. Manifeste Xanthämien mit dauernder Xanthodermie ohne sonstige Störungen, etwa bei übermäßigem Mandarinengenuß (Aurantiasis cutis).

3. Dekompensierte atoxische Xanthosen bei Leberschwächlingen. Dabei soll es je nach dem Zustand der Leber zu sogenannten oszillierenden Xanthosen kommen, d.h. je besser die Leber imstande ist, Karotin (Provitamin A) in Vitamin A umzuwandeln, desto weniger gelb ist bei Luxuskonsumtion von Karotin die Haut gefärbt. Henschen nimmt also die Leber als den Ort der Konversion von Karotin in Vitamin A an.

4. nennt Henschen die dekompensierten Xanthosen, die Xanthosen also, um die es sich hier speziell handelt. Er berichtet über eine 49jährige Frau, bei der er die Diagnose „Pseudoikterus durch alimentäre Xanthodermie infolge übermäßigen Mohrrübengenusses" stellte. Im einzelnen ergab die Vorgeschichte:

Bei der wegen Anämie „erfolglos mit Eisentinktur behandelten" in schlechtem Allgemeinzustand befindlichen Patientin kam es spontan zu übermäßigem Genuß von Mohrrüben; es stellte sich ein picamäßiges Verlangen danach ein. Die Patientin verzehrte monatelang täglich bis 1 kg Mohrrüben, meist roh und ungereinigt. Mohrrüben, die von den besorgten Angehörigen in den Mülleimer geworfen worden waren, nahm sie heimlich wieder heraus. Außerdem aß sie täglich 3 bis 4 Salatköpfe, hatte Abneigung gegen Süßigkeiten und frisches Obst. Nach einigen Monaten Xanthodermie, zunehmende Müdigkeit, dumpfer Schmerz in der Lebergegend, Knöchelödem, Bewegungsdyspnoe. Sogar die Mundschleimhaut war gelb angefärbt, die Skleren aber weiß. Vergrößerung von Leber und Milz. BB. sekundäre Anämie mit 57% Hb. Bilirubin i. S. nicht erhöht. Karotin i. S. 349 γ %, Gallenfarbstoffe im Urin nicht vermehrt. Ferner wurden ein Uterusmyom und eine Katarakt festgestellt. Behandelt wurde mit karotinfreier Kost. Danach Verschwinden der Xanthodermie. 4 Monate später betrug das Hb. 64% und das Karotin i. S. 306 γ % (Pat. war nicht nüchtern). Nach Aderlaß und Blutauffüllung 4 Jahre später 72% Hb. Nach Myomektomie normales Blutbild. Henschen schließt aus der Pica auf Karotin, der Anämie, dem schlechten Allgemeinzustand in Verbindung mit dem erhöhten Karotingehalt des Blutes auf eine toxische Karotinwirkung und glaubt, daß es sich bei der Patientin um einen Leberschwächling handelte, bei dem die Konversion Karotin in Vitamin A gestört sei, so daß sich das Karotin im Körper anhäufe.

Mc Conaghey (12) sind zwei Fälle aufgefallen, die die Bedeutung des übermäßigen Karotingenusses in das rechte Licht rücken. Wieder handelt es sich um Frauen.

Bei dem einen Fall ist es eine 42jährige Frau, die im Krankenhause mit Lebensmitteln zu tun hatte. Nach ihren Angaben war sie lebensfreudig und konnte nicht fett essen. Da sie an einem Katarrh litt und gelesen hatte, daß Karotten gut für Katarrh

selbst, aß [illegible] Mohrrüben, die im [illegible] Mitteln. Einige Wochen [illegible] der Handinnenflächen und [illegible] Weiß, Depression, Müdigkeit, Faeces normal, [illegible] Druckempfindlichkeit im Epigastrium. Klagen über [illegible] 88 % Hb und 4,9 Mill. Ery. Karotin [illegible] % (γ % der Verfasser) erhöht. Nach 6 Wochen [illegible] war die Gelbsucht abgeblaßt.

[illegible] handelte es sich um eine 40jährige [illegible] ihrer ersten Gravidität, aß sie große Mengen [illegible] 3 Jahre später während der zweiten Gravidität hatte [illegible] dauernde Begierde nach Karotten. Nach [illegible] vor ärztlicher Beobachtung — täglich [illegible] Gelbfärbung der Hände, allgemeine [illegible] schwäche. Bb. 70 % Hb. bei 4,4 Mill. Ery. [illegible] 500 mg % (γ % γ der Verfasser). Da [illegible] nicht über sich bringen konnte, auf Karotten zu [illegible] blieb sie weiterhin gelb.

[illegible] Heiligen z. T. nicht eindeutigen Fällen von toxischer Karotinwirkung wäre noch ein weiterer selbst beobachteter Fall hinzuzufügen:

[illegible]jährige Hausfrau, verwitwet mit 4 Kindern. 1923 nach [illegible] Dysenteriearzt 1/2 Jahr Gelbsucht. 1947 Gallenblasenentzündung (nach reichlicher Ernährung) mit Schmerzen, die 14 Wochen anhielten. Keine Gallensteine. Schmerzen in der Gallenblasengegend treten auch jetzt bei Diätfehlern auf. IV.—VIII. 1956 zur Erholung nach Herzbeschwerden Kur in einem Waerlandheim, wo Anämie von 50 % Hb. festgestellt wurde. Seit dieser Kur hat Frau R. ihre Ernährungsweise geändert. In dem Waerlandheim gab es folgende Kost: 7 Uhr 1/4 l Gemüsebrühe mit eingeweichtem Leinsamen, Mohrrüben und Suppengrün. 9 Uhr 1/4 l einer Art Buttermilch, darin Weizenbrot und ein Löffel gemahlener Leinsamen sowie getrocknete Früchte. 11 Uhr Kräutertee. 12 Uhr Gemüse (Salat, Rettiche), dazu eine Mohrrübe, Radieschen und rote Beete. Kaum Petersilienwurzel. Danach Reibekartoffeln, Quark und Leinöl mit Buttermilch und 1 bis 2 Scheiben Waerlandbrot. 16 Uhr eine Tasse Kräutertee oder Malzkaffee. Abends Kruska, Reis mit Rosinen oder geschmorten Obst und Hirse. Kartoffeln roh, gerieben. Leinöl oder Vitaquell oder Butter.

Frau R. wog damals bei 169 cm Größe 63 kg und nahm während der Kur nicht an Gewicht ab. Sie nahm im Kurheim zusätzlich Eisen ein, worauf der Hämoglobingehalt auf über 60 % anstieg. Später, zu Hause, führte sie obige Kostform bis zum Frühjahr 1957 durch, doch nicht mehr so streng wie im Waerlandheim. Sie aß wegen familiärer Schwierigkeiten nicht mehr regelmäßig. Erstmals im Winter 1957 beim Heizen im Keller aß sie ab und zu von den dort eingelagerten Mohrrüben und brachte es seit Herbst 1957 auf den Verbrauch von 1 kg roher Mohrrüben täglich. Daneben aß sie viel Kopfsalat und Petersilienwurzeln. Seit dem Herbst 1957 bemerkte sie eine gelbe Verfärbung der Haut; eine gelbe Verfärbung der Skleren will sie nicht gesehen haben. Das Hämoglobin soll 50 % betragen haben. Es setzte auch ein Heißhunger auf Gemüse, besonders auf Karotten und Petersilienwurzeln, ein; sie fühlte sich matt und elend und klagte über Brennen der Zungenspitze. Kein Appetit auf Süßigkeiten. Krankenhauseinweisung am 24. [illegible] 1958 nicht wegen der gelben Hautverfärbung, sondern zur Bandwurmkur (die Taeniasis konnte nicht bestätigt werden).

Befund: Reduzierter E. Z. und A. Z. Größe 169 cm, Gewicht 58 kg. Gelbrötliche Verfärbung der Haut, besonders an den Handinnenflächen und an den Fußsohlen. Skleren weiß. Schleimhäute nicht verfärbt. Kein Juckreiz. Schweiß in den Achselhöhlen zeigte ebenfalls keine gelbliche Verfärbung. Kein besonders intensiv gefärbter Urin. Kein Hautjucken. Leber um 2 Querfinger unter dem Rippenbogen zu tasten ohne Druckschmerzhaftigkeit. Kein Milztumor. Gallenfarbstoffe im Urin nicht vermehrt. Gesamtbilirubin im gelblichen Serum nach Jendrassik 0,33 mg %, direktes Bilirubin nicht nachweisbar. Meulengrachtsche Zahl 15. Gesamtlipide = 1,42 g %. β-Karotin i. S. = 286 γ % (bestimmt im Institut für Lebensmittelchemie Berlin), Gesamtcholesterin 143 mg %, Cholesterinester 95 mg %. Thymol ++. Mod. Takata-Ara-Reaktion nach Mancke-Sommer = 60 mg %. Elektrophorese: 52,5 % Albumine, 3,4 % α₁-, 9,1 % α₂-, 13,5 % β-, 21,5 % γ-Globuline bei 7,16 % Gesamteiweiß. Bromthaleintest fiel pathologisch aus. Osmotische Erythrozytenresistenz ohne pathol. Befund. Blutbild: 54 % Hb, 2,51 Mill. Erythrozyten, 6900 Leukozyten. Mäßige Anisozytose. Diff. BB normal. Kurve nach Price-Jones: regelrechter Ery-Durchmesser. Magensaft: histaminrefraktäre Anämie. Gallensonde: positiver Reflex, kein pathol. zytologischer und bakteriologischer Befund. Röntgenologische Gallenblasenuntersuchung: kein pathologischer Befund. Thoraxaufnahme ⊘. Grundumsatz: + 5 %.

Die Aufnahmediagnose „Hyperkarotinämie" wurde bereits aus der gelben Verfärbung der Haut bei frei bleibenden Skleren und aus Fragen nach den Eßgewohnheiten der Patientin gestellt. Therapeutisch wurden aus der Kost alle karotinhaltigen Nahrungsmittel praktisch fortgelassen. Medikamentös wurde mit Vit. B, Vit. C und Eisenpräparaten behandelt.

Der Carotinentzug in der Nahrung bewirkte zunächst kein Abblassen der gelben Hautfarbe. Dem dringenden, geradezu süchtigen Verlangen der andauernd in der Stationsküche erscheinenden Patientin nach Mohrrüben wurde nicht stattgegeben. 15 Tage später Absinken der Meulengrachtschen Zahl auf 8; die Serumfarbe sieht heller aus. Nach 23 Tagen Karotinspiegel 170 γ %, deutliches Abblassen der Haut, nicht aber der Haut an den Handinnenflächen und Fußsohlen. Da der Einseitigkeit wegen die Kost nicht mehr ganz karotinfrei gereicht werden konnte, wurde die Diätform gelockert; lediglich Mohrrüben und Petersilienwurzeln wurden nicht gestattet. Nach 2 Monaten Diät bestand auch an den Handflächen und Fußsohlen keine gelbe Verfärbung mehr. Die Pica auf Karotin verschwand. Das Hämoglobin stieg auf über 70 % bei 3,51 Mill. Erythrozyten. Eine Karotinspiegelkontrolle einen Monat nach Entlassung aus stationärer Behandlung (10. 5. 58) ergab wiederum einen normalen Karotinspiegel i. S. (170 γ %). Die Leberfunktionsproben fielen mit einer positiven Thymolprobe, einer Mancke-Sommer-Reaktion von 50 mg % und leicht erhöhten β- und γ-Globulinwerten immer noch pathologisch aus. Leichte Gewichtszunahme und Besserung des Allgemeinzustandes konnten erzielt werden.

Übersieht man die hier mitgeteilten Fälle, so kann man feststellen, daß reichlicher Karotingenuß zu gelber Verfärbung der Haut und eventuell auch der Schleimhäute führt; die Skleren bleiben auffälligerweise stets frei. Da der Bedarf des Menschen an Karotinen auf ca. 30—60 γ/kg Körpergewicht (5, 13, 14, 15, 16) geschätzt wird, hat Frau R. mit einem Gewicht von 58 kg und einem Verzehr p. d. von 1 kg Mohrrüben (18—72 mg Karotin enthaltend) sich täglich 310—1240 γ Karotin, also das zehn- bis zwanzigfache der physiologischen Menge, einverleibt. Ähnlich liegt es bei den in der Literatur beschriebenen Fällen. Es liegt also zweifellos eine Luxuskonsumtion von Karotinen vor. Sicherlich liegen die einverleibten Karotinmengen viel höher, wenn man den reichlichen Genuß von Kopfsalat (ca. 0,32 mg Karotin 100 g) und Petersilienwurzel mit berücksichtigt. Die ins Rötliche spielende Hautverfärbung ist in ihrer Lokalisa-

tion bei allen Fällen die gleiche und hält sich am längsten in der Haut der Handinnenflächen und Fußsohlen, offenbar weil der Stoffwechsel bzw. der Abtransport in diesen Hautpartien langsamer vor sich geht.

Die Ursachen des überreichlichen Karotingenusses sind verschiedene. Einmal sind es Zufälligkeiten wie bei Frau R., oder es handelt sich um neurotische Patienten (Fall von Tokay), oder eine Gravidität ist die mögliche Ursache, oder aber eine Diät führt zu reichlichem Genuß von Karotin (besonders bei Kindern).

Der Karotinspiegel im Serum ist immer erhöht. Bei normalem oder niedrigem ([5], Fall Frau R.) Bilirubinspiegel hat das Serum eine stark gelbe Farbe. Die Meulengrachtsche Zahl (17) ist erhöht, d. h. bis zur Farbgleichheit mit einer Kaliumbichromatlösung 1 : 10 000 muß das Serum auf über 1 : 6 verdünnt werden, wobei die Verhältniszahl der nötigen Menge der Verdünnungsflüssigkeit als Grad für die Gelbfärbung gilt. Der endgültige Beweis für den erhöhten Karotinspiegel bei normalem Serumbilirubin und erhöhter Meulengrachtscher Zahl wird photometrisch erbracht. Im Falle der Frau R. wurde das Serum mit Alkohol und Petroläther geschüttelt. In der oberen Petrolätherschicht sammeln sich die Karotine, während die andere (Alkohol) Phase die Gallenfarbstoffe und Xanthophylle enthält.

Die abzupipettierende Petrolätherschicht wird photometrisch gemessen und der gefundene Wert mit einer Eichkurve, hergestellt aus Petrolätherverdünnungen einer öligen Karotinlösung, verglichen (18). 0,6γ β-Carotin = 1 I. E. und entspricht in seiner biologischen Aktivität ca. 0,3 γ Vitamin A-Alkohol bzw. ca. 0,344 γ Vitamin-A-Azetat. Die Normalwerte i. S. liegen bei 60 bis 180 γ % Karotin (19).

Man findet einen erhöhten Karotinspiegel i. S. bei Hyperthyreose und Kropf (20,21,22), da Carotin und Thyroxin Antagonisten sind; ferner bei Diabetes mellitus, wobei einmal erhöhte Karotinzufuhr bei gemüsereicher Zuckerdiät oder Leberschädigung oder Pankreasinsuffizienz mit mangelhafter Conversion von Carotin in Vitamin A angeschuldigt werden (23).

Auch bei Hyperlipämie ist ein erhöhter Karotinspiegel i. S. gefunden worden (23, 24). Obige Veränderungen wurden bei den beschriebenen Fällen, abgesehen von Leberschädigungen und der Hyperlipämie im Falle der Frau R., nicht festgestellt.

Nach Henschen sollen Leberschädigungen bei erhöhtem Karotinangebot in der Nahrung zum Versagen der Einregulierung des Gleichgewichts zwischen Karotin und Vitamin A führen. Aber nach neueren Untersuchungen wird das Karotin in der Darmwand in Vitamin A umgewandelt (25, 26, 27, 28). Danach muß man wohl annehmen, daß irgendwelche Leberschädigungen, die im übrigen bei Henschen und in früheren Arbeiten nicht genügend präzisiert sind, keine Rolle bei der Entstehung der Hyperkarotinämie spielen. Auch bei Frau R., bei der zweifellos eine Leberschädigung vorliegt (erhöhter Bromthaleintest, Thymol + + etc.) dürfte diese Tatsache keine Rolle spielen.

Neben der Xanthodermie und der Hyperkarotinämie fällt die häufige Erwähnung einer (hypochromen) Anämie auf. Doch lassen sich auch andere Erklärungen als gerade eine toxische Wirkung des Karotins für die Anämie finden. Im Falle Henschen (5) lag ein Myom vor, nach dessen Exstirpation die Anämie verschwand. Bei Frau R. bestand die Anämie schon vor der Luxuskonsumtion von Karotinen und dürfte durch unzulängliche Ernährung bzw. Resorptionsstörungen bedingt gewesen sein. Bei den beiden Fällen von McConaghey (12) bestand nur bei einer Patientin eine Anämie, doch fehlen Angaben über sonstige Untersuchungsbefunde über mögliche andere Ursachen der Anämie. Der reduzierte Körperzustand und die Mattigkeit, die meist erwähnt werden, sind wohl durch kachierte Unterernährung bei einseitiger vegetabilischer Diät bedingt.

Die Pica auf Karotin bzw. karotinhaltige Nahrungsmittel ist in allen Fällen vorhanden, z. T. auf neurotischer Grundlage, in anderen Fällen als Ausdruck der Karotinintoxikation (Fälle: Henschen [5], McConaghey [12], Frau R.). Warum diese Pica auftritt, bleibt ungeklärt. Es scheint fraglich, ob nur der süßliche Geschmack der Karotten dazu verführt, denn im Falle R. bestand auch Heißhunger auf Petersilienwurzel, deren Geschmack doch ein ganz anderer ist.

Zusammenfassung

Außer den in der Literatur niedergelegten Beobachtungen über toxische Erscheinungen nach Luxuskonsumtion karotinhaltiger Nahrungsmittel wird ein eigener Fall von Karotinintoxikation mitgeteilt. Es wird gefolgert, daß neben der harmlosen Gelbfärbung von Haut und Schleimhäuten sich lediglich eine Pica auf karotinhaltige Nahrungsmittel — insbesondere Karotten — entwickelt, die nicht immer beherrscht werden kann.

Die Pica bleibt das einzige immer wiederkehrende Symptom der Intoxikation, während weitere bisher beobachtete pathologische Veränderungen wie Mattigkeit und Anämie andere Ursachen haben können.

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BETA-CAROTENE

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*These studies have been published and are included elsewhere in the monograph.

**Translation included.

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TRANSLATION

0088

Interoffice Memo

From: Dr. R. Jürgens To: Dept. VI June 23, 1954

To the question of V/Chem. Dr. Brauchli to Dept. VI, 6/21/54, concerning results of toxicity test of synthetic β-carotene, the following information has been received from VI/Med.:

According to the experiments of Dr. Pfaltz in growth tests with young vitamin A-deficient rats, synthetic β-carotene (Preparation Ro 1-8300/2) is tolerated for 6 months without toxic symptoms in daily oral doses of 20 mg/kg, corresponding to 30,000 I.U. of vitamin A. Especially, it was observed that no hypervitaminosis occurred as after the administration of corresponding doses of vitamin A. The rats maintained for the entire 6 months a weight of 240 grams, which corresponded to the weight of normally fed animals. Also the general appearance of all the animals was very good.

According to the experiments of Dr. F. Studer in normal-growing rats which had received daily 30,000 to 50,000 I.U. of vitamin A per os for 5 weeks, severe hypervitaminosis symptoms appeared in contrast to the findings after administration of synthetic β-carotene (corresponds to 50,000 I.U. vitamin A), where the animals thrived and had no hypervitaminosis.

In the determination of vitamin A content in the liver after daily dosage of 50,000 I.U. of β-carotene for 20 days, Dr. Harkees found a definite storage (between 4000 and 7000 I.U. of vitamin A) of vitamin A above the normal, but definitely a relatively lower storage than would have been expected after administration of this high dose of β-carotene (corresponds to 50,000 I.U. of vitamin A). Similar experiments after administration of single doses of 300 to 1200 I.U. of vitamin A yielded in liver storage test of Guggenheim and Koch storage of vitamin A in the liver of about 30 to 50% of administered vitamin A (Dr. Pfaltz).

Synthetic β-carotene possesses in small doses (corresponding to 1.5 to 2.5 I.U.vitamin A) the same growth activity in growing vitamin A-deficient rats as natural β-carotene and synthetic vitamin A.

After 6 months of administration of 20 mg/kg orally, corresponding to 30,000 I.U. vitamin A, to growing vitamin A-deficient rats, no hypervitaminosis A appeared as after administration of similar doses of vitamin A.

The storage of vitamin A in the liver appears according to orienting experiments to be considerably less after administration of high doses of β-carotene than after similar doses of vitamin A.

No toxicity of β-carotene has appeared in doses tested to date after a half year of daily administration in contrast to vitamin A. The organism appears to split only as much vitamin A from the ingested β-carotene as it requires.

Interne M i t t e i l u n g Basel, den 23. Juni 1954

Abteilung Prof. Dr. R. Jürgens

Betrifft: β-Carotin

Auf die Anfrage von V/Chem. Dr. Brauchli an Abteilung VI vom 21.6.54, betreffend die Ergebnisse der Verträglichkeit von synthetischem β-Carotin, wird von VI/Med. Folgendes mitgeteilt:

Nach Untersuchungen von Frau Dr. Pfaltz im Wachstumstest an jungen Vitamin A-Mangelratten wird synthetisches β-Carotin (Präparat Ro 1-8300/2) in Dosen von 20 mg/kg, entsprechend 30'000 I.E. Vitamin A, täglich oral verabreicht, während 6 Monaten ohne Nebenerscheinungen ertragen. Besonders entwickelt sich keine Hypervitaminose, wie sie nach Verabreichung gleicher Dosen von Vitamin A beobachtet wird. Die Ratten nahmen während 6 Monaten durchschnittlich 240 g an Gewicht zu, was der Gewichtszunahme normal ernährter Tiere entspricht. Zudem war der Allgemeinzustand bei allen Tieren sehr gut.

Nach Untersuchungen von Dr. F. Studer an normalen erwachsenen Ratten, die täglich 30'000 bis 50'000 I.E. Vitamin A per os während 5 Wochen erhalten hatten, entwickelten sich schwere Hypervitaminoseerscheinungen im Gegensatz zu den Befunden nach Verabreichung von synthetischem β-Carotin (entsprechend 50'000 I.E. Vitamin A), wobei die Tiere überlebten und keine Hypervitaminose hatten.

Bei der Bestimmung des Vitamin A-Gehaltes in der Leber nach Verabreichung von 50'000 I.E. β-Carotin, täglich während 20 Tagen, fand Dr. Markees zwar eine erhebliche Anreicherung (zwischen 4'000 und 7'000 I.E. Vitamin A) von Vitamin A gegenüber der Norm, hingegen eine relativ geringere Speicherung, als sie bei Verabreichung derartig hoher Dosen von β-Carotin (entsprechend 50'000 I.E. Vitamin A) zu erwarten gewesen wäre. Entsprechende Untersuchungen nach Verabreichung einmaliger Dosen von 300 - 1'200 I.E. Vitamin A, ergaben im Leberspei-cherungstest nach Guggenheim und Koch eine Anreicherung von Vitamin A in der Leber von ca. 30 - 50 % der verabreichten Vitamin A-Mengen. (Dr. Pfaltz).

Synthetisches β-Carotin bewirkt in kleinen Dosen (entsprechend 1,5 - 2,5 I.E. Vitamin A) dieselbe Gewichtszunahme an wachsenden Vitamin A-Mangelratten wie natürliches β-Carotin und synthetisches Vitamin A.

Nach 6 Monate langer Verabreichung von 20 mg/kg oral, entsprechend 30'000 I.E. Vitamin A, an wachsende Vitamin A-Mangelratten entwickelt sich keine A-Hypervitaminose wie nach Verabreichung gleicher Dosen von Vitamin A.

9-fach

Die Speicherung von Vitamin A in der Leber scheint nach orientierenden Untersuchungen nach Verabreichung hoher Dosen von β-Carotin erheblich geringer zu sein als nach Verabreichung entsprechender Dosen Vitamin A.

Eine Toxizität von β-Carotin besteht in den bisherggeprüften Dosen selbst nach halbjähriger täglicher Verabreichung im Gegensatz zu Vitamin A nicht. Der Organismus scheint demnach nur soviel Vitamin A aus dem aufgenommenen β-Carotin abzuspalten, wie er nötig hat.

Jg/Mey
Basel, 23.6.54

Jüngner

Date May 4, 1960

Report from R. E. Bagdon

Department to Management

Chronic Toxicity Studies of β-Carotene:

A Comparison of Pure β-Carotene and 75% β-Carotene

Summary:

Studies of a mixture of 75% β-carotene-25% degradation products revealed this material to be non-toxic. No untoward effects were observed on growth, general condition, food consumption or hemopoietic tissues. Gross and microscopic findings were not unusual.

RBK 5/5/60

R.E.Bagdon

Chronic Toxicity Studies of β-Carotene:

A Comparison of Pure β-Carotene and 75% β-Carotene

Previous chronic toxicity studies of β-carotene have demonstrated the non-toxic nature of this naturally occurring pigment (G. Zbinden and A. Studer. Z. Lebensm-Untersuch Forsch. 108, 113-134, 1958; R. E. Bagdon, G. Zbinden and A. Studer. Toxicol. Applied Pharmacol. 2, 225-235, 1960). To determine if impurities or degradation products of β-carotene showed significant differences in toxicity than have been observed with the parent compound of 100% purity, material of 75% purity was prepared by oxidation of crystalline β-carotene and this mixture, containing 25% degradation products, was administered to rats at high dietary levels of 1.0% and 0.5% for 13 weeks; one group received 2.4S β-carotene beadlets and another group of rats was administered pure crystalline β-carotene for a similar period; an additional group was given the basal diet alone to serve as controls. The results of these experiments are reported in this communication.

Methods

Five groups of 40 Sprague-Dawley rats, 20 males and 20 females, were employed in these experiments. One group was given 100% pure β-carotene at a dietary level of 1.0%; 2 additional groups were administered a mixture of 75% β-carotene-25% degradation products in dietary concentrations of 1.0% and 0.5%; another group of 20 rats received type 2.4S β-carotene beadlets at a dietary concentration of 1.0%; a 5th group received the basal diet consisting of ground Purina Lab Chow Pellets in order to serve as a control. The duration of this feeding experiment was 13 weeks.

The mixture of β-carotene and degradation products was prepared by spreading the crystalline compound in thin layers contained in trays and placing the material exposed to air in an oven at 45°C for 96 hours; this procedure resulted in approximately 25% degradation of the parent compound. The mixture of β-carotene and degradation products and the pure crystalline β-carotene was stored in sealed bottles under nitrogen in lots of about 100 grams; the diets were freshly made each week. Each week, when a bottle was opened, an aliquot was taken for assay of β-carotene content to determine the stability of the mixture or pure crystalline compound; also, samples of the pure β-carotene and the mixture of degradation products admixed in the diet were assayed for β-carotene content after standing at room temperature exposed to air for 1 week. These measurements carried out by Dr. A. Hutchens and Dr. R. Dowell, are shown in the following compilation:

Experimental Week	Sample Under Nitrogen		Sample Admixed in the Diet at Room Temperature and Exposed to Air		
	Crystalline	Degraded	Crystalline	Degraded 1% In Diet	Degraded 0.5% In Diet
	Per Cent		Per Cent		
0	100.0	67.9	100.0	-	-
1	-	67.7	96.0	75.0	72.0
2	100.0	71.6	99.0	70.0	60.0
3	99.3	65.6	101.0	60.0	68.0
4	99.3	72.2	94.0	64.0	66.0
5	100.0	70.5	105.0	71.0	70.0
6	-	69.8	-	-	-
7	99.0	70.8	97.0	70.0	66.0
8	99.3	69.3	-	-	-
9	100.0	68.4	-	-	-
10	99.3	70.0	-	-	-
11	-	69.7	-	-	-
12	-	67.1	-	-	-
13	-	67.6	-	-	-
14	99.3	67.9	96.0	66.0	65.0

Thus, it is evident that under conditions of this experiment, there was only a slight additional breakdown of the β-carotene-degradation products when admixed in the diet and exposed to the air for 1 week; crystalline β-carotene was essentially stable under the same conditions.

The animals were closely observed daily and measurements of body weights and feed consumption were obtained at weekly intervals. Determinations of the hematocrit, hemoglobin, total and differential leukocyte counts were performed during the 0, 7th and 13th experimental weeks. At the completion of the 13 weeks treatment period, the animals were sacrificed and the tissues and organs examined for evidence of gross pathology. Weights of livers and kidneys were obtained. Sections of brain, peripheral nerve, heart, lungs, liver, kidneys, adrenals, thyroid, pituitary, stomach, small and large intestines, bladder, pancreas, bone marrow and gonads were fixed in 10% formalin and stained with hematoxylin-eosin. Also, frozen sections of liver were stained with hematoxylin-Sudan IV or oil red O in order to demonstrate fat deposition.

Results

1. The Effects on Growth, Food Consumption and General Condition

Administration of pure β-carotene admixed in the diet, and a concentration of 1.0% and 0.5% of a mixture of 75% β-carotene, 25% degradation products was well tolerated when given to rats continuously for 13 weeks. Also, rats given β-carotene in the form of beadlets at a dietary concentration of 1.0% did not show differences from controls.

This is evident from the data presented graphically in Figure 1.

Despite the high dietary concentrations administered, food intake was unimpaired. The weekly food consumption of each group is tabulated in Table 1; these data do not differ significantly from controls.

Table 1. Average Weekly Food Consumption (grams) of Rats Given β-Carotene For 13 Weeks

Group and Dietary Concentration	Experimental Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Grams	% of Controls
Pure β-Carotene 1.0%															
1 - 20 F	90	155	122	120	134	138	137	129	130	141	113	128	123	1660	103.8
21 - 40 M	100	18	157	170	189	178	195	185	187	183	164	171	169	2238	103.3
Degraded β-Carotene 1.0%															
41 - 60 F	96	159	108	118	136	129	120	113	139	139	117	122	110	1606	104.2
61 - 80 M	108	187	154	170	190	178	196	187	193	188	175	177	171	2274	104.9
Degraded β-Carotene 0.5%															
81 - 100 F	85	152	111	123	128	129	118	124	142	137	119	122	117	1617	104.9
101 - 120 M	94	186	154	173	190	179	194	181	188	186	171	167	161	2224	102.6
β-Carotene Beadlets 1.0%															
161 - 170 F	85	138	106	117	136	117	127	112	119	127	118	123	109	1529	99.2
171 - 180 M	105	181	153	171	199	176	183	177	183	180	182	180	168	2238	103.3
Controls															
121 - 140 F	89	143	114	111	142	123	123	119	120	119	114	109	113	1541	-
141 - 160 M	99	183	151	159	202	178	183	171	172	172	167	154	166	2167	-

Calculations of the amounts of β-carotene consumed by each group are shown in Table 2; dietary concentrations of 1.0% β-carotene as the pure material, as beadlets, or as a mixture containing 75% degradation products permitted an average daily intake of carotenoid of approximately 1000 mg/kg/day; a concentration of 0.5% added in the diet corresponds to an average intake of about 500 mg/kg/day.

Table 2. **Calculation of the Average Daily Intake of β-Carotene Given To Rats For 13 Weeks**

Group & Dietary Concentration	Average Daily Food Consumption (grams)	Average Body Weight (Grams)	Total Daily Drug Intake (mg)	Average Daily Drug Intake (mg/kg/day)
Pure β-Carotene 1.0%				
1 - 20 F	18.2	174	182	1046.0
21 - 40 M	24.6	267	246	921.3
Degraded β-Carotene 1.0%				
41 - 60 F	17.6	177	176	994.4
61 - 80 M	25.0	273	250	915.8
Degraded β-Carotene 0.5%				
81 - 100 F	17.8	175	89	508.6
101 - 120 M	24.4	272	122	448.5
β-Carotene Needlets 1.0%				
161 - 170 F	16.8	176	168	954.5
171 - 180 M	24.6	278	246	884.9
Controls				
121 - 140 F	16.9	173	-	-
141 - 160 M	23.8	270	-	-

2. Hematologic Findings

Blood counts were obtained on every rat before treatment and during the 7th and 12th experimental weeks; these measurements consisted of determinations of the hematocrit, hemoglobin, total and differential leukocyte counts. In agreement with previous findings, high concentrations of β-carotene, given in the crystalline or needlet form, or as a mixture containing 25% of degradation products, did not exert untoward effects on circulating blood cells or hemopoietic tissues. The average blood counts of each group are compiled in Table 3.

3. Gross Autopsy Findings

Administration of high concentrations of β-carotene and degradation products to rats for 13 weeks did not cause pathological tissue changes which were grossly visible. Organ changes observed were those commonly seen in laboratory rats and do not constitute untoward effects produced by carotenoids.

In the control group, inflammatory changes of the lungs were seen in rats Nos. 121, 130, 131, 133-135, 138, 146, 147, 152, 153, 157, 160; the intestines of No. 144 were markedly hyperemic; rat No. 132 exhibited poor alimentation due to dental malformations and was eliminated from the study.

The only changes seen in rats given 1% pure β-carotene were chronic pneumonitis; this was noted in rats Nos. 1-4, 7,9, 13, 15, 17, 19, 22, 23, 25, 26, 28, 37 and 39; the heart of rat No. 4 appeared to be enlarged. The intestinal contents of all rats were colored orange due to unabsorbed carotene.

Similar inflammatory lung changes were seen in rats given 1.0% or 0.5% degraded carotene; this was especially marked in rats Nos. 41, 85, 89, 97 and 103; the pneumonitis was more diffuse in the remaining rats. The left adrenal of rat No. 105 was somewhat fibrous. Gastrointestinal contents were stained orange.

Similar non-specific changes were seen in rats given carotene beadlets; hyperemia of the gastrointestinal tract was seen in rats Nos. 161 and 177 in addition to diffuse inflammatory lung changes. Liver and kidney weights are shown in Table 4; rats treated with β-carotene or the mixture of degradation products did not differ from controls in this respect.

Table 4. Liver and Kidney Weights of Rats Given β-Carotene and Degradation Products for 13 Weeks

Group & Dietary Concentration	Liver (% of Body Weight)	Kidneys (% of Body Weight)
Pure β-Carotene 1.0%		
1 - 20 F	3.87	.852
21 - 40 M	4.27	.817
Degraded β-Carotene 1.0%		
41 - 60 F	4.02	.834
61 - 80 M	4.51	.851
Degraded β-Carotene 0.5%		
81 - 100 F	4.12	.872
101 - 120 M	4.47	.839
β-Carotene Beadlets 1.0%		
161 - 170 F	4.40	.877
171 - 180 M	4.56	.854
Controls		
121 - 140 F	4.10	.856
141 - 160 M	4.25	.797

4. Histopathologic Findings

Microscopic examination of sections of brain, peripheral nerve, heart, lungs, liver, kidneys, adrenals, thyroid, pituitary, stomach, small and large intestines, bladder, pancreas, bone marrow and spleen was performed by Dr. S. J. Schnitzer. As observed in earlier toxicologic investigations of β-carotene, chronic administration of high doses of this carotenoid did not cause pathological changes in the organs of the digestive system, the renal and genito-urinary system, the cardiovascular, hemopoietic, nervous or endocrine tissues and organs.

The only important histologic change seen was the deposition of large Sudan IV positive or oil red O positive droplets in the Kupffer cells of the livers of rats given pure crystalline β-carotene; this fat-staining material, identified in the earlier studies as vitamin A (Zbinden and Studer 1958) was much less prevalent in rats given the mixture of β-carotene and degradation products. Little or no deposition was observed in the livers of rats Nos. 60, 83, 84, 85, 87, 91-99, 102, 105, 109, 110, 111, 112, 119, 142, 144, 146, 148, 150, 151, 153-155, 158-162, 164, 166, 168, 170, 172 and 178. This finding suggests that the degradation products are not absorbed from the gastrointestinal tract or cannot readily serve as precursors of vitamin A in rats. The occurrence of pneumonitis in a large number of rats is judged to be unrelated to administration of β-carotene.

Discussion

The results of chronic toxicity studies in rats for 13 weeks of a mixture consisting of 75% β-carotene-25% degradation products demonstrated the non-toxic nature of this material. Adverse reactions on growth, general condition, food consumption, hemopoietic tissues, gross and microscopic appearance of organs and tissues was not observed. Of considerable interest is the finding that the mixture containing degradation products does not cause deposition of fat-staining material in the Kupffer cells of treated rats. This is in contrast to the results obtained with pure crystalline β-carotene and indicates that either the breakdown products of β-carotene are not absorbed from the gastrointestinal tract or cannot serve as precursors for the biosynthesis of vitamin A.

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Table 3.

Average Blood Counts of Rats Given β-Carotene and Degradation Products For 13 Weeks Before Treatment

Group and Dietary Concentration	WBC in Thousands	Hematocrit %Vol. RBC	HGB in gms/100 mls.	DIFFERENTIAL NSN	SN	L	M	E	B	Pl. Cells	%Nc RB
Pure β-Carotene 1%											
1 - 20 F	14.1	39.9	11.6	0.0	14.6	83.9	1.7	0.2	0.2	0.1	0.
21 - 40 M	13.5	38.7	11.3	0.5	16.6	81.4	1.5	0.3	0.2	0.05	0.
Degraded β-Carotene 1.0%											
41 - 60 F	14.4	39.4	11.7	0.0	14.4	83.0	1.8	0.6	0.2	0.05	0.
61 - 80 M	13.4	38.2	11.2	0.0	16.8	81.5	1.3	0.2	0.05	0.3	0.
Degraded β-Carotene 0.5%											
81 -100 F	14.7	39.1	11.5	0.05	16.2	81.9	1.3	0.4	0.1	0.1	0.
101 -120 M	14.4	39.0	11.4	0.0	13.3	79.9	1.4	0.3	0.1	0.1	0.
β-Carotene Beadlets 1.0%											
161 - 170 F	14.1	39.4	11.7	0.0	15.7	81.8	1.6	0.5	0.1	0.3	0.
171 - 180 M	13.8	38.6	11.3	0.0	18.1	80.5	0.7	0.1	0.3	0.3	0.
Controls											
121 - 140 F	14.2	39.0	11.5	0.0	16.8	81.5	1.2	0.3	0.2	0.1	0.
141 - 160 M	13.6	38.3	11.2	0.0	17.9	80.4	1.3	0.05	0.3	0.2	0.

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Table 3.
(Continued)

Average Blood Counts of Rats Given β-Carotene and Degradation Products for 13 Weeks After 7 Weeks of Treatment

Group and Dietary Concentration	WBC in Thousands	Hematocrit %Vol. RBC	HGB in gms/100 mls.	DIFFERENTIAL MSN	SN	L	M	E	B	Pl. Cells	% R
Pure β-Carotene 1%											
1 - 20 F	14.2	44.9	14.2	0.0	11.5	87.4	0.7	0.4	0.05	0.0	0.
21 - 40 M	20.5	46.3	14.4	0.0	11.8	88.9	0.0	0.3	0.0	0.0	0.
Degraded β-Carotene 1.0%											
41 - 60 F	17.2	45.1	14.2	0.0	10.7	89.1	0.2	0.1	0.0	0.0	0.
61 - 80 M	19.3	46.0	14.4	0.0	11.9	87.9	0.3	0.1	0.0	0.0	0.
Degraded β-Carotene 0.5%											
81 - 100 F	14.7	44.8	14.2	0.0	13.3	86.5	0.2	0.0	0.0	0.0	0.
101 - 120 M	20.6	45.6	14.2	0.0	10.2	89.2	0.4	0.3	0.0	0.1	0.
β-Carotene Beadlets 1.0%											
161 - 170 F	14.7	44.7	14.1	0.0	11.3	86.4	1.5	0.8	0.0	0.0	0.
171 - 180 M	19.3	45.0	14.1	0.0	10.7	87.3	1.0	1.0	0.0	0.0	0.
Controls											
121 - 140 F	14.6	44.4	13.9	0.0	14.8	83.4	1.1	0.6	0.1	0.0	0.
141 - 160 M	20.1	44.7	13.9	0.0	11.7	86.5	1.1	0.8	0.1	0.0	0.

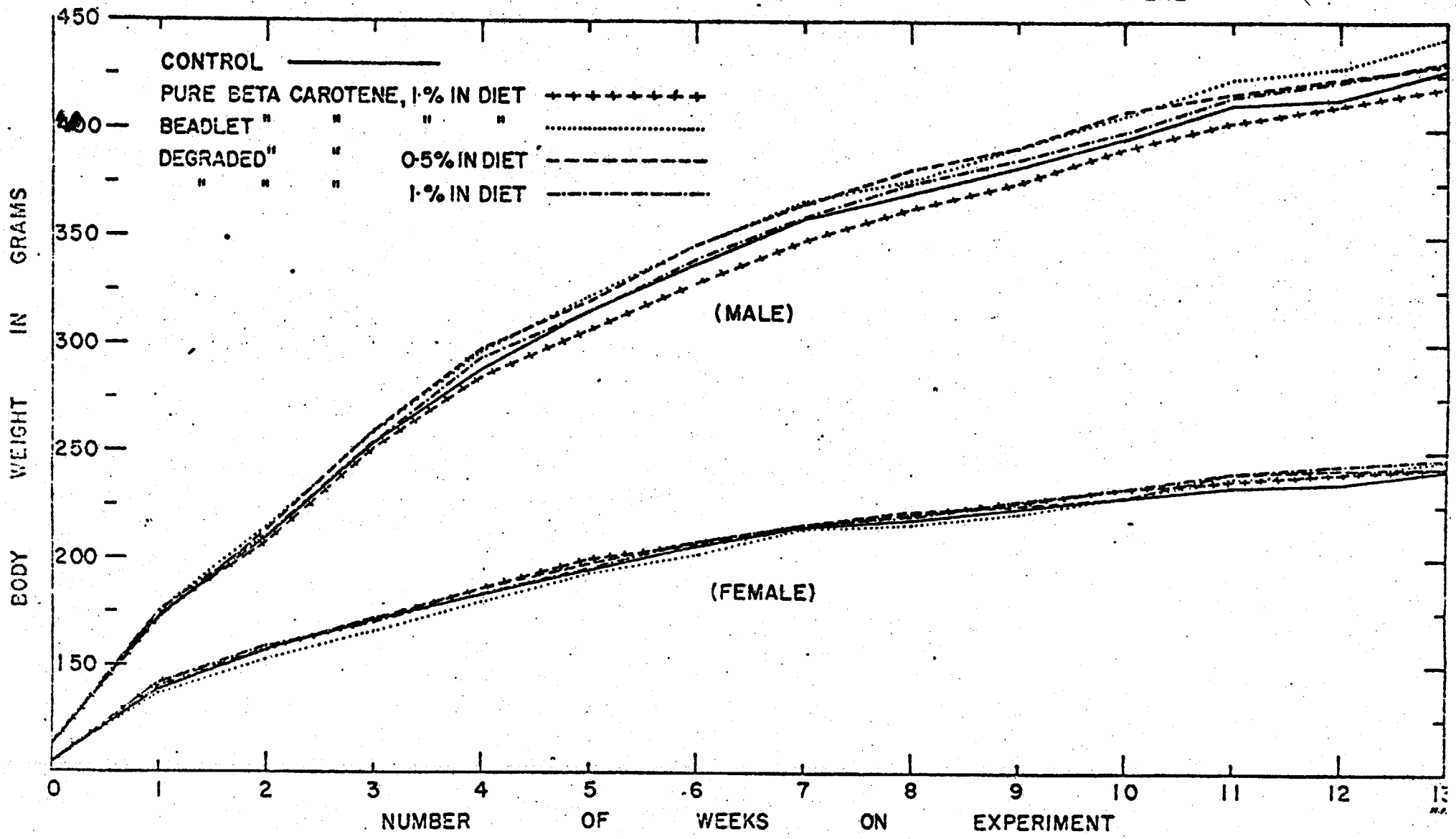
ble 3.
(Continued)

Average Blood Counts of Rats Given β-Carotene and Degradation Products For 13 Weeks After 12 Weeks of Treatment

roup and ietary oncentration	WBC in Thousands	Hematocrit %Vol. RBC	HGB in gms/100 mls.	DIFFERENTIAL NGN	SN	L	M	E	B	Pl. Cells	%Hemol. RBC
ure β-Carotene 1.0%											
1 - 20 F	11.7	46.7	14.8	0.0	13.2	86.2	0.4	0.2	0.0	0.0	0.3
21 - 40 M	13.3	47.1	14.7	0.0	12.0	86.9	0.5	0.3	0.05	0.5	0.05
egraded β-Carotene 1.0%											
41 - 60 F	12.2	46.7	14.8	0.0	10.8	87.3	0.4	1.0	0.05	0.0	0.0
61 - 80 M	14.9	45.8	14.3	0.0	11.1	86.9	0.8	0.7	0.1	0.05	0.05
egraded β-Carotene 0.5%											
81 - 100 F	14.6	46.6	14.9	0.0	12.5	85.9	0.5	0.9	0.0	0.2	0.05
101 - 120 M	17.0	47.1	14.5	0.0	12.9	85.8	0.5	0.7	0.05	0.05	0.05
Carotene Beadlets 1.0%											
161 - 170 F	14.0	45.8	14.4	0.0	13.4	84.6	1.0	0.8	0.2	0.0	0.0
171 - 180 M	18.0	46.4	14.3	0.0	13.2	84.8	1.1	0.9	0.0	0.0	0.0
Controls											
121 - 140 F	13.2	46.6	14.6	0.0	16.2	82.4	0.7	0.6	0.05	0.2	0.05
141 - 160 M	19.0	45.4	14.1	0.0	12.4	84.8	1.4	1.3	0.2	0.0	0.05

(fig.no.1)

THIRTEEN WEEK RAT STUDY OF PURE AND DEGRADED BETA CAROTENE



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68

Red Corner Report

By: Dr. K. Schürer/Dr. F. Studer and
Dr. H. Thommen

Dept.: VI/Med. and VEF

Date: November 14, 1961

Concerns: Themes 2140, 6002, 2128

Ro 4-1259 = β-Apo-8'-carotenal

Ro 4-2000 = β-Apo-8'-carotinic acid (C_{30})-methyl ester.

β-Carotene

Comparative Subacute Toxicity in Administration of Very High Daily Doses.

Summary:

Ro 4-1259, Ro 4-2000 and β-Carotene were administered by stomach tube to young male rats in daily doses of 1 g/kg on 5 week days for a period of 4 weeks.

These dosages were tolerated very well. There were no indications that the weight development of the animals was impaired. Liver function (bromsulphalein test) proved normal after a 4-week experiment period. No damaging effects on the testes were observed in any of the treated animals. Pigment deposits were found in the kidneys of animals treated with Ro 4-1259 and Ro 4-2000, respectively; but these were not present in animals treated with β-carotene. No pigment deposits were detected in the liver, although this organ was somewhat heavier in treated animals than in controls. It was estimated that the pigment deposits in the kidney were still present in unaltered form 14 days after completion of the experiment.

Chemical examination showed only fractions of the administered drug doses to be present in the organs. While in β-carotene administration, traces of pigment were detected only in the liver following treatment with Ro 4-1259 and Ro 4-2000 traces of pigment were also observed in the kidneys

In chronic toxicity studies of the vegetable and feed-coloring agents Ro 4-1259 and Ro 4-2000, certain harmful effects on the testes occurred more frequently in treated animals than in the controls. Due to the small number of animals tested, it is difficult to assess the effects of β-carotene in this respect. In order to determine whether these drugs have deleterious effects on the testes and if so, whether there are notable differences in the degree of damage, a 4-week subacute toxicity experiment was carried out during which the drugs were administered in very high doses (1g/kg/day). Moreover, as part of the study, the animals were sacrificed at different stages of the experiment to obtain data on pigment deposits in the various organs and, specifically, as to how long these deposits are present after the drug has been discontinued. In addition, chemical examination of the affected organs was expected to supply information concerning deposition of the three drugs in the organism.

Procedure:

Groups consisting of 24 male rats, weighing initially app. 80-90 g. received 1 g/kg/day of Ro 4-1259, Ro 4-2000 and β-carotene, respectively, on 5 week days for a period of 4 weeks. The drugs, dissolved in peanut oil were administered by stomach tube. The control group, treated with peanut oil alone, consisted of 12 animals. The animals were maintained on the usual breeding diet (biscuits, composition see Appendix to this Report).*)

On the 1st, 8th and 15th day after completion of the drug administrations, 1/3 of the animals were killed.

Weight Checks:	twice a week
Liver Function Tests:	bromsulphalein tests at completion of experiment.
Organ Weights:	liver, spleen, kidney, testes
Histological Examinations:	liver, kidney, spleen, testis, heart, lung, small intestine, large intestine, adrenals.
Chemical Examination:	liver, kidney, spleen, heart, lung, testes.

Results:

1. Weight Gains:

The following weight gains were recorded in the course of the 4-week experiment:

Ro 4-1259:	86.9 ± 17.5 g.
Ro 4-2000:	91.8 ± 18.5 g.
β-Carotene:	83.8 ± 18.1 g.
Controls:	88.8 ± 14.4 g.

Accordingly, with respect to weight gain, there were no differences between the 4 groups.

2. Bromsulphalein Tests at Completion of Experiment:

The bromsulphalein tests yielded the following results in 5 animals of each experimental group and 4 controls:

Preparation:	Blood Level 30 Min. after injection of 25 mg/kg BSP	BSP-Retention (20 mg% = 100%)
Ro 4-2159	0.26 mg%	1.3%
Ro 4-2000	0.23 mg%	1.1%
β-Carotene	0.21 mg%	1.1%
Controls	0.30 mg%	1.5%

These results indicate that at the termination of the 4-week experiment bromsulphalein eliminations proved normal in all animals examined. There were no indications of hepatic damage.

3. Autopsy Findings:

In animals treated with Ro 4-1259 and Ro 4-2000, respectively, autopsies revealed a very slight yellow discoloration of the body fat. Apart from this finding, all organs appeared completely normal.

4. Organ Weights:

The mean weights and variations of the liver, kidney, spleen and testes of animals

in the various experiment groups are compiled in Table 1.

The livers of animals treated with the pigments are in general somewhat heavier than those of the controls; this is particularly true of animals treated with Ro 4-1259 and Ro 4-2000, respectively. When the drug administrations are discontinued these differences between treated animals and controls become less distinct.

With respect to the kidney, the picture is approximately the same; however the weight differences between organs of control and treated animals are only small. The average weights of the spleen exhibited greater variation in the various groups, however no specific differences can be detected between treated and untreated animals.

5. Histological Examination:

Liver, Spleen, Testes, Heart, Lungs, Small Intestine, Large Intestine, Adrenals: These organs were found to be completely normal and, particularly, no pigments were detected, after the 4-week experiment period. The protoplasm in the livers of animals treated with Ro 4-1259 and Ro 4-2000, respectively, appears to be somewhat coarser than in animals of the other groups. In the testes, maturation of spermatozoa proceeds unimpaired.

Kidneys:

Pigment granules ranging in size from small to fairly large are observed regularly in the epithelium of a few tubuli contorti or in larger areas in animals treated with Ro 4-1259 and Ro 4-2000, respectively. In the unstained sections, these pigments are deep yellow; in frozen sections stained with hemalum they appear in yellow-greenish color (but are easily covered over by the other stain), and in the H.E.-sections they sometimes occur as brightly eosiniphile-stained particles. As a result, the epithelium is stained irregularly in the H.E.-sections and, for the most part, is somewhat inflated. No pigment granules are found in the glomeruli and in the other tubular sections.

In animals sacrificed 8 and 15 days, respectively, after the last treatment, these pigments are likewise present in the same form and to the same extent as in the animals killed immediately after the completion of the experiment. In animals treated with β-carotene and those in the control group no pigment deposits were found in the kidney tubules.

6. Chemical Examinations:

The Table below records the chemically determined amounts of carotenoid found in the various organs.

Prep.	Carotenoid	γ per 100 g of fresh substance in Liver	Kidney	Spleen	Lung	Heart	Testis
4-1259	β-Apo-8'-carotenal	130	110	<25	50	0	0
	β-Apo-8'-carotinic acid	<10					
4-2000	β-Apo-8'-carotinic acid	20	60	0	30	0	0
β-Carotene	β-Carotene	150	0	0	0	0	0

(0 = Determination not possible with specimen available).

This Table shows that

1. only very small portions of the administered drug are detectable in the organism since the largest part of the administered amount is not resorbed;
2. Ro 4-2000 and Ro 4-1259 behave differently with respect to storing than does β-carotene; in the case of the latter, deposits of the drug are found only in the liver while in the case of Ro 4-2000 and Ro 4-1259 other organs as well, particularly the kidney and lungs also contain rather large quantities of the drug. Moreover, differences also seem to exist between Ro 4-1259 and Ro 4-2000; while the pigments are fairly evenly distributed in the liver and kidney in the case of Ro 4-1259, after treatment with Ro 4-2000 the pigments are deposited in significantly greater amounts in the kidney than in the liver.

The relatively high degree of deposition in the lungs is striking. Since histological examinations in the course of earlier chronic toxicity studies never revealed pigment deposits in the lungs it can be assumed that the drug particles observed in this experiment were aspirated during feeding by stomach tube and reached the lungs in this manner.

No pigment deposits whatsoever were detected in the testes. Consequently, pigment deposition is probably not responsible for the testicular damage observed in other experiments.

Discussion of the Results.

In earlier experiments during which 0.2% and 0.5%, respectively, of Ro 4-1259 was administered in the form of dietary supplements, yellow pigment deposits in the liver and kidneys were detected after a 16-week experiment period; in addition, 1 out of 4 animals sacrificed from each group exhibited testicular damages. During the 16-week experiment, the rats had consumed a total of 13 g/kg and 30 g/kg, respectively, of the drug. In the experiment presented here the amount of drug administered by stomach tube during a 4-week period reached app. 20 g/kg. The pigment depositions were expected to be approximately the same as in the experiments in which the drug was administered as dietary supplement.

As anticipated, histological examination revealed the presence of yellow pigment granules in the kidneys of rats treated with Ro 4-1259. But, surprisingly, no pigment granules could be found in the liver although the livers of treated animals were somewhat heavier than those of the controls. The same applies in rats treated with Ro 4-2000 while those treated with β-carotene exhibited no pigments connected with administration of the drug in the various organs examined.

These differences in pigment deposition between the previously mentioned dietary supplement experiment and the present experiment in which the drugs were administered by stomach tube are possibly due to the different forms of

while in single daily administration by stomach tube it seems likely that larger quantities of the drug are excreted in the feces. It appears, moreover, that not only the absolute quantity of the drug administered is responsible for the formation of pigment deposits but that duration of administration is also a factor.

In animals of the β-carotene group, chemical examination showed pigment particles to be present only in the liver while in treatment with Ro 4-1259 and Ro 4-2000, respectively, rather large pigment portions were found also in other organs, particularly in the kidney and sometimes in the lungs. This indicates that between the three preparations fairly important differences exist with respect to accumulative capacity. On the other hand, the drug portions detected in the various organs show that only the smallest part of the drug quantities administered are in fact deposited later in the organism while the largest part is probably not resorbed at all.

However, in this experiment daily doses of 1 g/kg administered on the 5 week days for a period of 4 weeks were tolerated very well; in particular, no differences with regard to weight increases were observed between treated animals and controls. No untoward effects on the testes were noted upon completion of the 4-week experiment. It can therefore be stated that even very high daily doses of Ro 4-2000 and of Ro 4-1259 did not primarily produce harmful effects on the testes.

The results of this experiment added very little information to clarify how long these pigments are retained in the organism. In any case, the pigment deposits observed do not disappear at once since approximately the same amounts were detected in the kidneys 14 days after the last administration of the drug.

(Signed) Schärer

Hist. No. 1927 - 2147/61
2311 - 2534/61
2719 - 2941/61

Nutley, New Jersey, December 19, 1961
Translated from the German
Translator: Irene J. Ryby

IJR:mb

Appendix

Composition of the Breeding Diet

Corn, ground	20.--- kg
Wheat "	18.750 kg
Oats "	17.500 kg
Dry yeast	8.--- kg
Meat meal	7.500 kg
Hay meal	3.--- kg
Skim milk powder	5.250 kg
Fat	2.--- kg
Coconut oil	1.--- kg
NaCl	0.750 kg
Ferrous citrate	0.075 kg
Manganese sulfate	0.007 kg

Time (days after the last administration)	1000 mg/kg/day	Body Weight g	Liver g	Liver % B.W.	Kidney g	Kidney % B.W.	Spleen g	Spleen % B.W.	Testes g	Testes % B.W.
1	4-1259	177.4	9.45	5.3	1.48	0.84	1.23	0.69	2.38	1.34
	4-2000	177.6	8.77	4.9	1.39	0.78	1.19	0.67	2.39	1.36
	β-Carotene	169.1	7.90	4.7	1.27	0.75	1.45	0.88	2.22	1.32
	Controls	180.1	7.18	4.0	1.28	0.72	1.32	0.73	2.23	1.24
8	4-1259	196.4	9.29	4.7	1.56	0.79	1.20	0.61	2.32	1.18
	4-2000	203.2	10.01	4.9	1.48	0.73	0.98	0.48	2.33	1.15
	β-Carotene	196.5	8.82	4.5	1.48	0.75	1.16	0.66	2.41	1.15
	Controls	180.0	7.08	3.9	1.31	0.73	1.15	0.64	2.37	1.31
15	4-1259	220.1	9.86	4.5	1.55	0.77	1.39	0.63	2.56	1.16
	4-2000	225.2	10.09	4.5	1.68	0.75	1.38	0.61	2.36	1.17
	β-Carotene	209.3	8.85	4.2	1.60	0.76	1.38	0.66	2.41	1.15
	Controls	202.0	8.08	4.0	1.42	0.70	1.30	0.64	2.16	1.07

Table 1: Mean absolute and relative organ weights 1, 8 and 15 days after the last administration of Ro 4-1259, Ro 4-2000 and β-carotene, respectively. (1 g/kg/day during 4 weeks).

ROTECKER-REPORT

Sector: VI/med.

Date: February 28, 1962

Re: Subject 2140, 6002, 2128

Preparation Ro 4-1259 = β-Apo-S'-carotenal

Preparation Ro 4-2000 = β-Apo-S'-carotenic acid (C_{30})-... *) ethylester

β-carotene.

FERTILITY OF MALE RATS AND GENERAL TEST COURSE WITH 34-WEEK ADMINISTRATION OF 100 AND 500 mg/kg/workday OF PREPARATIONS 4-1259, 4-2000 AND -CAROTENE.

*) illegible

RCR SCHÄRER/STUDER No. Nov. 11, 1961 reports on the fertility of male rats during prolonged administration of large Prep. 4-1259 doses.
Fertility tests of this type with carotenoids were scheduled since testicle lesions were frequently found in chronic tolerance experiments with Preparations 4-2000 and 4-1259.

Present report includes the results of fertility tests with Prep. 4-2000 and β-carotene, whereby the results of such tests with Preparation 4-1259 are listed as well for reasons of comparison.

Testing method.

Groups of 16 male rats each (Wistar-progeny from our own breed), with a weight of 80 to 90 g, were tested. The following preparation volumes were administered with the gastric tube on 5 workdays per week:

Group 1: 100 mg/kg/day of Prep. 4-1259 in 0,5 ml per 100 g arachis oil;

Group 2: 500 mg/kg/day of Prep. 4-1259 in 0,5 ml per 100 g arachis oil;

Groups 3: control group, same volume of arachis oil;

Group 4: 100 mg/kg/day of Prep. 4-2000 in 0,5 ml per 100 g arachis oil;

Group 5: 500 mg/kg/day of Prep. 4-2000 in 0,5 ml per 100 g arachis oil;

Group 6: 100 mg/kg/day of β-carotene (20% oily solution, prepared with arachis oil to obtain 0,5 ml per 100 g);

Group 7: 500 mg/kg/day of β-carotene, " "

Composition: the fodder customary for our breed was supplied.

Mating:

When the rats reached an approximate weight of 250 g after a 10-week testing period, 13 to 14 animals from each group were mated each month with untreated virgin females of the breed. 4 females were placed with each male; they were separated again after giving birth. The following was determined:

a) the precentage of fertile males from each group;

b) the number of pregnant females per fertile male ;

c) the number of progeny per litter (litter count).

Test duration . A total of 34 weeks up to, and including the 6th mating.

The animals were killed 3 weeks later. The discontinuation of the experiment became necessary because the resistance of the animals against probing intensified and losses increased as a result of lung puncture.

Besides said fertility tests, the following investigations were performed to clarify the overall aspect of tolerance:

a) weight controls, each week;

b) blood counts, condition at the termination of the test;

c) liver function tests, BSP, SGOT, SGPT, before termination of the test;

d) kidney function tests, blood level determination of para-aminohippuric acid and inulin;

e) determination of organ weights (heart, liver, kidney, suprarenal gland, spleen, testicles);

f) histologic investigations: heart, lung, liver, kidneys, spleen, bone marrow, thyroid glands, suprarenal gland , testicles, esophagus, stomach, jejunum, rectum, pancreas, skeletal muscle system, skin, brain, cerebral membrane, nervus ischiadicus.

Results.

1. Mating tests.

The results of the mating tests are recorded on Tables 1 to 3.

a) Fertility of the males (Table 1)

2 of the 112 used rats were sterile from the first mating on (Rat 3, 100 mg/kg/day of 4-1259; Rat 27, 500 mg/kg/day of 4-1259).

The values entered on Table 1 in parenthesis for these two groups do not include the above-mentioned two animals.

The following percentages of fertile males resulted from 6 matings for the various test groups:

Prep. 4-1259: 100 mg/kg/day: 93%;
Prep. 4-1259: 500 mg/kg/day: 97%;
Prep. 4-2000: 100 mg/kg/day: 94%;
Prep. 4-2000:500 mg/kg/day: 100%;
β-carotene: 100 mg/kg/day: 99%;
β-carotene: 500 mg/kg/day: 99%;
Controls: 99%.

Accordingly, no differences between individual groups resulted regarding fertility. The sporadic occurence of sterility was correlated with disorders of the general condition, probably as a result of lung probing in most cases.

b) Number of pregnant females per fertile male: (Table 2)

If disorders of the testicle function had occurred, a decrease of the pregnant female count could have been expected. Average number of pregnant females per fertile male and per mating, among the 4 females used per male:

Prep. 4-1259:	100 mg/kg/day:	2,65	pregnent	females/fertile	male;	
	500 mg/kg/day:	2,45	"	"	"	"
Prep. 4-2000:	100 mg/kg/day:	2,90	"	"	"	"
	500 mg/kg/day:	2,85	"	"	"	"
β-carotene :	100 mg/kg/day:	2,95	"	"	"	"
	500 mg/kg/day:	2,8	"	"	"	"
Controls:		2,85	"	"	"	"

For rats treated with Prep. 4-1259, this figure is slightly lower than for the other groups. However, the differences between the 0,5%-group and the controls are by no means statistically significant (p Student = 0,7).

c) Litter count (Table 3)

Deteriorating fertility of the males would presumably have resulted in a somewhat lower count per litter, since a less elevated sperm count would probably have reduced the number of fertilized ova.

Average litter size according to Table 3:

Prep. 4-1259:	100 mg/kg/day:	8,87	young	rats	per	litter;
	500 mg/kg/day:	9,45	"	"	"	"
Prep. 4-2000:	100 mg/kg/day:	9,01	"	"	"	"
	500 mg/kg/day:	9,17	"	"	"	"
β-carotene:	100 mg/kg/day:	9,31	"	"	"	"
	500 mg/kg/day:	9,11	"	"	"	"
Controls:		9,25	"	"	"	"

Accordingly, no differences whatsoever exist concerning litter size between the various test groups.

2. General course of the tests.

All preparations were very well tolerated. The following synopsis shows the number of spontaneous deaths up to the last mating; insofar as determinable, the cause of death in these cases was mostly lung probing (puncture).

3. Weight analysis.

Since the weight curves of the various test groups are practically identical, they cannot be graphically represented. The following Table shows a survey of the weight gains in 10, 20 and 35 testing weeks.

	Gewichtszunahmen (g)						
	4-1259		4-2000		β-Carotin		Kontr.
	100 mg/kg/Tg	500 mg/kg/Tg	100 mg/kg/Tg	500 mg/kg/Tg	100 mg/kg/Tg	500 mg/kg/Tg	
10 Wochen 4	165.0	164.0	157.6	163.3	162.1	164.8	160.4
20 Wochen	205.5	216.5	208.8	213.6	215.2	211.2	219.3
35 Wochen	246.6	249.5	238.8	263.6	243.7	242.0	244.4

1) Weight gains (g); 2) controls; 3) Tg. - day;
4) weeks.

4. Hematologic investigations.

The values compiled on Table 4 (single values and average values with simple deviation) were determined after 34 testing weeks for the investigated blood samples .

No differences were found in the red cell count of the various test groups. The leukocyte counts are also nearly identical for all groups: significantly, 4 to 5 animals were found in each group with a leukocyte count above 20,000. The differential blood count results indicated no differences between the various test groups.

5. Organ function tests.

a) Bromsulfalein tests.

The following resulted from the BSP elimination of 6 rats each (with large dose) and the control groups respectively:

Versuchsgruppe	n	mg % BSP (30 Min. n. Inj. von 25 mg/kg BSP)	% BSP-Retention (20 mg % = 3 Min. = 100 %)
4-1259, 500 mg/kg/Tag[4]	6	0.45 ± 0.29	2.2 %
4-2000, 500 mg/kg/Tag	6	0.30 ± 0.15	1.5 %
β-Carotin, 500 mg/kg/Tag	6	0.25 ± 0.10	1.25 %
Kontrollen[5]	6	0.18 ± 0.06	0.91 %

1) Test group; 2) mg % BSP (30 minutes after the injection of 25 mg/kg BSP); 3) BSP retention (20 mg % = 3 min = 100%); 4) day; 5) controls.

A single animal from the 4-1259 group showed slightly delayed bromsulfalein elimination, with a BSP retention of 5 %. The average values supplied no criteria indicating liver damge ,since according to my findings, BSP-retentions up to approx. 5 % are normal.

b) Serum transaminases.

See the Table on p. 6 (of original text) for serum transanimase-values as determined by the end of the testing period.

While no differences whatsoever existed between the various test groups regarding SGPT-values, the SGOT -values with Preparation 4-1259 were slightly higher than for the other groups.

Präparat	mg/kg/Tag	n	SGOT (SF-Einheiten)	SGPT (SF-Einheiten)
4-1259	100	9	135.0 ± 35.3	49.1 ± 11.3
	500	8	119.5 ± 25.1	47.1 ± 22.9
4-2000	100	14	108.6 ± 19.0	37.8 ± 8.5
	500	9	105.6 ± 16.3	36.0 ± 7.6
β-Carotin 4	100	12	100.0 ± 17.0	40.3 ± 6.9
	500	10 (11)	112.5 ± 43.2	35.4 ± 12.3
Kontrollen 5		12 (14)	85.0 ± 9.6	37.1 ± 7.6

1) Preparation; 2) day; 3) (sf-units; 4) carotene; 5) controls.

Präparat 1	mg/kg/Tag 2	n	Inulin mg %	PAH mg %
4-1259	500	8	6.1 ± 0.64	0.60 ± 0.18
4-2000	500	6	6.2 ± 1.17	0.66 ± 0.15
β-Carotin 3	500	7	5.9 ± 1.32	0.66 ± 0.14
Kontrollen 4	-	8	6.7 ± 1.4	0.59 ± 0.16

1) Preparation; 2) day; 3) -carotene; 4) controls.

c) Kidney function tests.

60 minutes after the injection of 100 mg/kg inulin and 200 mg/kg paramino-hippuric acid the blood levels shown on the above Table were determined. Accordingly, no criteria resulted for the presence of kidney function disorders.

6. Dissection results.

A pronounced discoloration of the organic fats to yellowish-orange was present in the animals treated with the Preparations 4-1259 and 4-2000, whereby the orange hue seemed somewhat stronger in the 4-2000 rats. No well-defined differences existed between the two doses. The fat pigment was normal (see Figure 1) in the β-carotene animals and in the controls. The livers of the 4-1259 and 4-2000 animals likewise showed a slight orange tint. Slight traces of yellow discoloration were observed in the brain of these animals.

Pneumonic foci were found in the lung of numerous animals, frequently with the color of the pigment (sequelae of accidental preparation aspiration)

Liver atrophy with humped surface was verified in 2 rats (Rat 15, 100 mg/kg/day, and Rat 29, 500 mg/kg/day; both sterile during the last mating). The pancreas was embedded in a gelatinous mass. Besides pronounced testicle atrophies, both animals showed pneumonic foci.

7. Organ weights.

Table 5 shows the synopsis of the average weights and their simple deviation for heart, liver, kidney, spleen, suprarenal gland and testicles. The same Table also shows the relative weight of these organs.

No significant differences exist between various test groups concerning the absolute and relative weights of heart, liver, kidney, spleen and suprarenal glands.

It is evident that the weight of the testicles in case of the 500 mg/kg 4-1259 group is relatively lower than for the controls, the β-carotene animals , and with small doses of 4-1259 and 4-2000. With the large dose of 4-2000, the relative testicle weight is below the (relative) weight in other groups. The above indicates that the testicle weight is reduced by the larger doses of Preparations 4-2000 and 4-1259.

Histologic investigations.

Findings for the 18 examined organs will be subject to a brief discussion here only.

Lung: no differences between groups; frequently extensive peribronchial lymph clusters. Occasionally minor pneumonic foci and larger spots with foamy-pitted alveolar epitheliums. Sporadic bronchopneumonias(aspiration during probing).

Heart:Occasional enlargement of the interstitial connective tissues.

Liver: no conspicuous deviation from the normal pattern. Quite regularly, minor infectious necrotizing granulomas occur in all test groups. Very fine golden-brownish pigment granula are found sporadically in liver cells and star cells of animals from the 4-1259 and 4-2000 groups. No dose-related difference could be determinded because the occurrence of the pigments is extremely scarce. According to the administered pigment volumes, much more extensive pigment deposits could have been expected.

Muscle system: muscular fibers somewhat broad; transverse stripes not clearly evident. Trend towards longitudinal fibrous structure. No differences between the various groups.

Spleen: moderate to pronounced hyperemia, Variably high content of hemosiderin pigment. Other pigments not in evidence.

Kidney: In animals treated with Preparation 4-1259 and 4-2000, larger pigment lumps are found in the kidney cortex, in the tubuli. The untreated cross-section of the same shows yellow self-pigment; they are likewise determinable in the HE-sections as homogeneous orange or greyish eosinophil particles. No difference exists between the color of both pigments; no dosage-related dependence of its intensity was determinable. As a result of their pigment content, the epitheliums frequently appear to be considerably frayed. The above pigmentation is missing in rats treated with β-carotene and in the controls. No other histologic differences result between the various test groups.

Testicles: findings in killed male rats which proved to be fertile during the last mating; in the testicles and epididymis, large masses of mature sperm were found. The course of sperm maturation is normal in all cases.

Rat 214, 500 mg/kg/day of 4-2000: strong atrophy of the semen ducts in one testicle, while the weight of the other testicle indicated slight hypertrophy.

The following resulted for rats which were sterile during the last mating or earlier:

Rat 15, 100 mg/kg/day of 4-1259: minor reversible maturation disorders, probably connected with upset of general condition due to pneumonia.

Rat 29, 500 mg/kg/day of 4-1259: germ ("Keim") epithelials partially somewhat rudimentary. No mature sperm ; testicle lesion connected with pneumonia and liver atrophy.

Rat 308, control: normal sperm maturation; relatively numerous cellular elements in the lumina of the epididymis ducts.

Rat 102, 100 mg/kg/day of 4-2000: somewhat small testicles with normal sperm production, numerous mature spermia in the testicles and epididymis.

Rat 103, 100 mg/kg/day of 4-2000: 1 testicle normal with moderate sperm count in the epididymis, but mixed with quite numerous cellular elements. In the other testicle, the epitheliums are partially rudimentary, Moderate mature sperm count in the epididymis with numerous cellular elements,

No significant differences between the various test groups resulted for the rest of the investigated organs.

Discussion of the results.

Male rats received for 34 weeks doses of 100 and 500 mg/kg/ per workday of Preparations 4-1259, 4-2000 and β-carotene, administered with the gastric probe. The following findings resulted:

1. General condition. No differences between the various test groups and the controls.

2. Weight analysis: " "

3. Blood counts: " "

4. BSP, para-amino-hippuric acid and inulin blood level decline: " "

5. SGPT: " "

SGOT: insignificant increase in the 100 mg/kg/day 4-1259 group.

6. Organ weights: no relative and absolute differences for heart, liver, kidney, spleen, suprarenal gland.

Testicles: minor degree of absolute and relative weight reduction with 500 mg/kg/day of 4-1259; minor degree of relative weight reduction with 500 mg/kg/day of 4-2000.

7. Dissection findings: intensified orange-yellow adiposis of the organic fat with 4-1259 and 4-2000, not depending on dosage.

Slight ochre discoloration of the liver with 4-1259 and 4-2000, as well as slight yellow pigmentation of the brain.

8. Histologic findings: sporadic pigment deposits in the liver cells and star cells with 4-1259 and 4-2000; moderate pigment deposits in the kidney tubuli with 4-1259 and 4-2000, without dosage correlation.

9. Fertility: no differences between the various test groups.

These tests were scheduled because frequent testicle damage occurred in the course of chronic toxicity investigations of Preparations 4-1259 and 4-2000. In present test series, said damege could not be confirmed either by means of fertility tests or by means of histologic investigations.

The following fact might be interpreted as the only indication for disorders of this type: with large doses of Preparations 4-2000 and 4-1259, the testicles were found to be somewhat lighter than in other groups and with smaller dosages of the above-mentioned preparations . Since in case of Preparation 4-1259 testicle damage was present already after 16-week administration of 0,2 % and 0,5% fodder additives (= approx. 100 to 170 mg/kg/day or respectively, 210 to 400 mg/kg/day), while the duration of present test was 34 weeks with daily doses up to 500 mg/kg/day, the time factor is not at issue regarding the discrepancy as compared with earlier tests. On the other hand, the tests differ concerning application method and basic diet from earlier tests.

1. Application method: while the preparations were supplied as fodder additives during chronic toxicity tests and therefore were continuously available for the animals, the administration in this case was performed with the gastric probe. Since said method involves intermittent application, it is conceivable that the preparations were resorbed much less extensively than in case of administration in the fodder. Said difference can be considered confirmed because, for example, with preparation 4-1259 much fewer pigment deposits were evident in the liver in present test than in earlier tests with reduced and weaker additional dosages. We neverthless believe that said difference is less relevant for the occurrence of testicle lesions than

2. the basic diet: during the chronic toxicity test, a synthetic diet was applied, with vitamin additions twice each week. In present tests, a natural diet was supplied. Investigations to be reported in the near future showed that with a synthetic diet, the dialuric acid hemolysis of the erythrocytes increases considerably even without pigment additives, i.e. the diet does not contain sufficient Vitamin E. It is therefore reasonable to assume that testicle damage which occurred during chronic tolerance tests is ascribable to chronic vitamin E deficiency.

The age of the animals at the start of the test is probably likewise an important factor (80 to 90 g in present tests, approx. 50 g in chronic tolerance tests). Experiments are in course at this time to prove a chronic vitamin E-deficiency. They will be reported in the near future.

February 2,1962.

SCHA/bir (signature)

Hist. No. 10458 - 11505/61

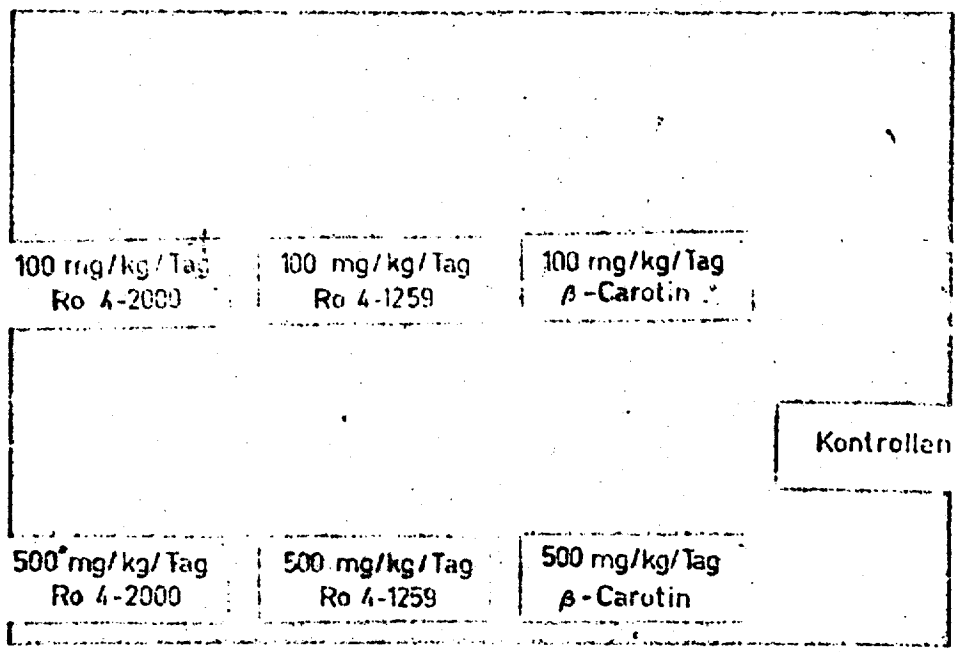

FIGURE 1: Discoloration of organic fats after 34-week administration of carotenoids.

1) day; 2) - carotene; 3) controls.

TABLE 1: Percentage of fertile male rats following the administration of large carotenoid doses.

Präparat	mg/kg/Tag	% fertile Männchen bei den Paarungen in den Monaten				
		April	Mai	Juni	Juli	August
A-1250	100	93 % (100% *)	86 % (93 % *)	83 % (91 %)	100 %	83 %
	500	86 % (93 % *)	93 % (100% *)	91 % 6100% *)	89 % (100% *)	89 % (100% *)
A-2000	100	100 %	100 %	93 %	93 %	93 %
	500	100 %	100 %	100 %	100 %	100 %
β-Carotin	100	100 %	100 %	100 %	100 %	93 %
	500	100 %	100 %	100 %	93 %	93 %
Kontrollen		100 %	100 %	100 %	100 %	100 %

September
90 % 78 % (83% *)
85 % 100 %
93 % 100 %
93 %

1) % of fertile males during matings in (month) . . .
2) Preparation
3) day
4) - carotene
5) controls

*) not including males which were sterile prior to the start of the test.

TABLE 2 Number of pregnant females per fertile male (used for test: 4 females per 1 male)

		Von 4 eingesetzten Normal-Weibchen wurden durchschnittlich pro fertilen Bock trächtig:					
Präparat	mg/kg/Tag	April	Mai	Juni	Juli	Aug.	Sept.
4-1259	100	2.8	3.3	2.4	2.3	2.4	2.7
	500	2.8	2.4	2.3	2.2	2.4	2.6
4-2000	100	3.6	2.7	2.5	3.0	2.8	2.8
	500	3.1	3.1	3.0	2.6	2.5	2.8
β-Carotin	100	2.5	3.1	3.1	3.4	3.1	2.5
	500	2.6	3.3	3.1	3.0	2.8	2.2
Kontrollen		3.1	2.6	2.7	3.0	3.0	2.7

1) Average per fertile male of standard females for the 4 tested, which become pregnant:

2) preparation
3) -carotene
4) controls
5) day

TABLE 3:

Average litter size per litter after treatment of the males with carotenoids

		durchschnittliche Zahl der Jungen pro Wurf:					
Präparat	mg/kg/Tag	April	Mai	Juni	Juli	Aug.	Sept.
4-1259	100	9.1	8.6	7.9	7.9	9.9	8.8
	500	9.3	9.2	8.9	9.8	9.5	10.0
4-2000	100	9.2	9.0	8.7	8.7	9.5	9.0
	500	8.3	8.6	8.7	10.0	9.4	10.3
β-Carotin	100	8.9	9.5	8.8	9.3	10.3	9.1
	500	9.2	9.1	8.5	9.3	8.8	10.0
Kontrollen		9.2	8.7	9.3	9.1	9.7	9.5

1) Average litter size per litter:
2) Preparation
3) - carotene
4) controls
5) day

Table 4 (cont.)

Ko.	301	12.9	40	14.0	1	15	74	-	10	-
	303	13.2	44	20.2	-	28	62	-	10	-
	305	13.5	44	10.5	2	40	49	-	9	-
	306	13.8	44	9.4	3	17	74	2	4	-
	307	12.6	41	25.0	-	30	68	-	2	-
	308	14.4	48	31.6	-	35	59	-	6	-
	309	13.5	43	26.0	-	40	56	-	4	-
	310	13.2	44	21.0	2	23	67	1	7	-
	311	14.7	46	16.9	3	18	76	-	3	-
	312	14.1	46	9.1	-	30	63	-	7	-
	313	15.1	47	10.7	-	38	59	-	3	-
	314	12.9	42	21.3	1	48	47	1	3	-
	315	14.4	44	9.7	2	31	63	-	4	-
	316	14.7	47	10.3	-	42	51	-	7	-
	M	13.8 ± 0.8	44.1 ± 4.4	16.8 ± 7.4	32.1 ± 9.6		62.0 ± 9.3	0.3	5.6	-
4-2000	101	14,1	46	24.5	2	41	55	1	1	-
100	102	12.0	44	18.2	-	32	62	-	6	-
	103	12.9	41	34.4	3	51	38	-	8	-
	104	14.1	44	22.2	1	21	76	-	2	-
	106	14.4	46	11.8	-	22	69	-	9	-
	107	13.8	47	13.1	1	29	69	1	-	-
	108	14.1	46	16.0	-	31	65	-	4	-
	109	13.5	44	22.6	3	35	60	-	2	-
	110	15.6	48	13.8	-	50	44	-	6	-

Table 4 (cont.)

	112	13.8	44	33.2	-	19	75	2	4	-
	113	15.3	48	12.8	-	27	68	-	5	-
	114	14.4	44	16.0	4	28	66	-	2	-
	115	14.4	44	15.3	1	23	66	-	10	-
	116	15.1	45	8.7	-	35	59	-	6	-
	M	14.1 ± 0.9	45.1 ± 1.9	18.8 ± 7.8	32.8 ± 10.5		62.3 ± 12.3	0.3	4.6	-
4-2000	124	14.1	46	21.2	5	50	44	-	1	-
500	128	14.1	47	21.8	2	19	70	2	7	-
	129	13.2	46	24.5	-	23	75	-	2	-
	131	13.5	44	24.6	-	19	74	-	7	-
	133	13.8	42	16.3	3	27	60	-	10	-
	134	13.5	44	19.6	1	34	55	-	10	-
	136	13.5	46	15.5	3	48	45	-	4	-
	138	14.1	48	11.3	-	28	67	1	4	-
	139	15.1	46	9.4	-	23	76	1	-	-
	M	13.9 ± 0.6	45.4 ± 1.9	18.2 ± 5.5	31.7 ± 13.1		62.9 ± 12.5	0.4	5.0	-
β-Carotin	201	14.1	42	38.2	1	42	53	-	4	-
100	202	13.8	43	20.0	1	26	71	-	2	-
	203	15.1	48	29.6	-	31	61	-	8	-
	204	15.1	43	26.0	-	27	70	-	3	-
	205	14.1	43	31.3	3	24	64	1	[illegible]	

Table 4 (cont.)

	206	13.5	44	13.2	1	23	72	-	4	-
	207	15.1	44	12.1	2	40	57	-	1	-
	208	15.3	46	14.8	3	48	41	-	8	-
	209	15.1	44	16.3	-	25	66	1	8	-
	210	13.2	40	12.7	-	37	56	-	7	-
	211	13.5	42	16.8	1	21	74	-	4	-
	214	11.4	38	40.6	7	33	53	2	5	-
	215	13.2	46	16.4	-	29	69	-	2	-
	216	14.7	46	19.3	2	36	53	-	9	-
	M	14.1 ± 1.1	43.9 ± 2.9	21.9 ± 9.5	33.1 ± 8.7		61.4 ± 9.6	0.3	5.3	-
β-Carotin 500	223	13.2	42	12.0	1	18	79	-	2	-
	226	14.1	46	21.2	-	28	62	1	9	-
	227	15.6	48	18.4	-	25	63	-	12	-
	228	13.2	44	32.1	-	60	38	-	2	-
	230	12.3	42	21.1	-	36	62	1	1	-
	232	14.7	46	16.0	2	35	57	-	8	-
	233	14.4	44	8.2	-	23	76	-	1	-
	234	14.4	44	8.9	-	26	66	-	8	-
	235	14.4	44	12.3	1	29	69	1	-	-
	236	15.3	48	12.9	-	34	63	1	2	-
	238	12.3	42	26.1	2	46	52	-	-	-
	237	14.4	45	22.8	-	30	68	-	2	-
	M	14.0 ± 1.1	44.6 ± 2.1	17.7 ± 7.3	33.0 ± 11.4		62.8 ± 10.9	0.3	3.9	-

von 100 mg/kg/Tag und 500 mg/kg/Tag Prüp. 4-1259, 4-2000 + β-Carotin

Daily dose mg/kg	Rat No.	Haemoglobin in gms/100 mls.	Haematocrit %	WBC in 1000	Differential NSN	SN	L	M	E	B
4-1259 100	1	14.1	46	23.3	2	46	36	1	15	-
	4	12.6	42	8.3	-	25	67	-	8	-
	6	12.6	45	19.1	1	21	66	-	12	-
	7	13.5	44	21.5	-	34	59	-	7	-
	11	13.5	46	23.6	3	30	61	2	4	-
	12	15.3	48	9.1	-	31	57	-	12	-
	13	13.2	45	8.8	1	33	61	-	5	-
	15	13.5	46	47.9	-	39	49	-	12	-
	16	14.1	47	12.0	-	32	64	-	4	-
	M	13.6 ± 0.8	45.4 ± 1.8	19.3 ± 12.4	33.1 ± 7.5		57.8 ± 9.8	0.3	8.7	-
4-1259 500	24	15.3	48	15.2	-	29	67	-	4	-
	25	12.3	44	21.3	3	24	70	1	2	-
	27	14.4	44	12.2	-	30	66	-	4	-
	28	14.1	45	18.7	-	22	74	-	4	-
	29	10.8	40	60.9	6	36	54	1	3	-
	30	12.3	44	20.0	-	36	60	-	4	-
	33	15.9	48	10.7	-	25	64	1	10	-
	34	13.5	44	8.8	2	40	46	-	12	-
	M	13.6 ± 1.7	44.5 ± 2.6	21.0 ± 16.8	31.6 ± 7.6		62.6	0.4	5.3	-

21d

TABLE 4 (94 A to 94 D)

Blood count values of male rats after 34-week administration of 100 mg/kg/day and 500 mg/kg/day of Preparation 4-1259, 4-2000 and β-carotene.

TABLE 5: Organ weights of male rats after 34-week administration of carotenoids.

	Präparat und Dosierung (mg/kg/Tag)				
	4-1259		4-2000		
	100	500	100	500	
...ergewicht (g)	330.6	336.1	328.6	359.4	
...z g	0.89 ± 0.12	0.98 ± 0.09	0.97 ± 0.08	0.91 ± 0.13	
% KG	0.27 %	0.29 %	0.29 %	0.25 %	
...ber g	10.04 ± 2.27	10.98 ± 3.25	11.00 ± 1.37	11.14 ± 1.37	
% KG	3.03 %	3.27 %	3.35 %	3.10 %	
...ere g	2.23 ± 0.20	2.18 ± 0.28	2.25 ± 0.19	2.31 ± 0.18	
% KG	0.67 %	0.65 %	0.68 %	0.64 %	
...lz g	0.96 ± 0.18	0.89 ± 0.19	0.96 ± 0.18	0.92 ± 0.21	
% KG	0.29 %	0.26 %	0.29 %	0.26 %	
...bennieren mg	38.7 ± 3.8	40.1 ± 7.1	38.2 ± 7.6	44.7 ± 12.1	
% KG x 10^{-3}	1.17	1.19	1.16	1.24	
...den g	2.82 ± 0.34	2.58 ± 0.32	2.85 ± 0.27	2.87 ± 0.27	
...hne die bei ... % KG ...r letzten Paarung ...erilen Ratten)	0.85 %	0.77 %	0.87 %	0.80 %	

1) Preparation and dosage (mg/kg/day)
2) - carotene
3) controls
4) body weight
5) heart
6) liver
7) kidney
8) spleen
9) suprarenal glands
0) testicles

1) not including rats which were sterile during the last mating)

	β-Carotin		Kontrollen
	100	500	
	341.2	342.2	330.7
	0.94 ± 0.13	0.93 ± 0.10	0.95 ± 0.05
	0.28 %	0.27 %	0.29 %
	11.06 ± 1.87	11.31 ± 1.59	10.57 ± 1.60
	3.24 %	3.30 %	3.20 %
	2.12 ± 0.28	2.07 ± 0.29	2.09 ± 0.24
	0.62 %	0.60 %	0.63 %
	0.92 ± 0.20	0.93 ± 0.15	0.98 ± 0.09
	0.27 %	0.27 %	0.30 %
	38.2 ± 5.0	37.2 ± 7.2	35.4 ± 5.5
	1.12	1.09	1.07
	2.93 ± 0.28	3.01 ± 0.18	2.94 ± 0.33
	0.86 %	0.88 %	0.89 %

Drs. Schürer and Studer Report February 28, 1962

Fertility of Male Rats and General Progress of Investigation on 34 Weeks Administration of 100 and 500 mg/kg/working day of Preparations Ro 4-1259, Ro 4-2000 and β-Carotene

Translation of Authors' Summary

Since in the chronic tolerance studies of Preparations Ro 4-1259 and Ro 4-2000 (other carotenoids. Translator's note), some overall damage to testicles was observed, male rats received 100 and/or 500 mg/kg/day of Ro 4-1259, Ro 4-2000 and β-carotene 5 days per week for 34 weeks. These doses were tolerated without any disturbances of general condition, growth, blood picture or liver and kidney function. In addition to a microscopically strong yellow-orange coloration of body fat, histological studies showed slight pigment storage in the liver and moderate pigment storage in the kidneys of rats treated with Ro 4-1259 and Ro 4-2000.

In the monthly pairings of these bucks with normally fed females, no effects on fertility were ascertained. No testicular damage could be demonstrated histologically. This difference from the chronic tolerance trials may be due to a lesser absorption when administered by stomach tube. It seems more likely however, that the switch from a synthetic diet in the chronic trial to a natural diet in this experiment has prevented the occurrence of testicular damage, since this might possibly be referable to a chronic vitamin E deficiency.

Translated by WRSullivan/mw
Oct. 4, 1971

[redacted]

0063

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ROTECKENRAPPORT

von : Dr. K. Schärer und Dr. F. Studer

Abteilung : VI/Med.

Datum : 28. Februar 1962

Betrifft : Thema 2140, 6002, 2128

Präp. Ro 4-1259 = β-Apo-8'-carotinal
Präp. Ro 4-2000 = β-Apo-8'-carotinsäure (C_{30})-methylester
β-Carotin

Fertilität männlicher Ratten und allgemeiner Versuchsverlauf bei 34-wöchiger Verabreichung von 100 und 500 mg/kg/Arbeitstag der Präp. 4-1259, 4-2000 und β-Carotin.

Zusammenfassung:

Da bei den chronischen Verträglichkeitsprüfungen der Präparate 4-1259 und 4-2000 gehäuft Hodenschädigungen zu beobachten waren, erhielten männliche Ratten während 34 Wochen an 5 Arbeitstagen pro Woche 100 bzw. 500 mg/kg/Tag 4-1259, 4-2000 und β-Carotin.

Diese Präparatmengen wurden ohne Störungen des Allgemeinzustandes, der Gewichtsentwicklung, des Blutbildes und der Funktion von Leber und Niere vertragen. Neben einer makroskopisch stark gelb-orangen Verfärbung des Körperfettes konnten bei den histologischen Untersuchungen geringe Pigmentablagerungen in der Leber und mässige Pigmentablagerungen in der Niere bei den mit Ro 4-1259 und Ro 4-2000 behandelten Ratten gefunden werden.

Bei den monatlichen Paarungen dieser Böcke mit normal ernährten Weibchen aus der Zucht wurde keine Beeinträchtigung der Fertilität festgestellt. Histologisch konnten keine Hodenschädigungen nachgewiesen werden. Dieser Unterschied gegenüber den chronischen Verträglichkeitsversuchen ist eventuell bedingt durch eine geringere Präparatresorption bei der Schlundsondenverabreichung. Wahrscheinlicher erscheint aber, dass der Wechsel von der synthetischen Diät im chronischen Verträglichkeitsversuch auf eine natürliche Diät im vorliegenden Versuch das Auftreten von Hodenschädigungen verhindert hat, da diese möglicherweise auf einen chronischen Vit. E-Mangel zurückzuführen sind.

Studer Schärer

Im RCR Schärer/Studer Nr. 32 353 vom 17.11.61 wurde über die Fertilität von männlichen Ratten bei lange dauernder Verabreichung von grossen Dosen Präp. 4-1259 berichtet. Derartige Fertilitätsversuche mit Carotinoiden wurden angesetzt, weil bei den chronischen Verträglichkeitsversuchen mit Präparat 4-2000 und 4-1259 histologisch häufig Hodenschädigungen beobachtet wurden.

Im vorliegenden Rapport sind die Ergebnisse von Fertilitätsversuchen mit Präp. 4-2000 und β-Carotin zusammengestellt, wobei vergleichsweise auch diejenigen des Präparates 4-1259 aufgegriffen werden.

Versuchsanordnung.

Gruppen mit je 16 männlichen Ratten (Wistarabkömmlinge aus eigener Zucht) wurden im Gewichte von 80-90 g in den Versuch genommen. An 5 Arbeitstagen pro Woche wurden mit der Schlundsonde folgende Präparatmengen verabreicht:

Gruppe 1: 100 mg/kg/Tag Präp. 4-1259 in 0,5 ml pro 100 g Arachisöl
Gruppe 2: 500 mg/kg/Tag Präp. 4-1259 in 0,5 ml pro 100 g Arachisöl
Gruppe 3: Kontrollgruppe, Arachisöl in gleicher Menge
Gruppe 4: 100 mg/kg/Tag Präp. 4-2000 in 0,5 ml pro 100 g Arachisöl
Gruppe 5: 500 mg/kg/Tag Präp. 4-2000 in 0,5 ml pro 100 g Arachisöl
Gruppe 6: 100 mg/kg/Tag β-Carotin (20%ige ölige Lösung mit Arachisöl auf 0,5 ml pro 100 g gebracht)
Gruppe 7: 500 mg/kg/Tag β-Carotin " " "

Zusammensetzung: Es wurde das in unserer Zucht gebräuchliche Futter verabreicht.

Paarungen: Nachdem die Ratten nach 10-wöchiger Versuchszeit ein Gewicht von ca. 250 g erreicht hatten, wurden monatlich aus jeder Gruppe 13 bis 14 Tiere mit unbehandelten, virginellen Weibchen aus der Zucht gepaart. Zu jedem Männchen wurden 4 Weibchen gesetzt, welche nachher bis zur Geburt separiert wurden. Bestimmt wurden:

a) Der Prozentsatz der fertilen Böcke aus jeder Gruppe,
b) die Anzahl der trächtigen Weibchen pro fertilem Männchen,
c) die Anzahl der geworfenen Jungen pro Wurf.

Versuchszeit. Total 34 Wochen bis und mit 6. Paarung.
Tötung der Tiere 3 Wochen später. Ein Abbruch des Versuches wurde nötig, weil die Tiere der Sondierung immer grösseren Widerstand entgegensetzten und sich die Ausfälle wegen Lungensondierungen mehrten.

Neben diesen Fertilitätsversuchen wurden noch folgende Untersuchungen zur Abklärung der Allgemeinverträglichkeit durchgeführt:

a) Gewichtskontrollen, wöchentlich;
b) Blutuntersuchungen, Status bei Versuchsabschluss;
c) Leberfunktionsproben, BSP, SGOT und SGPT vor Versuchsabschluss;
d) Nierenfunktionsproben, Blutspiegelbestimmungen von Para-Aminohippursäure und Inulin;
e) Bestimmung der Organgewichte (Herz, Leber, Niere, Nebenniere, Milz, Hoden.)
f) Histologische Untersuchungen: Herz, Lunge, Leber, Nieren, Milz, Knochenmark, Schilddrüsen, Nebennieren, Hoden, Oesophagus, Magen, Jejunum, Enddarm, Pankreas, Skelettmuskulatur, Haut, Gehirn, Haut, Nervus ischiadicus.

Ergebnisse

1. Paarungsversuche

Die Ergebnisse der Paarungsversuche sind in den Tabellen 1 - 3 eingetragen.

a) Fertilität der Männchen (Tabelle 1)

Von den 112 eingesetzten Ratten waren 2 von der ersten Paarung an steril (Ratte 3, 100 mg/kg/Tag 4-1259; Ratte 27, 500 mg/kg/Tag 4-1259).

Die in Tabelle 1 bei diesen Gruppen in Klammern eingetragenen Werte zählen diese beiden Tiere nicht mit.

Es ergaben sich bei den verschiedenen Versuchsgruppen aus 6 Paarungen folgende durchschnittliche Prozentsätze an fertilen Böcken:

Präp. 4-1259:	100 mg/kg/Tag:	93 %
Präp. 4-1259:	500 mg/kg/Tag:	97 %
Präp. 4-2000:	100 mg/kg/Tag:	94 %
Präp. 4-2000:	500 mg/kg/Tag:	100 %
β-Carotin :	100 mg/kg/Tag:	99 %
β-Carotin :	500 mg/kg/Tag:	99 %
Kontrollen :		99 %

Es entstanden demnach zwischen den einzelnen Gruppen keine Unterschiede bezüglich Fertilität. Die vereinzelt aufgetretenen Sterilitäten standen in Zusammenhang mit

Störungen des Allgemeinzustandes, wohl meist als Folge von Lungensondierungen.

b) Anzahl der trächtigen Weibchen pro fertilem Bock: (Tabelle 2)

Falls Störungen der Hodenfunktion eingetreten wären, so hätte man ein Absinken der Zahl der trächtigen Weibchen erwarten können. Von 4 Weibchen, die pro Männchen eingesetzt worden waren, wurden durchschnittlich pro fertilem Bock pro Paarung trächtig:

Präp. 4-1259:	100 mg/kg/Tag:	2,65	trächtige Weibchen/fert. Bock.
	500 mg/kg/Tag:	2,45	" " " "
Präp. 4-2000:	100 mg/kg/Tag:	2,90	" " " "
	500 mg/kg/Tag:	2,85	" " " "
β-Carotin:	100 mg/kg/Tag:	2,95	" " " "
	500 mg/kg/Tag:	2,83	" " " "
Kontrollen:		2,85	" " " "

Bei den mit Präp. 4-1259 behandelten Ratten liegt diese Zahl etwas tiefer als bei den übrigen Gruppen. Die Unterschiede zwischen der 0,5%-Gruppe und den Kontrollen sind aber statistisch keineswegs signifikant (p Student = 0,7).

c) Anzahl Junge pro Wurf (Tabelle 3).

Bei einem Absinken der Fertilität der Böcke wäre wahrscheinlich die Zahl der Jungen pro Wurf auch etwas gefallen, weil eine weniger hohe Spermienzahl auch wahrscheinlich zu weniger befruchteten Eiern geführt hätte.

Nach Tabelle 3 wurden aber durchschnittlich geboren:

Präp. 4-1259:	100 mg/kg/Tag:	8,87	Junge pro Wurf
	500 mg/kg/Tag:	9,45	" " "
Präp. 4-2000:	100 mg/kg/Tag:	9,01	" " "
	500 mg/kg/Tag:	9,17	" " "
β-Carotin:	100 mg/kg/Tag:	9,31	" " "
	500 mg/kg/Tag:	9,11	" " "
Kontrollen:		9,25	" " "

Somit bestehen bezüglich der Anzahl der Jungen pro Wurf keinerlei Unterschiede zwischen den verschiedenen Versuchsgruppen.

2. Allgemeiner Versuchsverlauf.

Alle Präparatdosen wurden sehr gut vertragen. Die Zahl der Spontaneingänge bis zur letzten Paarung, die, soweit sich eine Todesursache feststellen liess, meist auf Lungensondierungen zurückzuführen waren, geht aus folgender Zusammenstellung hervor:

Präp. 4-1259: 100 mg/kg/Tag: 6 Eingänge; 500 mg/kg/Tag: 7 Eingänge
Präp. 4-2000: 100 mg/kg/Tag: 3 Eingänge; 500 mg/kg/Tag: 6 Eingänge
β-Carotin : 100 mg/kg/Tag: 3 Eingänge; 500 mg/kg/Tag: 5 Eingänge
Kontrollen : 3 Eingänge

3. Gewichtsentwicklung.

Da die Gewichtskurven der verschiedenen Versuchsgruppen praktisch gleich verlaufen, lassen sie sich nicht gut graphisch darstellen. Die folgende Tabelle gibt einen Ueberblick über die Gewichtszunahmen in 10, 20 und 35 Versuchswochen.

	Gewichtszunahmen (g)						
	4-1259		4-2000		β-Carotin		Kontr.
	100 mg/kg/Tg	500 mg/kg/Tg	100 mg/kg/Tg	500 mg/kg/Tg	100 mg/kg/Tg	500 mg/kg/Tg	
10 Wochen	165.0	164.0	157.6	163.3	162.1	164.8	160.4
20 Wochen	205.5	216.5	208.8	213.6	215.2	211.2	219.3
35 Wochen	246.6	249.5	238.8	263.6	243.7	242.0	244.4

4. Haematologische Untersuchungen.

Bei den nach 34 Versuchswochen untersuchten Blutstaten wurden die in Tabelle 4 (Einzelwerte und Durchschnittswerte mit einfacher Streuung) zusammengestellten Werte ermittelt.

Bei den roten Blutwerten zeigten sich zwischen den verschiedenen Versuchsgruppen keine Unterschiede. Auch die Leukozytenzahlen sind in allen Gruppen fast gleich: Auffälligerweise treten in jeder Versuchsgruppe 4-5 Tiere auf, die mehr als 20 000 Leukozyten aufweisen.

Im Differentialblutbild ergeben sich zwischen den verschiedenen Versuchsgruppen keine Unterschiede.

5. Organfunktionsprüfungen.

a) Bromsulphaleinproben.

Von je 6 Ratten der grossen Dosis bzw. Kontrollgruppe ergab die BSP Ausscheidung nach 34 Versuchswochen das Folgende:

Versuchsgruppe	n	mg % BSP (30 Min. n. Inj. von 25 mg/kg BSP)	% BSP-Retention (20 mg % = 3 Min. = 100 %)
4-1259, 500 mg/kg/Tag	6	0.45 ± 0.29	2.2 %
4-2000, 500 mg/kg/Tag	6	0.30 ± 0.15	1.5 %
β-Carotin, 500 mg/kg/Tag	6	0.25 ± 0.10	1.25 %
Kontrollen	6	0.18 ± 0.06	0.91 %

Ein einziges Tier aus der 4-1259-Gruppe wies mit 5% BSP-Retention eine leicht verzögerte Bromsulphaleinausscheidung auf. Bei den Durchschnittswerten ergaben sich keine Anhaltspunkte für eine Leberschädigung, da nach unseren Erfahrungen BSP-Retentionen bis ca. 5 % normal sind.

b) Serumtransaminasen.

Bei Versuchsabschluss wurden folgende Serumtransaminasenwerte ermittelt:
Tabelle siehe S. 6

Während bei der SGPT überhaupt keine Unterschiede zwischen den verschiedenen Versuchsgruppen vorhanden waren, lagen die SGOT-Werte bei Präp. 4-1259 etwas höher als bei den übrigen Gruppen.

Präparat	mg/kg/Tag	n	SGOT (SF-Einheiten)	SGPT (SF-Einheiten)
4-1259	100	9	135.0 ± 35.3	49.1 ± 11.3
	500	8	119.5 ± 25.1	47.1 ± 22.9
4-2000	100	14	108.6 ± 19.0	37.8 ± 8.5
	500	9	105.6 ± 16.3	36.0 ± 7.6
β-Carotin	100	12	100.0 ± 17.0	40.3 ± 6.9
	500	10 (11)	112.5 ± 43.2	35.4 ± 12.3
Kontrollen		12 (14)	85.0 ± 9.6	37.1 ± 7.6

c) Nierenfunktionsprüfungen

60 Minuten nach der Injektion von 100 mg/kg Inulin und 200 mg/kg Paraminohippursäure wurden folgende Blutspiegel bestimmt:

Präparat	mg/kg/Tag	n	Inulin mg %	PAH mg %
4-1259	500	8	6.1 ± 0.64	0.60 ± 0.18
4-2000	500	6	6.2 ± 1.17	0.66 ± 0.15
β-Carotin	500	7	5.9 ± 1.32	0.66 ± 0.14
Kontrollen	-	8	6.7 ± 1.4	0.59 ± 0.16

Es ergaben sich somit keine Anhaltspunkte für das Vorliegen von Nierenfunktionsstörungen.

6. Sektionsbefunde.

Bei den mit den Präparaten 4-1259 und 4-2000 behandelten Tieren bestand eine deutliche Verfärbung des Körperfettes ins Gelborange, wobei der Orangefarbton bei den 4-2000-Ratten etwas stärker schien. Deutliche Unterschiede

zwischen den beiden Dosen bestanden nicht. Bei den β-Carotintieren und den Kontrollen lag eine normale Fettfarbe vor (siehe Abb. 1). Ebenfalls wiesen die Lebern der 4-1259 und der 4-2000-Tiere einen leichten Stich ins Okerfarbige auf. Im Gehirn dieser Tiere war eine leichte Andeutung einer Gelbverfärbung zu beobachten.

Bei vielen Tieren fanden sich pneumonische Stellen in der Lunge, häufig mit der Farbe des Farbstoffes verfärbt (Folgen von akzidentellen Präparataspirationen).

Bei 2 Ratten der 4-1259-Gruppe (Ratte 15, 100 mg/kg/Tag und Ratte 29, 500 mg/kg/Tag; beide steril bei den letzten Paarungen) bestand eine Atrophie der Leber mit höckeriger Oberfläche. Das Pankreas war in eine gallertartige Masse eingebettet. Neben deutlichen Hodenatrophien wiesen beide Tiere pneumonische Herde auf.

7. Organgewichte

Die durchschnittlichen Gewichte und deren einfache Streuung von Herz, Leber, Niere, Milz, Nebenniere und Hoden sind in Tabelle 5 zusammengestellt. Die gleiche Tabelle zeigt auch das relative Gewicht dieser Organe.

Bei Herz, Leber, Niere, Milz und Nebennieren bestehen keine wesentlichen Unterschiede in den absoluten und relativen Gewichten bei den verschiedenen Versuchsgruppen.

Bei den Hoden fällt auf, dass bei der 500 mg/kg 4-1259-Gruppe das Gewicht absolut und relativ geringer ist als bei den Kontrollen, den β-Carotin-Tieren und bei kleinen Dosen von 4-1259 und 4-2000. Bei der grossen Dosis 4-2000 liegt das relative Hodengewicht tiefer als in den übrigen Gruppen. Es besteht demnach eine Andeutung, dass durch die grösseren Dosen der Präparate 4-2000 und 4-1259 das Hodengewicht verkleinert wird.

8. Histologische Untersuchungen

Die Befunde der 18 untersuchten Organe sollen hier nur ganz kurz abgehandelt werden.

Lunge: Ohne Gruppenunterschiede häufig sehr umfangreiche peribronchiale Lymphhaufen. Gelegentlich kleinere pneumonische Herde und grössere Stellen mit schaumig-wabigen Alveolarepithelien. Vereinzelt Bronchopneumonien (Aspiration bei der Sondierung).

Herz: Gelegentlich Verbreiterung des interstitiellen Bindegewebes.

Leber: Keine auffälligen Abweichungen vom Normalbild. Ziemlich regelmässig kleinere infektiös nekrotisierende Granulome in allen Versuchsgruppen. Bei den Tieren der

4-1259 und der 4-2000-Gruppen findet man in vereinzelten Leberzellen und Sternzellen sehr feine goldbräunliche Pigmentgranula. Ein Dosenunterschied lässt sich, da die Pigmente nur äusserst spärlich vorkommen, nicht feststellen. Nach den verabreichten Präparatmengen hätte man eine viel umfangreichere Pigmentablagerung erwartet.

Muskulatur: Muskelfasern etwas breit, Querstreifung nicht sehr deutlich. Tendenz zu Längsfaserung. Keine Unterschiede zwischen den verschiedenen Gruppen.

Milz: Mässige bis deutliche Hyperaemie. Wechselnd starker Gehalt an Haemosiderinpigment. Andere Pigmente nicht erkennbar.

Niere: Bei den mit dem Präparat 4-1259 und 4-2000 behandelten Tieren findet man in der Nierenrinde in den Tubuli grössere Pigmentbrocken. Diese zeigen im Nativschnitt eine gelbe Eigenfarbe und sind auch in den HE-Schnitten als homogen orange oder gräulich eosinophile Partikel zu finden. Farbmässig besteht zwischen den beiden Farbstoffen kein Unterschied. Bezüglich der Intensität lassen sich keine Dosenabhängigkeiten feststellen. Die Epithelien erscheinen wegen des Pigmentgehaltes häufig stark ausgefranst. Bei den mit β-Carotin behandelten Ratten und bei den Kontrollen fehlt diese Pigmentierung. Im Uebrigen ergeben sich zwischen den verschiedenen Versuchsgruppen keine histologischen Unterschiede.

Hoden: Bei den getöteten Böcken, die sich in der letzten Paarung als fertil erwiesen hatten, fanden sich in Hoden und Nebenhoden massenhaft reife Spermien. In allen Fällen vollzieht sich die Spermienreifung normal.

Bei Ratte 214, 500 mg/kg/Tag 4-2000 bestand eine starke Atrophie der Samenkanälchen in einem Hoden, während der andere Hoden sich nach dem Gewicht als leicht hypertrophisch erwies.

Bei den Ratten, die bei der letzten Paarung oder schon früher steril waren, ergab sich das Folgende:

Ratte 15, 100 mg/kg/Tag 4-1259: geringgradige reversible Reifungsstörungen, wahrscheinlich in Zusammenhang mit einer Störung des Allgemeinzustandes wegen Pneumonie.

Ratte 29, 500 mg/kg/Tag 4-1259: Keimepithelien teilweise etwas rudimentär. Keine reifen Spermien vorhanden. Hodenschaden in Zusammenhang mit Pneumonie und Leberatrophie.

Ratte 308, Kontrolle: Normale Spermienreifung, relativ viele zellige Elemente in den Lumina der Nebenhodenkanälchen.

Ratte 102, 100 mg/kg/Tag 4-2000: Etwas kleine Hoden mit normaler Spermienproduktion, zahlreiche reife Spermien in Hoden und Nebenhoden.

Ratte 103, 100 mg/kg/Tag 4-2000: 1 Hoden normal mit mässig vielen Spermien im Nebenhoden, aber mit ziemlich vielen zelligen Elementen vermischt. Im andern Hoden Epithelien teilweise rudimentär. Reife Spermien in mässiger Zahl im Nebenhoden mit vielen zelligen Elementen.

Bei den übrigen untersuchten Organen ergaben sich keine nennenswerten Unterschiede zwischen den verschiedenen Versuchsgruppen.

Besprechung der Ergebnisse

Männliche Ratten erhielten während 34 Wochen Dosen von 100 und 500 mg/kg pro Arbeitstag der Präparate 4-1259, 4-2000 und β-Carotin mit der Schlundsonde. Dabei wurden folgende Befunde erhoben:

1. Allgemeinzustand. Keine Unterschiede zwischen den verschiedenen Versuchsgruppen und den Kontrollen.

2. Gewichtsentwicklung: " " " "

3. Blutbilder: " " " "

4. RSP, Para-Aminohippursäure und Inulin-Blutspiegelabfall: "

5. SGPT: " " " "
   SGOT: Unmerkliche Erhöhung in der 100 mg/kg/Tag 4-1259-Gruppe.

6. Organgewichte: Keine relativen und absoluten Unterschiede bei Herz, Leber, Niere, Milz, Nebenniere.

   Hoden: Geringgradige absolute und relative Gewichtsverminderung bei 500 mg/kg/Tag 4-1259, geringgradige relative Gewichtsverminderung bei 500 mg/kg/Tag 4-2000.

7. Sektionsbefunde: Stärkere orangegelbe Verfettung des Körperfettes bei 4-1259 und 4-2000, ohne Dosenabhängigkeit.
   Leichte Okerverfärbung der Leber bei 4-1259 und 4-2000 sowie leichte Gelbfärbung

des Gehirns.

8. Histologische Befunde: Vereinzelte Pigmentablagerungen in Leberzellen und Sternzellen bei 5-1259 und 4-2000, mässige Pigmentablagerungen in den Nierentubuli bei 4-1259 und 4-2000, ohne Dosenabhängigkeit.

9. Fertilität: Keine Unterschiede zwischen den verschiedenen Versuchsgruppen.

Diese Versuche wurden angesetzt, weil bei den chronischen Toxizitätsprüfungen der Präparate 4-1259 und 4-2000 gehäuft Hodenschädigungen auftraten. In dieser Serie konnten sie weder durch die Fertilitätsprüfung noch durch die histologische Untersuchung bestätigt werden.

Ein einziger Hinweis auf eine derartige Störung deutet vielleicht die Tatsache an, dass in der grossen Dosis bei den Präparaten 4-2000 und 4-1259 etwas leichtere Hoden beobachtet wurden als bei den übrigen Gruppen und bei den kleineren Dosierungen der beiden erwähnten Präparate. Da bei Präparat 4-1259 schon nach 16-wöchiger Verabreichung von 0,2%igen und 0,5%igen Futterzusätzen (= ca. 100-170 mg/kg/Tag bzw. 210 - 400 mg/kg/Tag) Hodenschädigungen vorhanden waren, während der vorliegende Versuch 34 Wochen mit Tagesdosen bis zu 500 mg/kg/Tag dauerte, kommt der Zeitfaktor für die Diskrepanz zu den früheren Versuchen nicht in Frage, dagegen bestehen zu den früheren Versuchen Untersuchungen bezüglich Applikationsform und Grundfutter.

1. Applikationsform: Während bei den chronischen Toxizitätsversuchen die Präparate als Futterzusätze verabreicht wurden und den Tieren somit dauernd zur Verfügung standen, erfolgte die Verabreichung hier durch die Schlundsonde. Da mit dieser Applikation eine Verabreichung stossartig erfolgt, ist es denkbar, dass die Präparate zu einem viel kleineren Teil resorbiert wurden als bei der Futterzusatzverabreichung. Dieser Unterschied kann als gesichert betrachtet werden, da beispielsweise beim Präparat 4-1259 in der Leber in diesem Versuch bedeutend weniger Pigmentablagerungen zu sehen waren, als bei den früheren Versuchen mit kürzeren und schwächeren Zusatzdosen. Trotzdem scheint uns dieser Unterschied weniger massgebend zu sein für das Auftreten von Hodenschädigungen als

2. das Grundfutter: Beim chronischen Toxizitätsversuch wurde eine synthetische Diät mit wöchentlicher zweimaliger Vitaminzugabe verwendet, in den vorliegenden Versuchen eine natürliche Diät. In demnächst zu rapportierenden Untersuchungen konnte festgestellt werden, dass bei der synthetischen Diät die Dialursäurehaemolyse der Erythrozyten auch ohne Farb-

stoffzusätze stark erhöht ist, d.h. dass das Futter zu wenig Vitamin E enthält. Es ist deshalb naheliegend, die bei den chronischen Verträglichkeitsversuchen aufgetretenen Hodenschädigungen auf einen chronischen Vitamin-E-Mangel zurückzuführen. Wahrscheinlich spielt auch das Alter der Tiere bei Versuchsbeginn zusätzlich noch eine grosse Rolle (80 bis 90 g in den vorliegenden Versuchen, ca. 50 g bei den chronischen Verträglichkeitsversuchen). Für den Beweis eines chronischen Vitamin E-Mangels laufen gegenwärtig noch Versuche, die in Kürze rapportiert werden sollen.

28.2.62
Schä/bir

Hist. Nr. 10458 - 11505/61

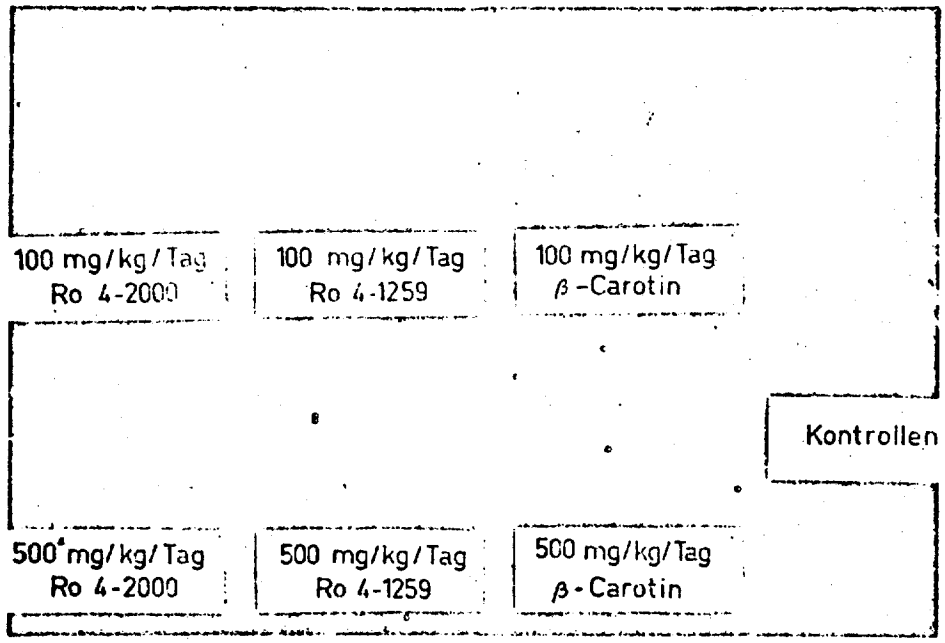

Abb. 1: Verfärbung der Körperfette nach 34-wöchiger Verabreichung von Carotinoiden.

ab. 1: Prozentsatz der fertilen Ratten-Männchen nach Verabreichung von grossen Carotinoiddosen

		% fertile Männchen bei den Paarungen in den Monaten					
Präparat	mg/kg/Tag	April	Mai	Juni	Juli	August	September
4-1259	100	93 % (100% *)	86 % (93 % *)	83 % (91 %)	100 %	83 %	90 %
	500	86 % (93 % *)	93 % (100% *)	91 % 6100% *)	89 % (100% *)	89 % (100% *)	78 % (89% *)
4-2000	100	100 %	100 %	93 %	93 %	93 %	85 %
	500	100 %	100 %	100 %	100 %	100 %	100 %
β-Carotin	100	100 %	100 %	100 %	100 %	93 %	93 %
	500	100 %	100 %	100 %	93 %	93 %	100 %
Kontrollen		100 %	100 %	100 %	100 %	100 %	93 %

* ohne die von Anfang des Versuches an sterilen Männchen.

Tab. 2: Anzahl der trächtigen Weibchen pro fertilen Männchen (eingesetzt 4 ♀/1 ♂), nach Behandlung der Männchen mit Carotinoiden

		Von 4 eingesetzten Normal-Weibchen wurden durchschnittlich pro fertilen Bock trächtig:					
Präparat	mg/kg/Tag	April	Mai	Juni	Juli	Aug.	Sept.
4-1259	100	2.8	3.3	2.4	2.3	2.4	2.7
	500	2.8	2.4	2.3	2.2	2.4	2.6
4-2000	100	3.6	2.7	2.5	3.0	2.8	2.8
	500	3.1	3.1	3.0	2.6	2.5	2.8
β-Carotin	100	2.5	3.1	3.1	3.4	3.1	2.5
	500	2.6	3.3	3.1	3.0	2.8	2.2
Kontrollen		3.1	2.6	2.7	3.0	3.0	2.7

Tab. 3: Durchschnittliche Zahl der Jungen pro Wurf nach Behandlung der Männchen mit Carotinoiden

		durchschnittliche Zahl der Jungen pro Wurf:					
Präparat	mg/kg/Tag	April	Mai	Juni	Juli	Aug.	Sept.
4-1259	100	9.1	8.6	7.9	7.9	9.9	8.8
	500	9.3	9.2	8.9	9.8	9.5	10.0
4-2000	100	9.2	9.0	8.7	8.7	9.5	9.0
	500	8.3	8.6	8.7	10.0	9.4	10.3
β-Carotin	100	8.9	9.5	8.8	9.3	10.3	9.1
	500	9.2	9.1	8.5	9.3	8.8	10.0
Kontrollen		9.2	8.7	9.3	9.1	9.7	9.5

Tab. 45

Blutwerte von männlichen Ratten nach 34-wöchiger Verabreichung

von 100 mg/kg/Tag und 500 mg/kg/Tag Präp. 4-1259, 4-2000 + β-Carotin

daily dose mg/kg	Rat No.	Haemoglobin in gms/100 mls.	Haematocrit %	WBC in 1000	Differential NSN	SN	L	M	E	B
4-1259 100	1	14.1	46	23.3	2	46	36	1	15	-
	4	12.6	42	8.3	-	25	67	-	8	-
	6	12.6	45	19.1	1	21	66	-	12	-
	7	13.5	44	21.5	-	34	59	-	7	-
	11	13.5	46	23.6	3	30	61	2	4	-
	12	15.3	48	9.1	-	31	57	-	12	-
	13	13.2	45	8.8	1	33	61	-	5	-
	15	13.5	46	47.9	-	39	49	-	12	-
	16	14.1	47	12.0	-	32	64	-	4	-
	M	13.6 ± 0.8	45.4 ± 1.8	19.3 ± 12.4	33.1 ± 7.5		57.8 ± 9.8	0.3	8.7	-
4-1259 500	24	15.3	48	15.2	-	29	67	-	4	-
	25	12.3	44	21.3	3	24	70	1	2	-
	27	14.4	44	12.2	-	30	66	-	4	-
	28	14.1	45	18.7	-	22	74	-	4	-
	29	10.8	40	60.9	6	36	54	1	3	-
	30	12.3	44	20.0	-	36	60	-	4	-
	33	15.9	48	10.7	-	25	64	1	10	-
	34	13.5	44	8.8	2	40	46	-	12	-
	M	13.6 ± 1.7	44.6 ± 2.6	21.0 ± 16.8	31.6 ± 7.6		62.6	0.4	5.3	-

Table 4 (cont.)

Ko.	301	12.9	40	14.0	1	15	74	-	10	-
	303	13.2	44	20.2	-	28	62	-	10	-
	305	13.5	44	10.5	2	40	49	-	9	-
	306	13.8	44	9.4	3	17	74	2	4	-
	307	12.6	41	25.0	-	30	68	-	2	-
	308	14.4	48	31.6	-	35	59	-	6	-
	309	13.5	43	26.0	-	40	56	-	4	-
	310	13.2	44	21.0	2	23	67	1	7	-
	311	14.7	46	16.9	3	18	76	-	3	-
	312	14.1	46	9.1	-	30	63	-	7	-
	313	15.1	47	10.7	-	38	59	-	3	-
	314	12.9	42	21.3	1	48	47	1	3	-
	315	14.4	44	9.7	2	31	63	-	4	-
	316	14.7	47	10.3	-	42	51	-	7	-
	M	13.8 ± 0.8	44.1 ± 4.4	16.8 ± 7.4	32.1 ± 9.6		62.0 ± 9.3	0.3	5.6	-
4-2000	101	14.1	46	24.5	2	41	55	1	1	-
100	102	12.0	44	18.2	-	32	62	-	6	-
	103	12.9	41	34.4	3	51	38	-	8	-
	104	14.1	44	22.2	1	21	76	-	2	-
	106	14.4	46	11.8	-	22	69	-	9	-
	107	13.8	47	13.1	1	29	69	1	-	-
	108	14.1	46	16.0	-	31	65	-	4	-
	109	13.5	44	22.6	3	35	60	-	2	-
	110	15.6	48	13.8	-	50	44	-	6	-

Table 4 (cont.)

	112	13.8	44	33.2	-	19	75	2	4	-
	113	15.3	48	12.8	-	27	68	-	5	-
	114	14.4	44	16.0	4	28	66	-	2	-
	115	14.4	44	15.3	1	23	66	-	10	-
	116	15.1	45	8.7	-	35	59	-	6	-
	M	14.1 ± 0.9	45.1 ± 1.9	18.8 ± 7.8	32.8 ± 10.5		62.3 ± 12.3	0.3	4.6	-
4-2000 500	124	14.1	46	21.2	5	50	44	-	1	-
	128	14.1	47	21.8	2	19	70	2	7	-
	129	13.2	46	24.5	-	23	75	-	2	-
	131	13.5	44	24.6	-	19	74	-	7	-
	133	13.8	42	16.3	3	27	60	-	10	-
	134	13.5	44	19.6	1	34	55	-	10	-
	136	13.5	46	15.5	3	48	45	-	4	-
	138	14.1	48	11.3	-	28	67	1	4	-
	139	15.1	46	9.4	-	23	76	1	-	-
	M	13.9 ± 0.6	45.4 ± 1.9	18.2 ± 5.5	31.7 ± 13.1		62.9 ± 12.5	0.4	5.0	-
β-Carotin 100	201	14.1	42	38.2	1	42	53	-	4	-
	202	13.8	43	20.0	1	26	71	-	2	-
	203	15.1	48	29.6	-	31	61	-	8	-
	204	15.1	48	26.0	-	27	70	-	3	-
	205	14.1	43	31.3	3	24	64	1	8	-

Table 4 (cont.)

	206	13.5	44	13.2	1	23	72	-	4	-
	207	15.1	44	12.1	2	40	57	-	1	-
	208	15.3	46	14.8	3	48	41	-	8	-
	209	15.1	44	16.3	-	25	66	1	8	-
	210	13.2	40	12.7	-	37	56	-	7	-
	211	13.5	42	16.8	1	21	74	-	4	-
	214	11.4	38	40.6	7	33	53	2	5	-
	215	13.2	46	16.4	-	29	69	-	2	-
	216	14.7	46	19.3	2	36	53	-	9	-
	M	14.1 ± 1.1	43.9 ± 2.9	21.9 ± 9.5	33.1 ± 8.7		61.4 ± 9.6	0.3	5.3	-
β-Carotin 500	223	13.2	42	12.0	1	18	79	-	2	-
	226	14.1	46	21.2	-	28	62	1	9	-
	227	15.6	48	18.4	-	25	63	-	12	-
	228	13.2	44	32.1	-	60	38	-	2	-
	230	12.3	42	21.1	-	36	62	1	1	-
	232	14.7	46	16.0	2	35	55	-	9	-
	233	14.4	44	8.0	-	23	76	-	1	-
	234	14.4	44	8.0	-	26	66	-	[illegible]	-
	235	14.4	44	12.3	1	29	69	1	-	-
	236	15.3	48	12.9	-	34	63	1	2	-
	238	12.3	42	26.1	2	46	52	-	-	-
	237	14.4	45	22.8	-	30	68	-	2	-
	M	14.0 ± 1.1	44.6 ± 2.1	17.7 ± 7.3	33.0 ± 11.4		62.8 ± 10.9	0.3	3.9	-

Tab. 5: **Organgewichte von männlichen Ratten nach 34-wöchiger Verabreichung von Carotinoiden**

	Präparat und Dosierung (mg/kg/Tag)						
	4-1259		4-2000		β-Carotin		Kontrollen
	100	500	100	500	100	500	
Körpergewicht (g)	330.6	336.1	328.6	359.4	341.2	342.2	330.7
Herz g	0.89 ± 0.12	0.98 ± 0.09	0.97 ± 0.08	0.91 ± 0.13	0.94 ± 0.13	0.93 ± 0.10	0.95 ± 0.05
% KG	0.27 %	0.29 %	0.29 %	0.25 %	0.28 %	0.27 %	0.29 %
Leber g	10.04 ± 2.27	10.98 ± 3.25	11.00 ± 1.37	11.14 ± 1.37	11.06 ± 1.87	11.31 ± 1.59	10.57 ± 1.60
% KG	3.03 %	3.27 %	3.35 %	3.10 %	3.24 %	3.30 %	3.20 %
Niere g	2.23 ± 0.20	2.18 ± 0.28	2.25 ± 0.19	2.31 ± 0.18	2.12 ± 0.28	2.07 ± 0.29	2.09 ± 0.24
% KG	0.67 %	0.65 %	0.68 %	0.64 %	0.62 %	0.60 %	0.63 %
Milz g	0.96 ± 0.18	0.89 ± 0.19	0.96 ± 0.18	0.92 ± 0.21	0.92 ± 0.20	0.93 ± 0.15	0.98 ± 0.09
% KG	0.29 %	0.26 %	0.29 %	0.26 %	0.27 %	0.27 %	0.30 %
Nebennieren mg	38.7 ± 3.8	40.1 ± 7.1	38.2 ± 7.6	44.7 ± 12.1	38.2 ± 5.0	37.2 ± 7.2	35.4 ± 5.5
% KG x 10^{-3}	1.17	1.19	1.16	1.24	1.12	1.09	1.07
Hoden g	2.82 ± 0.34	2.58 ± 0.32	2.85 ± 0.27	2.87 ± 0.27	2.93 ± 0.28	3.01 ± 0.18	2.94 ± 0.33
(ohne die bei der letzten Paarung sterilen Ratten) % KG	0.85 %	0.77 %	0.87 %	0.80 %	0.86 %	0.88 %	0.89 %

INTERCOFFICE CORRESPONDENCE

TO Dr. J. D. Surmatis DATE February 6, 1967

FROM Dr. R. Banziger and Miss D. Hane

SUBJECT Acute Mouse Toxicity of Four Trans-β-Carotene Samples.

Summary

All preparations are considered to be non-toxic. No differences in toxicity were observed between the four samples.

Samples

Trans-β-Carotene - pilot plant batches PP112866 and PP081166
Trans-β-Carotene - lots 890 and 891

Procedures

CF-1 mice of both sexes weighing 17-22 grams were orally administered the samples. Each sample was suspended in 5% gum acacia solution for administration. Ten mice were used per dose level and were observed 72 hours for mortality.

Results

Trans-β-Carotene Sample	LD_{50} ± S.E. (mg/kg, p.o.)
PP112866	> 4000
PP081166	> 4000
Lot 890	> 4000
Lot 891	> 4000

R. Banziger

D. Hane

INTEROFFICE CORRESPONDENCE

TO Mr. H. Newmark DATE June 12, 1967

FROM Dr. R. Banziger and Miss D. Hane

SUBJECT Acute Oral Toxicity of β-Carotene Samples in Mice.

Summary

All samples give an LD_{50} value greater than 10,000 mg/kg, p.o. in mice.

Samples

Product	Regular Production (Cis)	New Production (Trans)
Dry β-Carotene 2.4S yellow	400016	5751-114
Dry β-Carotene 2.4S orange	5751-157	M-313
Emulsion β-Carotene type 3.6	313126	68-3-87
Dry β-Carotene 10%	352057	354057

Procedure

CF-1 mice of both sexes weighing 17-25 grams were orally administered the samples. All β-Carotene samples were suspended in 5% gum acacia solution for administration. Ten mice were used per dose level and were observed 72 hours for mortality.

Results

Product	Dose (mg/kg, p.o.)	# Dead/ # Dosed	LD_{50} (mg/kg, p.o.)
Dry β-Carotene 2.4S yellow (cis) #40016	10,000	0/10	> 10,000
Dry β-Carotene 2.4S yellow (trans) #5751-114	10,000	0/10	> 10,000
Dry β-Carotene 2.4S orange (cis) #5751-157	10,000	1/10	> 10,000
Dry β-Carotene 2.4S orange (trans) M-313	10,000	0/10	> 10,000
Emulsion β-Carotene type 3.6 (cis) #313126	10,000	0/10	> 10,000
Emulsion β-Carotene type 3.6 (trans) #68-3-87	10,000	0/10	> 10,000
Dry β-Carotene 10%(cis)#352057	10,000	0/10	> 10,000
Dry β-Carotene 10%(trans)#354057	10,000	0/10	> 10,000

R. Banziger

D. Hane

By : Dr. W. F. Körner and M. Hammer

Department: VI/VEF

Date : May 4, 1970

Concerning: Studies on the protective effect of β-carotene and carotenoids against ultra violet rays and a review of the existing experience with vitamin A for the same indication

Theme : 2128, 2140, 2141, 2118

Summary

A review is given on the experience with vitamin A for the prevention of sunburn and UV-erythemas up to the present time. There is no significant proof of any UV-ray protective effect of retinol. On the other hand, experimental studies showed favorable radiation protection of carotenes in biological systems. The preliminary results with β-carotene in human beings suffering from erythropoietic porphyria combined with high photosensitivity are summarized.

On the basis of this encouraging experience, we tested a series of carotenoid preparations in comparison with β-carotene, using the method of Wucherpfennig, which allows the determination of a possible effect of drugs on the UV-erythema threshold value. A three weeks' oral intake of the preparations Ro 01-8300/076 F, Ro 01-8300/077 F, Ro 04-1259/040 F and Ro 04-2463/010 F provoked a statistically significant increase of the UV-erythema threshold value in human beings, while Ro 01-9915/038 F showed a slighter effect. In general, the preparations were well tolerated. The intolerance of the gastrointestinal tract was mostly transient,

and possibly caused by the dose which might have been slightly too high in some cases. Only one female volunteer considered the yellowish coloring of the skin in the facial area and of the hands as inconvenient. Most of the other female individuals attributed a certain cosmetic effect to the preparations. A modified combination of Ro 01-8300 and Ro 01-9915 is brought up for discussion in the case of developing a sunburn prophylactic for internal application.

(signatures)

M. Hammer

W. F. Körner

Kn/MH/gr

Studies on the protective effect of β-carotene and carotenoids against ultraviolet rays and a review of the existing experience with vitamin A

1. Introduction

The ultraviolet component of sunlight causes the natural tan as well as erythema solare. The UV-range includes the wave-lengths from 200 to 400 nm and is divided into 3 subranges according the intensity of its biologic radiation effect (Figure 1). Pigmentation occurs mainly at wave-lengths from 315 to 400 nm (UV-A), where practically no UV-erythema is observed. In contrast, the wave-lengths from 280 to 315 nm (UV-B) and 220 to 280 nm (UV-C) definitely produce erythema, and severe burns are sustained at wave-lengths of $\lambda < 250$ nm.

Figure 1 shows that the erythema-producine UV-component of sunlight producing amounts approximately 5% in the UV-B-range. The UV-C-band is not contained in sunlight. Normally, it is absorbed together with a small part of the UV-B-band by the ozone layer of the atmosphere. However, the amount of radiation in the range from 290 to 310 nm rises with increasing altitude and may amount to 50% of the total UV-radiation at an altitude of 2,000 meters (cause of higher incidence of sunburns in the high mountains, e.g. glacial sunburn).

Hence, a suitable protective agent against sunlight must be able to filter or absorb the rays of the wave-lengths from approximately 295 to 315 nm that produce erythema. At the same time, it may not influence the wave-lengths from 315 to 390 to permit natural tanning. A compound with an absorption maximum between 296 and 297 nm could be considered ideal. Additional requirements are

a) light stability
b) moisture stability
c) pH stability (skin pH~5.5).

Topical protective agents against sunlight may not meet these requirements. Systemically administered compounds that are stored mainly in the skin should be more suitable.

2. Experience with vitamin A

2.1. The close correlation between vitamin A and the epithelial function raises the question whether vitamin A has a protective effect against radiation, particularly against the UV-component of sunlight. In the meantime, several teams worked on this problem, mainly using vitamin A in combination with colloidal calcium carbonate, as indicated by the available studies. The preparation Sylvasun was used; it contains 25,000 IU vitamin A and approximately 200 mg $CaCO_3$.

2.2. In a preliminary investigation in South Africa, Cluver (6) studied the effect of a single dose of 25,000 IU vitamin A on sun-bathing the following day, with a group of 12 fair-skinned students. These subjects had suffered painful sunburns during preceding vacations at the ocean. Depending on the response of the test subjects to the single administration of vitamin A, they received additional vitamin A doses on subsequent days. Cluver (6) wanted to determine whether the stress due to UV-radiation leads to a deficit of vitamin A in the skin and whether the additional administration of vitamin A reduces radiation stress. This study cannot be used as proof for a protective effect of vitamin A against radiation since it was conducted without controls and the results were not uniform.

2.3. In a second study, Cluver (6) determined the protective effect of Sylvasun against sunlight with 104 fair-skinned students. The plan called for the administration of 2 tablets (50,000 IU vitamin A) every evening before sun-bathing on the following day. Additional doses of 50,000 IU vitamin A were given every 2 hours when painful erythemas appeared during exposure to the sun. Again, this is not a controlled study. Placebos were not

used.. The results are:

Mild erythema or no changes were observed in 62 test subjects. These did not receive any additional vitamin A medication. Adequate natural tanning occurred relatively quickly.

28 Subjects received an additional dose of 50,000 IU vitamin A on the first day of sun-bathing because of indications of erythema. A natural tan also developed quickly in these cases.

Pronounced erythema was observed in 14 subjects on the first day, with slight pain in some cases. With the additional administration of 150,000-200,000 IU vitamin A during the first day, the erythema disappeared and a tan developed.

In the group of 22 students who neglected to take the tablets some cases of severe sunburn were observed.

2.4. In another study, Cluver and Politzer (7) investigated the correlation between vitamin A plasma levels and the appearance of sunburn. An average decrease of the naturally low vitamin A plasma concentration from 64 IU/100 ml to 43 IU/100 ml was observed in 11 fair-skinned test persons after 1 hour of sun-bathing. The analysis was conducted according to the method by Varley[22]; the values must be considered with reservations. Furthermore, the results are not statistically reliable. It cannot be determined whether these figures reflect actual losses of vitamin A or simply fluctuations in the daily rhythm.

2.5. Finally, we would like to mention the preliminary results by Camps (3) with artificial UV-radiation. The right inner arms of 6 subjects were exposed to a 250 watt UV-lamp (λ>280 nm) at a distance of 30 cm. After 4 minutes of radiation, third and second degree erythemas formed in 3 cases

each. The experiment was repeated after administration (how long?) of vitamin A (dose? probably >25,000 IU); this time, the degree of severity of the erythema was reduced by one degree in all cases. It was mentioned that vitamin A prevented sunburn in longer term studies although erythema developed. The tanning effect of exposure to sun was not altered.

2.6. Anderson (1) conducted a double-blind study with 64 volunteers. The dosage of the vitamin A preparation (Sylvasun) was similar to that in the tests by Cluver (6). Results were similar with both groups. The UV-erythema threshold dose was determined in another double-blind test with 10 different volunteers, but a protective effect against sunlight could not be demonstrated for vitamin A.

2.7. In a skeptical, but critical article, Carruthers (4) demands new controlled investigations to determine the value or uselessness of vitamin A as protective agent against sunlight. A negative opinion is given in an editorial of the British Medical Journal (17), whereas O'Donnell (19) reaches the conclusion that the vitamin A preparation Sylvasun has at least a slight protective effect against sunlight.

2.8. Starting from the consideration that exposure to sunlight lowers the plasma level of vitamin A and that this may be a cause of sunburn (analogous to the opinions by Cluver and Politzer [7]), Nobile (18) determined in a controlled study the plasma level of vitamin A under the influence of sun rays and in the shade. Volunteers received totals of 100,000 IU vitamin A in the form of the preparation Sylvasun, or corresponding placebos before and during the study. No correlation can be established between the determined plasma values and exposure to sunlight.

2.9. These results are in contrast to the observation by Marchionini and Patel (14). These authors exposed large areas of skin to sunlight, producing changes in the serum level of vitamin A in relation to the dose of the UV-radiation. Low UV-doses raised the level on the second day after radiation, high UV-doses reduced the serum level of vitamin A.

2.10. Finally, Findlay (8) observed that the increase in the vitamin A level in the blood caused by the administration of vitamin A does not influence skin pigmentation. Page et al. (20) used animal experiments to demonstrate that the reduction in the vitamin A blood level is caused by the heat stress rather than by the activity of UV-rays.

2.11. In another study, Findlay and van der Merwe (10) found no correlation between the vitamin A plasma level and the occurrence of radiation erythema after administration of identical UV-erythema doses. The authors reached the conclusion that sunburn does not inactivate vitamin A directly in vivo, but that a biphase behavior of the blood level (rise followed by drop) could be related to the development of the erythema. However, beyond this relation UV-radiation does not affect the concentration of vitamin A in the epidermis, independent of preceding administrations of vitamin A.

2.12. Finally, Parkes and Lessin (21) tested vitamin A and 48 other compounds in guinea pigs to determine their protective effect against UV-radiation. No effect could be observed after oral administration of the toxic dose of 2 g vitamin A palmitate per day.

Although the results are, in part, contradictory, they demonstrate that vitamin A does not possess any protective effect against sunburn.

3. β-Carotene as protective agent against UV-rays

3.1. Present results

In contrast to vitamin A, there are certain indications that β-carotene, and possibly other carotenoids, can prevent erythema solare.

An early report of the UV filter effect of β-carotene was published by Kesten (12). The author tested a 44-year old female patient with high photosensitivity. After brief exposure to sunlight, she suffered from erythema, urticarial weals with intense itching, followed by chills, paleness, exhaustion and a feeling of tension at the base of the skull. Tests with various light sources and filtered light of various wave-lengths showed that erythema, urticaria and itching were produced by light of wave-lengths ranging from 420 and 490 nm, i.e., from the visible spectrum. Various substances with a filter effect as well as different antihistamines were applied topically and tested for a protective effect. Erythema and urticaria remained uninfluenced in all cases, whereas itching was prevented by the antihistamines, as could be expected. The only exception was observed after the application of a β-carotene cream (30 mg/g), in which case urticaria and itching did not appear, while the erythema developed after an initial delay and reached only approximately 1/3 of the former intensity.

The studies by Mathews (15) and Kunisawa (13) furnish additional evidence of a protective effect of β-carotene. The investigations of both groups indicate that the carotenoid pigments of certain bacteria are capable of protecting the microorganisms against lethal photosensitization. An animal experiment demonstrated that lethal sensitivity to visible light can be induced in mice if they were pretreated with hematoporphyrin i.p. Mathews (15) used this model to test the protective effect of β-carotene against lethal photosensitization. A dose of 3 mg β-carotene (dissolved in 0.2 ml Tween 80-NaCl) was administered intraperitoneally 18-24 hours

before the exposure to light. Shortly before the exposure, 1 mg hematoporphyrin (0.2 ml solution) was applied. The light used for the radiation ranged in wave-length from 340 to 440 nm, i.e., light containing approximately 60% UV-rays. When the animals were exposed to the light, 7/9 of those that received hematoporphyrin exclusively died, whereas 2/3 of the animals that received β-carotene survived. (The Tween-NaCl solution was atoxic and without any protective effect.) - Since carotenoids frequently occur in combination with chlorophyll in green plants, it is assumed that these carotenoid pigments protect chlorophyll and other plant porphyrin compounds against photolysis, thereby preventing the destruction of cells. Carotenoid pigments are also found in the skin, which contains photosensitizers such as porphyrin and cytochrome in its cells. Thus, we may assume that β-carotene has a protective function against damaging influences of visible light on the plant and animal cell. These investigations indicate that β-carotene and similar compounds may be effective in patients suffering from light dermatosis or photosensitivity, which could be correlated with the absorption maxima of β-carotene at 520,485 and 450 nm. A certain filter effect of β-carotene may be the explanation. If the carotenoids also develop a distinct protective effect against erythema solare in the UV-B-range, the effect would have to be based on a different mechanism.

Winterhalter (23) treated 2 children, one a 9-year old girl with erythropoietic porphyria, with good results. This child received daily doses of 50 mg β-carotene (100 mg at times) in the form of our 10% WS preparation for a period of one year. Later, Ro 01-8300/076 F (capsules containing 25 mg active substance) was used instead and made the administration much more pleasant. The β-carotene has been tolerated without side effects and the therapy has been continued for 2 years. Before treatment with β-carotene, the child suffered from dermatitis solaris on the skin areas exposed to sunlight. After approximately 4 weeks of therapy with carotene, the sensitivity to diffuse

daylight disappeared and, after several weeks of continued administration of carotene, the child was able to sun-bath without suffering skin reactions. The skin had a slightly brownish-yellow coloring, but only those who knew of the treatment were aware that the yellowish complexion was caused by the accumulation of carotene in the skin.

A second case of erythropoietic porphyria was treated by Findlay (9), but the results have not become available. Only recently did we read of the observations by Kass (11) who treated 3 patients with erythropoietic porphyria and light sensitivity. The light sensitivity improved after the administration of 60 mg β-carotene per day (β-carotene 10% WS) per day for 2 months; 2 of these patients were able to spend the summer out-of-doors. Skin discoloration or other side effects were not observed in any of the described cases.

These results indicate that β-carotene is stored in adequate amounts in the human skin, and that certain components of the light are absorbed to a degree that prevents radiation damage. Based on the available observations, the tanning effect is not reduced. - The accumulation of carotene or carotenoids in the skin has been documented with an adequate number of observations in the animal experiments, which led to the use of β-carotene and other carotenoids, such as β-apo-8'-carotenal and canthaxanthin, as pigment for broilers (2, 16).

3.2. Own studies of the protective effect of β-carotene and carotenoid against UV-radiation

3.2.1. Test center

Dr. H.-A. Ladner, lecturer, Radiation Clinic of the University Mainz and Dr. K. Nürnberger, Dermatological Clinic of the University Mainz

3.2.2. Tested preparations

1) Ro 01-8300/076 F

Capsules containing 250 mg β-carotene 10%, water soluble

2) Ro 01-8300/077 F

Capsules containing 250 mg β-carotene 10%, water soluble;
50 mg DL-α-tocopherol acetate;
100 mg ascorbic acid

3) Ro 04-1259/040 F

Capsules containing 250 mg β-apo-8'-carotenal 10%, water soluble

4) Ro 04-2463/010 F

Capsules containing 250 mg β-apo-8'-carotinic acid ethyl ester 10%, water-soluble

5) Ro 01-9915/038 F

Capsules containing 250 mg canthaxanthin 10%, water-soluble

3.2.3. Test procedure

A slightly modified version of the method by Wucherpfennig (24) was used to determine the UV-erythema threshold values (UV-ESW) in 5 groups of 20 volunteers each (male and female students). Subsequently, the volunteers received daily doses of 4 capsules of the preparations mentioned above for 20 days, corresponding to a daily amount of 100 mg β-carotene or carotenoid. The UV-ESW was determined again on the 21st day.

3.2.4. Method

The UV-ESW is defined as follows:

The UV-ESW is the erythema that is produced after 24 hours by the lowest amount of radiation on the skin of the back, or the upper part of the buttock, and barely outlines the exposed field. It must be greater by 20% than that of the preceding field that is not visible.

A template called a staircase is attached to the upper part of the buttock. It consists of pairs of fields 9 x 9 mm in size, one lying below the other, that can be exposed automatically at given intervals by removing cover strips. The template must adhere closely to the skin to result in well-delimited erythema.

A constant UV-light source with a precisely defined wave-length (λ=295 nm) is used for the radiation. The maximum time of exposure is 4 minutes and is reached over 12 increasing intervals of exposure. Thus, the lowest phase (Phase 12) has an exposure time of 32 seconds; the exposure time of each subsequent phase is increased by 20%. The 12 phases produce a geometric series shown below.

1 t_{UV} = 238 sec.	2 t_{UV} = 198 sec.
3 t_{UV} = 165 sec.	4 t_{UV} = 138 sec.
5 t_{UV} = 115 sec.	6 t_{UV} = 96 sec.
7 t_{UV} = 80 sec.	8 t_{UV} = 66 sec.
9 t_{UV} = 55 sec.	10 t_{UV} = 46 sec.
11 t_{UV} = 38 sec.	12 t_{UV} = 32 sec.

<u>Note:</u> Increasing light sensitivity corresponds to the increasing field numbers; Decreasing light sensitivity corresponds to increasing t_{UV} values.

3.2.5. Evaluation

Since each volunteer is his own control, the UV-ESW values can be compared directly, i.e., the differences can be used as a measure of the protective effect of the carotene or the carotenoids.

3.2.6. Results

The values determined before and after administration of the preparation are compiled in the following tables (table 1, 2, 3, 4 and 5).

Table 1

Preparation Ro 01-8300/076 F						
Volunteer No.	UV-ESN				$\Delta F_I/F_{II}$	$\Delta t_I/t_{II}$
	I before treatment		II after treatment			
	Field No.	t [sec.]	Field No.	t [sec.]		
1	3	165	3	165	0	0
2	2	198	1	238	-1	+40
3	3	165	2	198	-1	+33
4	3	165	2	198	-1	+33
5	4	138	4	138	0	0
6	5	115	3	165	-2	+50
7	5	115	3	165	-2	+50
8	5	115	3	165	-2	+50
9	6	96	4	138	-2	+42
10	3	165	2	198	-1	+33
11	5	115	2	198	-3	+83
12	6	96	4	138	-2	+42
13	2	198	1	238	-1	+40
14	6	96	4	138	-2	+42
15	5	115	2	198	-3	+83
16	2	198	3	165	+1	-33
17	4	138	2	198	-2	+60
18	3	165	3	165	0	0
19	6	96	5	115	-1	+19
20	4	138	0	>238	-4	>+100

Table 2

Preparation: Ro 01-8300/077 F						
Volunteer-No.	UV-ESW				$\Delta F_I/F_{II}$	$\Delta t_I/t_{II}$
	I before treatment		II after treatment			
	Field No.	t/sec./	Field No.	t/sec./		
1	5	115	3	165	-2	+50
2	4	138	2	198	-2	+60
3	4	138	1	238	-3	+100
4	3	165	3	165	0	0
5	5	115	5	115	0	0
6	4	138	3	165	-1	+27
7	7	80	4	138	-3	+58
8	5	115	0	>238	-5	>+123
9	4	138	3	165	-1	+27
10	3	165	1	238	-2	+73
11	4	138	1	238	-3	+100
12	4	138	4	138	0	0
13	7	80	4	138	-3	+58
14	5	115	2	198	-3	+83
15	5	115	3	165	-2	+50
16	3	165	0	>238	-3	>+73
17	5	115	3	165	-2	+50
18	3	165	3	165	0	0
19	5	115	2	198	-3	+83
20	5	115	3	165	-2	+50
21	5	115	4	138	-1	+23

Table 3

Preparation Ro 04-1259/040 F						
	UV-ESN					
	I before treatment		II after treatment			
Volunteer No.	Field No.	t [sec.]	Field No.	t [sec.]	$\Delta F_I/F_{II}$	$\Delta t_I/t_{II}$
1	4	138	1	238	-3	+100
2	3	165	2	198	-1	+33
3	5	115	3	165	-2	+50
4	6	96	*	*	*	*
5	4	138	4	138	0	0
6	6	96	4	138	-2	+42
7	5	115	3	165	-2	+50
8	4	138	3	165	-1	+27
9	8	66	3	165	-5	+99
10	4	138	2	198	-2	+60
11	3	165	2	198	-1	+33
12	1	238	1	238	0	0
13	8	66	6	96	-2	+30
14	3	165	3	165	0	0
15	5	115	3	165	-2	+50
16	4	138	4	138	0	0
17	4	138	1	238	-3	+100
18	4	138	4	138	0	0
19	3	165	2	198	-1	+33
20	4	138	2	198	-2	+60
21	6	96	3	165	-3	+69
22	4	138	2	198	-2	+60

* Discontinued treatment early

Table 4 -

Preparation Ro 04-2463/010 F						
	UV-ESM					
	I before treatment		II after treatment			
Volunteer No.	Field No.	t [sec.]	Field No.	t [sec.]	$\Delta F_I/F_{II}$	$\Delta t_I/t_{II}$
1	2	198	0	>238	-2	>+40
2	5	115	*	*	*	*
3	5	115	*	*	*	*
4	4	138	2	198	-2	+60
5	3	165	*	*	*	*
6	6	96	3	165	-3	+69
7	3	165	*	*	*	*
8	5	115	4	138	-1	+23
9	7	80	*	*	*	*
10	5	115	2	198	-3	+83
11	4	138	2	198	-2	+60
12	4	138	2	198	-2	+60
13	4	138	2	198	-2	+60
14	5	115	5	115	0	0
15	3	165	2	198	-1	+33
16	7	80	6	96	-1	+16
17	5	115	3	165	-2	+50
18	5	115	5	115	0	0
19	2	198	1	238	-1	+40
20	5	115	3	165	-2	+50

* Discontinued treatment early

Table 5

Preparation Ro 01-9915/038 F						
	UV-ESM					
	I before treatment		II after treatment			
Volunteer No.	Field No.	t [sec.]	Field No.	t [sec.]	$\Delta F_I/F_{II}$	$\Delta t_I/t_{II}$
1	6	96	5	115	-1	+19
2	6	96	4	138	-2	+42
3	5	115	4	138	-1	+23
4	6	96	6	96	0	0
5	4	138	4	138	0	0
6	3	165	2	198	-1	+33
7	6	96	5	115	-1	+19
8	5	115	5	115	0	0
9	5	115	4	138	-1	+23
10	3	165	3	165	0	0
11	2	198	2	198	0	0
12	6	96	6	96	0	0
13	3	165	2	198	-1	+33
14	4	138	4	138	0	0
15	6	96	4	138	-2	+42
16	4	138	1	238	-3	+100
17	6	96	4	138	-2	+42
18	6	96	4	138	-2	+42
19	5	115	5	115	0	0
20	6	96	4	138	-2	+42
21	3	165	3	165	0	0
22	5	115	3	165	-2	+50

3.2.7. Statistical evaluation

Two series of values (two for the field numbers and two for the t_{UV} times) were available for the statistical evaluation of each preparation. The following methods were used therefore (5):

Sign test for T_{UV} values

Random 2-sample test by Kolmogorov-Smirnov for the frequency determination of the field numbers

Checking for differences between the volunteer groups at the beginning of the study, method by Kruskal-Wallis

Testing for differences between the preparations.

In the sign test, the effect of preparations Ro 01-8300/076 F, Ro 01-8300/077 F, Ro 04-1259/040 F and Ro 04-2463/010 F is highly significant with an error possibility of $p = 0.005$, whereas the effect of Ro 01-9915/038 F cannot be considered significant with a confidence limit α of 0.05. The 2-sample test produces the same result. The test by Kruskal-Wallis showed that the groups were identical before the study. This evaluation indicated that Ro 01-9915/038 F is inferior in activity to the other four tested preparations. The contrast test shows that the mean of the position of Ro 01-9915/038 F differs significantly from those of the 4 other preparations. Thus, the protective effect of Ro 01-9915/038 F against UV-radiation is weaker than that of the other 4 preparations. These 4 preparations are identical in effect.

The protective effect of

1) Ro 01-8300/076 F
2) Ro 01-8300/077 F
3) Ro 04-1259/040 F and
4) Ro 04-2463/010 F

against UV-radiation can be considered reliable. In contrast, the effect of

Ro 01-9915/038 F

is not reliable.

3.2.8. Tolerance

In general, the preparations were well tolerated. The following additional symptoms were observed during the administration of carotenes and carotenoids, but their presence should not be overrated.

Symptoms	Preparation				
	Ro 01-8300/076 F	Ro 01-8300/077 F	Ro 04-1259/040 F	Ro 04-2463/010 F	Ro 01-9915/038 F
1) Yellowish coloring of skin of face and/or hands	8	10	11	16	8
2) Gastrointestinal disturbances					
a) pressure over stomach	3	1	2	1	1
b) belching	1		1		
c) nausea	1	1	1		
d) diarrhea	5	1	2	4	1
3) colored feces	4				2
4) Drowsiness					2
5) Disturbed sleep	1			1	1
6) No symptoms	5	10	10	4	14
N=	20	21	22	20	22

Ro 01-8300/077 F is tolerated best. Striking is the spontaneous comment, made by 4 volunteers who received preparation Ro 01-9915/038 F, that their faces had a "healthy" coloring during the treatment. At the end of the study, most volunteers asked for a supply of the respective preparation since they (mainly women) had noticed a better complexion. A certain cosmetic effect apparently is attributed to the carotenoids.

4. Discussion of the results

4.1. As reported inearlier observations, β-carotene, and possibly other carotenoids, provide, when administered topically and systemically, a protective effect in cases of photosensitivity apparently produced by rays of wave-lengths ranging from 420 to 490 nm. In addition, it protects mice with induced hematoporphyria against lethal radiation doses from the range of wave-lengths between 340 and 440 nm. The effects can be explained in 2 ways:

4.1.1. β-carotene develops in or on the skin a filtering effect for wave-lengths ranging from 340 to 490 nm.

4.1.2. β-carotene protects porphyrin and porphyrin derivatives against photolysis, preventing cell damage caused by photolysis products.

4.2. β-carotene, β-apo-8'-carotinal, β-apo-8'-carotinic acid ethyl ester and canthaxanthin demonstrate a marked protective effect for the highly erythema-producing range of wave-lengths around 295 nm in the UV-erythema test. However, this effect is observed only when the skin has a certain yellowish or yellow reddish coloring, i.e., when a certain amount of carotenoid has been stored in the epidermis. We do not know whether this is a purely physical absorption effect of the carotenoids although a situation could be created, due to the accumulation in the skin and the combined activity of various factors, that permits the absorption of UV-B-rays.

4.3. The advantage of the prophylactic systemic administration of carotenoid for a period of 2 to 3 weeks is the sustained protective effect (approximately 6-7 weeks) and the elimination of daily, frequently repeated, topical applications. Natural tanning is not inhibited and covers up a strong yellowish coloring of the skin within a relatively short time.

5. Conclusions

5.1. The results of this study demonstrate that β-carotene, β-carotene combined with vitamin E and C, β-apo-8'-carotenal and β-apo-8'-carotinic acid ethyl ester increase tolerance to UV-rays significantly and could be used as internal protective agents against sunlight. Although canthaxanthin is active, its effect is not statistically significant.

5.2. The doses used in these studies are based on information obtained with cases of erythropoietic porphyria on the basis of these observations; smaller doses, administered for 2-3 weeks, may provide adequate internal protection against sunlight. A daily dose of 40 mg active substance should be considered. Additional investigations are needed to establish the relation between dose and effect, and possibly the effect in comparison to topically applied sun oils and ointments.

5.3. The following combination seems to be suitable for further studies since no significant difference exists between β-carotene, β-apo-8'-carotenal and β-apo-8'- carotinic acid ethyl ester, and a desirable cosmetic effect was attributed spontaneously to canthaxanthin:

4 mg β-carotene (40 mg β-carotene 10% WS)
+ 4 mg canthaxanthin (40 mg canthaxanthin 10% WS)

A daily dose of 3-5 capsules could be recommended.

5.4. In addition to these studies, orientational tests should determine whether other carotenoids, possibly combined with canthaxanthin, could produce more favorable effects. The following carotenoids should be considered:

5.4.1. Bixin (Ro 1-9887)

Zeaxanthine (Ro 1-9371)

Crocetin (Ro 6-9322) as well as

5.4.2. Echinenone (Ro 4-2847)

Citranaxanthin (Ro 4-3799)

Torularhodinaldehyde = β-C_{40}-apo carotenal (Ro 4-2779)

Apo-4'-β-carotenal (Ro 4-2011)

C_{30}-dialdehyde (Ro 6-3391)

Lycopene (Ro 1-9251) and possibly

5.4.3. Capsanthin (Ro 4-1242)

Rhodoxanthin (Ro 7-5256)

Astaxanthin (Ro 4-3395)

Kn/Mh/gr

Translated from the German
Translator: E. Harmel
Aug. 26, 1970

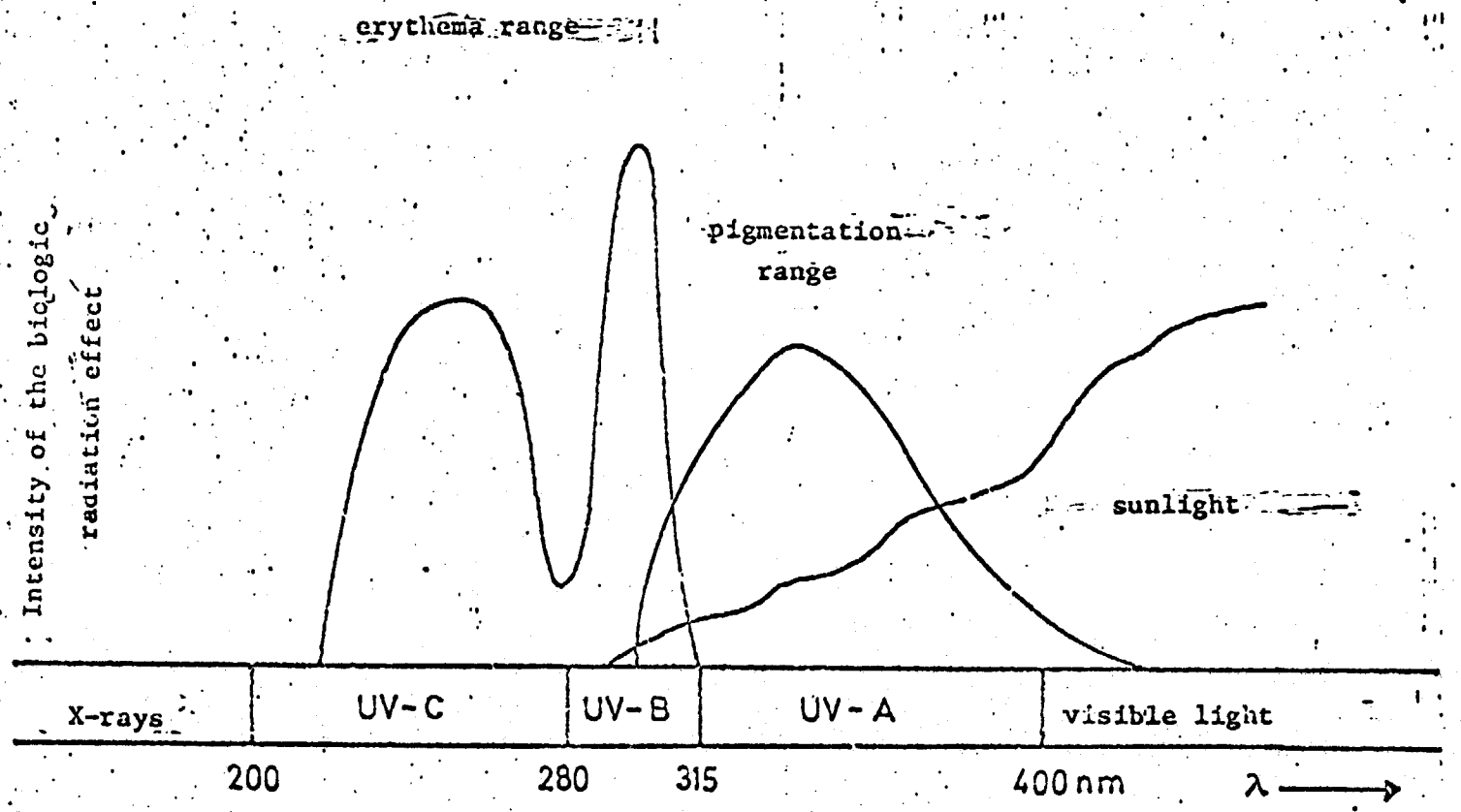
erythema range
pigmentation range
sunlight
Intensity of the biologic radiation effect
X-rays
UV-C
UV-B
UV-A
visible light
200
280
315
400 nm
λ

Figure 1

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Ocana-'35
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Ocana, T., Histological Study of the Effects Produced by High Doses of Carotene, Carrot Lipids, and Vitamin A (C. R. Soc. Biol., 1935, 120, 267-269)

We have studied the liver, kidneys, and skin of rats treated by Lewis and Reti (1) with high doses of carotene, carrot lipids and vitamin A (vogan).

Each rat in a series of 5 received by a gastric catheter 0.04 gr. of crystallized carotene (168 degrees), dissolved in 1 cc. of peanut oil for 10 days; 5 others received 1 cc. of peanut oil, and 3 served as the control group. All were fed bread and milk. The histological examination of the liver showed the presence of cellular hepatic modifications in a few cases in each series. A first degree of deterioration was established by the granular appearance of the protoplasm (marked by +). Other times there were the formations of fat vacuoles (++); and in others the cells were loaded with fat, voluminous, and rounded (+++). The nucleus always remained normal and necrobiosis was never observed. The overload (of fat) began at the periphery of the lobe in the vicinity of Kiernan's Spaces, and in the more pronounced cases spread up to the central cells (++++). The lobular structure was not modified. The cells of Kupffer were sometimes very visible, saturated with a brown pigment. There was no inflammatory reaction. The livers of the animals presented the following appearance:

Carotene: ++++, +++, +++, -, -,.

Peanut Oil: ++, +, +, -, -.

Control: ++, +, +.

The kidneys were never changed.

(1) C.R. de la Soc. de. Biol., 1935, +. 118, p. 552.

The rats who received carrot lipids (fraction soluble to venzol) dissolved in peanut oil (1.5 cc = 0.04 gr. of carotene per day) were studied as soon as possible after death, which happened after 3 to 5 days of treatment. The lobular structure of the liver was normal; there was a central lipoidosis, without signs of necrobiosis. The kidneys were always normal. Those who received the unsaponifiable of the carrot lipids survived from 3 to 13 days. In the liver was found a periphery lipoidosis entirely alike that of the preceding series, except for the distribution of the fatty overloads. The controls of these series, who received peanut oil, did not have lipoidosis, but the cells of Kupffer were saturated with fine brown granulations, probably of a lipoid nature, since they took on a brownish color with the Soudan III.

The skin of the occipital region of the thorax was studied in 4 rats treated with vogan (1 c.c. = 40,000 U. per day). One found a considerable thickening of the Malpighian layer (achantosis) with tendency to papillomatosis, evident peratosis and a parakeratosis. The hairy follicules were wasted away. The skin of the same regions of the animals treated with carrot lipids (2) and of the 3 controls who received only peanut oil, was entirely normal.

Summary

The high doses of carotene produced in some animals a fatty overload of hepatic cells, which were also seen somewhat less pronounced, in the controls. The rats treated with the carrot lipids presented a central establishment lipoidosis of hepatic cellules. Those who received the fraction not saponifiable showed a periphecy lipoidosis. Local application of a concentration of vitamin A (vogan) produced a chantosis with tendancy towards papillomatosis, a keratosis with parakeratosis, and atrophy of the hairy skin follicules of the occipital regions of the thoraxic anterior.

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SOCIÉTÉ DE BIOLOGIE DE ROSARIO

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Présidence de M. E. Hug.

ETUDE HISTOLOGIQUE DES EFFETS PRODUITS PAR LES HAUTES DOSES DE CAROTÈNE, DE LIPIDES DE CAROTTE ET DE VITAMINE A,

par T. OCANA.

Nous avons étudié le foie, le rein et la peau des rats traités par Lewis et Reti (1) par de hautes doses de carotène, de lipides de carotte et de vitamine A (vogan).

Chaque rat d'une série de 5 a reçu par sonde gastrique 0,04 gr. de carotène cristallisé (p. f. 168°), dissous dans 1 c.c. d'huile d'arachide, pendant 10 jours ; 5 autres reçurent 1 c.c. d'huile d'arachide et 5 servirent comme témoins ; tous furent alimentés avec du pain et du lait. L'examen histologique du foie montra la présence de modifications cellulaires hépatiques dans quelques

(1) *C. R. de la Soc. de biol.*, 1935, t. *118*, p. 557.

cas de chaque série. Un premier degré de l'altération était constitué par l'aspect granuleux du protoplasme (signalé par +) ; d'autres fois il y avait formation de vacuoles de graisse (+ +) ; dans d'autres les cellules étaient surchargées de graisse, augmentées de volume et arrondies (+ + +). Le noyau restait toujours normal et on n'observa jamais de signes de nécrobiose. La surcharge commençait par la périphérie du lobule au voisinage des espaces de Kiernan et dans les cas plus accentués s'étendait jusqu'aux cellules centrales (+ + + +). La structure lobulaire n'était pas modifiée. Les cellules de Kupffer étaient parfois bien visibles, chargées d'un pigment brun. Il n'y avait pas de réaction inflammatoire. Le foie des animaux présentait l'aspect suivant :

Carotène : + + + +, + + +, + + +, —, —.
Huile d'arachide : + +, +, +, —, —.
Témoins : + +, +, +.
Les reins n'étaient jamais modifiés.

Les rats qui reçurent les lipides de carotte (fraction soluble au benzol) dissous dans l'huile d'arachide (1,5 c.c. = 0,04 gr. de carotène par jour) furent étudiés aussitôt que possible après la mort, qui est survenue après 3 à 5 jours de traitement. La structure lobulaire du foie était normale, il y avait une lipoïdose centrale, sans signes de nécrobiose. Le rein était toujours normal. Ceux qui reçurent l'insaponifiable des lipides de carotte survécurent de 3 à 13 jours. On trouva dans le foie une lipoïdose périphérique en tout semblable à celle de la série précédente, sauf la distribution de la surcharge graisseuse. Les témoins de ces séries, qui reçurent l'huile d'arachide, n'avaient pas de lipoïdose, mais les cellules de Kupffer étaient chargées de fines granulations brunes, probablement de nature lipoïde, puisqu'elles prenaient une couleur brune avec le Soudan III.

La peau de la région occipitale et du thorax fut étudiée chez 4 rats traités avec du vogan (1 c.c. = 40.000 U. par jour), on trouva un épaississement considérable de la couche malpighienne (achantose) avec tendance à la papillomatose, une kératose marquée et une parakératose, les follicules pileux étaient atrophiés. La peau des mêmes régions des animaux traités avec les lipides de carotte (2) et de 3 témoins qui reçurent seulement de l'huile d'arachide était tout à fait normale.

Résumé. — Les hautes doses de carotène produisent chez quelques animaux une surcharge graisseuse des cellules hépatiques qui a été vue aussi, quoique moins prononcée, chez les témoins. Les rats traités avec les lipides de carotte présentaient une lipoïdose centrale des cellules hépatiques ; ceux qui reçurent la frac-

tion non saponifiable montraient une lipoïdose périphérique. L'application locale d'un concentré de vitamine A (vogan) produit une achantose avec tendance à la papillomatose, une kératose avec parakératose et l'atrophie des follicules pileux de la peau des régions occipitale et thoracique antérieure.

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(1971) 11: 32

Hypothesis

Golden Ovaries

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Princess Alexandra Hospital, Brisbane

Summary: This report concerns 6 women who presented with an excessive intake of carotene-containing foodstuffs and menstrual disturbance. Four of these were well nourished with respect to protein intake, while the other 2 were undernourished (anorexia nervosa type syndrome).

Four of the patients were in an older age group (range 35 - 58) with excessive or irregular vaginal bleeding; 2 of the patients were younger (22, 23) and presented with oligo-amenorrhoea. The ovaries in all patients were golden yellow in colour and possessed a characteristic histological appearance. Fat stains revealed abundant, highly-dispersed, lipid granules occurring throughout the ovarian cortex, not restricted to areas around the corpora lutea; special stains for carotenoid pigments were strongly positive, the latter giving the ovary its uniformly yellow colour, which is usually only seen in relation to the corpus luteum.

It is proposed that those women with a normal protein and a high carotene intake suffered from a hypervitaminosis A syndrome leading to a bleeding tendency. Those women with a sub-normal protein intake may have had a suboptimal conversion of carotene to vitamin A and no bleeding tendency.

Carotenaemia is defined as an excessive amount of carotene in the serum produced by the ingestion of large quantities of food rich in this yellow pigment — carrots, pumpkins, pawpaw and squash (Hess and Myers, 1919). In susceptible individuals this high carotene intake causes yellow discolouration of the mucosal surfaces and skin, especially the horny layers of the palms and the soles. The sclera are never affected and the urine is not darker than normal. In such individuals the ingestion of large quantities of carotene can cause an elevation of serum cholesterol and vitamin A (Crisp and Stonehill, 1967).

Hypercarotenaemia is most commonly seen following addiction to the above foods, but can also be seen in hypercholesterolaemic states such as hypothyroidism (Stannus, 1924) diabetes mellitus (Niehaus, 1924; Boech and Yater, 1929), and the nephrotic syndromes (Josephs, 1939; Jeghers, 1944); it can occur in cases of hypopituitarism and conditions associated with lipoprotein abnormalities.

The occurrence of hypercarotenaemia leading to bright golden ovaries and menstrual abnormalities, an association not previously encountered in the literature, forms the basis of the present report.

CLINICAL FEATURES

Four patients in the older age group presented with irregular menstrual bleeding or postmenopausal bleeding for which no other cause could be found. There were 2 who suffered from primary weight phobia and ingested large amounts of carotene-containing food-stuffs to aid in weight reduction; these patients were underweight and developed irregular periods and finally amenorrhoea.

The clinical and laboratory findings in the 6 patients are summarised in table 1.

Illustrative Case Reports

One case from each group is recorded as the patients were very similar in all clinical respects.

Case 1 (table 1). This patient, aged 51 years, presented because her periods had become increasingly heavy and irregular. She also

Table 1. Summary of Clinical and Laboratory Findings

Case	Age	Presenting features	Serum carotenes (μg./100ml.)	Serum vitamin A (μg./100ml.)	Serum cholesterol (mg./100ml.)	Remarks
1	51	Menorrhagia, tiredness, depression	300	125	370	High intake of carrots and pumpkin.
2	45	Postmenopausal bleeding, tiredness, depression	200	—	—	High intake of carrots and pumpkin.
3	35	Irregular bleeding, tiredness, depression, sensory skin changes, loss of hair	220	170	300	Diabetic, caloric restriction. High intake of carrots, pumpkin and pawpaw.
4	58	Intermittent vaginal bleeding for 5 years	200	60	250	High intake of squash and pumpkin.
5	22	Amenorrhoea for 7 months	250	15	330	12 kg. underweight. Psychiatric history. Diet largely carrots and pumpkin.
6	23	Amenorrhoea for 3 years	280	—	—	19 kg. under weight. Anorexia nervosa. Diet largely carrots and pumpkin.

complained of tiredness and depression. She was obese and obviously yellow in colour; on close questioning she stated that for some years she had eaten 6 lb. of carrots and up to 7 lb. of pumpkin per week.

Investigation revealed a haemoglobin level of 9 g. per 100 ml., a serum carotene level of 330 μg./100 ml. (normal range 50 to 200); and a serum vitamin A level of 125 μg./100 ml. (normal range 20 to 60).

The patient was curetted and histological examination of the endometrium showed focal cystic glandular hyperplasia. Because of the persistence of heavy menstrual loss an abdominal hysterectomy and bilateral salpingo-oophorectomy was performed. At operation the ovaries were found to be golden yellow in colour (figure 1). This colour was uniform throughout the substance of the divided ovary and also the uterus. On further investigation, the patient's serum cholesterol was 370 mg./100 ml. and serum triglycerides were 288 mg./100 ml.

Case 5 (table 1). This patient, aged 22 years, presented with amenorrhoea of 7 months' duration. Normal menarche occurred at 13 years. The patient was married and had a primary weight phobia, being 26 lb. under standard for age and height. She admitted to eating mainly carrots — 10 to 14 per day and much pumpkin.

On examination, the patient had the obvious yellow colour of carotenaemia. Her serum carotene was elevated to 250μg./100 ml. and serum cholesterol was 330 mg./100 ml. Under anaesthesia, pelvic examination and diagnostic curettage was performed. There were no abnormal findings, but no endometrium was obtained. Culdoscopy revealed bright yellow ovaries—a laparotomy was performed because we had never seen this picture before and a wedge resection of both ovaries was then performed.

Four months after ceasing her carotene-rich diet, and with psychiatric treatment, her weight and periods returned to normal.

Figure 1. Typical appearance of golden ovary.

Ovarian Morphology

The ovaries in these 2 groups of patients were histologically identical. They were normal in size and shape and uniformly bright yellow in colour. The uterus and the surrounding fat showed a similar discolouration.

Follicular cysts were present in the premenopausal patients and there was a tendency to hyperthecosis. The cortical stromal tissue appeared to be fibrosed, but the tunica of the ovary was not thickened as in the Stein-Leventhal syndrome.

Developing follicles were absent or reduced in number whereas atretic follicles, corpora lutea, and corpora albicantia were present. Fat stains revealed abundant, finely dispersed lipid granules occurring throughout the ovarian cortex, not restricted to the area around the corpora lutea.

Fat stains for carotenoid pigments were strongly positive.

DISCUSSION

The ovarian pathology in all patients appears to be identical and has not been seen in any other condition previously. In the older patients there was an elevated serum vitamin A level and irregular or heavy bleeding (figure 2). The vitamin A level was only estimated in 1 of the 2 younger patients and it was found to be subnormal. Both of these patients experienced oligomenorrhoea leading to amenorrhoea. It is realized that amenorrhoea is one of the primary features with food phobias (anorexia nervosa). However, it seems reasonable to suggest that serum vitamin A may play some part in the difference of menstrual behaviour in the 2 groups. It is known that vitamin A and protein nutrition are closely related, both clinically and biochemically. For example, vitamin A cannot be metabolised or transported efficiently when there is protein deficiency (Olson, 1969). The results of different intakes of carotene and protein are shown diagramatically in figure 3.

Carotene is the principal lipochrome in human serum. It is fat soluble and when it is eaten to excess gives a yellow discolouration of the skin, particularly the horny layers of the palms and the soles. The dissolved carotenoids in fresh tissue give it its yellow colour and of course it gives the normal colour to the corpus luteum, the adrenal cortical tissue

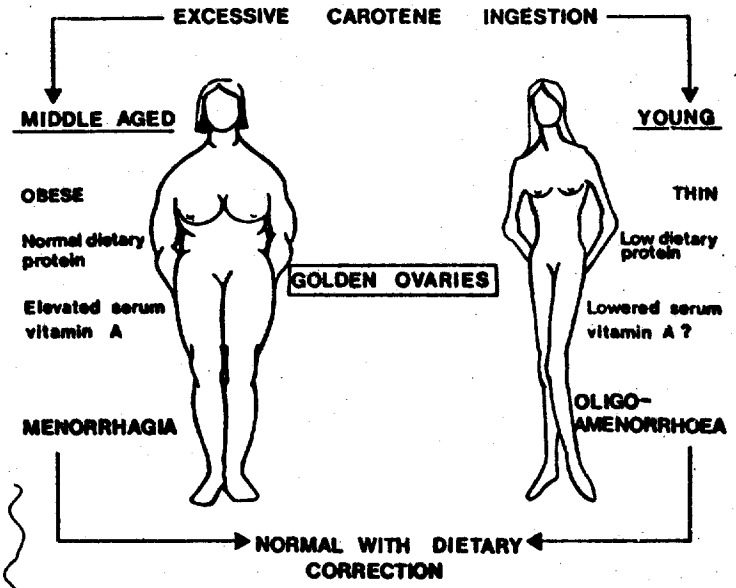

Figure 2. Postulated syndromes in patients with hypercarotenaemia.

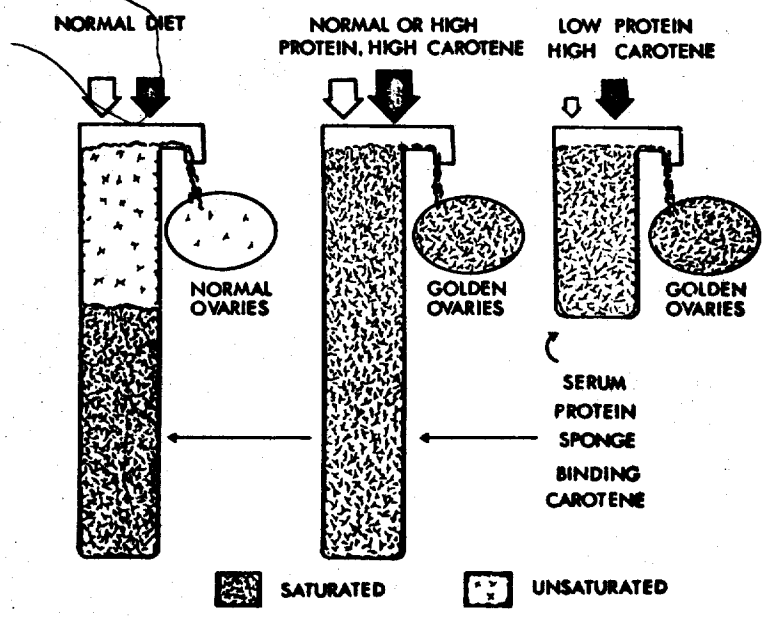

Figure 3. Diagram illustrating the possible relationship of dietary protein intake to the presence of carotenes in tissues such as the ovary. Tissue staining would occur more readily where protein intake was markedly curtailed.

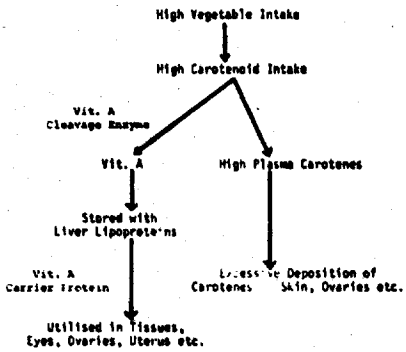

Figure 4. Metabolism of carotenes in the body.

and the body fat, so it is normally deposited in lipid-rich tissues, but in the patients in this study it was deposited throughout the substance of the ovary.

Patients eating abnormal amounts of carotenoids can convert them to large amounts of vitamin A. The normal daily requirement of vitamin A is 4,000 units and there are approximately 8000 units of vitamin A activity in a quarter pound of carrots, so from their dietary intake one can see that these people would have excessive amounts of vitamin A in their serum. Formerly, the only importance attached to carotenaemia lay in its recognition, since it was regarded as harmless (Abrahamson and Abrahamson, 1962).

Vitamin A has a complex absorption and storage mechanism, being totally dependent on carrier protein complexes for transport and storage (figure 4). Vegetables containing carotene form an important part of vitamin A metabolism. The amount of beta-carotene equivalent to one international unit of vitamin A differs according to the source of the carotene and is not constant for each individual (Davidson and Passmore, 1967).

It is not unreasonable to suggest that the 4 patients in Group 1 were transporting and storing excessive amounts of vitamin A and it is well documented that haemorrhage may occur in patients with vitamin A intoxication. The exact reason for this has not been explained but has been postulated that it is due to hypoprothrombinaemia (Light et al., 1944).

Carotenaemia is often described in association with elevated cholesterol levels and most of our patients showed evidence of this, but carotenaemia has been described without elevated serum vitamin A levels.

The association of hypercarotenaemia and anorexia nervosa was first described by Pops and Schwabe (1968) who reported a series of 12 cases. In 8 of these patients no history of unusual ingestion of carotene-containing foods could be obtained. It is important, in patients with primary weight phobias, to enquire carefully into their nutritional as well as their psychiatric history. An investigation of these patients will often reveal elevated serum carotene and cholesterol levels. If culdoscopy or laparoscopy is carried out in the investigation of diminished or absent menstrual function, the typical golden appearance of the ovaries may be seen.

Acknowledgements

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DDT AND THE METABOLISM OF VITAMIN A AND CAROTENE IN THE RAT[1]

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Abstract

The utilization of vitamin A and carotene has been studied in the rat following the feeding of DDT in the diet over a range of concentrations from 0 to 100 p.p.m. for periods up to 72 days. In two of three experiments, the feeding of over 10 p.p.m. DDT decreased the utilization of orally administered carotene. In two experiments, the feeding of 10 p.p.m. DDT or over decreased liver storage of vitamin A when vitamin A per se was administered. The storage of vitamin A was similarly reduced when a diet containing both DDT and carotene or DDT and vitamin A was fed.

Introduction

The requirement of an animal for vitamin A may be modified by a number of stress conditions. This is an important consideration when estimating the nutrients required by livestock. Field reports suggest that the incidence of vitamin A deficiency in bovines may be greater than previously suspected. It is recognized that dietary factors may influence the absorption or utilization of vitamin A or the provitamins and thus contribute to problems of vitamin A deficiency. Recently, attention has been directed towards the influence of nitrates and nitrites (1, 2), energy level (2), and protein content of the diet (3). The consumption of naturally occurring goitrogenic substances or feed additives having antithyroid activity are also known to influence vitamin A metabolism. Page *et al.* (4) have studied the effects of high temperature environmental stress on vitamin A metabolism and low temperature stress has been studied by Ershoff (5) and Phillips (6). It is probable that the increased incidence of vitamin A deficiency is not related to a single factor but is of multiple etiology.

It is interesting to note that the bovine hyperkeratosis observed in the States of New York, Pennsylvania, and Maryland, and fully described by Olafson (7), could be produced experimentally by the administration of chlorinated naphthalene compounds (8), or lubricants containing naphthalene additives (9). Hoekstra *et al.* (10) have also produced hyperkeratosis in calves by the topical application of an oil-based insecticide carrier. Whether DDT (2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane) would influence vitamin A metabolism in a manner similar to the chlorinated naphthalenes is not known. Because of the increased use of chlorinated hydrocarbons as insecticides in North American agriculture during the past decade, it was considered essential that their relationship to vitamin A metabolism be studied. The results reported here are

[1]Contribution No. 126 from the Animal Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

those of initial studies in a project on the effect of pesticides and herbicides on vitamin A metabolism.

Experimental

Materials and Methods

Albino rats of the Sprague–Dawley strain from the Institute colony were used in all experiments. The animals were weaned at 21 days of age and placed on a semipurified diet (11), to which was added stabilized vitamin A[2] to give a final potency equivalent to 121 μg of vitamin A alcohol/kg basal diet until the initiation of the experimental period as shown below. The diet was known to be adequate for growth without permitting appreciable liver storage. The treated animals received the above diet to which was added designated amounts of the para–para isomer of DDT.[3] Dosing solutions of crystalline carotene (100% beta) and of crystalline vitamin A alcohol were prepared in cottonseed oil. The volume of the dosing solution in experiments 1 to 5 was 0.5 ml. Animals were dosed orally by stomach tube and, following a specified time, the livers were excised. Liver vitamin A was determined as previously described (6). All animals exhibiting any evidence of an upper respiratory infection during the experimental period were immediately discarded. The data for liver weight, μg of vitamin A per g of liver, and μg of vitamin A per liver were subjected to an analysis of variance using the method outlined by Snedecor (12).

Experiment 1

Eighty male rats, 30 days of age, were divided into four groups. Each group was fed one of four diets containing DDT at a level of 0, 1.0, 10.0, or 100 p.p.m. for a period of 72 days. On day 71, 15 animals from each group were dosed orally with a cottonseed oil solution containing 1.28 mg of β-carotene. All animals were killed 24 hours after dosing.

Experiment 2

Sixty female rats, 24 days of age at the initiation of the experiment, were fed for 31 days on one of four experimental diets. DDT was added to the basal diet at levels of 0, 0.2, 2.0, or 20.0 p.p.m. Three animals in each group served as non-dosed controls. The remaining animals received an oral dose of cottonseed oil containing 0.91 mg β-carotene. Livers were excised 24 hours after dosing.

Experiment 3

This experiment, using 60 female rats, was similar to experiments 1 and 2 except that the duration of the experimental feeding period was 70 days and the levels of dietary DDT were 0, 0.7, 7.0, or 70 p.p.m. Animals were 32 days of age at the initiation of the experiment. The animals were dosed on day 69 with a cottonseed oil solution containing 0.729 mg of β-carotene and 0.5 mg of α-tocopherol. Livers were excised 24 hours after dosing.

[2]Nopcay "30" type V, Nopco Chemical Co., Harrison, N.J.
[3]Nutritional Biochemicals Corp., Cleveland, Ohio.

Experiments 4 and 5

In experiment 4, 80 male rats, 30 days of age, were divided into four groups and fed one of four diets containing DDT at a level of 0, 1.0, 10.0, or 100 p.p.m. for 63 days. On day 62, all animals received an oral dose of 260 μg all-*trans*-vitamin A alcohol and 0.5 mg α-tocopherol in cottonseed oil. Livers were excised 24 hours after dosing. Experiment 5 was a replicate of experiment 4 except that the dosing solution contained only 190 μg of all-*trans*-vitamin A alcohol with no tocopherol.

Experiments 6 and 7

These experiments were designed to study the effects of concomitant feeding of DDT and β-carotene. The basal diet of experiment 6 contained 3.84 mg β-carotene in crystalline form per kg of diet while in experiment 7 the diet contained 6.0 mg β-carotene. In each experiment, 30 female rats, 22 days of age in experiment 6 and 33 days of age in experiment 7, were assigned to each of four treatments of DDT (0, 1.0, 10.0, or 100 p.p.m. of diet). Half the number of animals were killed on the 30th day of feeding and the remainder on the 60th day of feeding.

Experiment 8

This experiment, using 120 female rats, was similar to experiments 6 and 7 except that vitamin A (Nopcay 30) was added to the basal diet to give a potency equivalent to 1800 μg vitamin A alcohol/kg diet. The animals were 29 days of age when the feeding of the experimental diets was begun.

Results

Table I demonstrates that the feeding of up to 100 p.p.m. of DDT in the diet of rats did not impair growth to 73 days of age. A physiological response to all levels of DDT was apparent, however, in that the liver weight increased as a percentage of body weight. Treatment significantly increased liver weight ($P = 0.01$) and the level of treatment evoked highly significant increases. The liver weight of animals receiving 100 p.p.m. DDT was significantly greater than when 10 p.p.m. DDT was fed, which in turn was greater than when 1.0 p.p.m. DDT was fed. Utilization of orally administered carotene as estimated by liver storage of vitamin A was less in those animals receiving 100 p.p.m. DDT in the diet. Both the concentration of vitamin A (μg per g liver) and the total amount of vitamin A (μg per liver) were significantly lower ($P = 0.01$) in those animals receiving 100 p.p.m. but no significant differences were observed between treatment levels of 0, 1.0, or 10.0 p.p.m. DDT. The total amount of vitamin A stored from a given dose of carotene was approximately 26% less in animals fed diets containing 100 p.p.m. of DDT than in those whose diets contained 0, 1.0, or 10 p.p.m. of DDT.

In experiment 2, the concentrations of DDT in the diet were reduced by a factor of 5 and the duration of feeding was only 31 days. Observations similar to experiment 1 were recorded confirming the previous experiment. Table II shows that, although the rate of growth was not influenced by DDT, the

TABLE I

Effect of ingestion* of DDT on the utilization of orally administered carotene (experiment 1)

Group	DDT in diet (p.p.m.)	No. animals	Dose†	Initial body wt. (g)	Live wt. gain (g)	Liver wt. (g) ± S.E.‡	Liver as % body wt.	μg vitamin A per g liver	μg vitamin A per liver	μg vit. A stored§
A	Nil	5	—	51	235	11.42±0.69	4.00	0.4±0.05	4.2±0.4	
A		14	+	52	244	13.28±0.25	4.48	6.2±0.3	82.2±4.1	78.0
B	1.0	5	—	51	234	13.19±0.59	4.62	0.4±0.08	5.3±0.9	
B		13	+	52	246	13.50±0.24	4.53	6.5±0.5	87.7±6.0	81.2
C	10.0	5	—	51	223	13.12±0.21	4.80	0.4±0.04	5.0±0.6	
C		13	+	52	248	14.67±0.35	4.88	5.5±0.4	84.9±4.3	79.9
D	100.0	5	—	51	235	14.99±0.54	5.24	0.4±0.06	6.1±1.1	
D		14	+	52	231	15.83±0.47	5.60	3.7±0.3	59.0±6.3	52.9

*Duration of feeding, 72 days.
†Dose: minus sign indicates no dose, plus sign indicates 1.28 mg β-carotene.
‡S.E., standard error of the mean.
§Total liver vitamin A of dosed animals minus total liver vitamin A of non-dosed animals.

TABLE II

Effect of ingestion* of DDT on the utilization of β-carotene (experiment 2)

Group	DDT in diet (p.p.m.)	No. animals	Dose†	Initial body wt. (g)	Live wt. gain (g)	Liver wt. (g) ± S.E.‡	Liver as % body wt.	μg vitamin A per g liver	μg vitamin A per liver	μg vit. A stored
A	Nil	3	—	46	101	6.80±.66	4.63	0.3±0.1	1.8±1.0	
A		9	+	45	104	6.67±.30	4.48	7.1±0.9	47.4±6.6	45.6
B	0.2	3	—	45	94	6.68±.57	4.81	0.1±0.1	0.9±0.6	
B		12	+	45	96	6.71±.21	4.76	5.7±0.6	38.1±3.3	37.2
C	2.0	3	—	44	100	6.88±.20	4.78	0.2±0.03	1.2±0.2	
C		12	+	46	97	6.94±.20	4.85	6.4±0.5	44.4±3.9	43.2
D	20.0	3	—	47	101	8.53±.36	5.76	0.1±0.1	0.9±0.6	
D		12	+	45	99	7.99±.21	5.55	3.9±0.6	29.7±4.0	28.8

*Duration of feeding, 31 days.
†Dose: minus sign indicates no dose, plus sign indicates 0.91 mg β-carotene.
‡S.E., standard error of the mean.

liver weight as a percentage of body weight did increase and the feeding of 20 p.p.m. DDT significantly ($P = 0.01$) increased liver weight. No significant differences were observed between the liver weights of animals fed 0, 0.2, or 2.0 p.p.m. DDT. Both the concentration and total amount of vitamin A in the liver were decreased by treatment. Twenty p.p.m. of DDT in the diet exerted a highly significant effect, greater than that exerted by 0, 0.2, or 2.0 p.p.m.

The results of experiment 3 are shown in Table III. Again, body weight and liver weight responded as in the previous experiments. The feeding of up to 70 p.p.m. DDT in the diet for 70 days did not influence the concentration or total amount of vitamin A stored when carotene was dosed orally. The carotene dosing solution contained 0.5 mg of α-tocopherol in experiment 3 but only β-carotene in cottonseed oil was administered in experiments 1 and 2. The reason for a lack of an effect of DDT on carotene metabolism in experiment 3 is not known. It is doubtful that the presence of tocopherol was significant, since it will be observed in experiment 4 that tocopherol did not modify the effect of DDT.

From the previous experiments, it could not be determined whether the effect of DDT was to decrease the conversion of β-carotene to vitamin A or to modify the transport or storage of vitamin A per se. Table IV shows the influence of feeding up to 100 p.p.m. of DDT for 63 days on the utilization of orally administered vitamin A (experiment 4). Of prime significance is the observation that feeding 10 or 100 p.p.m. DDT (groups C and D) significantly reduced the concentration and total amount of vitamin A stored in the liver 24 hours after an oral dose of vitamin A. The results of experiment 5 are shown in Table V. The feeding of DDT significantly reduced the concentration and total liver vitamin A when vitamin A per se was dosed orally; however, there was no significant difference between the various treatment levels.

The ad libitum feeding of a diet containing both DDT and β-carotene produced effects on the liver storage of vitamin A (Table VI) similar to those previously shown when a single dose of carotene was administered orally. Experiments 6 and 7 differed in the amount of carotene added to the basal diet. The vitamin A activity of the diet in experiment 6 was intentionally low to magnify an effect, if any, of DDT. The dramatic effect of DDT was apparent on both the concentration and total amount of vitamin A stored in the liver. At a higher dietary level of vitamin A activity (experiment 7), a similar effect was apparent but to a lesser degree. Similarly, when vitamin A was fed simultaneously with DDT (Table VII), the concentration of liver vitamin A was decreased but the hypertrophy of the liver compensated for this and the total amount of vitamin A stored was not significantly decreased.

Discussion

The initial experiments (1–5) are primarily of academic interest. They demonstrate very clearly that the feeding of over 10 p.p.m. DDT in the diet

TABLE III

Effect of ingestion* of DDT on the metabolism of β-carotene (experiment 3)

Group	DDT in diet† (p.p.m.)	No. animals	Initial body wt. (g)	Live wt. gain (g)	Liver wt. (g) ± S.E.‡	Liver as % body wt.	μg vitamin A per g liver	μg vitamin A per liver
A	Nil	14	87	134	8.46±0.21	3.83	4.6±0.4	38±3
B	0.7	15	87	131	9.26±0.23	4.25	3.7±0.3	34±3
C	7.0	10	87	127	9.39±0.24	4.43	4.1±0.5	39±5
D	70.0	13	87	128	11.61±0.31	5.40	3.7±0.4	42±4

*Duration of experiment, 70 days.
†All animals dosed with 0.729 mg β-carotene and 0.5 mg α-tocopherol.
‡S.E., standard error of the mean.

TABLE IV

Effect of ingestion* of DDT on the utilization of orally administered vitamin A† (experiment 4)

Group	DDT in diet (p.p.m.)	No. animals	Initial body wt. (g)	Final body wt. (g)	Liver wt. (g) ± S.E.‡	Liver as % body wt.	μg vitamin A per g liver	μg vitamin A per liver
A	Nil	15	80	297	12.87±0.40	4.33	2.8±0.3	35.4±4.1
B	1.0	20	80	296	13.01±0.24	4.40	2.9±0.3	36.9±3.2
C	10.0	20	80	297	14.26±0.28	4.80	1.7±0.2	23.1±2.2
D	100.0	20	80	295	16.97±0.46	5.75	0.7±0.1	11.4±1.6

*Duration of feeding, 63 days.
†All animals received an oral dose containing 260 μg of vitamin A alcohol plus 0.5 mg α-tocopherol.
‡S.E., standard error of the mean.

TABLE V

Effect of ingestion* of DDT on the utilization of orally administered vitamin A† (experiment 5)

Group	DDT in diet (p.p.m.)	No. animals	Initial body wt. (g)	Final body wt. (g)	Liver wt. (g) ± S.E.‡	Liver as % body wt.	μg vitamin A per g liver	μg vitamin A per liver
A	Nil	17	55	173	8.63±0.27	4.99	1.3±0.2	11.0±1.3
B	1.0	14	55	166	9.58±0.54	5.77	1.1±0.1	9.7±0.9
C	10.0	18	55	169	8.85±0.24	5.24	0.9±0.1	7.8±0.5
D	100.0	20	55	164	10.37±0.34	6.33	0.7±0.1	6.7±0.8

*Duration of feeding, 63 days.
†Dose, 190 μg of vitamin A alcohol.
‡S.E., standard error of the mean.

TABLE VI

The effect of concomitant feeding of DDT and β-carotene*

Group	DDT in diet (p.p.m.)	Duration (days)	No. animals		Initial body wt. (g)		Final body wt. (g)		Liver wt. (g)		Liver wt. as % body wt.		μg vitamin A per g liver		μg vitamin A per liver	
			Expt. 6	Expt. 7	Expt. 6	Expt. 7	Expt. 6	Expt. 7	Expt. 6	Expt. 7	Expt. 6	Expt. 7	Expt. 6	Expt. 7	Expt. 6	Expt. 7
A	Nil	30	15	11	45	62	129	157	8.34	8.00	6.47	5.10	0.9	5.0	7.3	39.3
B	1.0	30	15	13	45	62	130	154	8.21	8.04	6.32	5.22	1.3	4.1	10.9	33.3
C	10.0	30	15	15	45	62	134	163	7.75	8.87	5.78	5.44	0.7	3.3	5.4	29.7
D	100.0	30	15	15	46	63	129	158	9.60	9.58	7.44	6.06	0.3	2.6	3.2	24.8
A	Nil	60	15	14	45	62	193	199	8.23	8.89	4.26	4.42	0.8	3.8	6.3	33.0
B	1.0	60	15	15	45	62	198	202	7.93	8.46	4.01	4.15	1.1	6.0	8.6	49.2
C	10.0	60	15	15	46	63	203	197	9.07	9.06	4.47	4.60	0.8	2.9	6.8	25.8
D	100.0	60	15	15	46	63	191	202	9.37	9.77	4.91	4.64	0.4	3.0	3.5	29.7

*The diet contained 3.84 mg β-carotene per kg of diet in experiment 6 and 6.0 mg β-carotene per kg of diet in experiment 7.

TABLE VII

The effect of concomitant feeding of DDT and vitamin A* (experiment 8)

Group	DDT in diet (p.p.m.)	Duration (days)	No. animals	Initial body wt. (g)	Final body wt. (g)	Liver wt. (g) ± S.E.†	Liver as % body wt.	μg vitamin A per g liver	μg vitamin A per liver
A	0	30	15	53	154	8.10±0.26	5.26	14.3±0.6	116±5.2
B	1.0	30	15	52	155	8.72±0.25	5.63	14.4±0.5	124±3.5
C	10.0	30	14	53	160	9.09±0.29	5.68	13.8±0.6	125±6.0
D	100.0	30	15	52	153	10.11±0.33	6.61	9.5±0.5	96±5.4
A	0	60	15	53	189	8.10±0.25	4.29	20.9±0.5	169±5.8
B	1.0	60	15	53	198	9.03±0.28	4.56	20.8±0.5	188±7.0
C	10.0	60	15	53	195	8.94±0.22	4.58	20.0±0.4	179±6.1
D	100.0	60	15	53	198	10.09±0.21	5.10	16.4±0.7	165±7.0

*The diet contained the equivalent of 1800 μg vitamin A alcohol per kg of diet.
†S.E., standard error of the mean.

impairs the utilization or storage of vitamin A and β-carotene. It is recognized that the repeated exposure to DDT results in the storage of DDT and its metabolic products in fatty tissue and evokes a non-specific degenerative change in liver tissue. The magnitude of the dose required to produce histological changes in the hepatic tissue is subject to controversy. A morphological effect of DDT was reported by Laug *et al.* (16), when rats were fed 5 p.p.m. DDT in the diet. Changes included cytoplasmic hypertrophy, increased cytoplasmic oxyphilia, and margination of the basophilic granules. Haag *et al.* (17), however, did not demonstrate such changes when rats were fed DDT up to 100 p.p.m. When still higher doses of up to 36.0 mg/kg per day, corresponding to 350 p.p.m. of DDT in the total diet, were administered for 63 weeks (18), liver damage characteristic of DDT poisoning could not be detected in the experimental animals. In the present experiments, DDT was fed in the diet up to a corresponding value of approximately 5 mg/kg body wt. per day, and since gross pathological changes in the liver would not be expected at such levels, no histopathological examination of the liver was undertaken. However, DDT at all levels did affect liver mass. The increase in liver size has been reported by many groups of workers (19, 20). Sarett and Jandorf (20) have shown the increase to be due to an increase in the ether-soluble fraction of the liver while no increase was found in the percentage of water or glycogen. Since effects of DDT were similar whether carotene or vitamin A was administered, it can be concluded that the action of DDT is not primarily on the conversion of carotene to vitamin A but at some stage in their metabolism similar to both. The decrease in liver vitamin A following DDT ingestion may result from an aberration of lipid metabolism rather than from hepatic damage per se.

Although vitamin A has been implicated in detoxication mechanisms such as sodium benzoate (21), and monobromobenzene (22), there is no indication that the decrease in liver vitamin A following DDT ingestion is due to an increased requirement for vitamin A. Preliminary unpublished results on the rate of metabolism of hepatic vitamin A in control and DDT-fed animals do not show significant differences.

Of more practical significance are the feeding trials in which the diet contained both DDT and β-carotene or vitamin A. In a recent review by Dicker (13), it is pointed out that when DDT is sprayed at normally recommended rates (1½ lb/acre), the initial residue on the forage is 211 p.p.m. which decreases to 30 p.p.m. at 7 days and 15 p.p.m. at 13 days. Analysis of restaurant and institutional meals in the United States in 1954 and 1956 (14, 15) demonstrated that the average human consumed approximately 200 μg of DDT and smaller amounts of DDE (2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene) daily in his food. More recently, Read and McKinley (23) have shown from Canadian studies that human fat stores contained up to 14.3 p.p.m. of DDT and DDE combined. There is no evidence that either storage or ingestion of DDT at the levels cited has any harmful effects on the human. The present results suggest that further research on the effects of DDT and other insecticides on the

metabolism of the fat-soluble vitamins should be conducted with animal species of economic importance (and perhaps also with humans), in view of the increasing use of insecticides in the agricultural industry. It is recognized that, in contrast to the rat, the efficacy of carotene is low in bovines and humans; hence the effects of DDT may be more significant in these species, particularly if they are consuming marginal amounts of provitamin or vitamin A. It must be noted that Hoekstra *et al.* (10) demonstrated that an oil-based insecticide vehicle produced a hyperkeratosis-like disease in calves but the addition of active insect toxicants to the oil did not enhance the toxicity; however, the excellent study did not include DDT.

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Absorption of Dietary Carotenes in Human Subjects[1]

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CAROTENE DERIVED FROM plant foods forms the chief source of vitamin A in the diets of many population groups in developing countries, including India. It has been reported by several investigators that the plant carotenes are not fully absorbed by human subjects and the absorption has been found to vary over a wide range (1). The extent of availability of dietary carotenes, therefore, assumes importance in the context of recommending dietary allowance for vitamin A for Indians. Based on the published values for carotene absorption, the FAO/WHO Expert Group (1) on vitamin requirements assumed that one third of β-carotene present in foods is available to the human system. The Group, however, considered that the available data on carotene absorption are few and variable and indicated the necessity for further research. Studies relating to the utilization of carotenes from different vegetable foodstuffs by Indian subjects have so far been limited (2).

The results of an investigation undertaken to determine the absorption of carotenes from different food sources commonly present in habitual Indian diets are presented here.

MATERIALS AND METHODS

Subjects

Four healthy young male adults served as subjects of the study and were kept in the metabolism ward of the Laboratories during the entire period.

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Diets

Throughout the experiment, the subjects received a basal diet, the composition of which is given in Table I. The basal diet was constituted so as to contain minimal amounts of carotene. On analysis, the total daily diet was found to contain 1,125 μg of carotene of which 124 μg was β-carotene, most of which was derived from condiments in the diet. The amount of noncarotene foods in the basal diet fed to each subject was adjusted according to the individual preference and this amount was kept constant throughout the experiment. The protein and carotene intake in the four subjects was, however, kept the same. The diet for each subject was cooked separately in the metabolic kitchen, and it was ensured that the subjects ate the entire meals served to them.

Experimental Procedure

The experiment was divided into six periods of 4 days duration each. During the first period, only the basal diet was fed. In subsequent periods, each of the following foods, which served as a source of carotene, was added to the basal diet, in this order: *a*) amaranth (*Amarantus tricolor*), *b*) carrot (*Daucus carota*), *c*) papaya (*Carica papaya*), *d*) crystalline β-carotene (E. Merck Ag. Darmastadt), and *e*) mixed source of carotene, namely, corn, spinach, orange, and peas. Intake of total carotenes from these sources as fed ranged from 4,253 to 9,840 μg. This level was chosen so as to provide approximately a day's requirement of carotene equivalents as recommended by the FAO/WHO Expert Group (1). Each of the supplements were included as a single dose in the first meal of the 1st day of the respective experimental period, except in the case of the mixed source of β-carotene (e), which was distributed over the entire 1st day's meals. All the supplements except fruits and β-carotene were fed as

TABLE I

Composition of the diets, g

Food article	Basal diet[a]	Diet with mixed sources of carotene
Rice	340	
Corn		300
Wheat flour		100
Bread	100	
Sago	100	75
Greengram dal	25	
Redgram dal	30	15
Skimmed milk powder	30	10
White radish or cauliflower	100	
Calabash cucumber or cucumber	100	
Potato	100	
Spinach		30
Orange		190
Peas		120
Onions	125	125
Groundnut oil	15	15
Jam	40	60
Sugar	60	137

[a] The carotene supplement given in addition to the basal diet was amaranth, 100 g; carrot, 100 g; crystalline β-carotene, 4,500 μg; or papaya, 600 g.

cooked preparations. Fruits were consumed fresh, and the β-carotene was dissolved in about 2 ml of oil, which the subjects consumed along with breakfast.

Collection of Feces

Stool samples were collected on the basal diet for 4 days. Following the carotene supplementation, fecal samples were collected until the fecal content of carotene was the same, on 2 consecutive days, as that observed on the basal diet alone. It was observed that most of the unabsorbed carotene from the ingested sources was excreted within 48 hr after consumption and on the 3rd day the fecal excretion had returned to basal levels. Fecal samples were collected daily and were weighed and blended. Aliquots were analyzed for total carotene and β-carotene, the analysis for total carotene being completed on the day of collection. A known portion of each day's stools was also separately taken in an equal volume of ethanol, and the pooled samples for each period were refrigerated for subsequent analysis.

Analytical Methods

Total carotene was estimated by the method described by Belavady and Balasubramanian (3) employing calcium carbonate for adsorbing non-carotene pigments. Carotenes in the preserved pooled samples of the stools for each period were also determined by chromatography on alumina column (4). The β-carotene content was determined chromatographically using a calcium hydroxide column (5). The total and β-carotene content of amaranth, carrot, papaya, the basal diet, and the diet containing mixed sources of carotene were also determined by a similar procedure as was the β-carotene content of the crystalline β-carotene preparation. Purity was found to be 82%. The β-carotene value as determined was employed for computing absorption. The α-carotene content of carrots and of the corresponding stool samples was determined chromatographically using an alumina column (4).

RESULTS

The total carotene content in the feces collected during the first period on the basal diet varied from 488 to 844 μg/day in the four subjects. The amount of carotene excreted on the basal diet over a period of 4 days was subtracted from the total carotene excreted over a similar period of 4 days after the supplement was given and the difference was taken as carotene unabsorbed from the dietary supplement. From this data, the percent absorption of carotene was calculated. This may represent only apparent absorption since the possibility of bacterial destruction of carotenes in the intestinal tract was not taken into consideration. This is true of all the earlier studies on carotene absorption.

The results obtained regarding the absorption of carotene from different sources in the four subjects are shown in Table II.

There was considerable individual variation in the absorption of total carotene from a given source, as well as from different food sources. Crystalline β-carotene (*d*) was almost completely absorbed by all the four subjects; next in order were carotene from amaranth (*a*), papaya (*c*), carrots (*b*), and the

TABLE II

Absorption of carotene from different sources

Source	Ingested, μg		% Absorption	
	Total carotene	β-Carotene	Total carotene	β-Carotene
Amaranth	5,151	2,926	58.1 (44.1–79.6)	75.7 (52.4–99.8)
Papaya	9,840	3,319	45.8 (19.3–63.5)	90.3 (73.3–99.4)
Carrot	7,288	1,381	35.5 (15.9–67.3)	81.1 (73.2–96.8)
Crystalline β-carotene	4,500	3,679[a]	97.7 (93.2–100.0)	98.8 (95.4–100.0)
Diet with mixed sources[b]	4,253		33.4 (15.7–59.2)	

Values are means of four subjects with range of values in parentheses. [a] Determined by column chromatography on calcium hydroxide (5). [b] Contained yellow maize, spinach, orange, and peas as sources of carotene.

mixed source (*c*). It was observed that the values obtained for the absorption of β-carotene from these sources was much higher than that obtained for total carotene, indicating thereby that among the various carotene fractions present in these foods, β-carotene was better absorbed than the others. Even so, the absorption of β-carotene from foods was not as complete as that observed with pure crystalline β-carotene. The order of absorption of β-carotene from food sources were papaya (*c*), carrot (*b*), and amaranth (*a*).

The values obtained for absorption levels of the two principal carotenoids, α- and β-carotene present in carrots are shown in Table III. It will be seen that absorption of α-carotene was much lower than that of β-carotene.

Chromatography of the fecal samples on alumina showed the presence of several pigment bands that were not present either in the basal diet or in the ingested carotene sources and could have been derived from bacteria present in stools.

The values for carotene absorption levels from different sources were similar whether daily stool samples were analyzed for carotene content immediately, or after storing.

TABLE III

Absorption of α-carotene and β-carotene from carrots

Subject number	β-Carotene, %	α-Carotene, %
1	73.2	31.9
3	96.8	43.5
4	73.3	57.0
Average	81.1	44.1

Results obtained by employing the calcium carbonate method and the chromatographic method using alumina were similar.

DISCUSSION

The results of this study show that the absorption of carotene from various dietary sources varies considerably. This appeared to be true of even β-carotene present in these foods; however, the absorption of β-carotene was found to be much higher than the total carotene. It was observed that crystalline β-carotene was almost completely absorbed—a finding in line with the reported observations of Fujita et al. (6). Also noted here was the fact that the absorption of β-carotene from food sources was not as complete as with

crystalline β-carotene. One of the reasons for the variation in the absorption of total carotene may be that not all carotenes are absorbed to the same extent, and the relative proportions of the various carotenes in a given food may, therefore, influence the absorption. In the foods studied, differences in the extent of absorption of total carotene would appear to be related to the amount of β-carotene present. Thus, the absorption of total carotene was highest from amaranth, which has a high proportion of β-carotene content, whereas the absorption of total carotene from carrots was lowest, which had the lowest amounts of β-carotene. The observation that the other fractions of carotenes are not as well absorbed as β-carotene is supported by a comparison of the absorption of α-carotene and β-carotene from carrots, the absorption of α-carotene being only 50% of that of the β-fraction. Absorption studies in infants by Kubler (7) have shown similar differences.

The finding that the absorption of β-carotene present in food is less complete than that of crystalline β-carotene has practical implications. It would seem significant that β-carotene from papaya is absorbed much better than β-carotene from either carrots or amaranth. The fiber content of the latter two foods is much higher than that in papayas and may be the basis for the observed differences.

While some of the extra pigment bands that appeared on the column in the case of the fecal samples could be attributed to pigments present in the condiments and spices used, the source of others is not clear. These bands were, however, not found in any of the carotene sources. They may be of bacterial origin and preliminary studies suggest that the principal band observed in these fecal samples may be sarcinaxanthin—a pigment reported to be derived from bacteria (8). The identity of other pigment bands needs to be established.

The results of the study show that the leafy vegetable, amaranth, and the fruit, papaya, both readily available and inexpensive, serve as good sources of vitamin A in Indian diets that have a poor content of preformed vitamin A. Absorption of total carotene from these two sources is around 50 to 60% of the intake while the absorption of β-carotene present in them is more than 75%. In light of these observations, the value of 33% for the absorption of dietary β-carotene adopted by the FAO/WHO Expert Group (1) appears to be low. As far as Indian diets are concerned, since the chief source of carotene may be leafy vegetables or fruits, an average of 50% absorption is more realistic. Assuming that the efficiency of conversion of β-carotene into retinol is about 50%, a factor of 0.25 can be suggested for use in converting the dietary β-carotene into retinol, taking into consideration the vitamin A allowance for Indian subjects.

SUMMARY

The absorption of carotenes from a leafy vegetable, a root vegetable, a fruit, crystalline β-carotene, and a mixed vegetable source in four adult male volunteers on diets low in fat was studied.

While crystalline β-carotene was completely absorbed, the mean percentage absorption of carotene from amaranth (*Amarantus tricolor*), carrot (*Daucus carota*), papaya (*Carica papaya*), and the mixed vegetable source were 58, 36, 46, and 33, respectively.

Although absorption of β-carotene from foodstuffs was much better than the other carotenes, it was not as high as pure β-carotene, and that of α-carotene from carrots was found to be less than the β-carotene.

It appears from the present study that availability of carotene from vegetable foods may be 50% or more.

The authors are grateful to Dr. C. Gopalan, Director, for his keen interest and encouragement during this work.

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Ross-'62
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Serum Carotene Concentration in Normal Individuals and Its Clinical Interpretation

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STEATORRHEA and other evidence of malabsorption due to primary or secondary bowel disease is but slowly acquiring recognition. This stems in great part from the fact that the definitive diagnosis of steatorrhea hinges on a quantitative stool fat determination. Such a chemical analysis is a specialized procedure usually not available in routine clinical laboratories. A simpler procedure, readily performed, indicating in very many instances the presence of steatorrhea, and possibly indicating malabsorption of other substances as well, is the determination of the serum carotene concentration. This study presents the normal values in the largest group of individuals thus far reported in the literature.

The reliability of this single, easily performed determination as an indicator of small-bowel dysfunction depends on a variety of factors. The lack of ingestion of a proper diet containing foods rich in carotene such as spinach, lettuce, carrots, tomatoes, liver, and kidney will depress the serum carotene levels even in the presence of normal gastrointestinal function. Abnormal bowel continuity will have the same effect because of the bypassing of a proper absorbing surface, even in the presence of a proper diet. Fever, hyperthyroidism, liver disease, and chronic illnesses tend to lower the serum carotene values. Other diseases such as hypothyroidism, diabetes, nephrosis, and hyperlipemia cause increased serum levels. Excess carotene intake has the same effect on raising serum concentration. It thus becomes important to know the normal serum carotene levels and the range, to use this as a simple screening procedure to detect suspected malabsorption. A 75 per cent correlation between increased stool fat and decreased serum carotene concentration was indicated in a recent study.[1] This is indicative of the value of a simple serum determination as a guide to uncover malabsorptive states and would seem to warrant its more widespread use in clinical medicine.

Method and materials

The method used in this study was that of Yudkin.[2] This procedure was carried out as follows: 2 ml. of 95 per cent ethyl alcohol were added by drops and with shaking to 2 ml. of serum, followed by the addition of 4 ml. of petroleum ether (boiling point 30 to 60 C.). A glass-stoppered 15-ml. tube was used. The contents were shaken vigorously for ten minutes and then centrifuged for five minutes at 1,800 revolutions per minute. An aliquot of the colored supernatant petroleum ether solution containing the extracted carotene was removed and transferred to a suitable cuvette, which was immediately stoppered with a clean cork. The optical density was determined at 440 millimicrons, using the Coleman Jr. spectrophotometer with a 12-mm. cuvette. Its equivalent in micrograms of carotene per 100 ml. serum was evaluated by reference to a standard curve, which was prepared by reading the optical densities of petroleum ether solutions con-

taining known concentrations of carotene (90 per cent beta, 10 per cent alpha).

As a rule, the blood samples were collected between 5:00 and 6:00 P.M. and stored in the refrigerator until the following day, when they were analyzed. Harris *et al.*[3] and Kimble[4] have shown that ordinary meals do not cause a measurable increase in carotene within two to six hours. Yudkin[2] demonstrated that samples of blood may be stored in the refrigerator for forty-eight hours without change. He also showed that slight hemolysis had no effect on the results.

Subjects included in this study were selected at random from a cancer detection clinic. All received a complete physical examination as well as routine blood and urine examinations during their cancer detection investigation. With a few exceptions there was no evidence to indicate disease states which are known to influence the serum carotene level. The exceptions were 9 subjects who were essentially healthy and asymptomatic but whose low serum carotene levels resulted in establishing in 5 a diagnosis of steatorrhea. The remaining 4 were unwilling to submit to further study.

Of the 227 individuals examined, 91 were males and 136 females. These were further subdivided into five-year age groups ranging from fifteen to seventy-five years. Arithmetic means, standard deviations, and medians were calculated for the whole group, for males and females alone, and

SERUM CAROTENE *levels may be used as a simple screening procedure to detect suspected malabsorption. The normal range for serum carotene in 227 apparently healthy individuals on a normal mixed diet was found to be 70 to 300 micrograms per 100 ml. with a mean of 153 and a standard deviation of ± 57. No statistical variation was found according to sex, age, or season. For a proper interpretation of serum carotene values it is important to know the patient's dietary intake for at least the preceding two weeks.*

TABLE I. Summary of values (micrograms per 100 ml. of serum) found in the present study

Age Group	Sex	Number	Median	Mean	Standard Deviation
..	Total group	227	145	153	±57
..	Females	136	152	158	±50
..	Males	91	132	137	±49
15 to 20	F	0	..	..	...
	M	2	..	145	...
21 to 25	F	1	..	162	...
	M	3	104	100	...
26 to 30	F	14	142	144	±32
	M	6	127	125	±23
31 to 35	F	9	148	150	±32
	M	11	109	120	±42
36 to 40	F	11	178	171	±45
	M	8	134	134	±24
41 to 45	F	17	148	147	±37
	M	13	132	139	±32
46 to 50	F	21	172	169	±36
	M	12	160	166	±57
51 to 55	F	23	157	172	±55
	M	10	158	150	±34
56 to 60	F	14	138	146	±47
	M	13	116	125	±33
61 to 65	F	9	125	132	±55
	M	3	132	126	...
66 to 70	F	8	160	182	±50
	M	2	..	150	...
71 to 75	F	2	..	147	...
	M	3	156	139	...

TABLE II. Seasonal variation in normal serum carotene (micrograms per 100 ml. of serum)

Sex and Season	Number of Cases	Mean	Standard Deviation	Median
Males				
Spring	36	138	±37	132
Summer	20	152	±47	148
Fall	30	139	±43	135
Females				
Spring	54	162	±46	152
Summer	35	163	±51	150
Fall	40	159	±48	154
Total group				
Spring	90	152	±44	146
Summer	55	159	±49	150
Fall	70	150	±47	149

for each age group (Table I). Since it has been demonstrated that serum carotene concentrations vary with the season,[2,3,5,6] the values for the entire group were divided into the observations made in the spring, summer, and fall, and the same values were calculated as for the age groups (Table II). Finally, the values were arranged according to the number of observations in each 10-microgram range, starting with 51 to 60 and going up to 411 to 420 micrograms. These data are presented graphically in Figure 1.

Results

Of the 227 values in Figure 1, 215 or 95 per cent fall between 70 and 300 micrograms per 100 ml. There are five values (1.8 per cent) in the 51 to 60 range and seven values (3.1 per cent) between 311 and 470 micrograms. The mean for the total group is 153 micrograms per 100 ml. serum, with a standard deviation of ±57 (Table I). It therefore seems reasonable to exclude from the normal range the five lowest values, which are almost 2 standard deviations below the mean, and the seven highest values, which are 3 to 5 standard deviations above the mean. In calculating the mean, standard deviation, and median for the total group as shown in Table I, all the values were used. However, in calculating these figures for males and females and for the various age groups, these twelve extreme values were omitted. In addition, there are data which indicate that the persons in whom the five low values were found have gastrointestinal disease.[7] In some groups the number of individuals was too small to calculate medians, means, and standard deviations.

The results obtained in the spring, summer, and fall are seen in Table II. The values for females and for the total group in each season are the same, while the mean for males in the summer is somewhat higher than that found in the spring and fall. However, the difference is only one-half a standard deviation and is not significant.

Table III lists the results of other investigations in which the normal serum carotene was determined. Our values agree most closely with those reported by Kimble,[4] Harris *et al.*,[3] and Yudkin,[2] if one excludes Yudkin's winter observations.

Comment

In a study which aims to establish the range of serum carotene in normal individuals, such factors as age, sex, diet, and season must be considered as possible determinants. It can be seen from the data in Table I that between the ages of fifteen and seventy-five, age apparently does not play a role in influencing the level of serum carotene. No important differences in the means for the various age groups are seen. Yarbrough and Dann[8] studied 197 persons between the ages of five months and sixty-eight years. These authors do not present their data so that one may see whether or not any differences related to age were found.

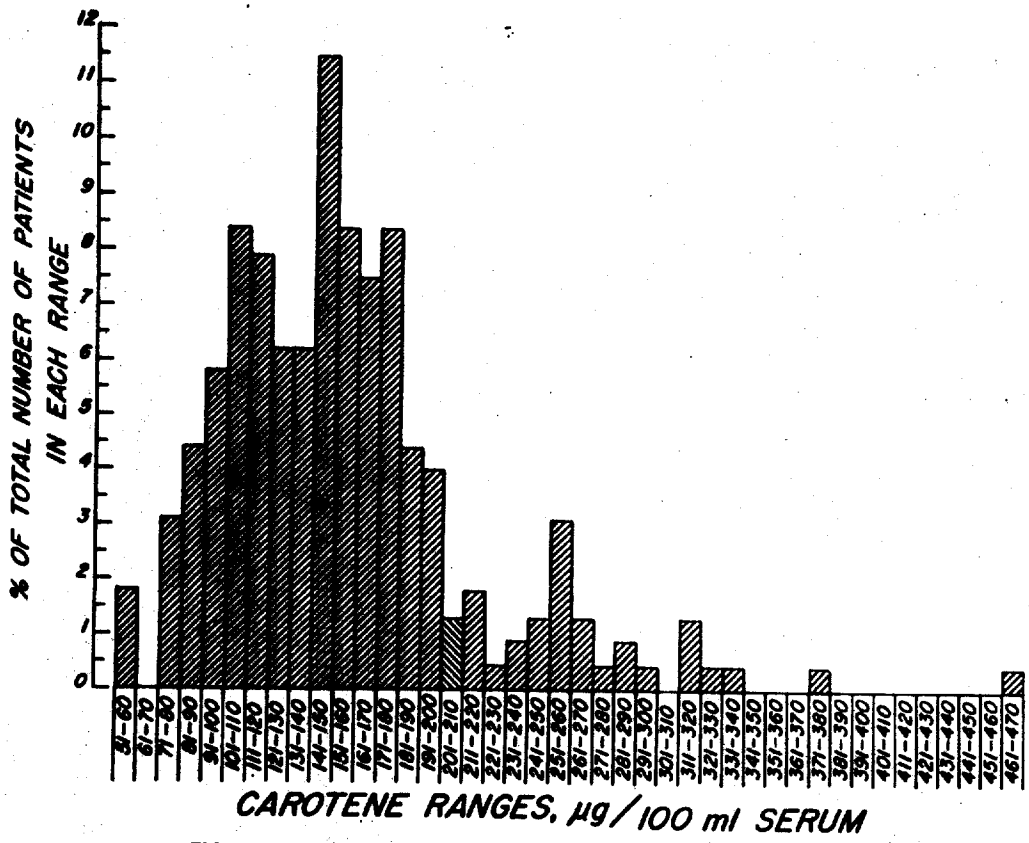

FIGURE 1. Carotene ranges (in micrograms per 100 ml. of serum).

TABLE III. Serum carotene values (in micrograms per 100 ml. of serum) as reported by other investigators

Investigator	Range	Mean	Standard-Deviation	Comment
Murrill *et al.*[11]	100 to 348	199	...	25 males
	136 to 420	227	...	20 females
Harris *et al.*[3]	80 to 370	210	±31	70 subjects
Haworth, Moschette, and Tucker[14]	49 to 202	120	±34	40 females 15 to 22 years of age
Kimble[4]	50 to 300	166	±59	30 males
	90 to 340	187	±77	34 females
Yudkin[5]	117 to 241	158	...	11 subjects, summer
	50 to 118	85	...	12 subjects, winter
Wenger, Kirsner, and Palmer[1]	70 to 282	123	±47	110 subjects
Caveness, Satterfield, and Dann[15]	18 to 278	138	±62	71 healthy persons, chiefly college students on ordinary diet
Yarbrough and Dann[8]	34 to 422	131		197 subjects 5 months to 68 years
Kirk and Chieffi[9]	200 to 320			155 middle-aged and old individuals; 47 younger subjects
Highman[6]	110 to 370	207	...	20 European males
	113 to 340	244	...	20 European females
	100 to 310	162	...	11 Indian males
	30 to 260	131	...	8 Bantu males

Their results are merely stated as a mean for the entire group, with the standard error and range. One may perhaps assume that no differences were found. Kirk and Chieffi[9] investigated 155 middle-aged and old persons and 47 younger ones, and found that while individual values varied greatly, there were no statistically significant differences between different age groups. Their values ranged from 200 to 320 micrograms per 100 ml. May *et al.*[10] studied serum carotene in infants and children ranging in age from four days to twelve years. Their results are expressed in "440 units," which is directly related to the optical density (measured at 440 millimicrons in an Evelyn photoelectric colorimeter) of the petroleum-ether-extracted carotene solution. For this reason, their figures are not quoted. These authors, however, did show that the serum carotene in infants up to five months of age is only about 20 per cent that of infants of nine months and of children up to twelve years. Age, then, seems to be a factor in serum carotene levels only in infants up to about nine months of age.

As Table I shows, the mean for females is 25 micrograms higher than that for males. However, the difference is less than one-half a standard deviation and is probably not significant. Murrill *et al.*,[11] Kimble,[4] and Kirk and Chieffi[9] found similar differences and also concluded that they were of doubtful significance. It would seem, therefore, that there is no important difference between the sexes in carotene concentrations.

Although serum carotene is not subject to important variations as a result of diet in any one day, concentrations may drop sharply within one week as a result of a markedly deficient intake of carotene-containing food. Thus Wald, Brouha, and Johnson[12] have demonstrated that shortly after one week on a diet deficient in carotene, the serum level was found to have decreased from 100 to 200 micrograms per 100 ml. to almost 50 micrograms per 100 ml. and in some instances to traces which could not be measured accurately. Similarly, Brenner and Roberts[13] found, on placing 6 subjects (4 females and 2 males, aged twenty to twenty-four years) on a low carotene diet for twenty to thirty-one weeks, that the values which were 139 to 310 micrograms at the start fell steeply during the first week to low values and then decreased slowly until a plateau was reached. Even at the end of the experimental period, however, the serum was not completely free of carotene, containing, as it did, 30 to 44 micrograms per 100 ml. The diet used provided some carotene, and perhaps this is why the depletion was not more complete. These data seem to indicate that in the normal individual the serum carotene concentration reflects to a great extent its daily intake, and that carotene is not stored to any extent in the body. This is probably what one sees in the seasonal variations found by many investigators.[2,3,6] Harris *et al.*,[3] quote a personal communication from Clausen and McCord, who found that serum carotenoids rose from a May low of 154 micrograms per 100 ml. to a maximum of over 280 micrograms per 100 ml. in September and gradually returned to a minimum the following May. In the present study, no seasonal differences between April and October were found (Table II). This would seem to indicate that in New York City during this period a supply of carotene-containing foods was obtained at all times. For a proper interpretation of serum carotene values it is, therefore, important to know the patients' dietary intake for at least the preceding two weeks.

Summary

1. The normal range for serum carotene in an apparently healthy adult population on a normal mixed diet was found to be 70 to 300 micrograms per 100 ml. of serum with a mean of 153 and a standard deviation of ±57. No seasonal variations were noted in this group from spring into the fall.

2. No statistical variation was found between males and females.

3. Age did not influence the serum carotene concentration in adults.

4. Dietary history is important in the proper interpretation of the serum carotene levels.

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Sharvill-'70
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Meeting November 20 1969

Cases

Familial Hypercarotinæmia and Hypovitaminosis A

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Mrs J W, aged 41. Housewife

History: She complained of increasing yellowness of the skin for a year, her general health remaining good. She had always disliked carrots and had eaten them only in very small quantities and infrequently, none during the previous eight months. She took normal helpings of green vegetables and no excess of any food rich in carotene. No member of her family had yellow skin or any illness which appeared relevant.

On examination: The skin generally presented a yellow appearance, more orange than that seen in jaundice and varying from day to day and according to ambient light. It was most marked on palms and soles, especially pressure areas, sparing the creases. The palms showed a few small callosities, but no diffuse hyperkeratosis. There were thickened, yellow plaques over the dorsa of the ankle joints, suggesting pigmented lichen simplex. The skin did not fluoresce under Wood's light. The scleræ were white and specialist examination showed no abnormality suggestive of vitamin A deficiency. She was overweight but general examination revealed no other abnormality. She appeared to be of above average intelligence and of normal personality.

Investigations: The following tests were normal or negative: Hb, blood films, urinary glucose, glucose tolerance, serum proteins and electrophoresis, PBI, liver function tests, serum cholesterol and triglycerides, fæcal fats, serum folate and vitamin B_{12}, blood urea, calcium, phosphorus.

Fresh serum fluoresced bright green under Wood's light. Electrophoresis of serum lipoproteins showed an increase in α-lipoprotein. Low density lipoprotein 790 mg/100 ml (normal 450–650).

Serum carotene (10 tests): mean 795, range 568–975 μg/100 ml (normal for sample of local population 70–180, published normals 50–200). Serum vitamin A:74–143 i.u./100 ml (normal 80–200). Vitamin A absorption test normal. Chromatography: see report below by Dr I M Sharman.

Investigation of family: Her mother, one of her three siblings and her only son had serum carotene levels persistently above 200 μg/100 ml. A maternal aunt and two siblings were normal. On occasions some of these relatives had low or very low vitamin A levels, but these were on other occasions normal, without obvious dietary explanation. Her mother and son had single readings as low as 18 and 20 i.u./100 ml and it seems unlikely that these were due to technical error.

Comment

This patient is unusual because of the persistently very high carotene while on a normal diet; her levels are among the highest ever recorded. In Cohen's extensive review (1958) patients eating as much as 4–8 lb (1·8–3·6 kg) of carrots daily had levels up to 500 µg/100 ml, associated with high vitamin A. In this family four members out of six examined in three generations had persistently high carotene, and occasionally low vitamin A. Cohen described a 'unique' case, a woman of 38 with raised carotene and low vitamin A. Frenk (1966) described a family in which 2 of 3 children of normal parents had similar biochemical abnormalities, but of lesser degree, associated with a diffuse keratodermic state, and mentioned one other patient who said that her mother had similar skin. It seems possible that this is a genetic disorder in which moderately elevated serum carotene causes no clinical disturbance. Possibly, too, there is an abnormality of conversion of β-carotene into vitamin A, or, perhaps, of storage of vitamin A. Unfortunately, it seems doubtful whether this can be pursued further without unjustifiable disturbance to the patient and her family.

Acknowledgments: I wish to thank Mr N Saunders for numerous, time-consuming investigations, and Dr I M Sharman for his special investigations.

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Dr I M Sharman (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*): I analysed blood specimens from the mother in this case, and found very high figures for total carotenoids but low normal values for vitamin A. A recent specimen contained carotenoids 481 and vitamin A 47·5 µg/100 ml plasma. Of the carotenoids 333 µg β-carotene was found by chromatographic analysis. The high percentage (69·3%) of β-carotene is in keeping with other reported cases of hypercarotinæmia. The β-carotene is identified by the three characteristic absorption peaks (Fig 1).

Examination of the extract in the ultraviolet region showed a remarkably high absorption at about 275 nm. Similar observations were reported by Le Page & Pett (1941, *J. biol. Chem.* 141, 747), who suggested that at this wavelength an oxidation product of vitamin A might occur in extracts of blood from human subjects heavily dosed with vitamin A.

High carotene levels found in the blood in this case might be explained by failure of conversion to vitamin A. A build-up from the small dietary intakes of carotene might then be possible. Normally carotene is converted to vitamin A in the intestinal wall. There are two current theories for the breakdown of the carotene molecule to vitamin A.

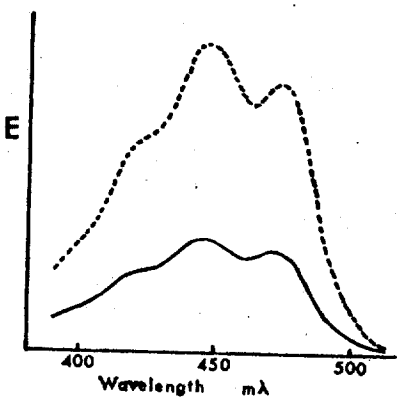

Fig 1 *Extinction curves of extract prepared from patient's plasma (———) compared with that for pure β-carotene (- - - -)*

According to one theory (Karrer *et al.*, 1931, *Helv. chim. Acta* 13, 1036, 1431) vitamin A is formed by a hydrolytic cleavage of the central double bond of the β-carotene molecule whereas in the other, the so-called 'β-oxidation theory' (Glover & Redfern, 1954, *Biochem. J.* 58, xv) cleavage may start at either end of the ethylenic chain. The oxidation continues progressively removing two-carbon units until a C_{20} unit is reached.

Dr Sharvill: I was advised by an ophthalmologist colleague that tests of night vision are not simple and might give misleading results if carried out by those unfamiliar with them. The patient and members of her family have had no difficulty getting about at night, nor during the wartime blackout.

Dr B C Tate: In cœliac disease and other cases of fat intolerance carotene used to be given instead of vitamin A. By this means adequate blood levels of vitamin A were maintained and no hypercarotinæmia resulted.

Dr M L Johnson: Since the conversion of carotene to vitamin A occurs mainly in the intestine, there is an analogy here with Fabry's disease where the absence of ceramidetrihexosidase in the gut can be demonstrated in jejunal biopsy specimens (Brady *et al.*, 1967, *New Engl. J. Med.* 276, 1163). A jejunal biopsy in this patient might be expected to show lowered activity of the relevant enzymes when compared with normal controls.

Sherwood-'36
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Jour. Nutrition 11: 593-597.

CHANGES IN THE VAGINAL EPITHELIUM OF THE RAT ON AN EXCESSIVE VITAMIN A DIET[1]

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(Received for publication February 26, 1936)

Many investigators have reported that excessive cornification, in the vaginal epithelium of the albino rat, is an early manifestation of vitamin A deficiency, Evans and Bishop ('22), Baumann and Steenbock ('32), Aberle ('33), Mason and Ellison ('35).

Evans ('28) noticed that four-fifths of the copulations were unsuccessful due to a lack of proper implantation. He also reported that only about one-fifth of the copulations lead to the birth of litters.

Sherwood, Toth and Carr ('34), Sherwood and Luckner ('35) reported a change in the colloidal content of the thyroid gland and a change in the histological picture when large amounts of cod liver oil, haliver oil and, carotene were administered. It was therefore concluded that the excessive vitamin A added to the normal diet was responsible for a greater activity of the epithelial tissues.

EXPERIMENTAL PROCEDURE

Since the vaginal epithelium was altered as reported by Mason and Ellison ('35) when a diet deficient in vitamin A was administered, it was deemed of sufficient interest to investigate the effect of large amounts of vitamin A on the vaginal tissues.

[1] The carotene used in the present investigation was generously furnished by S. M. A. Corporation, Cleveland, Ohio.

The technic of Long and Evans ('22) was used in the study of the vaginal smear picture. The source of vitamin A was carotene. Corn oil as well as cottonseed oil was used for the control animals. Since cottonseed oil is used as a base for carotene, it was thought necessary to investigate the effect of this oil in order that the results could be interpreted as being due to the carotene or provitamin, and not to the oil.

A group of eighteen animals, 150 days of age, were examined at 8-hour intervals for the normal vaginal smear content until eight complete oestrous cycles had been determined. These animals were then placed on 1500 international units of carotene daily, in addition to the normal diet, for a period of 15 days. The vaginal smear technic was continued during the carotene administration. Vaginal smears were examined after the carotene was discontinued until eight normal oestrous cycles had again been observed.

A second group of sixteen albino rats, 150 days of age, were used and the procedure repeated as mentioned above with the exception that 3750 international units of carotene were given daily for a period of 15 days.

Both experimental and control animals were fed dried skim milk, dried meat, cod liver oil, wheat germ, yellow corn grits, black-strap molasses, iodized salt, organic minerals, wheat cereal, oat cereal, corn cereal and vegetable fiber. This material was given in the form of a stock dog food[2]. This diet was supplemented with fresh whole milk and green lettuce.

RESULTS

The control animals showed no change in the vaginal smear even though they had received 0.5 cc. of cottonseed oil daily for a period of 15 days. The rats that were administered corn oil did not show a change in the vaginal picture. These rats demonstrated an oestrous cycle of approximately 4½ days in every case.

[2] Purina dog chow obtained from the Purina Co., St. Louis.

Table 1 represents the averages of both control and experimental animals. Five individual animals of each experimental group are also presented for the purpose of showing animal variations. The vaginal smears were observed to be abnormal within 2 days following carotene administration. The smears did not progress from the nucleated epithelial to the cornified cell stage.

TABLE 1

The effect of vitamin A given in the form of carotene on the vaginal smear picture

RAT NO.	NORMAL CYCLES	AVERAGE DAYS	DAYS ABNORMAL	NORMAL CYCLES	AVERAGE DAYS	REMARKS
1	8	4.5	32	7	4.5	Animals 1–5 1500 international units daily for 15 days
2	4	4.4	33	5	4.5	
3	6	4.5	34	4	4.6	
4	6	4.5	34	5	4.7	
5	7	4.4	34	5	4.6	
6	8	4.5	34	8	4.4	Animals 6–10 3750 international units daily for 15 days
7	8	4.5	31	5	4.0	
8	5	4.5	31	5	4.0	
9	5	4.5	31	5	4.4	
10	7	4.5	31	5	4.2	
11	5	4.2	—	5	4.2	Cottonseed oil controls
12	5	4.2	—	5	4.2	
Average of animals on 1500 units of vitamin A—18 rats						
	7	4.3	34	6	4.5	
Average of animals on 3750 units of vitamin A—16 rats						
	6	4.4	31	5	4.2	

Upon examination of the vaginal smears an excess of nucleated epithelial cells were seen, regardless of the phase of the oestrous cycle. Leucocytes were also observed continuously but were not as numerous as were the epithelial cells. Occasionally a few scattered cornified cells were observed which might have been obtained from the orifice of the vagina.

The normal oestrous cycle was not observed for approximately 20 days after the carotene feeding had been discontinued.

DISCUSSION

Baumann and Steenbock ('32) observed dioestrous smears within 1 week and oestrum in 2 weeks after the administration of carotene to rats that had been held on a diet deficient in vitamin A. The rapid return to normal seems to indicate a powerful vitamin effect on the growth of epithelial tissues.

The results obtained in the present investigation seem to be due to a rapid cell growth as indicated by the large number of young nucleated epithelial cells which predominated throughout the experimental phase of the problem.

The carotene must have been converted into vitamin A in excessive amounts since the rapid growth was noticed within 2 days after experimentation had begun.

Since the control rats gave no indication of a changed vaginal smear picture, it is clearly demonstrated that the change must have been brought about through the effect of large amounts of vitamin A in the experimental animals. This phenomenon must be expected since vitamin A has an effect on the growth processes of the animal in general. However, it is not to be expected that any normal growing process would cause a change in the oestrous cycle as abnormal as reported in the present investigation.

Further studies are being made for the purpose of finding a possible effect elsewhere in the organism which might be directly or indirectly responsible for the changes observed in the vaginal smears. The animals did not show a desire to copulate in most instances. When the animals did copulate, no pregnancy resulted until the vitamin effect had disappeared. One rat was left with the male for a period of 10 days and still gave the abnormal vaginal smear picture.

SUMMARY

Carotene in oil given daily for 15 days and representing as many as 3750 international units of vitamin A prevented directly or indirectly a normal vaginal smear picture.

Control rats that had received only the normal diet showed a normal oestrous cycle throughout the investigation.

Control rats given cottonseed oil and corn oil also exhibited a normal vaginal picture as observed by the smear technic.

Carotene in large amounts produced a rapid growth of the epithelium for a period of approximately 30 days. This was indicated by the large number of young nucleated epithelial cells which predominated in the vaginal smears.

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Sherwood-'37
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FURTHER STUDIES ON THE EFFECT OF EXCESSIVE VITAMIN A ON THE OESTROUS CYCLE OF THE RAT

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Baumann and Steenbock ('32), Mason and Ellison ('35) showed the effect of avitaminosis A; Sherwood, Brend and Roper ('36) showed the effect of hypervitaminosis A on the vaginal smear of rats. It was therefore deemed advisable in the present investigation to study the effect of excessive vitamin A administration upon the oestrous cycle. A study of the vaginal smear, the uterine and ovarian picture was made to show this phenomenon. The procedure of this investigation was designed to show if possible, the mechanism responsible for these changes.

EXPERIMENTAL

Vaginal smears were obtained on a group of twenty-six rats approximately 150 days of age. From four to thirteen oestrous cycles were observed for the purpose of determining the length of the normal cycle in these animals. Fifteen of the rats were then injected daily with 1500 international units of carotene [1] for a period of 5 to 15 days. Eleven animals were fed 1500 international units of carotene daily for a period of 5 to 32 days. The smears were taken on all animals during the period of vitamin A administration.

Five of the animals were killed after the carotene had been administered 5 to 10 days. Sixteen animals were killed at

[1] The carotene used in the present investigation was generously furnished by S.M.A. Corporation, Cleveland. Ohio.

intervals after carotene had been administered 11 to 32 days. Five rats were allowed to return to the normal oestrous cycle following carotene administration.

In order to prevent a cyclic disturbance due to an upset in nutrition and environment, the animals were kept in a room of constant temperature and given a well-balanced stock diet (Purina chow).

TABLE 1

The effect of carotene injections upon the vaginal smear, uterus and ovary

RAT NO.	NORMAL CYCLES	AVERAGE CYCLE	DAYS OF THERAPY	ABNORMAL	UTERUS	OVARY	ANIMAL KILLED
		days		*days*			
1	10	4.4	5	3	Proestrus 3.0 mm.	Follicles med. and large	5th day
2	13	4.2	7	5	Proestrus 2.3 mm.	Follicles med. and C.L.	7th day
3	13	4.1	10	8	Proestrus 2.7 mm.	Follicles large	10th day
4	8	4.6	10	8	Diocstrus 2.2 mm.	C.L. large	10th day
5	11	4.5	12	10	Diocstrus 2.0 mm.	C.L. large	12th day
6	12	4.2	12	10	Proestrus 2.7 mm.	Follicles small C.L.	12th day
7	12	4.3	15	16	Proestrus 2.9 mm.	Follicles small C.L.	18th day
8	8	4.1	15	16	Proestrus 2.7 mm.	Follicles small C.L.	18th day
9	7	3.9	15	16	Diocstrus 1.7 mm.	Follicles small C.L.	18th day
10	12	4.4	15	15	Proestrus 3.1 mm.	Follicles large C.L.	18th day
11	7	4.2	15	15			18th day
12	7	4.5	15	15	Diocstrus 1.7 mm.	Follicles vary C.L.	18th day
13	8	4.3	15	36			Returned to normal
14	6	4.4	15	33			Returned to normal
15	6	4.4	15	33			Returned to normal

RESULTS

The control animals that had received cottonseed oil showed no upset in the oestrous cycle. The animals that had received carotene continued to show a vaginal smear picture of leucocytes and nucleated epithelial cells during the period of carotene therapy. The injected rats rarely showed cornified

TABLE 2

The effect of carotene feeding upon the vaginal smear, uterus and ovary

RAT NO.	NORMAL CYCLES	AVERAGE CYCLE	DAYS OF THERAPY	ABNORMAL	UTERUS	OVARY	ANIMAL KILLED
		days		*days*			
16	10	4.7	5	3	Proestrus 2.5 mm.	Follicles small C.L.	5th day
17	6	3.8	11	8	Dioestrus 2.1 mm.	Follicles small C.L.	11th day
18	4	4.7	11	9	Dioestrus 2.5 mm.	Follicles vary C.L.	11th day
19	6	4.1	13	11	Proestrus 5.0 mm.	Follicles large C.L.	13th day
20	6	4.1	15	12	Proestrus 4.0 mm.		15th day
21	6	4.1	29	27	Dioestrus 1.7 mm.	Follicles vary C.L.	29th day
22	4	4.7	29	27	Oestrus 6.0 mm.	Follicles large C.L.	29th day
23	5	4.7	29	27	Proestrus 4.0 mm.	Follicles med. and C.L.	29th day
24	6	3.8	32	30	Dioestrus 1.7 mm.	Follicles small C.L.	32nd day
25	8	4.5	15	34			Returned to normal
26	4	5.2	15	17			Returned to normal

cells and the fed animals presented slightly greater numbers of cornified cells in the vaginal smear. In no case were the cornified cells typical of oestrus.

The average length of the oestrous cycle was determined from the control series obtained preceding the carotene administration. Vaginal smear records were obtained approximately 10 minutes prior to the death of each animal.

These smears were compared with the expected normal smears that should have been obtained had the animals not received carotene.

Table 3 presents the data obtained from the vaginal smear studies. Thirty to 80 hours would have been required for the animals to reach the calculated stage as determined from the control series. Some variation existed in the length of time for the various cycles in the control series. However,

TABLE 3

The effect of vitamin A upon the oestrous cycle of the albino rat

RAT NO.	AVERAGE CYCLE	VAGINAL SMEAR			CAROTENE THERAPY	SMEAR VARIED
		Beginning of injection	Day killed	Expected smear		
			Carotene injected			
	days				*days*	*hours*
1	4.4	Cor+++[1] L+[1]	L+ Ne+++[1]	Cor++ L+	5	61
2	4.2	Cor+++	Ne++ Cor+	L+++	7	45
3	4.1	L+ Ne++	Ne++ Cor+	Cor++ L+	10	35
4	4.6	Cor++ L+	L+ Ne++	Cor++ L+	10	55
5	4.5	L+ Ne++	L+ Ne++	L+++	12	40
6	4.2	L+ Ne++	L+ Ne++	L+++	12	30
7	4.3	L++ Ne+	L++ Ne+	L++ Ne+	17	00
8	4.1	L+ Ne++	L++ Ne+	Ne++ Cor+	17	80
			Carotene fed			
20	4.1	Cor+ L++	Ne+++	L+++	13	55
21	4.1	L++ Ne+	L++ Ne+	Cor+++	28	70
23	4.7	Cor+ L++	Ne++ Cor+	Cor++ L+	28	50
24	3.8	L++ Ne+	L++ Ne+	Ne++ Cor+	31	37

[1] The abbreviations are to designate the presence of cornified, leucocytes and nucleated epithelial cells and the plus sign indicates the cell type in the majority.

normal oestrous cycles were never observed during the carotene administration. After the second day of carotene therapy, all animals showed great numbers of nucleated epithelial cells and leucocytes. The majority of animals gave no indication of going through the cornified cell stage. Several animals were not sacrificed and showed normal cycles approximately 20 days following the cessation of carotene administration.

In the study of the uteri as shown in tables 1 and 2, it was found that only five of the twenty-six animals demonstrated

uteri above 3.0 mm. in diameter, the majority of them being apparently in late dioestrus and early proestrus. The ovaries presented a similar picture. There were few large follicles and many corpora lutea in various stages.

DISCUSSION

Since Baumann and Steenbock ('32) reported that excessive cornification, in the vaginal epithelium of the albino rat, was an early manifestation of vitamin A deficiency, and were able to produce dioestrous smears within 1 week and oestrus in 2 weeks after the administration of carotene, it was thought advisable to study the effect of excessive vitamin A since the vitamin has a decided effect upon epithelial cells.

Mason and Ellison ('35) reported a marked increase in cornified cells in avitaminosis A. This effect may have been an indication that the epithelial cells died early and that the body was not able to remove the cornified cells as rapidly as produced. One might have expected therefore a normal or even an excessive amount of vitamin A to have kept these cells in a healthy condition and have prevented cornification.

The excessive vitamin A administration in the present investigation apparently produced an upset in the oestrous cycle as shown from the study of the uteri and ovaries as well as the vaginal smears. Since the control rats gave no indication of a changed vaginal smear picture, it would seem possible that the vitamin A was responsible for an upset of the oestrous cycle, or brought about marked mitosis, thus producing greater numbers of nucleated epithelial cells.

The animals that had received carotene by subcutaneous injection may have shown a more marked effect due to the fact that the vitamin was more efficiently utilized because of a slow absorption and no loss through the alimentary canal.

Further studies on the changed uterine picture are being made for the purpose of checking more adequately the vitamin A effect. The possibility of a hormone-vitamin relationship is also being investigated.

SUMMARY

Vitamin A given in the form of carotene orally and subcutaneously produced a change in the oestrous cycle of the albino rat. The vaginal smears showed nucleated epithelial cells and leucocytes during the entire period of carotene therapy. Very few cornified cells were observed during the experimental period.

Vitamin A given subcutaneously produced a greater effect upon the vaginal smear and uterine pictures than did oral administration.

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Skaloud-'47
Bib No. 1611

Intern. Z. Vitaminforsch. 19:20-34. 1947.

The Relationship of Vitamin A to Dental Structure and the Surrounding Soft Tissues

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Through experiments, I studied the influence of the lack as well as of the excess of Vitamin A on the dental structure and on the soft tissues and solid parts surrounding it. For this purpose, I carried out three series of tests on 136 rats. All animals of these three test series were housed in the same room, under exactly the same heat, light and humidity conditions. The first test series took place in March and April 1937. In it, the rats were divided into the following two groups: 1. Those with the usual food, as control rats, 2. those with food that was practically without Vitamin A. The second test series took place in February, March and April 1938, with the rats being divided in the same manner. The third test series took place in March, April and May 1939 and in May 1940. In this series, the rats were divided into four groups: I. The usual food with an excess of Vitamin A with 24 000 international units in the total dosage with 0,864 g carotin for each rat. II. The usual food with an excess of Vitamin A of 48 000 international units in the total dosage with 1,728 g carotin for each rat. III. The usual food with an excess of Vitamin A of 72 000 international units in the total dosage with 1,613 g carotin for each rat. IV. With the usual food as control animals.

Lack of Vitamin A

The animals lost their appetite, became weak and there was a loss of weight which was slow at first and later became very rapid. The rats were sluggish, apathetic to their surroundings, their breathing was fast and superficial; finally, ataxia of all four extremeties was observed. In contrast, the control rats were, before the completion of the test, lively, playful and their appetite was always good.

The observations by Simonnet, Orr and Richards who maintain

that the loss of weight signifies no cessation of growth, cannot be confirmed by me. It is true that the skeleton was not weighed, but the skiagrams of the spine, the extremeties and the head, in the case of the avitaminose rats, demonstrated to a sufficient degree that they had smaller dimensions. The enclosed photographs which were made one day before the test animals were killed, confirm this difference in size. It can thus be stated that the lack of Vitamin A in the food not only results in the loss of weight but also in a slow-down of the growth. Suzuki, Spinka, Javillier, Emerique, Hart de Ruytter and Rosenthal stated the same.

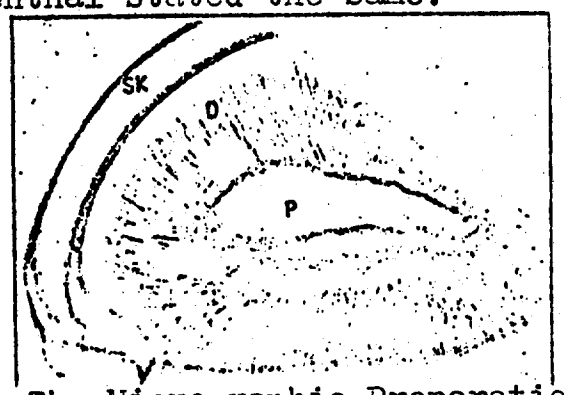

Illustration 1. The Micrographic Preparation of the Incisor Tooth of the Control Rat. SK = the Enamel, D = dentine, P = the Root Canal, Owens' Lines

Illustration 2. The Micrographic Preparations of the Incisor Tooth of a Rat. Food Lacking Vitamin A. SK = the Enamel, D = Dentine, P = the Root Canal, KP = Very clear concentric Owens' lines.

The fur of the avitaminose animals was thinner and dull, that of the control rats was in contrast thick, fine and smooth. The fur of the avitaminose animals was dry and brittle, especially in the area of the oral cavity. In this respect, my test confirms the observation by Schneider. The mucous memorane of the oral cavity of the avitaminose rats was pale and had a dirty coating, whereas that of the control rats was of a dark pink color.

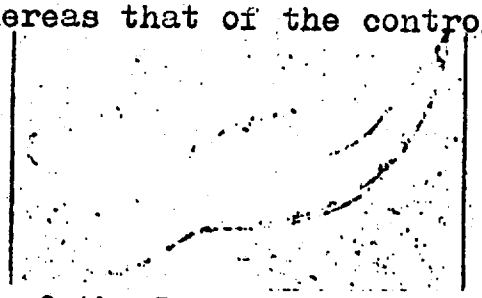

Illustration 3. Radiograph of the Lower Jaw of a Control Rat.

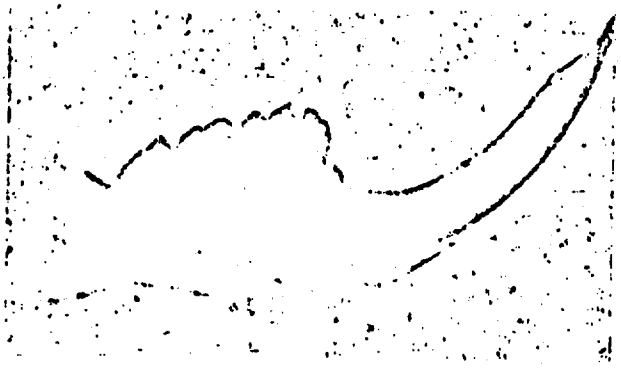

Illustration 4. Radiograph of the Lower Jaw of the Hypervitaminose Rat. The Spongiosa Structure Has Almost disappeared, namely in the Interdental Area as Well as in the Interradicular area. Processus Condyloideus et Coronoideus was Like Paper and Fell Off During the Preparation.

The incisor teeth of the avitaminose rats showed a slower growth (Suzuki, Fridericia), also a smaller size, a dirty yellow color and in two cases a slight loosening (Shibata, May-Mellanby, Howe). In these rats, one radiologically observed a retarded development of the bony parts of the head, as well as a significant difference in the size of the incisor teeth, also a change in the structure of the spongiosa bone (blurry marking of the small bone bars) and a change on the Corticalis (thinner).

Illustration 5. The Radiograph Shows a Thinner Corticalis at the Hollow Bones and Thinner Spongiosa Tissue at Ossa Iliaca. Caput Femoris Has an Unclear Marking. The Static Disturbances are at the Two Tibia and at the Right Femur. The Two Disturbances Develop into Fractures: At the Two Tibia and at the Right Femur, There is a New Fracture, at the Left Tibia, There is a Fracture with Healing Process.

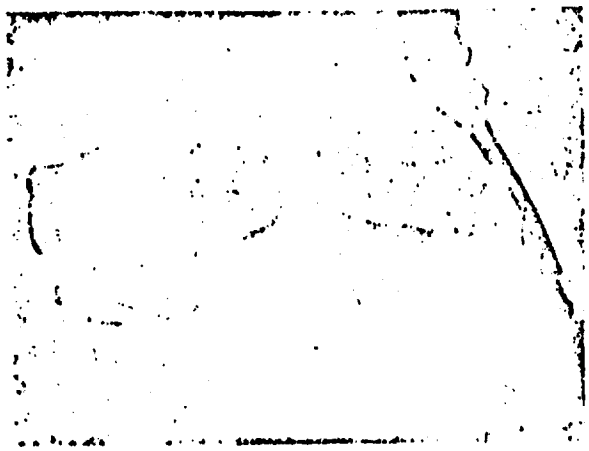

Illustration 6. In addition to the thin Corticalis and the thin spongiose tissue, the radiograph shows also considerable static changes - mainly at the femur and the tibia. Sympathetic Fractures were noted at the fibula which in the form of the "transformation zones" and crosswise extend over the oblong axis in the spots which respond to the mechanical stress of the bones.

Disturbances in the development of the bones are also described by Javillier, Simonnet and Randoin. The mucous membrane of the oral cavity was pale, had a dirty coating (Jeanneret) and was in most cases separated around the friction teeth. However, there was no deep pocket full of pus, as described by May-Mellanby. Richards also reports negatively in regard to this phenomenon.

Illustration 7. The radiograph shows a thin spongiose tissue and a thin corticalis. A pathological fracture with healing process is located at the femur. An artificial fracture is located at the tibia.

Illustration 8. A section of the micrographic series of the lower jaw of a control rat. H = the incisor tooth, ST = molar, P = pulpa, K = the bone tissue

The finding concerning the tartar by Shibata and May-Mellanby is also confirmed. However, the finding of an abnormal callosity of the gingival epithelium, as described by Shibata, May-Mellanby and others, cannot be confirmed, since, in the case of rats, the calloused epithelium is a completely normal symptom. Using all customary methods of investigation, I histologically found nothing specific at the incisor teeth.

Illustration 9. A section of the micrographic series of the lower jaw of a hypervitaminotic rat. The tooth pulpa and the marrow disappeared almost completely. H = the incisor tooth,

ST = Molar, K = the disappeared marrow, S = the mucous membrane, P = the disappeared tooth pulpa.

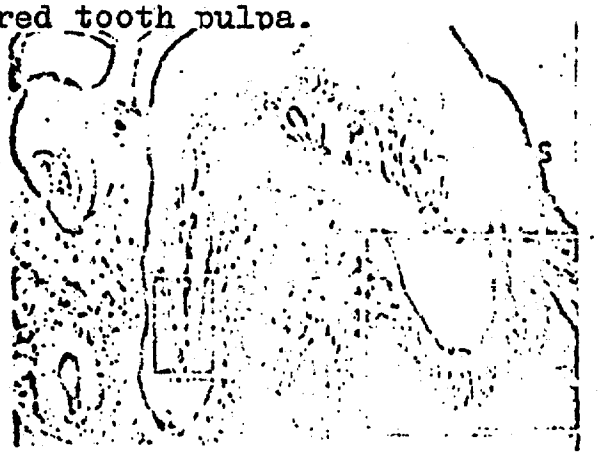

Illustration 10. A section of the micrographic series of the lower jaw of a control rat. A considerable enlargement of the molar and the surrounding soft and bony tissue from the illustration above. D =dentine, P = the fine gelatenous tissue of the pulpa with abundant mesh of the vessels. C = the cement, L = the ligamentous apparatus of the periodontium, K = the bone tissue with mass by Kerner, S = the mucous membrane.

The odontoblasts of the incisor teeth of the avitaminotic rats are usually lower, their row is sometimes interrupted and they are not parallel mainly on the lingual side. The interior edge of the dentine is wavy, the dentine usually does not have the same thickness along the whole length of the teeth (Pohto). However, these changes are not specific, but can also occur in the control rats (Neuwirt). The chemical composition points to a decrease of CaO (Shibata) as well as to a decrease of MgO.

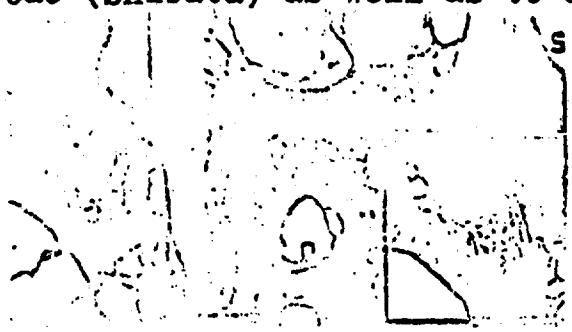

Illustration 11. A section of the micrographic series of the lower jaw of a hypervitaminotic rat. A stronger enlargement of the molar and the surrounding soft and bone tissue from the illustration above. D = the dentine shows no changes. P = the pulpa is shown with shrunken supports without cells.

C = the cement usurated by (from) - sic. translator - the surface, L = the ligamentous apparatus of the periodontium reduced to narrow skin-like septa. K = the bone is replaced by light spaces, B = skin-like separating walls between these spaces, S = the mucous membrane.

	Avitamin. Rat	Control Rat
CaO	32,60 %	36,22%
MgO	1,48 %	1,55%

(Shibata just recently found a larger amount). The total survey of the analyses points to a lower uniformity of the teeth and to a less complete mineralization of the teeth. The examination by means of the polarization microscope showed that the total structure had less compactness - uniform (sic) of the dentine. The concentric Owens' lines could be clearly observed. Debyescherer's diagrams were also made. Unfortunately, it was not possible to establish are more precise quantitive classification and obtain photometrograms of the two Illustrations.

Illustration 12. A section of the micrographic series of the lower jaw of a control rat. A more extensive enlargement of the distal root of the previous illustration. D = the dentine, P = the fine gelatinous pulpa tissue with abundant plexus of the vessels. A = the pulpa vessels, O = the cohesively located odontoblasts.

Excess of Vitamin A

In the case of the hypervitaminotic rats of the third test series (24 000 international units and especially those with 48 000 international units), the following was established: There was

no rapid loss of weight. The weight gain decreased a little in some, whereas in others, the weight increased constantly and gradually. However, the rats in this series in no case reached the weight of the control rats. Gradually, the rats lost interest in their surroundings and in the last phases, even in their food; they progressively lost weight until they died showing symptoms of cachexia which was preceded by diarrhea and paresis of the front and rear extremities. None of the females became pregnant, whereas the avitaminotic females usually became pregnant. All rats had trophic disturbances of the skin and loss of hair (Drigalski, Collazo and Rodriguez, Duvior, Moore, Finuci, Lewis and Reti have described those symptoms), especially on the side of the stomach, where in the case of some animals, the skin had no hair at all. The symptom usually and to the largest extent occurred in Group II. Within two weeks, almost all developed an only slight exophthalmus, mainly in the IInd group, however, only in one case, in Group III. Collazo and Rodriguez established the exophthalmus already at the end of the first week.

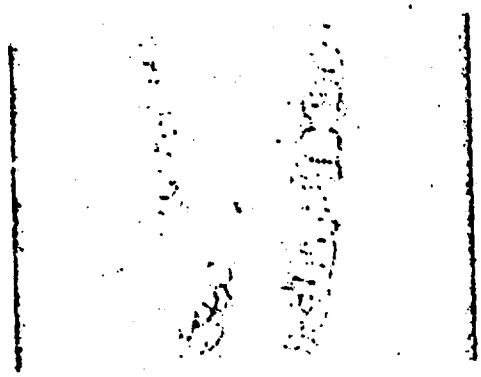

Illustration 13. A section of the micrographic series of a hypervitaminotic rat. A more extensive enlargement of the distal root of the previous illustration. D = the dentine with a clearer conturing of the canals. P = the shrunken notwwork of small skins which represents the pulpa. A = the rest of the pulpa vessels. O = the disappeared odontoblasts which were replaced by cohesive vacuoles located around the wall of the dentine.

The mucous membrane of the oral cavity was reddish and had a dirty coating. The incisor teeth were dirty, yellow, bendable, very brittle and significantly loosened. Loosening also took place in the case of the molar teeth, sometimes even to a larger extent than in the case of the incisor teeth.The increase of the incisor teeth (sic) is relatively smaller than in the control rats. Generally, it was radiologically determined that the head and the hollow bones as well as the spongiose bones have a thinner corticalis and a thinner spongiose tissue, especially in the case of Group II.

Illustration 14. A section of the micrographic series of the lower jaw of a control rat. A more extensive enlargement of the mesial root of Illustration 10. D = dentine, C = cement, L = the ligamentous periodontium apparatus, S = mucous membrane.

Illustration 15. A section of the micrographic series of the lower jaw of a hypervitaminotic rat. A more extensive enlargement of the mesial root of Illustration 11. D = dentine, C = the usurated cement, L = the disappeared periodontium tissue was replaced by skin-and rope-like forms, K = the alveolar bone which was usurated by a row of broad canals, S = mucous membrane.

Deformations are present at the epiphyses which are connected with static changes, in which case, there are, however, no changes which are characteristic of rickets. Spontaneous fractures at the femur, at the tibia and at the fibular are observed, as described by Collazo, Rodriguez, Davies and Moore. Sympathetic fractures were also found on the fibula, which extended in the form of the "transformation zone". It was noted that chemically, the relationship between Ca and Mg had worsened to the disadvantage of the magnesium.

	A Hypervitaminotic Rat	Control Rat
CaO	32,83 %	36,28%
MgO	1,17 %	1,55%

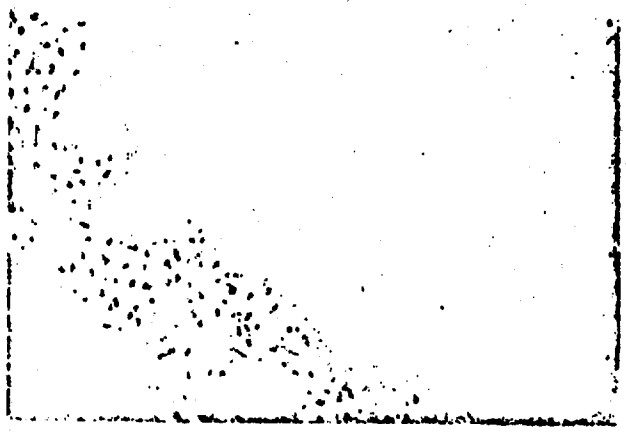

Illustration 16. A section of the micrographic series of the lower jaw of a control rat. The maximum enlargement of the apex of the mesial root of Illustration 14. D = dentine, C = cement, P = the ligamentous peridontium apparatus, K = alveolar bone.

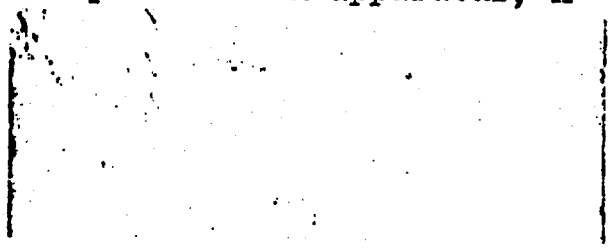

Illustration 17. A section of the micrographic series of the lower jaw of a hypervitaminotic rat. The maximum enlargement of the apex of the mesial root of Illustration 15. D - the dentine with clearer conturing of the canals. P = the periodontium is replaced by a row of vacuoles which on the side of the usurated cement are of the smallest size, and on the bone side of the largest. K= Alveolar bone, A = the previous vessel canals.

Especially in the Groups I and II the teeth are far less uniform and their mineralization in these groups is not complete. The examination by means of the polarization microscope has confirmed this incomplete mineralization and, in addition, a disturbance in the uniformity of the dentine in the whole structure was noted. Optically, the disturbance of the uniformity is expressed by a weakened double fracture and a very ununiform extinguishing (sic). Owens' concentric lines are very clear. Histologically, an almost complete loss of these tissues was also established: The tooth pulpa and the marrow almost completely disappeared, in which case the cells were completely lost, whereas the supporting connecting tissue apparatus (the collage and reticular lines) is pressed into the small skins and strings located between the vacuoles which, filled with a "clear" liquid, were all loosened because of the disappearance of the tissue. The pulpa vessels disappeared. However, the periodontium is reduced to a few weak septa through the vacuoles.
The alveolar bone is usurated and interspersed with wide bones. The cavities of the bone cells which are all filled with vacuoles press the cells against the wall. The dentine does not show many changes, with the exception of a more distinct conturing of the canals. The cement is usurated by the periodontium. This finding concerns the IIIrd test series and was described in detail after the excess of Vitamin A here resulted in the most pronounced damages to the tooth and bone tissue. In the Ist group of the III rd test series, the bone tissue has usurated edges and the bone structure has the appearance of a thin network. The marrow and the pulpa of the incisor as well as of the molar teeth is roughly vacuolized in places. The odontoblasts of the incisor teeth are in a connected row, but are thinner and not so high. In the IIIrd group of the IIIrd test, the picture is the same as in the case of the control rats (short duration of the experiment). Pohto (1930) made histological observations on hypervitaminose rats, but strangely did not note any changes in the microscopic structure of the incisor teeth.

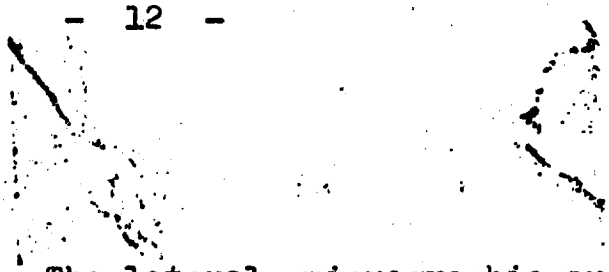

Illustration 18. The lateral micrographic preparation of the incisor tooth of a hypervitaminotic rat in normal light. SK = enamel, D = dentine, PK = very clear concentric Owens' lines.

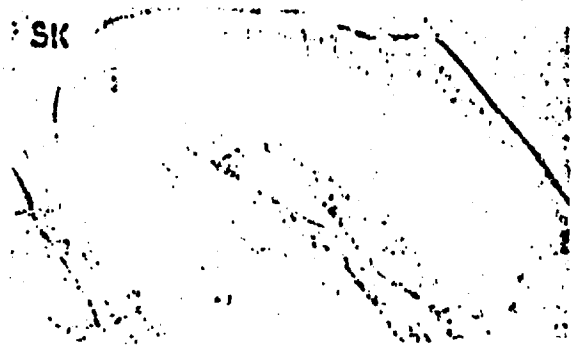

Illustration 19. The lateral micrographic preparation of the incisor tooth of a hypervitaminotic rat in the polarization light. SK = enamel, D = dentine, PK = Very clear concentric Owens' lines. The light parts around the root canal and in the enamel show areas with higher douole diffraction of rays which is caused by optical orientation.

The result shows that in rats an excess administration of Vitamin A or its lack , also with a sufficient dosis of Vitamin C and D, causes a disturbance in the development of the teeth, connected with damage to the general health. These results are in contrast to statements by Pappenheimer that Vitamin A has no influence at all on the tooth tissue.

In the case of the avitaminotic rats, the changes of the tooth structure were, to a certain extent, balanced by the fact that they had a sufficient quantity of Vitamin C. A sufficient quantity of Vitamin D was surely also not without influence. However, if there were disturbances of the support tissue, this is to be explained by an insufficient utilization of the reabsorbed salts, especially the calcium-hard salts (sic) and by the lack of Vitamin A.

In the case of the hypervitaminotic rats (II, III), changes in the dental structure were noted in spite of sufficient quantities of Vitamins C and D that were contained in the food. The flexibility of the teeth (not the brittleness) can be explained by the separation of salts, mainly of calcium-hard salt. The teeth are perhaps just like the bones. In regard to bones, it is known now that in them there may be inorganic components in different physical and chemical forms. In this respect, the indicated chemical analyses also had positive results as well as results which were made by means of the polarization microscope on micrographic preparations of the teeth.

The circumstance that the A-hypervitaminosis causes a complete decay in the dental tissue as well as in the bone tissue forces us to also turn our attention to other tissues and to determine how these are effected by the excess of Vitamin A. I am working on more experiments in this respect and hope to be able to report on them later.

By means of experiments with other vitamins (Bircher-Benner, Euler and others), one can state that Vitamin A also is at least important for the development of the teeth. Only future biological experiments and exact clinical studies can furnish details in regard to this problem. Up to now, only one case of an extreme A -avitaminosis in a human being has been known, the one that was observed and described by Fingerland.

Summary

The relationship of vitamin A to dental structure and the surrounding soft tissues. By F. Skaloud.
Vitamin A is among the substances necessary to dental development. Hypovitaminosis A leads to deterioration not only of the teeth, with malformation, but also of the bony tissues. Both vitamin C and vitamin D show compensating action in hypovitamentosis A.

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Die Beziehung des Vitamins A zum Zahngewebe und zu den umgebenden weichen Teilen

Von F. Škaloud, Praha

Eingegangen am 8. August 1946.

Ich studierte experimentell sowohl die Wirkungen des Mangels, als auch des Überflusses des Vitamins A auf das Zahngewebe und auf die dasselbe umgebenden weichen Gewebe und festen Teile. Zu diesem Zwecke nahm ich drei Reihen von Versuchen an 136 Ratten vor. Sämtliche Tiere dieser drei Versuchsreihen wurden in demselben Zimmer und unter genau denselben Wärme-, Licht- und Feuchtigkeitsbedingungen untergebracht. Die erste Versuchsreihe fand im März und April 1937 statt, wobei die Ratten in folgende zwei Gruppen eingeteilt wurden: 1. mit gewöhnlicher Nahrung als Kontrollratten, 2. mit Nahrung praktisch ohne Vitamin A. Die zweite Versuchsreihe fand im Februar, März und April 1938 statt mit derselben Einteilung der Ratten. Die dritte Versuchsreihe fand im März, April und Mai 1939 und im Mai 1940 statt, wobei die Ratten in vier Gruppen eingeteilt wurden: I. gewöhnliche Nahrung mit Überfluß von A-Vitamin zu 24 000 internat. Einheiten in der Gesamtdosierung à 0,864 g Carotin für jede Ratte, II. gewöhnliche Nahrung mit Überfluß von A-Vitamin zu 48 000 internat. Einheiten in der Gesamtdosierung à 1,728 g Carotin für jede Ratte, III. gewöhnliche Nahrung mit Überfluß von A-Vitamin zu 72 000 internat. Einheiten in der Gesamtdosierung à 1,613 g Carotin für jede Ratte, IV. mit gewöhnlicher Nahrung als Kontrolltiere.

Mangel an Vitamin A

Die Tiere verloren den Appetit, wurden matt und es stellte sich zuerst eine langsame, später eine sehr rapide Gewichtsabnahme ein. Die Ratten waren schwerfällig, apathisch gegen ihre Umgebung und Nahrung, atmeten oberflächlich und schnell; zum Schluß war Ataxie sämtlicher vier Glieder wahrnehmbar. Die Kontrollratten waren hingegen vor der Beendung des Versuches munter, spiellustig und hatten stets guten Appetit.

Die Feststellungen von Simonnet, Orr und Richards, welche behaupten, daß die Gewichtsabnahme keine Einstellung des Wachstums bedeutet, kann ich nicht bestätigen. Das Skelett wurde

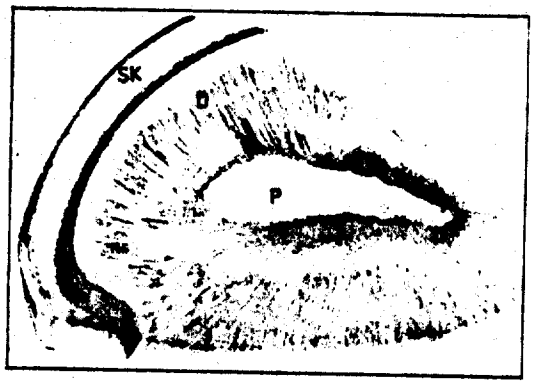

Abb. 1. Das Schliffpräparat des Nagezahnes bei der Kontrollratte. SK = der Schmelz, D = Deutin, P = der Wurzelkanal, Owens Linienfäden.

Abb. 2. Die Schliffpräparate des Nagezahnes bei einer Ratte. Mangel-Nahrung Vitamin A. SK = der Schmelz, D = Dentin, P = der Wurzelkanal, KP = Ganz deutliche Owensche konzentrische Linien.

zwar nicht gewogen, aber die Skiagramme der Wirbelsäule, der Gliedmaßen und des Kopfes zeigen bei den avitaminösen Ratten deutlich genug geringere Dimensionen. Die beigelegten Photographien, die einen Tag vor dem Töten der Versuchstiere hergestellt wurden, bestätigen den Größenunterschied. Es ist somit zu konstatieren, daß der Mangel des A-Vitamins in der Nahrung nicht nur eine Gewichtsabnahme, sondern auch eine Verlangsamung des Wachstums zur Folge hat. Dasselbe haben auch Suzuki, Spin-

Abb. 3. Röntgenogramm des Unterkiefers der Kontrollratte.

Abb. 4. Röntgenogramm des Unterkiefers der hypervitaminotischen Ratte. Die Spongiosastruktur ist fast verschwunden, und zwar in der Interdentalgegend sowie in der Interradikulargegend. Processus condyloideus et coronoideus war wie Papier und ist bei der Präparation abgefallen.

ka, Javillier, Emerique, Hart de Ruytter und Rosenthal konstatiert.

Das Fell der avitaminösen Tiere war dünner und matt, hingegen das der Kontrollratten dicht, fein und glatt. Das Fell der avitaminotischen Ratten war trocken und brüchig, besonders in der Umgebung der Mundhöhle. In dieser Beziehung bestätigt mein Versuch den Befund von Schneider. Die Schleimhaut der Mundhöhle der avitaminotischen Ratten war blaß und hatte einen schmutzigen Überzug, während die der Kontrollratten eine sattrosa Farbe aufwies.

An den Nagezähnen der avitaminotischen Ratten war ein langsameres Wachstum wahrzunehmen (Suzuki, Fridericia), ferner eine geringere Größe, eine schmutzig gelbliche Farbe und in zwei Fällen eine mäßige Lockerung (Shibata, May-Mollanby, Howe). Bei diesen Ratten wurden röntgenologisch eine ver-

Abb. 5. Röntgenogramm zeigt eine dünnere Corticalis an den Röhrenknochen und dünnere Spongiosagewebe an Ossa iliaca. Caput femoris hat undeutliche Zeichnung. Die statischen Störungen sind an den beiden Tibia und am rechten Femur. Die beiden Störungen führen zu Frakturen: an den beiden Tibia und am rechten Femur ist ein frischer Bruch, an der linken Tibia ist ein Bruch mit Heilprozeß.

Abb. 6. Röntgenogramm zeigt außer dünner Corticalis und dünnem spongiösem Gewebe auch große statische Veränderungen — hauptsächlich an Femur und Tibia. An der Fibula wurden sympathische Frakturen festgestellt, welche in Form der «Umbauzonen» und quer über die längliche Achse in den Stellen verliefen, die der mechanischen Belastung der Knochen antworten.

Abb. 7. Röntgenogramm zeigt ein dünnes spongiöses Gewebe und eine dünne Corticalis. Am Femur ist eine pathologische Fraktur mit einem Heilprozeß. An der Tibia findet man eine artifizielle Fraktur.

Abb. 8. Ein Teil der Schnittserie des Unterkiefers einer Kontrollratte. H = der Nagekahn. K = das Knochengewebe. ST = Molar. P = Pulpa.

spätete Entwicklung der knöchernen Kopfteile, sowie ein bedeutender Unterschied in der Größe der Nagezähne, ferner Veränderung in der Struktur des Spongiosaknochens (undeutliche Zeichnung der Knochenbälkchen) und Veränderung auf der Corticalis (dünner) beobachtet. Störungen in der Knochenentwicklung werden auch von Javillier, Simonnet und Randoin beschrieben. Die Schleimhaut der Mundhöhle war blaß, hatte einen schmutzigen Überzug (Jeanneret) und war in den meisten Fällen rund von den Reibzähnen losgetrennt. Es war jedoch keine tiefe Tasche vollen Eiters vorhanden, wie dies May-Mellanby schildert. Auch bei Richards ergibt sich ein negativer Befund dieser Erscheinung. Es wird auch der Befund des Zahnsteines von Shi-

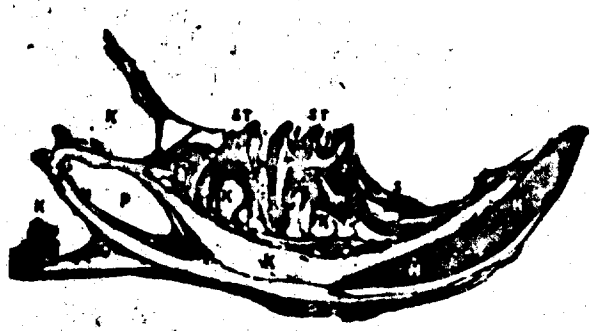

Abb. 9. Ein Teil der Schnittserie des Unterkiefers einer hypervitaminotischen Ratte. Die Zahnpulpa und das Knochenmark verschwunden beinahe gänzlich. H = der Nagezahn. ST = Molar. K = das verschwundene Knochenmark. S = die Schleimhaut. P = die verschwundene Zahnpulpa.

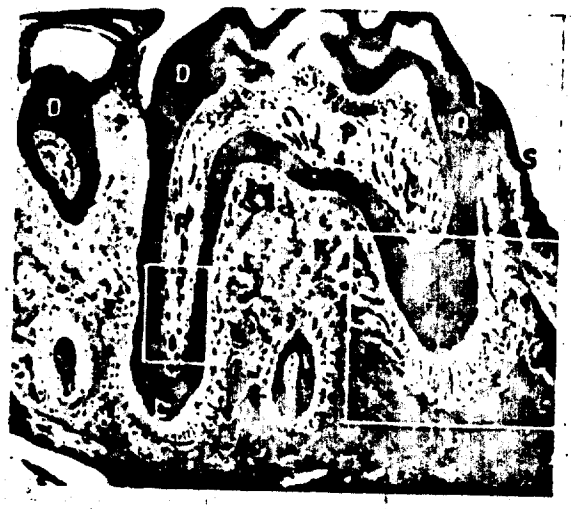

Abb. 10. Ein Teil der Schnittserie des Unterkiefers einer Kontrollratte. Eine stärkere Vergrößerung des Molars und der umgebenden weichen und Knochengewebe aus dem vorigen Bild. D = das Dentin. P = das feine, sulzige Gewebe der Pulpa mit reichem Geflecht der Gefäße. C = der Zement. L = der ligamentöse Apparat des Periodontiums. K = das Knochengewebe mit Menge von Kerner. S = die Schleimhaut.

bata und May-Mellanby bestätigt. Es kann jedoch der Befund einer abnormalen Verhornung des gingivalen Epithels so, wie denselben Shibata, May-Mellanby und andere beschrieben, nicht bestätigt werden. Bei Ratten ist nämlich das verhornte Epithel eine ganz normale Erscheinung. Ich fand bei sämtlichen

Abb. 11. Ein Teil der Schnittserie des Unterkiefers einer hypervitaminotischen Ratte. Eine stärkere Vergrößerung des Molars und des umgebenden weichen und Knochengewebes aus dem vorigen Bild. D = Das Dentin weist keine Veränderungen auf. P = Die Pulpa ist mit verschrumpftem Gebälke ohne Zellen dargestellt. C = Der von der Oberfläche usurierte Zement. L = Der ligamentöse, auf enge, häutchenartige Septen reduzierte Apparat des Periodontiums. K = Der Knochen ist durch helle Räume ersetzt. B = Häutchenartige Scheidewände zwischen diesen Räumen. S = Die Schleimhaut.

üblichen Untersuchungsmethoden histologisch nichts spezifisches an den Nagezähnen. Die Odontoblasten der Nagezähne der avitaminotischen Ratten pflegen öfters niedriger zu sein, ihre Reihe ist manchmal unterbrochen und dieselben sind hauptsächlich an der Lingualseite nicht parallel. Der innere Rand des Dentins ist wellig, das Dentin pflegt nicht dieselbe Stärke an der ganzen Länge der Zähne aufzuweisen (Pohto). Diese Veränderungen sind jedoch nicht spezifisch, sondern kommen auch bei den Kontrollratten vor (Neuwirt). Die chemische Zusammensetzung deutet zu eine Abnahme von CaO (Shibata), sowie auch an eine Abnahme von MgO.

	A avitamin. Ratte	Kontrollratte
CaO	32,60 %	36,22 %
MgO	1,48 %	1,55 %

(Shibata fand soeben eine größere Menge vor.) Das Gesamtbild der Analysen deutet eine geringere Einheitlichkeit der Zähne auf deren weniger vollkommene Mineralisation ab. Durch die Untersuchung mit dem Polarisationsmikroskop wurde im Gegenteil eine geringere Kompaktheit — Einheitlichkeit des Dentins — fest-

Abb. 12. Ein Teil der Schnittserie des Unterkiefers einer Kontrollratte. Eine stärkere Vergrößerung der Distalwurzel vom vorigen Bild. D = das Dentin. P = das feine, sulzige Pulpagewebe mit reichem Geflecht der Gefäße. A = die Pulpagefäße. O = die zusammenhängend angeordneten Odontoblasten.

gestellt. Die Owenschen konzentrischen Linien waren deutlich wahrnehmbar. Es wurden auch Debyeschererdiagramme verfertigt. Es war leider nicht möglich eine genauere quantitative Klassifikation und Photometrogramme der beiden Aufnahmen herzustellen.

Überfluß des A-Vitamins

Bei den hypervitaminotischen Ratten der dritten Versuchsreihe (24 000 internat. Einheiten und insbesondere bei denen, welche 48 000 internat. Einheiten hatten) wurde Folgendes konstatiert: Es hat keine rapide Gewichtsabnahme stattgefunden: bei einigen ist die Gewichtszunahme ein wenig gesunken, während bei anderen das Gewicht wiederum beständig und allmählich zunahm. Die Ratten dieser Reihe erreichten jedoch nicht in einem einzigen Falle das Gewicht der Kontrollratten. Die Ratten verloren allmählich Interesse für ihre Umgebung und in den letzten Stadien sogar auch für die Nahrung; sie magerten progressiv ab, bis sie unter Anzeichen von Kachexie, welcher Durchfall und Parese der Vorder- und Hinter-Gliedmaßen voranging, krepierten. Nicht ein einziges Weibchen wurde trächtig, wogegen die avitaminotischen

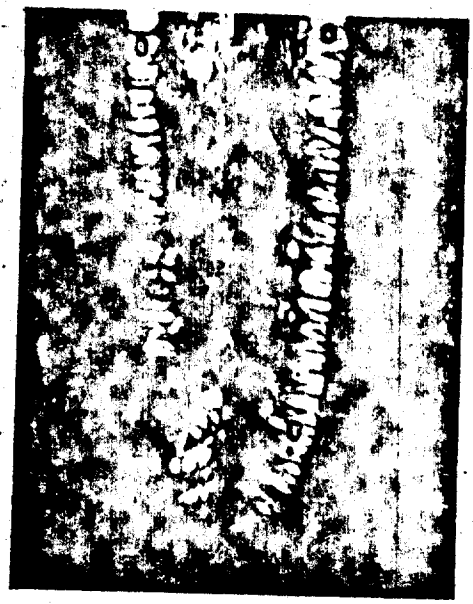

Abb. 13. Ein Teil der Schnittserie des Unterkiefers einer hypervitaminotischen Ratte. Eine stärkere Vergrößerung der Distalwurzel vom vorigen Bild. D = das Dentin mit deutlicherem Konturieren der Kanäle. P = das verschrumpfte Netz von Häutchen, das die Pulpa darstellt. A = der Rest von Pulpagefäßen. O = die verschwundenen Odontoblasten, die durch zusammenhängende und ringsum an die Dentinwand angeordnete Vakuolen ersetzt wurden.

Weibchen meistens trächtig wurden. Bei sämtlichen Ratten stellten sich trophische Störungen der Haut und Haarausfall ein (diese Erscheinungen haben Drigalski, Collazo und Rodriguez, Duvier, Moore, Finuci, Lewis und Reti beschrieben), insbesondere auf der Bauchseite, wo die Haut bei einigen Tieren ganz haarlos war. Die Erscheinung kam meist und im größten Ausmaße bei der II. Gruppe vor. Beinahe bei allen entwickelte sich binnen vierzehn Tagen nur ein mäßiger Exophthalmus, hauptsächlich in der II. Gruppe, jedoch nicht in einem einzigen Falle bei der Gruppe III. Collazo und Rodriguez haben den Exophthalmus bereits am Ende der ersten Woche konstatiert. Die Schleimhaut der Mundhöhle war rötlich und hatte einen schmutzigen Überzug. Die Nagezähne waren schmutzig, gelb, biegsam, sehr brüchig und bedeutend gelockert; Lockerung kam auch bei den Backenzähnen vor, manchmal sogar in einem noch größeren Maße als bei den Nagezähnen. Der Zuwachs der Nagezähne ist verhältnismäßig geringer als bei den Kontrollratten. Es wurde röntgenologisch allgemein festgestellt, daß sowohl die Schädel- und Röhren-

Abb. 14. Ein Teil der Schnittserie des Unterkiefers einer Kontrollratte. Eine stärkere Vergrößerung der Mesialwurzel von der Abb. 10. D = Dentin, C = Zement. L = Der ligamentöse Periodontiumapparat. S = Schleimhaut.

Abb. 15. Ein Teil der Schnittserie des Unterkiefers einer hypervitaminotischen Ratte. Eine stärkere Vergrößerung der Mesialwurzel von der Abb. 11. D = Dentin. C = der usurierte Zement. L = das verschwundene Periodontiumgewebe wurde mit häutchen- und seilartigen Formen ersetzt. K = Alveolarknochen, der durch eine Reihe breiter Kanäle usuriert wurde. S = Schleimhaut.

Abb. 16. Ein Teil der Schnittserie des Unterkiefers einer Kontrollratte. Die maximale Vergrößerung des Apex der Mesialwurzel von der Abb. 14. D = Dentin. C = Zement. P = Der ligamentöse Periodontiumapparat. K = Alveolarknochen.

Abb. 17. Ein Teil der Schnittserie des Unterkiefers einer hypervitaminotischen Ratte. Die maximale Vergrößerung des Apex der Mesialwurzel von der Abb. 15. D = das Dentin mit deutlicherem Konturfehlen der Kanäle. P = das Periodontium ist durch eine Reihe von Vakuolen ersetzt, die an der Seite des usurierten Zementes am kleinsten, an der Knochenseite am größten sind. K = Alveolarknochen. A = die vorigen Gefäßkanäle.

knochen, als auch die spongiösen Knochen eine dünnere Corticalis und ein dünneres spongioses Gewebe haben, besonders bei der Gruppe II. An den Epiphysen sind Deformationen vorhanden, welche mit den statischen Veränderungen zusammenhängen, wobei jedoch keine für Rachitis charakteristischen Veränderungen wahrzunehmen sind. Es werden spontane Frakturen am Femur, an der Tibia und an der Fibula festgestellt, wie dies von Collazo, Rodriguez, Davies und Moore beschrieben wurde. Außerdem wurden auf der Fibula sympathische Frakturen befunden, welche in Form der «Umbauzone» verliefen. Chemisch wurde das Verhältnis zwischen Ca und Mg zu Ungunsten des Magnesium verschlimmert festgestellt.

	A hypervitamin. Ratte	Kontrollratte
CaO	32,83 %	36,28 %
MgO	1,17 %	1,55 %

Die Zähne sind besonders bei den Gruppen I und II weit weniger einheitlich und deren Mineralisation darin ist nicht vollkommen. Die Untersuchung durch das Polarisationsmikroskop hat diese unvollkommene Mineralisation bestätigt und es wurde ferner eine Störung in der Einheitlichkeit des Dentins in der ganzen Struktur konstatiert. Die Störung der Einheitlichkeit äußert sich optisch durch geschwächten Doppelbruch und sehr uneinheitliches Auslöschen. Owens konzentrische Linien sind ganz deutlich. Histologisch wurde auch ein beinahe vollkommener Untergang dieser Gewebe konstatiert: die Zahnpulpe und das Knochenmark verschwanden beinahe gänzlich, wobei die Zellen sich vollkommen verloren, während der Stütz-Bindegewebsapparat (die Kollagen- und Retikularfaden) in die zwischen den Vakuolen vorhandenen Häutchen und Stränge gedrückt wird, welche mit «klarer» Flüssigkeit gefüllt, sämtlich infolge des Verschwindens des Gewebes gelockert wurden. Die Pulpagefäße verschwanden. Das Periodontium wird jedoch durch die Vakuolen auf wenige schwache Septen reduziert. Der Alveolarknochen ist usuriert und mit breiten Knochen durchgewebt. Die Höhlen der Knochenzellen, welche sämtlich mit Vakuolen ausgefüllt sind, drücken die Zellen an die Wand. Das Dentin weist nicht viel Veränderungen auf, abgesehen von einem deutlicheren Konturieren der Kanäle. Der Zement wird vom Periodontium usuriert. Dieser Befund betrifft die III. Versuchsreihe und wurde ausführlich beschrieben, nachdem der Überfluß von A-Vitamin sich hier in seinen schädlichen Wirkungen am Zahn- und Knochengewebe am markantesten zeigte. Bei der I. Gruppe der III. Versuchsreihe weist das Knochengewebe usurierte Ränder auf und das Knochengebälke hat das Aussehen eines dünnen Net-

Abb. 18. Das quere Schliffpräparat des Nagezahnes einer hypervitaminotischen Ratte in normalem Licht. SK = Schmelz. D = Dentin. PK = Ganz deutliche Owensche konzentrische Linien.

Abb. 19. Das quere Schliffpräparat des Nagezahnes einer hypervitaminotischen Ratte im Polarisationslicht. SK = Schmelz. D = Dentin. PK = Ganz deutliche Owensche konzentrische Linien. Die hellen Partien rings um den Wurzelkanal und im Schmelz zeigen Stellen mit höherer doppelter Strahlenbrechung, die durch optische Orientierung verursacht ist.

zes. Das Knochenmark und die Pulpa der Nage-, sowie der Bakkenzähne ist stellenweise grob vakuolisiert. Die Odontoblasten der Nagezähne befinden sich in einer zusammenhängenden Reihe, sind jedoch dünner und nicht so hoch. Bei der III. Gruppe des III. Versuches ergibt sich dasselbe Bild, wie bei den Kontrollratten (kurze Zeitdauer des Experimentes). Histologische Beobachtungen an hypervitaminösen Ratten hat Pohto (1930) gemacht, welcher sonderbarerweise überhaupt keine Veränderungen im mikroskopischen Bau der Nagezähne feststellte.

Aus dem Ergebnis zeigt sich, daß bei Ratten eine Überdosierung des A-Vitamins oder sein Mangel auch bei der genügenden Dosis von Vitamin C und D eine Störung in der Entwicklung der Zähne hervorruft, im Zusammenhang mit Schäden an der ganzen Gesundheit. Diese Ergebnisse sind entgegengesetzt den Behauptungen von Pappenheimer, daß das A-Vitamin überhaupt keinen Einfluß auf das Zahngewebe hat.

Bei avitaminotischen Ratten waren die Veränderungen der Zahnstruktur in gewissem Maße dadurch ausgeglichen, daß sie eine genügende Menge von C-Vitamin hatten. Eine genügende Menge von D-Vitamin war sicherlich auch nicht ohne Einfluß. Erfolgten jedoch Störungen des Unterstützungsgewebes, ist dies durch ungenügende Ausnützung der resorbierten Salze, hauptsächlich der kalkharten, und durch den Mangel von A-Vitamin zu erklären.

Bei hypervitaminotischen Ratten (II., III.) wurden trotz der genügenden Mengen von C- und D-Vitamin, die in der Nahrung enthalten waren, Veränderungen der Zahnstruktur festgestellt. Die Biegsamkeit der Zähne (nicht die Mürbheit) kann man sich durch das Abscheiden von Salzen erklären, hauptsächlich von kalkharten. Bei Zähnen verhält es sich vielleicht genau so wie bei den Knochen. Im Bezug auf Knochen ist bis jetzt bekannt, daß in ihnen die nichtorganischen Bestandteile in verschiedenen physikalischen und chemischen Formen vorkommen können. In dieser Richtung wiesen auch die angegebenen chemischen Analysen positive Resultate auf sowie auch Resultate, die mit Hilfe des Polarisationsmikroskop auf Schliffpräparaten der Zähne hergestellt wurden.

Der Umstand, daß die A-Hypervitaminose ein vollkommenes Verderben, sowohl im Zahngewebe als auch im Knochengewebe hervorruft, zwingt uns, unsere Aufmerksamkeit auch anderen Geweben zuzuwenden und festzustellen, wie auf diese der Überfluß von A-Vitamin wirkt. In dieser Richtung beschäftige ich mich mit weiteren Versuchen und hoffe, später darüber zu berichten.

Durch Erfahrungen mit anderen Vitaminen (Bircher-Benner, Euler und andere) kann man urteilen, daß auch das A-Vitamin zur Entwicklung der Zähne zu mindest wichtig ist. Erst

zukünftige biologische Versuche und genaue klinische Studien können dieses Problem in allen Details beleuchten. Bis jetzt ist nur ein einziger Fall einer extremen A-Avitaminose bei Menschen bekannt, und zwar der, der von Fingerland beobachtet und beschrieben wurde.

CONCLUSION

Les relations entre la vitamine A et le tissu dentaire, ainsi que les parties molles voisines. Par. F. Skaloud.

La vitamine A est nécessaire pour le développement du tissu dentaire. Sous l'hypervitaminose A les dents et aussi les os se courbent. Pendant l'hypovitaminose A les vitamines C et D peuvent aussi entrer en jeu et diminuer les symptomes de cette carence.

SUMMARY

The relationship of vitamin A to dental structure and the surrounding soft tissues. By F. Škaloud.

Vitamin A is among the substances necessary to dental development. Hypovitaminosis A leads to deterioration not only of the teeth, with malformation, but also of the bony tissues. Both vitamin C and vitamin D show compensating action in hypovitaminosis A.

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MASSIVE DOSES OF VITAMINS A AND D IN THE PREVENTION OF THE COMMON COLD

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During their various studies of the clinical use of massive doses of vitamin D, Reed and associates[1] noted that many patients receiving large doses of vitamin A and vitamin D claimed that both the incidence and the severity of their colds were appreciably reduced. No accurate statistics were kept, but it appeared to them that only those subjects receiving both vitamins were thus benefited.

This observation was not surprising since many investigators have noted great reduction in the incidence of colds and respiratory infections through the use of fairly liberal quantities of cod liver oil. Holmes and his colleagues,[2] for example, reviewed the subject and showed a reduction of about two thirds in the average "lost time" of industrial workers due to colds and respiratory diseases when cod liver oil was furnished. In general Holmes felt that the reduction in colds was due to the vitamin A content of the cod liver oil, although no proof of this could be offered. The doses used by Reed and associates, however, were many times larger than those used by previous workers, and the subjects could not be said to be deficient in vitamins A or D. Whatever effects appeared, therefore, were almost certainly due to some cause other than correction of vitamin deficiency.

In view of these facts, it was of interest to study the effects of vitamins A and D in large doses, both singly and combined, on a group of subjects. The purpose of this study was to determine, first, whether any amelioration as regards the incidence and severity of colds was achieved; second, whether one or the other of the vitamins was alone responsible for the effects or whether both were necessary.

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1. Reed, C. I.: Personal communication to the author. Reed, C. I.; Struck, H. C., and Steck, I. E.: Vitamin D, Chicago, University of Chicago Press, to be published.

2. Holmes, A. D.; Pigott, M. G.; Sawyer, W. A., and Comstock, L.: The Influence of Body Weight and the Administration of Cod Liver Oil on Industrial Absenteeism, J. Am. Dietet. A. **10**:208, 1934; The Value of Cod Liver Oil for Building Bodily Resistance of Industrial Workers, Oil and Soap **12**:86, 1935; Cod Liver Oil: A Five Year Study of Its Value for Reducing Industrial Absenteeism Caused by Colds and Respiratory Diseases, Indust. Med. **5**:359, 1936.

PROCEDURE

Fifty-four patients were studied during the three winters from 1934 to 1937. Thirty-two of these subjects were studied for all three winters, 18 for two years and 4 for only one winter. Thirty-four of the subjects were men, and the rest were women. Their ages ranged from 7 to 49 years, with 14 of the subjects 16 years old or younger. All the patients belonged to the group called "chronic or frequent cold sufferers" (Spiesman and Arnold [3])) and had previous records of five to seven colds each winter for several years previous to the present study. These colds were accompanied by fever and lasted for periods of from seven to fourteen days. Many patients required rest in bed. All patients chosen for this study were free from pathologic conditions as determined by close examination, particularly of the respiratory tract and nasal sinuses. It was felt that this precaution would insure results unobscured by extraneous factors.

Without regard to age or sex the subjects were divided into 3 groups as follows: Group 1 received only vitamin A; group 2 received only vitamin D, and group 3 received both vitamins A and D. There were 7 patients in group 1; 7 patients in group 2 and 40 patients in group 3. The explanation for the small number of subjects in groups 1 and 2 is that it was difficult to persuade the subjects in these groups, who derived no benefit from the use of vitamin A or D alone, to continue the treatment over longer periods.

The vitamin A used was in two forms: a solution of carotene in cottonseed oil [4] containing 7,000 international units of vitamin A per gram and a fish oil concentrate of vitamin A [5] containing 40,000 international units of this vitamin per cubic centimeter, stated to be free of vitamin D. The vitamin D [6] used was in the form of a solution of calciferol in oil, containing approximately 920,000 international units per cubic centimeter.

Treatment was started in all cases about September 1 and continued throughout the winter and spring until about June 1. The initial dose of vitamin A as carotene solution for subjects in group 1 was approximately 900 units per day. For those receiving the fish oil concentrate the dose was approximately 9,000 units per day, taken in two doses with meals. In group 2, the initial dose of vitamin D was 120,000 units per day. In group 3 the subjects started with 9,000 units of A and 120,000 units of D daily. In all cases, the dose was increased by gradual stages as follows: For patients taking the carotene solution the dose was increased by approximately 400 units every third day up to 7,000 units daily. For those receiving the fish oil concentrate the dose was increased by about 2,500 units every third day until the maximum dose of 40,000 units was reached. For subjects receiving vitamin D the dose was increased by 60,000 units every third day until a maximum of 300,000 units was reached. In a similar manner the doses of vitamin D, after varying periods, were gradually reduced to the initial dose. Children under 12 years of age received half these amounts.

All the subjects were examined weekly or semimonthly throughout the season, and records were kept of the number and severity of colds experienced by them.

3. Spiesman, I. G., and Arnold, L.: Host Susceptibility to Common Colds, Am. J. Digest. Dis. & Nutrition 4:438, 1937.

4. Furnished by the S. M. A. Corporation.

5. Furnished by the Glaxo Laboratories.

6. Furnished by Mead Johnson & Company.

RESULTS

The table summarizes the results of the study. It shows that none of the subjects who received either vitamin A or vitamin D alone derived any benefit from the treatment; that is to say, they experienced

Summary of Results

Subject	Age, Years	Sex	Vitamins	Result
1	6	F	A*	No benefit
2	41	M	A	
3	19	M	A*	
4	28	F	A	
5	25	F	A*	
6	31	F	A	
7	29	M	A	
8	19	F	D	
9	24	M	D	
10	27	M	D	
11	18	F	D	
12	47	M	D	
13	32	M	D	
14	23	F	D	
15	7	M	A and D	Free of colds during second and third years
16	10	M	A and D	
17	19	F	A and D	
18	23	M	A and D	
19	41	M	A and D	
20	33	F	A and D	
21	11	M	A and D	Colds reduced in number and severity
22	26	F	A and D	
23	49	M	A and D	
24	41	F	A and D	
25	27	F	A and D	
26	16	M	A and D	
27	29	M	A and D	
28	16	M	A and D	
29	9	M	A and D	
30	46	M	A and D	
31	27	F	A and D	
32	11	F	A and D	
33	22	M	A and D	
34	18	M	A and D	
35	30	M	A and D	
36	14	M	A and D	
37	12	M	A and D	
38	32	F	A and D	
39	25	F	A and D	
40	19	M	A and D	
41	42	M	A and D	
42	12	M	A and D	
43	27	M	A and D	
44	14	M	A and D	
45	29	M	A and D	
46	47	F	A and D	
47	38	F	A and D	No benefit
48	31	M	A and D	
49	27	M	A and D	
50	18	M	A and D	
51	14	M	A and D	
52	10	M	A and D	
53	9	F	A and D	
54	11	F	A and D	

* Vitamin A was given in the form of carotene solution; in all other instances this vitamin was given in the form of a fish oil concentrate.

just as many colds (five to seven) and the colds were just as severe as those of the seasons in which they did not take the vitamins. On the other hand, of the patients who received both vitamins, 6 (15 per cent) were completely free of colds during the second two years of

the treatment, and during the first winter experienced a marked reduction in number (three or less) as well as a definite decrease in severity. In the group which received both vitamins, 8 (20 per cent) received no benefit from the treatment, while 26 (65 per cent) experienced a reduction in both number and severity. This is to say that the number of colds per year dropped to three and that the average duration was five days, with but little elevation of temperature. When these conditions were not fulfilled, the subject was classified as having received no benefit from the treatment.

It was apparent that the combination of vitamins A and D reduced the incidence and severity of colds in the majority of susceptible persons. This is not to say that ingestion of vitamins in the doses mentioned is to be considered as curative or completely preventive. The results point only to the striking difference between those subjects treated with both vitamins and those treated with only one.

Symptoms of toxicity due to one or another of the vitamin preparations developed in a few of the subjects. All 3 of the patients who received the carotene solution had symptoms of toxicity after taking 7,000 units daily for periods of from four to fourteen weeks. The symptoms were carotenemia of high degree as evidenced by yellow coloration of the skin and eyes, anorexia, loss of weight and a general feeling of malaise. One patient (no. 6) receiving only vitamin A as fish oil concentrate showed similar symptoms except that hypercarotenemia was absent. Two patients (nos. 10 and 12) receiving only vitamin D also exhibited signs of toxicity, the symptoms being headache, polyuria, nausea and anorexia. This toxicity occurred six and eleven weeks, respectively, after treatment with 300,000 units daily. No subject in group 3, receiving both vitamins A and D, displayed any symptoms of toxicity at any time during the study.

In every case of toxicity the vitamin intake was stopped immediately, and a 10 per cent solution of calcium levulinate was given intravenously in doses of 10 to 20 cc. daily for five days, or a 10 per cent solution of calcium chloride was given by mouth, 1 tablespoonful (15 cc.) four times daily until symptoms vanished. In all cases the symptoms disappeared fairly rapidly under these conditions, requiring about four to ten days to vanish completely. No other injuries were apparent, and after several weeks of rest the patients were again given the vitamins but in doses only half as great as previously.

SUMMARY

Vitamins A and D in massive doses did not produce immunity to the common cold when given separately.

When massive doses of vitamins A and D were given together, 80 per cent of the subjects showed a significant reduction in both the

number and the severity of common colds. The number of colds per year dropped to three, and the average duration was five days, with but little elevation in temperature.

It must not be assumed here that the use of vitamins in the treatment of the common infectious cold is offered as a panacea for colds. Average susceptibility must be taken seriously into account, as well as the emotional state of the subject (Spiesman[7]) as affected by an innately unstable vasomotor mechanism.

7. Spiesman, I. G.: Vasomotor Responses of the Mucosa of the Upper Respiratory Tract to Thermal Stimuli, Am. J. Physiol. 115:181, 1936.

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Occurrence, distribution and absorption of provitamins A

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The term vitamin A activity has been used for many years to refer to all substances that can restore growth and other functions in experimental animals or man subsisting on a vitamin A-deficient diet. Among the many vitamin A-active substances are the carotenoids, whose importance becomes obvious when it is remembered that they are the ultimate source of all vitamin A in nature. In this paper the term provitamins is restricted to the biologically active carotenoids. For many animals, in particular ruminants and herbivores, these provitamins are the only source of vitamin A activity. For omnivores such as man the proportion of the vitamin A intake derived from provitamins varies widely. Carnivores get most of their vitamin A preformed; there is doubt if some of them, the cat in particular (Gershoff, Andrus & Hegsted, 1954), can convert carotene into vitamin A.

This paper deals mainly with the importance of the provitamins in human nutrition. First the various forms and their occurrence in plants and in animals are considered, then the importance of the vegetable and fruit sources in various parts of the world, and finally the many factors influencing absorption and utilization of the provitamins.

Carotenoid provitamins

In general to possess vitamin A activity a provitamin must have at least one β-ionone ring and a polyene side-chain.

β-Carotene and its provitamin derivatives. β-Carotene is the most important provitamin for two reasons. First, it is present in many plants that are important to man and his livestock; secondly, it possesses two β-ionone rings and polyene side-chains and is the provitamin A with the greatest activity. The β-apocarotenals, first prepared in the laboratory by Karrer & Solmssen (1937), have been found in nature by Winterstein, Studer & Rüegg (1960) and postulated to be the degradation products formed if terminal oxidation of the β-carotene molecule is the pathway for the conversion into vitamin A (Glover & Redfearn, 1954). The β-apocarotenals have around 50% of the activity of β-carotene when given by mouth (Karrer & Solmssen, 1937; Glover & Redfearn, 1954; Marusich, De Ritter, Vreeland & Krukar, 1960) or by injection (McGillivray, 1961).

Oxidation that opens both rings destroys activity but, when only one ring is attacked, β-hydroxycarotene and β-semicarotenone are formed. They have half the activity of β-carotene. The 5,3,6-mono- and 5,6-5′,6′- di-epoxide have been found in plants (Karrer, Jucker, Rutschmann & Steinlin, 1945) and are vitamin A precursors despite alteration to the β-ionone ring, the explanation being that the body can convert the epoxide into β-carotene. Another active oxide of β-carotene found in nature is mutatochrome; it has one intact β-ionone ring and the other changed to a furanoid structure. Hydrogenation, as would be used in the hardening of vegetable fats,

completely destroys the activity of β-carotene. Dehydrogenation also causes loss of activity.

α- and γ-carotenes. Since these compounds have only one β-ionone ring, they have only half the activity of β-carotene. Little is known about their derivatives, but the monoepoxides are active and occur in plants (Karrer *et al.* 1945). The apocarotenes are inactive, since degradation begins at the β-ionone ring and α-apocarotenals are formed.

Carotenoids containing oxygen. Cryptoxanthin, 3- or 4-hydroxy-β-carotene, is undoubtedly, as far as man is concerned, the most important provitamin next to β-carotene because of its occurrence in significant quantities in plants. As it has only one β-ionone ring intact, it has the same activity as α-carotene, but Johnson & Baumann (1948) found that it was as effective for deposition of vitamin A in the liver as β-carotene though it had only half the activity of β-carotene when assayed by the growth method. Because of the hydroxyl group, it often occurs as the ester.

Myxoxanthin occurs in freshwater and marine algae and is a provitamin (Karrer & Rutschmann, 1944). Other biologically active carotenoids are echinenone and aphanin. Lutein (3,3′-hydroxy-α-carotene) is biologically inactive, but Zechmeister & Petracek (1956) showed that on treatment with acids three breakdown products are formed, one of which is a provitamin. Recently, Budowski, Ascarelli, Gross & Nir (1963) have suggested that this compound is 3′-hydroxy-3,4-dehydro-β-carotene (anhydrolutein) and that it acts as a precursor of vitamin A_2 in the chick. It is possible that such compounds are formed in the preparation of food and animal feeds, e.g. in silage. Finally, astaxanthin ($C_{40}H_{52}O_4$) has been claimed to be a provitamin for fish (Grangaud & Massonet, 1950). Although this is unlikely, if it were true the presence of the pigment in considerable amounts in a wide range of crustaceans would make it an important source of vitamin A in the marine food chain.

Cis-trans *isomerism.* Molecules with structures such as the carotenoids can exist in several spatial forms. Gilham & El Ridi (1935) first drew attention to the problem. It was studied at great length by Zechmeister, Le Rosen, Schroeder, Polgar & Pauling (1943). Bending of the molecule only occurs at those double bonds in the central chain adjacent to a methyl group. Zechmeister (1949) then showed that the all-*trans* forms predominate in nature and have twice the biological activity of the corresponding *cis* forms.

Occurrence of the provitamins in plants and animals

This subject was reviewed comprehensively by Goodwin (1952).

Plants. Carotenoids are found in all green tissues of plants and are associated with chlorophyll in the chloroplasts, probably as water-soluble protein complexes and sometimes in lipid droplets. In green leaves, although xanthophylls predominate, β-carotene is always present. In fifty-nine species studied by MacKinney (1935) the range was 200–700 μg β-carotene/g dry matter. Forty of these species contain α-carotene as well in amounts varying from a trace to 35% of the total carotene fraction. In fruits, although the preponderant leaf carotenoids, β-carotene and lutein, do occur, they are often minor constituents; for instance, either α- or γ-carotene or lycopene

Table 1. *Proportion (mg/100 g fresh weight) of carotenoids in the more important sources of provitamins A for man and his livestock*

Source	Carotene β	Carotene α	Lycopene	Cryptoxanthin	Xanthophylls	Reference
Grass	8	Trace	—	—	16	Kon & Thompson (1940)
Carrots	7·6	4·8	—	—	1·4	Dark & Booth (1946)
Peas	0·3	—	—	—	0·6	Booth (1945)
Potatoes	0·006	—	—	—	0·04	Thompson (1941, unpublished)
Sweet potatoes	2·0	—	—	—	Trace	Ezell & Wilcox (1948)
Tomatoes	0·5	—	9	—	Trace	Kuhn & Grundmann (1932)
Maize	0·3	0·02	—	0·55	2·6	Sadana & Ahmad (1946*a*)
Mangoes	0·14	—	—	—	0·14	Sadana & Ahmad (1946*b*)
Water melons	0·05	—	0·6	—	0·1	Zechmeister & Polgar (1941)
Pumpkins	0·7	—	—	—	—	Fraps (1947)
Bananas	0·3	—	—	—	0·2	Sadana & Ahmad (1949*b*)
Red-palm oil	38	20	—	—	—	Hunter & Scott (1944)
Oranges	0·15	—	—	0·8	1·3	Sadana & Ahmad (1948)

is often the predominant carotenoid. Table 1 shows the proportions of carotenoids in some of the more important sources of provitamins A for man and his livestock. Although in many laboratories a large number of plant foods have been analysed as sources of provitamins A for man, the values given are generally the findings in terms of 'carotene' and do not give information about the proportion of the provitamins and other carotenoids (see, for instance, for food eaten in USA Fraps (1947) and in Central America (Guatemala) Munsell, Williams, Guild, Kelley, McNally & Harris (1950)). On the other hand, careful studies of the proportions of the carotenoids have been made on many plants, but they are not necessarily representative of the foods going into consumption as these investigations are often made in great detail on only one sample. For instance, the batch of water melons studied by Zechmeister & Polgar (1941) had the carotenoid distribution given in Table 1, but Fraps (1947) found a mean carotene content ten times greater for the many samples he studied. Clearly, variety and stage of maturity alter the value widely. Table 1 shows that the most important sources of provitamins are grass, carrots, maize, sweet potatoes and red-palm oil. The carotenoid content of many fruits increases on storage, for instance in mangoes (Sadana & Ahmad, 1949*a*; Chaudhary, 1950) and in tomatoes (Ellis & Hamner, 1943).

Lichens are a source of provitamins for reindeer, an important domestic animal in Lapland (Ellis, Palmer & Barnum, 1933). Fungi are generally devoid of carotenoids, but it is interesting to note that the carotenoid torularhodin ($C_{37}H_{48}O_2$) isolated by Karrer & Rutschmann (1946) from the red yeast *Rhodotorula rubra* has vitamin A activity. Probably more is known about carotenogenesis in the fungi that do contain carotenoids than in any other plants (cf. Goodwin & Wilmer, 1952).

Animals. There is little doubt that animals do not synthesize carotenoids *de novo* but acquire them from their food either directly from plants or indirectly from animal sources, or produce carotenoids characteristic for the animal species by altering those in their food. Crustaceans contain traces of β-carotene, large amounts of astaxanthin, and also preformed vitamin A (Kon & Thompson, 1949). Astaxanthin is often present as a protein complex, as in the lobster (Lwoff, 1925). Fish feed on zooplankton; they are capable of converting any β-carotene available (Morton & Creed, 1939), but may get most of their vitamin A preformed (from crustaceans, cephalopods, other fish). The skin and other tissues of fish are sometimes coloured, but the pigments in the edible parts are not provitamins; for instance, in salmon (Emmerie, van Eekelen, Josephy & Wolff, 1934) and trout (Steven, 1948) the flesh is coloured by astaxanthin.

Insects are not often thought of as food, but an entertaining paper by Bristowe (1953) shows that they are quite important in the Far East. In the Middle East locusts have been included in the diet since biblical times; they contain 10–15 μg β-carotene/g fresh weight (Goodwin, 1949).

Mammals can be divided into three groups according to whether they accumulate in the fatty tissues of the body (1) a mixture of carotenoids, (b) predominantly carotenes, or (c) no carotenoids (Goodwin, 1952). Man falls in the first category, for the blood, milk and many organs contain, in addition to carotenes, lycopene, an unknown pigment and lutein (Kon & Mawson, 1950). Cattle are the most important example of the second group. Palmer & Eckles (1914) first noted the preferential accumulation of carotene in the tissue of cows. However, this depends to some extent on breed and diet, for Thompson, Henry & Kon (1964) found that the percentage of active carotene in milk in Great Britain was around 80 in summer and 68 in winter. In winter in Scotland, cows eat yellow turnips, a source of lycopene, and the milk-fat carotenoids contain occasionally only 25% carotenes (Thompson & Kon, 1950). The horse, Indian buffalo, deer, antelope, water-buffalo, guinea-pig and hedgehog also come in this group. The sheep, dog and elephant sometimes have traces of carotenes as the only carotenoid (for more detailed information see Goodwin, 1952). In the last group are the goat, pig, rat, rabbit, hare (Goodwin, 1952), giraffe (Aschaffenburg, Gregory, Rowland, Thompson & Kon, 1962) and reindeer (Aschaffenburg, Gregory, Kon, Rowland & Thompson, 1962). Birds, like mammals, can convert provitamins into vitamin A, but they store preferentially xanthophylls. However, Steinegger, Streiff & Zeller (1957) showed that the hen is capable of passing to the egg a surprising number of oxygenated carotenoids including β-apo-8-carotenal which has vitamin A activity as well as colouring powers. Wald & Zussman (1938) found that hens can absorb astaxanthin, and Fox (1962) has shown that the pigments of the American and West Indian flamingo are mainly canthaxanthin, with some astaxanthin and numerous oxidation products of carotenoids not yet completely characterized.

Sources of provitamin A in man's diet in different parts of the world

Since the introduction of vitamin A acetate as the international standard for vitamin

A, it is agreed that 1 μg retinol has an activity of 3·33 i.u. (World Health Organization, 1950). It is also agreed that 0·6 μg all-*trans* β-carotene has the activity of 1 i.u. preformed vitamin A when the test is done at the low level used in the rat-growth method (League of Nations: Second Conference on Vitamin Standardisation, 1934), but for practical purposes, because of low digestibility, absorption or utilization, this conversion rate has to be lowered, and in this country McCance & Widdowson (1960) divide the i.u. calculated in this way by 3 and the (USA) National Research Council (1964) in America divide by 2. In Britain (British Medical Association, 1950) and in America (National Research Council, 1964) it is agreed that adults need about 3000 i.u. of preformed vitamin A daily or two to three times this amount as provitamin A.

Europe. In Britain the foods going into consumption supplied in 1962 4300 i.u. vitamin A activity daily per head (Ministry of Agriculture, Fisheries and Food: National Food Survey Committee, 1964: Appendix C). Of this total, 1130 i.u. was from provitamins in vegetables, fruits and cereals and another 290 i.u. from provitamins in dairy produce; hence about 33% of the total vitamin A intake is supplied as provitamins. During the war the contribution from provitamins was around 44%. The carrot supplied 68% of our carotene from vegetables and the tomato 72% of that from fruits (Table 2). In southern Europe melons, persimmons and pumpkins would contribute additionally or replace some of the sources listed in the table.

Table 2. *The importance of vegetables and fruits as sources of provitamins A in Great Britain*

Vegetable or fruit	Amount consumed* (g/head day)	Carotene content† (mg/100 g wet wt)	Contribution to intake (i.u./head day)
Brussels sprouts	9·2	0·4	52
Cabbages	20·0	0·3	
Carrots	11·2	9·0	566
Lettuces	4·9	1·0	55
Peas and green beans	15·3	0·3	16
Dried, canned and others	35·1	—	141
Apples	29·0	0·03	3
Pears		0·01	
Citrus fruits	17·0	0·05	10
Bananas	14·7	0·20	2
Tomatoes	17·0	0·50	170
Other, stone and soft fruits and canned	32·5	—	51

*Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1964).
†McCance & Widdowson (1960).

Some data of Aykroyd & Sukhatme (1960) were used to prepare Table 3. It reflects the big differences in feeding habits in the European countries listed. For instance, the Finns eat one-sixth the amount of vegetables and over three times the amount of milk consumed by the French.

North and South America. More detailed information is available for the United States than for any of the other countries. In the United States the general feeding

Table 3. *Yearly consumption (kg/head) of some foods in different parts of the world*

Country		Sweet potato	Vegetables	Fruits	Meat	Milk	Cereals
Europe:	Finland	0	19	32	33	346	119
	France	0	132	48	69	98	111
	Germany	0	45	70	48	146	96
	Greece	0	92	95	18	57	146
	United Kingdom	0	59	43	54	158	88
America:	United States	3·3	98	82	82	195	69
	Brazil	0	27	110	30	44	91
Africa:	Egypt	1·9	62	64	13	49	188
	South Africa	1·7	38	30	43	77	149
Middle East:	Israel	0	116	120	21	106	140
Far East:	China	65	58	15	17	0·4	148
	India	3·3	16	12	1·5	40	130
	Japan	28	67	16	3·2	13	148

Adapted from Aykroyd & Sukhatme (1960); mean values for 1954-7.

Table 4. *Comparison of food consumption and vitamin A intake in Great Britain and the United States*

Food	Great Britain* lb/week	Great Britain* i.u./day	USA† lb/week	USA† i.u./day
Milk	6·22	674	8·4	1344
Cheese	0·20			
Meat	2·40	959	3·8	1919
Fish	0·36	13		
Eggs	0·59	334	1·0	622
Butter	0·39	754	0·2	998
Margarine	0·20	387	0·2	
Sugar and jams	1·35	—	1·3	—
Vegetables	5·3	830	7·6	4475
Fruits	1·8	236	5·1	656
Cereals	4·30	61	3·0 calc.	64 calc.
Beverages and other foods	0·24	52	—	77
Total		4306		10 155

*Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1964).
†United States Department of Agriculture (1957).

pattern is the same as in Europe, but Table 4 shows that the total vitamin A intake (10 200 i.u. daily) is two and a half times that in Great Britain, and that over 50% of it comes from vegetable sources; the consumption of dairy produce (excluding butter) and of fruit and vegetables is much greater than in Britain. The sweet potato provides one-tenth of the provitamin A from vegetable sources. Other important sources, not eaten in such large amounts in Britain, are sweetcorn, pumpkins, spinach, squashes, apricots, peaches, avocado pears, melons, water melons and yellow maize. In sharp contrast, Eskimoes in 1855, according to calculations made by Sinclair (1953), were almost carnivorous; their intake per head daily of preformed vitamin A was about 10 000 i.u., mainly from seal flesh. Their only vegetable source, berries, supplied 15 i.u. provitamin A daily.

Probably typical of the situation that can be found in many parts of Central and South America is that in Guatemala described by Flores, García, Flores & Lara (1964), where not only does the diet supply often far less than the recommended allowance of vitamin A for adults, but 93% comes from provitamins and of this 34% from yellow maize.

Africa. The outstanding source of provitamin A on this continent is red-palm oil. As Table 1 shows, it is a very rich source of vitamin A activity. In western Nigeria the intake of 100–200 g of this oil supplies 60 000–120 000 i.u. daily, and this remarkably high intake causes the blood serum to be unusually rich in carotene (Carter & Cook, 1963). Apart from carrots and maize, other important sources are sweet potatoes, fresh leaves, red paprikas, cape gooseberries, paw-paws and mangoes. Despite these rich sources of provitamins, vitamin A deficiency is prevalent in many parts of Africa (Nicol, 1949; Roels, Trout & Dujaquier, 1958). Aykroyd & Sukhatme (1960) give food consumption data for only two countries in Africa, Egypt and South Africa; Table 3 shows that the intake of cereals in these countries is high, which is probably true for many parts of the African continent.

The Middle East. Table 3 shows that in Israel the diets are better balanced than in Africa or the Far East.

The Far East. In many parts of India, particularly the south, little meat and milk are eaten and very little vegetables and fruits. The diets, mainly of cereals such as millet, rice, wheat or tapioca, supply less vitamin A activity than the recommended allowance. A typical diet in the state of Mysore (Subrahmanyan, Narayanarao, Ramarao & Swaminathan, 1955) supplied only 1000 i.u. daily (mainly as carotene). Aykroyd (1944) said that Indians would have to increase their consumption of leafy vegetables six- to eight-fold to balance their diet. Other interesting differences in feeding habits in the East are seen in Table 3, for instance the low milk consumption in China and Japan.

Factors affecting the absorption and utilization of the provitamins

Since there is no evidence so far that the provitamins can act as vitamin A *per se*, the interest is not so much in their absorption as such as in their potential yield of vitamin A after absorption and conversion.

Digestibility. Provitamins A are present in most foods; in the cells of plants they are as solutions in fats or as protein complexes (Strauss, 1942) or in crystalline forms. During passage through the digestive tract, first the plant cells must be broken down, and the presence of carotene in faeces in intact plant cells shows that this can be far from complete (Hume & Krebs, 1949). Then in the small intestine the provitamins can be (a) absorbed as such, (b) converted into vitamin A, or (c) destroyed by enzymes, bacteria or oxidation. Clearly the difference between the amounts of the provitamin in the food and in the faeces does not represent that converted into vitamin A, but such studies have shown that carotene is better utilized from oily solution than from vegetable sources (De, 1937; Kemmerer & Fraps, 1938).

Level of intake. A recent example of the poor utilization at high dose level is shown in Fig. 1 (from Thompson, 1963). In the cow, increasing the dose of β-carotene

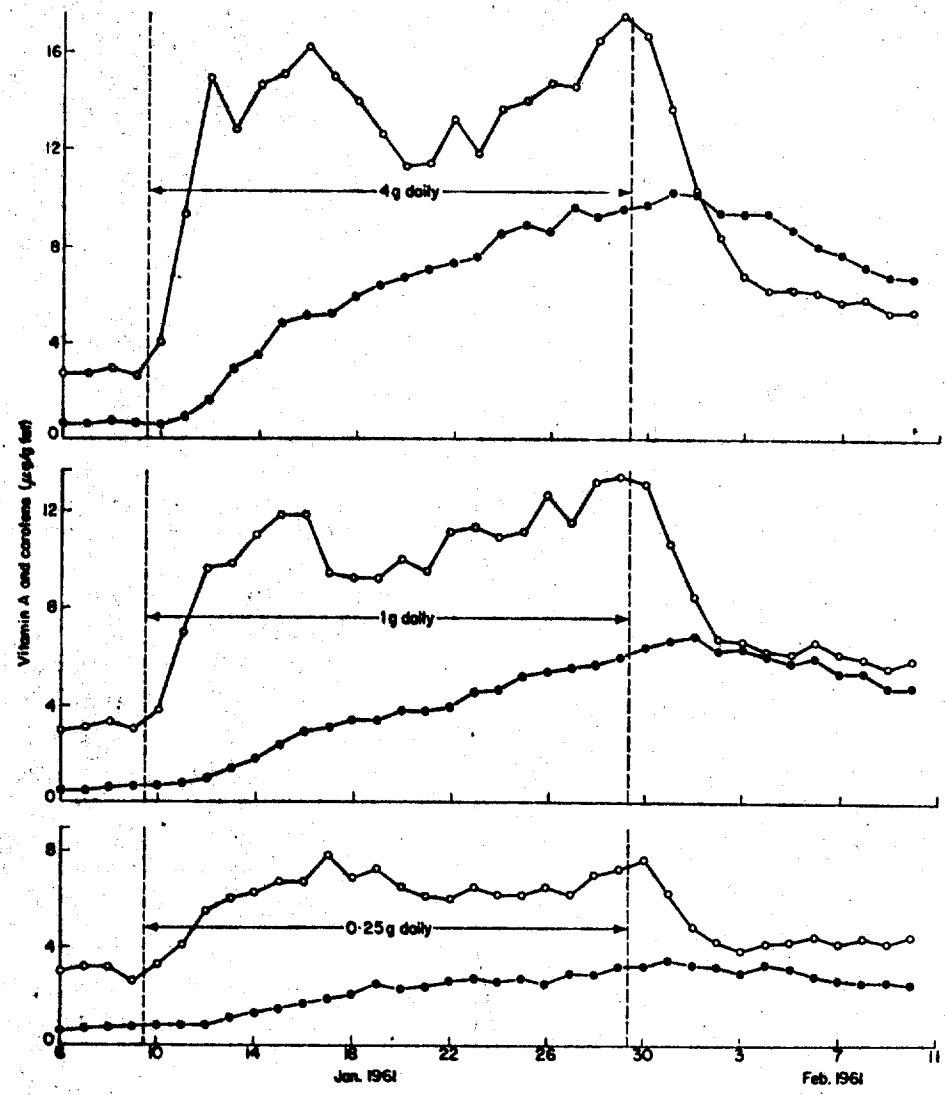

Fig. 1. Vitamin A and carotene in the milk fat of twelve Friesian cows on a carotene-low diet, supplemented during 21 days with 0·25, 1·0 or 4·0 g carotene daily as a 14·5% microcrystalline gelatin beadlet preparation. Each point represents the mean value for four cows. o—o, vitamin A; ●—●, carotene. (Thompson, 1963.)

fourfold produced a 2·5-fold increase in milk carotene and vitamin A content, and a sixteenfold increase in the dose produced only a fourfold increase.

Amount of dietary fat and state of dispersion. Animal experiments have shown the beneficial effect of fat in the utilization of provitamins, which has been shown to be true for man. Recently Roels *et al.* (1958) found that African boys receiving 200 g carrots in addition to their normal diet absorbed only 5% of the

carotene, but a supplement of 18 g olive oil increased the absorption to 25% and produced large and rapid increases in serum content of carotene and vitamin A. However, carotene can be used in the absence of fat, either in aqueous colloidal dispersions or, better still, as dispersions with surface-active agents such as Tweens (Tomarelli, Charney & Bernhart, 1946; Kon, McGillivray & Thompson, 1955). Particle size is very important since the introduction of dry powder preparations. For instance, Thompson (1963) found that if the particles were less than 1 μm the provitamin was very effectively utilized.

Nature of the dietary fat. An antagonism between the unsaturated fatty acids and vitamin E affects carotene metabolism (Sherman, 1941). The inclusion in the diets of fats that are poorly digested, such as paraffin oil or beeswax, lowers the utilization of carotene (McGillivray, 1957; McGillivray, Thompson & Worker, 1958). The levels of tocopherol and lecithin also are important. For a brief review of other fat-soluble factors see Thompson (1964).

Nature and level of the dietary protein. Since the original report on the effect of protein intake on the utilization of provitamin A (Indian Council of Medical Research, 1952), there has been evidence to show that a diet low in protein, or containing poor-quality proteins, hinders the utilization of the provitamins (Arroyave, Béhar, Wilson, Mendez & Scrimshaw, 1959; Friend, Heard, Platt, Stewart & Turner, 1961; Deshmukh, Malathi & Ganguly, 1964; Dietl, 1963).

Other dietary factors. Cattle feeds containing much nitrite or nitrate have been blamed for lowering the vitamin A status of the animal (Pugh & Garner, 1963). Raw soya bean contains an enzyme that oxidizes fat and destroys carotene (Squibb, Cannon & Allen, 1948). Since the original observations of von Euler & Klussman (1932) of an antagonism between thyroxine and carotene affecting the growth of rats deficient in vitamin A, and of Fasold & Heidemann (1933) that carotene appears in the otherwise colourless milk fat of the goat after thyroidectomy, there has been no aspect of carotene metabolism more controversial, and there is much evidence for and against such a relationship between carotene and thyroxine (cf. Moore, 1957).

It is evident that many factors affect the utilization of preformed vitamin A, and that even more affect that of the provitamins. The chemical estimation of the provitamin tells the dietician which forms are present and in what amounts; after that, like the policeman in Gilbert & Sullivan's *Pirates of Penzance*, his lot is not a happy one.

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CONCERNING THE TOXICITY OF VITAMIN A

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Several investigators have described a pathological condition which they called hypervitaminosis A. Von Drigalski and Laubman ('33) fed six rats 1 cc. of an oil concentrate of vitamin A. These rats died in from 5 to 14 days, and post mortem examination showed glomerular nephritis with calcification to which the death of the animals was attributed. Pathologic changes on a smaller scale were noted in the spleen, liver and testicles. Collazo and Rodriguez ('33) also fed several 50-gm. rats a vitamin A concentrate, Vogan, prepared by E. Merck of Darmstad, Germany. The rats were fed 0.5 cc. daily, or a dosage of 20,000 units of vitamin A. The outstanding features of the disease produced in about 10 days were failure to grow, inflammatory changes in the eyes with exophthalmos and changes in the bones resulting in spontaneous fractures. Bumskov and Seeman ('33) obtained similar results in eleven rats from the administration of Vogan, and somewhat similar observations have been published by Harris and Moore ('29) and others.

The results obtained were attributed to vitamin A by these authors chiefly because the administration of similar amounts of sesame oil and other vegetable oils containing no vitamin A are harmless, and because several observers had found that vitamin A was destroyed by ultra-violet irradiation and that the toxicity of the oil was also lost. Thus von Drigalski and Laubman ('33) submitted the toxic Vogan to ultra-violet

irradiation, and finding that this destroyed the toxic power of this concentrate, concluded that this proved that the toxic effect was produced by vitamin A. This conclusion does not necessarily follow because the toxic effect may have been due to some other constituent in the oil also destroyed by ultra-violet radiation. Peacock ('26) showed that irradiated cod liver oil lost its normal fluorescence in addition to the loss of vitamin A and that these two phenomena were different and independent. Irradiation of such an oil also produced vitamin D which is supposed to have an antagonistic action to vitamin A. For these reasons, the above experiments, performed moreover on very small series of rats, hardly prove that the toxicity of a complicated fish liver oil concentrate is caused by its vitamin A content. It is also known that the feeding of carotene, the pro-vitamin A, is entirely harmless, a fact that we have confirmed by feeding several young rats 40,000 international units both alone and in solution in vegetable oil for 100 days without ill effect.

Since Vogan is unobtainable in this country, we obtained a vitamin A concentrate from the British drug houses, having a Carr-Price blue value of not less than 50,000. Lathbury ('34) has shown that there is a constant ratio between the results of the biological, chemical and physical methods of testing purified vitamin A concentrates. Half a cubic centimeter of this concentrate was administered to 50-gm. rats daily, but this preparation was so objectionable that rats so treated refused any food and died of starvation in 4 days.

In the following experiments we have used 140 rats, of which 127 form the basis of this report, the remainder being various controls. These experiments were all performed with oil from the liver of the jewfish (Stereolepis gigas), which is the richest source of vitamin A known at present, being many times more potent than concentrates from ordinary oils. All of the oil so used was furnished us through the courtesy of Dr. Charles E. Bills, director of the Research Laboratory of Mead Johnson and Company.

Technic. Healthy male rats of the Wistar strain and of 50 gm. weight were used in all experiments, except that females were introduced at times to determine breeding capacity. The diet fed was Purina dog chow, which is a complete diet for rats. Stock rats so fed grew and bred normally. This diet was used for its convenience in weighing in order to determine the amount eaten daily. The oils used were administered daily by means of a pipette, and dosage was kept at 0.5 cc., changes in concentration being effected by proper dilution with oil of sesame, shown in controls to be inert. The vitamin A potency of the oil was determined by vitameter readings, and it had been planned to dose all rats with multiples of 25,000 international units. Unfortunately, it was found later that owing to some unknown constituent in the jewfish liver oil, the vitameter readings were about one-third higher than the results of the biological test. In experiments performed later, 100,000 units by biological test were used.

The experiments were all continued for 100 days unless death occurred earlier. It was assumed that if rats continued to grow normally for 100 days, the oil administered was non-toxic. A post mortem examination was made on all rats that died as a result of the treatment, and later all the surviving rats were killed not only for post mortem examination, but to determine the vitamin A content of their livers.

The number of units stored in the liver was determined by the blue reaction with antimony trichloride. The antimony trichloride is used in a 30% chloroform solution (weight in volume). The liver to be tested was dissolved in a 10% solution of potassium hydroxide; this solution was extracted in a separatory funnel with ether; the ether evaporated, and the residue redissolved in chloroform. To 0.2 cc. of this chloroform solution, which was usually made up to 500 cc., 2 cc. of the antimony trichloride was added, and the resultant blue color matched against the standard glasses of a Lovibond tintometer, in accordance with the method described in "The Vitamins: Symposium on the present Status of the Knowledge of Vitamins." J. Am. Med. Assn., April 23 to August 6, 1932.

We were unable to confirm the pathological findings of von Drigalski and Laubman ('33). At post mortem, all the organs except the bones appeared normal grossly. Microscopic sections showed that the stomach and intestines, the testes,

adrenals, thyroid and parathyroid were normal. There were often mild congestion of the lungs, marked congestion and some cloudy swelling and, at times, fatty infiltration of the liver, moderate congestion and cloudy swelling of the tubules of the kidney, the glomeruli appearing normal, and a spleen slightly congested. In some cases there appeared to be some swelling of the heart muscle fibers, and a loss of staining of their nuclei. While these findings were by no means invariable, they were frequent enough to indicate that death was probably caused by an insufficiency of the cardiac muscle with accompanying congestion of the abdominal viscera.

The symptoms agreed very well with those described by Collazo and Rodriguez ('33) except that they were only manifested by some of our rats. They included a diminished growth curve, hemorrhages from the eyes often with exophthalmos, hemorrhages from the nose, rales in the chest, and the bone lesions. The bone lesion is rarefaction with a pronounced tendency to spontaneous fracture. If the animal lived, the fractures healed in spite of the continuance of the large dosage of vitamin A.

In the following tables presenting the results of our experiments, both vitameter readings and the results of the biological test are given. Since three separate lots of jewfish liver oil were used, there are three series of experiments tabulated.

Series 1. This lot of oil contained 550,000 international units of vitamin A per cubic centimeter (vitameter) and 4500 units of vitamin D.

Results. Series 1

RATS	DOSAGE I.U.A.[1]	AVERAGE FINAL WEIGHT	REMARKS
1– 4	25,000	259	Normal except some decrease in weight
5– 8	50,000	241	One rat died in 40 days. All had fractures
9–20	100,000	45	No rat lived 100 days. Fractures and hemorrhages.

By biological test, these amounts were 16,000, 33,000 and 66,000 international units respectively.

Series 2. This lot of oil contained 663,000 international units of vitamin A per cubic centimeter (vitameter), and 4500 units of vitamin D.

Results. Series 2

RATS	DOSAGE I.U.A.[1]	AVERAGE FINAL WEIGHT	REMARKS
1– 4	25,000	250	Normal
5– 8	50,000	194	Fractures in two rats. All lived 100 days
9–16	100,000	127	Two rats lived 100 days. Six had fractures

[1] By biological test these amounts were 16,000, 33,000 and 66,000 international units, respectively.

Series 3. This lot of oil contained 600,000 international units of vitamin A per cubic centimeter (vitameter) and 5000 units of vitamin D.

Results. Series 3

RATS	DOSAGE I.U.A.[1]	AVERAGE FINAL WEIGHT	UNITS VITAMIN A PER GRAM LIVER	REMARKS
1– 4	50,000	319	1766	Normal. All rats lived 100 days
5–12	100,000	123	1704	Three rats lived 100 days. Two rats had fractures

[1] By biological test these amounts were 33,000 and 66,000 international units, respectively.

Series 4. One liter of jewfish oil having 530,000 international units of vitamin A per gram was submitted to molecular distillation by Dr. K. Hickman of the research laboratories of the Eastman Kodak Company. Six lots of oil were used as follows:

1. The original oil as a control containing 530,000 I.U. vitamin A per gram
2. Distilled fraction 1 containing 515,000 I.U. vitamin A per gram
3. Distilled fraction 2 containing 865,000 I.U. vitamin A per gram
4. Distilled fraction 3 containing 805,000 I.U. vitamin A per gram
5. Distilled fraction 4 containing 248,000 I.U. vitamin A per gram
6. The residue from distillation containing 53,000 I.U. vitamin A per gram

In the first five cases, the oil was so diluted with sesame oil that each 0.5 cc., the daily dosage, contained 100,000 international units of vitamin A. In the case of the residue, 2 cc. of undiluted oil were administered daily.

Results. Series 4

RATS	DOSAGE I.U.A. 100,000	AVERAGE FINAL WEIGHT	UNITS VITAMIN A PER GRAM LIVER	REMARKS
1– 4	Original oil	87	818	Only one lived 100 days. Fractures
5– 8	Fraction 1	135	4722	Three rats lived 100 days. No fractures
9–12	Fraction 2	97	3250	One rat lived 100 days. No fractures
13–16	Fraction 3	89	3250	One rat lived 100 days. Three had fractures
17–20	Fraction 4	38		Average life was 12 days. Hemorrhages
21–24	Residue	272	2199	All lived 100 days. Normal

Fraction 1 and the residue were less toxic than the other fractions. To be sure that this was the fact, each was repeated on a new lot of four rats, with precisely the same result. One female was introduced in the four receiving the residue, and nine young were born on the fifty-second day of the experiment. All nine lived.

Since jewfish oil was undoubtedly toxic in amounts equivalent to 100,000 international units of vitamin A, we next endeavored to ascertain whether this toxic effect was due to vitamin imbalance. For this purpose we used lot three of the jewfish oil, which was non-toxic in doses of 50,000 international units, and decidedly toxic in doses of 100,000 units (vitameter reading). Other vitamins were administered in addition. Vitamin D was administered from appropriate dilutions of a solution of irradiated ergosterol containing 1,000,000 units per cubic centimeter. Vitamin B_1 was used in two forms: an adsorbate received through the courtesy of the Eli Lilly Company, and crystalline vitamin synthesized and furnished us through the kindness of Merck and Company.

Vitamin C was also the synthetic product, furnished by Merck and Company. The results in the following table should be compared with the results in series 3 in which the same lot of jewfish oil was used alone.

Results from addition of other vitamins

RATS	DOSAGE	AVERAGE FINAL WEIGHT	UNITS VITAMIN A PER GRAM IN LIVER	REMARKS
1– 4	50,000A + 50,000D	44		Average life 27 days. No fractures
5– 8	50,000 + 25,000D	209	2731	Three lived 100 days. One rat had fractures
9–12	100,000A + 100,000D	39		Average life 10 days. No fractures
13–20	100,000A + 50,000D	190	2093	Five lived 100 days. No fractures
21–24	100,000A + 500 B_1	54		None lived 100 days. Average life 44 days. All had fractures
25–36	100,000A + 50,000D + 500 B_1	84	2750	Three lived 100 days. Two had fractures
37–48	100,000A[1] + 5 mg. ascorbic acid	241	2560	All lived 100 days. All normal and one raised ten young

[1] One hundred thousand international units vitamin A per cubic centimeter by biological test.

DISCUSSION

The term toxicity means poisonous. Many food materials such as sodium chloride or iodine can become deleterious in large quantities, but they are not called poisons. Neither should fish oils be called poisons because they are injurious when fed in enormous quantities. But some term is necessary to refer to this deleterious action in these experiments, and we have used toxicity, with the understanding that in the usual doses the various ingredients of such oils may be necessary in the diet.

The question as to the toxicity of large doses of vitamin A cannot be regarded as completely settled by these experiments, and the complete answer must await the isolation of

vitamin A in pure form. Nevertheless, several facts appear to indicate that the toxicity of jewfish liver oil is due to some constituent of the oil other than vitamin A. In the same dosage of vitamin A, the toxicity varies for each lot of oil. According to the European observers, Vogan is highly toxic, killing rats in about 2 weeks in doses containing 20,000 units of vitamin A. No sample of jewfish liver oil was toxic in less than 33,000 international units daily and the toxicity of lot 3 of the jewfish liver oil only commenced at 66,000 international units daily. Further, our 50-gm. rats receiving 33,000 units lived 100 days as a rule. It would seem that if vitamin A is the toxic factor, the toxicity of different samples of fish liver oil should be proportional to their vitamin A content. This is certainly not the case.

Still more significant is the fact that when lot 4 of the jewfish liver oil was submitted to molecular distillation, the toxic substance was separated from vitamin A. In the dosage of 100,000 international units of vitamin A daily, fraction four killed all rats in from 9 to 26 days, while three out of four rats receiving the same dosage of vitamin A from fraction one, lived 100 days. Finally, the four rats receiving 100,000 units of vitamin A from the residue from the distillation not only lived 100 days, but grew normally and were capable of bearing and rearing young. This result cannot be due to vitamin D which was only present in the original oil in doses of 5000 units. If it had all been distilled in one fraction, a most improbable supposition, it would have been quite insufficient to balance 100,000 units of vitamin A. We have shown that about 50,000 units of D are required for this purpose. We may conclude from this experiment, which was repeated with the same result, that if vitamin A is toxic, it must be given in higher dosage than 100,000 international units daily to 50-gm. rats, and that at least the greater part of the toxicity of the various fish liver oils must be due to some other factor than vitamin A. Since there is an increase of one-third in the vitameter reading over the result of the biological test as applied to jewfish liver oil, this discrepancy

is due to some unknown constituent in this particular oil. It is possible that this unknown constituent is the toxic fraction.

The greater part of vitamin A administered in these large doses must be either excreted or broken down in the body. In the course of 100 days at a dosage of 100,000 units daily, 10,000,000 units have been given. The maximum amount found in any liver per gram was 5000 units, which, with a 7-gm. liver was sufficient vitamin A to last this rat many years. The largest total amount found in the liver of any rat was rat no. 3 of series 3, with 2794 units per gram, which with 17 gm. of liver equalled 47,550 units. While lesser amounts are stored in other organs, only about 1 day's dosage, or one-hundredth part of the 10,000,000 units administered, can be accounted for. This fact is more obvious in our experiments than in those of other observers, because we have used much larger dosage, and kept our rats for much longer periods. No vitamin A is excreted in the urine, and of several rats tested, only from 500 to 600 units were excreted daily in the feces. Since the rat eliminates so little vitamin A, it seems certain that most of the vitamin A administered is destroyed in the body, and this constitutes another argument against the toxicity of this vitamin.

The neutralization of the toxic effect of fish liver oil by the administration of other vitamins has been suggested by numerous observers. Bell, Gregory and Drummond ('33) and others believe that the disturbance in the growth of rats that occurs when the diet contains a considerable percentage of fish liver oil may be compensated by an increased amount of the vitamin B complex. So far as our experiments with jew-fish liver oil are concerned, the administration of 500 international units of B_1 daily appeared to be not only of no value, but to be positively injurious. McHenry ('36) has shown that the addition of vitamin B_1 to rats on a basal diet practically doubles the percentage of fat in the liver. Whether used alone, or in combination with vitamin D, and whether used as an adsorbate or as crystalline vitamin, the results were poorer than control series without B_1. Of the rats receiving vitamin

A at 66,000 units, with vitamin D and vitamin B_1, only three out of twelve lived 100 days; but on the same dosage of A and D without B_1, five out of eight lived 100 days.

Thoenes ('35) and others have asserted that vitamin A and vitamin D are physiologically antagonistic; that is, that vitamin D is antagonistic to vitamin A concentrates. Our experiments afford some confirmation of this view, or at least that administration of considerable amounts of vitamin D relieves the toxic effects caused by jewfish liver oil. When the unitage of vitamin D exceeded that of vitamin A, the combination was far more toxic than the same amount of vitamin A alone. But vitamin D was of distinct benefit when its dosage was somewhat less than that of the vitamin A of jewfish oil. Thus when jewfish liver oil was given so as to administer 66,000 international units of vitamin A and 50,000 units of vitamin D, five out of eight rats lived 100 days; whereas, with the same lot of jewfish liver oil alone, administered in the same dosage, only two out of eight lived 100 days. Perhaps a still better proportion between the vitamin A containing fish liver oil and vitamin D dosage is possible, since we only tried two proportions.

The most striking result of these experiments, and one that so far as we are aware has never been suggested, is the preeminent protective action of pure crystalline ascorbic acid or vitamin C. Five milligrams of this vitamin given daily is apparently sufficient to protect all rats receiving such doses of jewfish oil as those containing 100,000 international units of vitamin A. Eleven out of twelve rats receiving such dosage of jewfish liver oil not only lived 100 days, but many of them grew normally. The only rat of this series that died earlier had a severe intercurrent infection which probably excludes it from consideration. No other dosage of vitamin C was used [illegible] it is possible that smaller doses would suffice, since ½ mg. daily should be approximately the amount required by an animal the size of a rat. On the other hand, larger doses may give a better result, as indicated by the fact that several of these rats developed fractures.

Since the rat is known to be capable of synthesizing vitamin C and is not susceptible to scurvy, it is difficult to explain this action of vitamin C. A possible explanation is that vitamin C destroys the toxic factor and that all the vitamin C synthesized by the rat is used up in this process, leaving it susceptible to scurvy. Scurvy of the rat has never been described, but the symptoms produced by fish liver oils are not unlike scurvy; they include failure to grow, hemorrhages, particularly from the eyes and nose but to some extent from other mucous membranes, and abnormal rarefaction and fragility of the bones.

In a few rats, the urine was collected and titrated with 2,6-dichlorophenol indophenol. After administration of jewfish liver oil equivalent to 100,000 units of vitamin A for about 3 weeks, the excretion of vitamin C decreased to the vanishing point, but reappeared in the urine in considerable amounts after the daily administration of 5 mg. of crystalline ascorbic acid. While this is suggestive, since the physiological action of vitamin C is quite unknown, the correct explanation of its remedial action for jewfish liver oil must await further experimentation.

CONCLUSIONS

If vitamin A is ever toxic, it is in doses in excess of 100,000 international units daily for 50-gm. rats.

The greater part of the vitamin A administered in such large dosage is destroyed in the body of the rat.

In addition to vitamin A, fish liver oils contain a toxic principle, at present not identified. The toxicity of various samples of these oils and their concentrates varies greatly for rats.

Vitamin B_1 was of no value in counteracting the toxicity of jewfish liver oil for rats.

Vitamin D in proper dosage at least partially counteracts this toxicity of jewfish liver oil as administered to rats.

Ascorbic acid (crystalline vitamin C) in doses of 5 mg. daily almost completely counteracted the toxicity of jewfish liver oil administered to rats.

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THE TOXICITY OF CAROTIN.

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In the course of an investigation of the effects of the bleaching of flour by chlorine gas, it became necessary to ascertain the possible toxicity of the pure pigment matter, both bleached and unbleached. As there could be found in the literature no report of any study of the possible physiological action of coloring matters of this class, it has seemed advisable to publish a brief account of our work, although the results are entirely negative.

It is quite certain that the coloring matter in flour is the same as that in carrots—carotin. Wesener and Teller ascribed the color in flour to carotin in 1911,[1] and suggested this in 1909. Monier-Williams[2] has advanced further proof that the coloring matter in flour is carotin, which is called "carrotene" by him.

The amount of carotin in flour is very small. Monier-Williams found in flour in parts per million as follows:

	Unbleached.	Bleached.
Flour A (photographic)	1.4	0.90
" A (colorimetric)	1.3	0.81
" B (")	2.0	1.28

The color is usually obtained from carrots, which contain it in relatively large quantities. Willstätter obtained 125 gm. from 5,000 kg. of carrots. Monier-Williams gives 2 gm. as the yield from 100 lbs. of carrots. 1.3 gm. coloring matter was obtained by us from 54 lbs. of carrots.

We obtained the coloring matter as follows: 54 lbs. of carrots yielded 21.2 kg. cleaned and trimmed material, which was ground

[1] Wesener, J. A., and Teller, G. L., *J. Ind. and Eng. Chem.*, 1911, iii. 912.

[2] Monier-Williams, G. W., *Reports, Local Gov't Board (Great Britain), Pub. Health and Med. Subjects, M Series, No. 73*, 1912.

in a meat grinder. The ground material was desiccated first by alcohol and then by distillation at 80–90° *in vacuo*. The dried mass was extracted with boiling 95 per cent alcohol. The alcohol extract yielded an extract with chloroform which was saponified with alcoholic potash. After the alcohol was removed by distillation, the residue was dissolved in water and extracted with ether. This ether extract weighed 11.2 gm. and consisted of colorless crystals mixed with carotin. The pigment was concentrated by dissolving the entire mass in carbon bisulfide and alcohol with the subsequent addition of enough water to cause the carbon bisulfide to separate, carrying with it most of the color. Several such treatments gave finally a highly colored mass that left practically nothing in the alcohol-water layer. This was now treated with alcoholic potash to remove with certainty all carbon bisulfide. After removing the alcohol the aqueous solution of the residue yielded an ether extract containing 1.3 gm. of concentrated pigment.

One-half of the pigment, 0.65 gm., was chlorinated as follows: The ether solution was shaken with a water solution of chlorine (6 cc. Cl_2 solution containing 0.01443 gm. Cl_2 per cc.). 0.65 gm. carotin, $C_{40}H_{56}$, requires by theory 0.0861 gm. Cl_2 to form $C_{40}H_{56}Cl_2$, and our material used 0.08658 gm. Cl_2. The color of the ether solution decreased and the Cl_2 odor vanished. The chlorinated product weighed about 0.09 gm. more than the initial amount.

In subsequent experiments we obtained but 1.2 gm. pigment from 200 lbs. of carrots, which vary greatly in pigmentation at different seasons.

This purified pigment was used in the following experiments. As it is soluble only in fat solvents, which are all more or less toxic, it was used dissolved in sterile olive oil. This would seem to be a suitable solvent, however, as shown by the efficiency of phlorhizin dissolved in oil when used in metabolism studies. Because of the extremely minute quantity of coloring matter contained in flour, it was not feasible to conduct experiments with carotin from flour, as only 1 gm. of color is contained in 1,000 kg. of flour, and in view of the demonstrated identity of the coloring matter of flour and carotin from carrots the investigation of the latter seemed sufficient.

Two pairs of male guinea pigs were given intraperitoneal injections of carotin dissolved in olive oil and sterilized by brief heating on the water bath.

N 1.	Wt. 339 gm.,	given	3 cc.	oil	with	0.100 gm.	natural carotin.
P 1.	Wt. 316 "	"	3 "	"	"	0.100 "	chlorinated carotin.
N 2.	Wt. 265 "	"	5 "	"	"	0.200 "	natural carotin.
P 2.	Wt. 245 "	"	5 "	"	"	0.200 "	chlorinated carotin.

These animals were kept under close observation for 12 hours, the temperature being taken every 2 hours. At the end of 4 days, during which they were frequently examined, they were killed with illuminating gas. Practically no effects were observed. All showed a rise of 1–2°F., 2 to 6 hours after the injection, but so did control pigs given olive oil alone. They all remained quiet but alert, and ate as well as the control pigs. The urine at no time showed albumin or sugar, but was of a deep reddish brown color. The autopsies showed no changes except the presence of a considerable amount of fatty yellow material in the peritoneum, but much less than the amount injected. There were no signs of inflammation or other local changes. All the viscera seemed normal in both gross and microscopic examination.

Evidently carotin possesses no considerable toxicity, if any, and chlorination does not make it demonstrably toxic. The quantities of carotin used in these experiments are enormous in comparison to the amounts that could be obtained from any food, even carrots. The larger dose used, 0.2 gm., is as much as is contained in 200 kg. of flour, or as lutein in 4,000 cows' ovaries (Escher[3]).

Local or Cellular Toxicity.

Although the absence of either symptoms or anatomic evidence of irritation after intraperitoneal injections of carotin indicated that it can have little or no local effect, this was determined more definitely by a series of intradermic tests. This method, devised and used in this laboratory by Dr. Corper[4] for the study of local toxicity, has been found very useful for this purpose. The principle and method are similar to those used in the intradermic

[3] Escher, H. H., *Z. Physiol. Chem.*, 1913, lxxxiii, 198.
[4] Corper, H. J., *J. Biol. Chem.*, 1915, xx, p. xxi.

injection of tuberculin or diphtheria toxin. As the two preparations to be compared are injected side by side in the same animal, no individual peculiarities of the animal enter as a source of confusion or error.

The carotin, dissolved in varying concentration in olive oil, was injected in parallel sites intradermically in 0.2 cc. quantities into two guinea pigs. The effects were the same in both.

Carotin dissolved in olive oil to the strength of 10 per cent seems to have a very slight local toxicity, as shown by the development of a little edoma and swelling. At 1 per cent the results are very little different from those with olive oil alone. These results are not appreciably different with chlorinated and natural carotin. Olive oil containing very small amounts of carotin (0.1 per cent) seems to cause, if anything, slightly less protracted swelling than olive oil alone; possibly a slight irritant effect from carotin hastens absorption.

These experiments indicate that even in relatively very large doses carotin, whether in its natural state or saturated with chlorine, is almost entirely devoid of toxicity. Such large amounts as 20 mg. injected intradermically cause only a local edema and inflammation, but no necrosis.

The extensive studies of Palmer and Eckles[5] indicate that carotin is almost universally distributed throughout all animal bodies, coming chiefly, if not solely, from the food. Our few experiments seem to be sufficient to warrant the assumption that any such quantities as can ever accumulate in the tissues have no harmful effects.

[5] Palmer, L. S., and Eckles, C. H., *J. Biol. Chem.*, 1914, xvii, 191.

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THE DISTRIBUTION OF RADIOACTIVE CARBON IN THE RAT AFTER THE ADMINISTRATION OF RANDOMLY LABELLED C^{14}-β-CAROTENE[1]

J. S. WILLMER[2] AND D. H. LAUGHLAND

Abstract

The distribution of C^{14} has been studied in rats following the oral administration of C^{14}-β-carotene, which is known to be readily converted to vitamin A in the intestine. The rats were maintained in a respiration chamber and individuals removed at various time intervals up to 28 hours after dosing, and the concentration of C^{14} in tissues was determined. The counts per million per gram of tissue was higher in the adrenal glands and hypophysis than in the other organs examined. The pattern of C^{14} distribution in intestine, liver, kidneys, and blood appears consistent with current knowledge of the metabolism of vitamin A.

Introduction

Although there are many references in the literature to the dramatic impairment of physiological and metabolic function imposed by a deficiency of vitamin A, there is little information concerning the specific manner in which the vitamin exerts its effect. An exception to this is the role of vitamin A in the visual process, which is reasonably well understood.

Laughland (10) studied the distribution of C^{14} in the chick after the administration of C^{14}-β-carotene, but it is now known that a contaminant, which was not separable by chromatography, was present in the dosing solution.

Krause, Coover, and Powell (8) claimed that after C^{14}-β-carotene was given orally to a rat, all of the absorbed C^{14} was found in the carotene-free, non-saponifiable fraction of the rat. These workers inferred that β-carotene was not changed into any water-soluble or saponifiable material. Kahn (7) administered vitamin A-2-C^{14} to rats and studied the distribution of C^{14} in the saponifiable and non-saponifiable fractions of various tissues. The saponifiable fractions contained appreciable amounts of radioactivity, and at least two metabolites of vitamin A were present in urine. Wolf, Kahn, and Johnson (18) administered vitamin A-2-C^{14} intraperitoneally to a rat and observed the following percentage distribution of radioactivity: expired carbon dioxide 5.2, urine 11.67, faeces 22.38, liver 13.79, skin 0.54, eyes 0.01, and remaining carcass 24.29. Water-soluble metabolites of vitamin A which were insoluble in petroleum ether but soluble in butanol were found in the urine.

The present work was conducted in an attempt to determine the distribution of C^{14} in the tissues of the rat after administration of an oral dose of randomly labelled C^{14}-β-carotene. It is obvious that in long term experiments the C^{14} would appear in most, if not all, tissues, once the C^{14} had found its way into the various pools of metabolically active carbon compounds. In an attempt to determine the rate of appearance of C^{14} in tissues the activity has been

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[2]National Research Council Postdoctorate Fellow, 1955–57.

determined at various time intervals after dosing. It was considered that the rate at which vitamin A or its degradation products appeared in a particular tissue and its relative abundance might, in relation to the metabolic function of this tissue, provide some indication of its mode of action.

Experimental

Methods

C^{14}-β-carotene was prepared from rye seedlings which were grown in an atmosphere of $C^{14}O_2$. After a germination period of 48 hours in the dark the seedlings were transplanted into moist sand in a growth chamber and maintained in an atmosphere of $C^{14}O_2$ for 7 to 10 days. A quantity of $C^{14}O_2$ based upon previously observed requirements was generated from $Na_2C^{14}O_3$ just prior to each photosynthetic period of 16 hours' duration. At the end of this time the residual $C^{14}O_2$ was removed from the chamber by pumping the gases through sodium hydroxide towers, and the seedlings were kept in the dark for 8 hours. A subirrigation technique was used to saturate the sand with the nutrient medium of Arnon and Hoagland (1) before and after each dark period.

At the end of the growth period the seedlings were removed, washed with water, and separated from the roots. The tissue was ground with sand and extracted in the cold with peroxide-free diethyl ether. After removal of solvent under reduced pressure the residue was saponified at room temperature in the dark for 18 hours by the addition of 20.0 ml. ethanol and 2.0 ml. of 50% potassium hydroxide. The non-saponifiable fraction was extracted with ether, and the extract washed free from alkali and dried over anhydrous sodium sulphate. Ether was removed under reduced pressure and the residue dissolved in 20.0 ml. of Skellysolve B.* Sterols were removed by cooling the solution to $-60°$ C. and filtering it through dry ice according to the technique of Glover, Goodwin, and Morton (5).

C^{14}-β-carotene was separated from the filtrate by chromatography on activated alumina. It has been observed in this laboratory and also by Glover and Redfearn (6) that it is impossible to obtain pure β-carotene by ordinary chromatographic means because an unidentified compound of high specific activity has been found to move with β-carotene on the column. The C^{14}-β-carotene used in the current experiments was recrystallized several times from chloroform by the addition of 80% methanol until a compound of constant specific activity was obtained.

The dosing solution was prepared by dissolving 2.05 mg. of C^{14}-β-carotene having a specific activity of 1.41×10^6 c.p.m. per mg. β-carotene in a few drops of diethyl ether. To this solution was added 9.5 ml. of 20% Tween 40 (polyoxyethylene sorbitan monopalmitate). The solution was mixed by means of a stream of nitrogen, and ether was removed by warming.

Eight male vitamin A-deficient albino rats (age 50 days) were given 1.0 ml. of the dosing solution by stomach tube. Four rats were then placed in each of two respiration chambers in which there was provision for the collection of urine and faeces. Drinking water was provided but no food was given.

*Skelly Oil Company, Kansas City, Missouri.

The respired carbon dioxide was collected in towers containing 0.5 *N* NaOH. Rats were removed from the chamber at 1, 2, 4, 6, 10, 16, 22, and 28 hours after dosing and the following organs removed for analysis: adrenals, heart, hypophysis, intestines, kidneys, liver, lungs, pancreas, spleen, stomach, testes, and thymus. A sample of blood was also taken at the time each rat was killed. Tissues were washed with physiological saline and blotted dry in order to remove as much blood as possible before weighing. Intestines were cut into lengths of approximately 10 cm. and flushed with saline; the washings were added to the excreta. The term "excreta" has been used to refer to the combined urine, faeces, stomach, and intestinal contents. Each sample was frozen and stored in an individual sealed container until analyzed for activity. The samples were saponified with alcoholic potassium hydroxide and the activity of the saponifiable and non-saponifiable fractions was determined. Aliquots of each extract were oxidized by the wet combustion method of Van Slyke, Plazin, and Weisiger (17) and counted as barium carbonate pads according to the technique of Migicovsky and Evans (14).

Results

The total activity recovered in each organ at various times after dosing is shown in Table I. It should be recognized that vitamin A or β-carotene would appear in the non-saponifiable fractions and the activity in the saponifiable residues represents degradation products. In view of the known ability of the rat to convert β-carotene to vitamin A in the intestinal mucosa, it is unlikely that β-carotene appeared in the systemic circulation in large amounts.

The total activity in the non-saponifiable fractions of tissues from eight rats over the 28-hour period was greatest in liver followed in descending order by

TABLE I

TOTAL ACTIVITY PER ORGAN,* C.P.M.

Tissue	Fraction	Hours after dosing							
		1	2	4	6	10	16	22	28
Adrenals	N.S.	N.a.†	3600	4896	6952	15000	6132	3536	2652
	Sap.	–	+	195	1272	1047	770	1760	1677
Heart	N.S.	188	209	363	327	174	307	186	–
	Sap.	195	137	139	175	279	227	177	128
Hypophysis	N.S.	117	110	120	615	135	105	120	200
	Sap.	+	197	180	1007	+	307	+	+
Intestines	N.S.	N.a.‡	7750	12334	13354	8875	1673	1626	–
	Sap.	2000	–	2750	–	–	–	–	–
Kidneys	N.S.	293	460	558	889	731	911	879	907
	Sap.	–	781	–	–	159	–	–	158
Liver	N.S.	2700	2700	8750	6650	13150	12450	13750	8800
	Sap.	1341	1177	1221	1302	1224	2436	679	1060
Lungs	N.S.	–	–	+	+	553	1038	855	667
	Sap.	–	–	–	–	–	–	–	–
Spleen	N.S.	150	+	116	119	204	128	+	+
	Sap.	–	–	–	–	–	–	–	–
Stomach	N.S.	546	275	144	–	–	–	–	–
	Sap.	–	–	–	+	+	+	–	–
Blood†	N.S.	1137	2920	1441	553	705	668	574	717
	Sap.	+	+	+	+	+	+	+	+

*+ Trace of radioactivity. – No radioactivity.
†Total activity calculated on basis of 6.0 ml. blood per 100 g. body weight.
‡Result not available.

TABLE II

DISTRIBUTION OF C^{14} IN NON-SAPONIFIABLE FRACTIONS AS A PERCENTAGE OF TOTAL ACTIVITY RECOVERED IN THE FIVE TISSUES

	Hours after dosing						
Tissue	2	4	6	10	16	22	28
Adrenal	20.80	17.49	24.47	39.00	28.08	17.36	20.27
Intestine	44.40	44.10	47.01	23.07	7.66	7.98	0.00
Kidney	2.60	2.00	3.13	1.90	4.17	4.32	6.96
Liver	15.47	31.27	23.41	34.19	57.02	67.51	67.29
Blood	16.73	5.16	1.98	1.84	3.07	2.83	5.48

intestines, adrenal glands, blood, kidneys, lungs, heart, hypophysis, stomach, and spleen. Barely detectable amounts of C^{14} were found in pancreas, testes, and thymus.

In order to assess more adequately the partitioning of activity, the percentage distribution of C^{14} in the non-saponifiable fractions of adrenals, intestines, kidneys, liver, and blood is shown in Table II. These values are expressed as percentages of the total activity recovered in these five tissues. It is apparent that over the 28-hour period after dosing there is the expected reallocation of radioactivity. During the early stages of absorption, activity is high in the intestine and blood. As the metabolic phase becomes superimposed upon the absorption process, increasing activity is observed in other tissues. The adrenal gland, however, appears unique in that C^{14} is rapidly assimilated and a high concentration is maintained during the entire experimental period.

The concentration of radioactivity in tissues (c.p.m./g. fresh tissue) is shown in Table III. The adrenal glands exhibited the greatest concentration and

TABLE III

CONCENTRATION OF ACTIVITY IN TISSUES (COUNTS PER MINUTE PER G. TISSUE $\times 10^{-3}$)*

		Hours after dosing							
Tissue	Fraction	1	2	4	6	10	16	22	28
Adrenals	N.S.	N.a.†	223.6	259.0	431.8	1041.6	406.0	221.0	144.9
	Sap.	—	+	10.3	79.0	72.7	50.9	110.0	91.6
Heart	N.S.	0.405	0.360	0.851	0.849	0.530	0.751	0.540	—
	Sap.	0.420	0.236	0.326	0.454	0.851	0.555	0.541	0.255
Hypophysis	N.S.	3.874	2.165	4.651	14.505	2.406	3.431	3.727	5.333
	Sap.	+	3.878	6.977	23.750	+	10.032	+	+
Intestines	N.S.	N.a.†	1.674	2.812	3.394	1.735	0.379	0.461	—
	Sap.	0.313	—	0.627	—	—	—	—	—
Kidneys	N.S.	0.255	0.338	0.571	0.904	0.882	0.955	1.059	0.785
	Sap.	—	0.574	—	—	0.192	—	—	0.137
Liver	N.S.	0.567	0.470	2.369	1.812	4.080	3.654	4.154	1.919
	Sap.	0.282	0.205	0.330	0.355	0.380	0.715	0.205	0.231
Lungs	N.S.	—	—	+	+	1.468	1.735	1.903	1.059
	Sap.	—	—	—	—	—	—	—	—
Spleen	N.S.	0.195	+	0.228	0.252	0.415	0.241	+	+
	Sap.	—	—	—	—	—	—	—	—
Stomach	N.S.	0.585	0.316	0.221	—	—	—	—	—
	Sap.	—	—	—	+	+	+	—	—
Blood, c.p.m./ml.	N.S.	0.166	0.312	0.237	0.094	0.153	0.105	0.123	0.100
	Sap.	+	+	+	+	+	+	+	+

*+ Trace of radioactivity. — No radioactivity.
†Result not available.

this was maintained throughout the experimental period of 28 hours. Following the adrenal glands in decreasing order of their activity were the hypophysis, liver, intestines, kidneys, lungs, heart, spleen, blood, and stomach.

In view of the fact that four rats were maintained in each of two metabolism chambers with a consequent pooling of excreta it was not possible to calculate the percentage absorption of C^{14} on an individual basis. The total radioactivity recovered from all samples obtained during the course of the experiment amounted to 70% of the administered C^{14}. $C^{14}O_2$ production by all animals accounted for 11.7% of the administered C^{14}-β-carotene, and the excreta 47.7%.

The total activity for the non-saponifiable fraction of intestines, blood, liver, kidneys, and adrenals was calculated as a percentage of the maximum value attained by the tissue after dosing and these results are shown in Figs. 1 and 2. Maximum activity in the intestinal mucosa was observed 6 hours after dosing

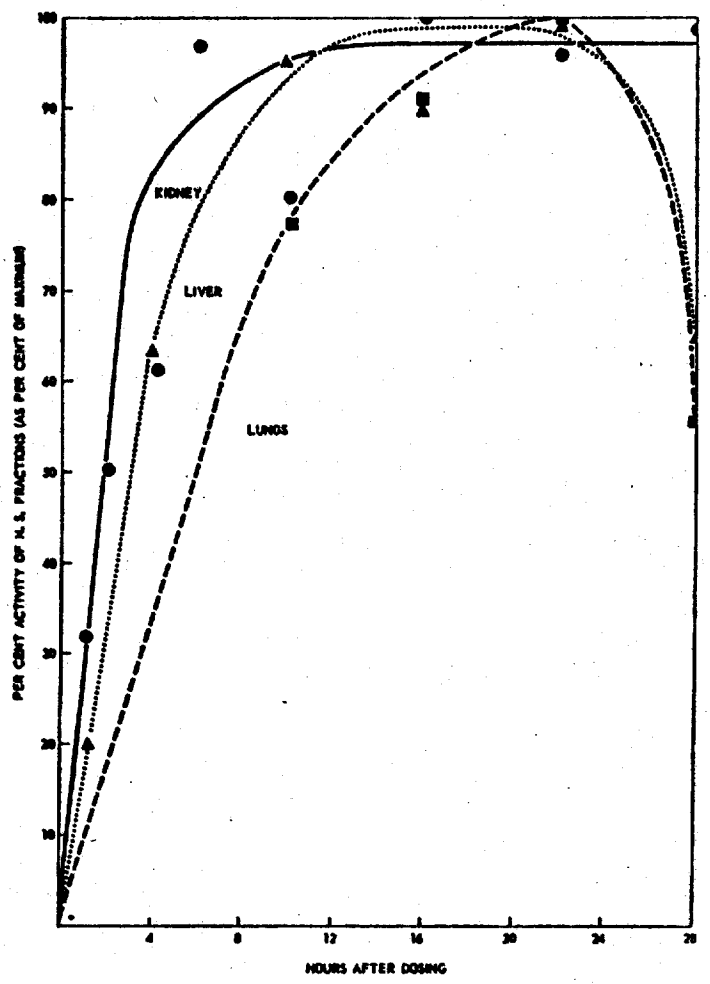

FIG. 1. C^{14} activity of tissues expressed as a percentage of maximum value observed.

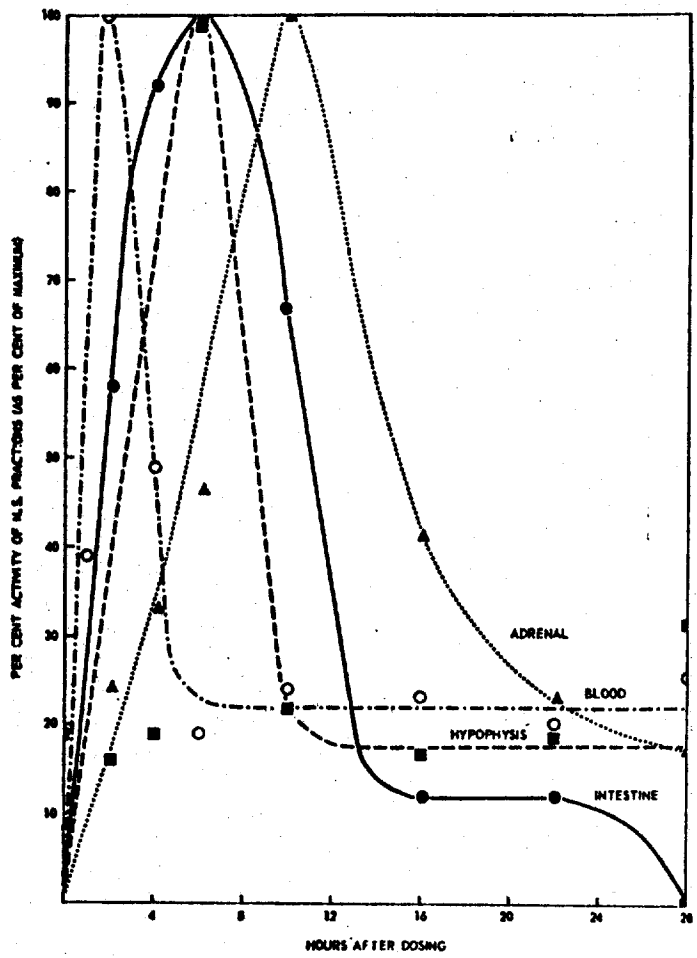

FIG. 2. C^{14} activity of tissues expressed as a percentage of maximum value observed.

and the amount rapidly decreased after this time. It may be concluded that the greater part of the C^{14}-β-carotene was absorbed during the 6-hour interval after dosing. In contrast to the foregoing, maximum blood levels were attained in 2 hours. The concentration decreased during the succeeding 4 hours to a constant level which was maintained for the remainder of the experiment. Activity in the kidney gradually increased to a maximum at about 8 hours and continued at this level for the duration of the experiment. The data for liver samples indicated that maximum activity was found at about 10 hours and was maintained until 22 hours, when a decrease was observed. Adrenal activity increased during the first 8 hours but fell rapidly after this time.

Of the total activity in the non-saponifiable fractions of the liver and adrenals, the proportion residing in vitamin A and β-carotene was determined in the following manner. The non-saponifiable extracts of each tissue were

combined and known amounts of vitamin A alcohol and β-carotene were added. The extracts were chromatographed on alumina columns to resolve vitamin A and β-carotene. In the case of liver it was found that 90.0% of the activity was present as vitamin A and 2.0% as β-carotene. The adrenal extracts had 91.8% of the activity in the form of vitamin A and 3.8% as β-carotene. If it can be assumed that other tissues resemble liver and adrenal in the above respect then the activity observed in the non-saponifiable fractions may be considered to be primarily vitamin A.

The data in Table III, with the exception of those for the adrenals and hypophysis, indicate that the activity of the saponifiable fractions was relatively low in comparison with the non-saponifiable fractions. The high activity observed in the saponifiable fractions of adrenals and hypophysis may suggest that these organs are particularly active in metabolizing vitamin A.

Discussion

The high concentration of activity found in the adrenal glands is of particular interest. It was demonstrated by Popper and Greenberg (15) that vitamin A occurs in the adrenal gland and is concentrated chiefly in the cortical zone. In contrast to the foregoing, the work of Bennett, Bennett, Shaver, and Gracht (2) did not reveal a high concentration of the vitamin in the adrenal gland after administration of oral doses of vitamin A.

The possibility of an association of vitamin A with steroid metabolism has been suggested by Bodansky and Markardt (3). Reichstein's compound L acetate (3-β-acetoxy-17-hydroxy-allopregnan-20-one) was found to markedly decrease the concentration of vitamin A in plasma. Lowe, Morton, and Harrison (12) have suggested that an abnormal secretion of glucocorticoids occurs in vitamin A deficiency. Further evidence of a possible interrelationship between vitamin A and steroid metabolism has been presented by Clark and Colburn (4); Migicovsky (13); Ralli and Waterhouse (16); Lane, Wolf, and Johnson (9); and Laughland and Phillips (11). This last work revealed that blood glucose rose to abnormally high levels when glucose was administered orally to vitamin A-deficient rats. There was no impairment in the absorption of glucose but studies *in vivo*, and *in vitro* with liver slices, showed that the ability of the liver to convert glucose to glycogen was impaired.

Attempts in this laboratory to demonstrate that vitamin A is directly involved in the respiration of adrenal tissue or in steroid synthesis have thus far given inconclusive results. It is, however, tempting to speculate that the avidity with which the vitamin is taken up by the adrenal gland is indicative of some functional relationship.

The rapidity with which a constant level of activity in blood was attained is further evidence of the homeostasis which is known to govern serum levels of the vitamin. The fact that the liver concentration of C^{14} increased for a period of 10 hours whereas the blood level was maximal at 2 hours must indicate a precise balance between absorption from the intestine and storage in the liver.

About 12% of the C^{14} administered as C^{14}-β-carotene was recovered in the expired air. This value represented the total output of eight rats which were metabolizing the administered C^{14}-β-carotene for various periods of time. It was observed that the production of $C^{14}O_2$ from the administered C^{14}-β-carotene was very low during the first few hours after dosing and appreciable amounts of $C^{14}O_2$ were only formed later on in the experiment when degradative changes were occurring owing to metabolic activity in the tissues.

It is apparent that the C^{14} activity of C^{14}-β-carotene is rapidly transferred (presumably as vitamin A) through the intestinal epithelium and stored in the liver. The adrenal glands have been found to be particularly active in the uptake of the C^{14}.

Acknowledgment

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ZBINDEN, A. and STUDER, A.:

ANIMAL TESTS ON THE CHRONIC COMPATIBILITY OF β-CAROTENE, LYCOPENE, 7,7'-DIHYDRO-β- CAROTENE AND BIXIN.

Report from the Research Department of the F. Hoffmann-La Roche Co. Ltd, Basel.

With 6 Figures in the text.

(Received on January 30, 1958).

Carotedoids are natural pigments which are extremely widespread in the vegetable kingdom[1]. Nutrients introduce them into the human and animal organism where they are partially resorbed and decomposed, and partially separated as vitamin A as well. Various animal species store carotinoids, either as pigment of visible body parts (for example: plumage of the canary, bill and skin of the flamingo, the shell of numerous crustaceans[2]), or in internal organs (liver, ovary, suprarenal gland, fatty tissues). The metabolims of the most important carotenoids was thoroughly researched in the course of the last 20 to 30 years, in particular regarding their significance as provitamin A. To the best of our knowledge, however, no toxicologic investigations were performed so far, possibly because carotenoids, as natural components of customary foods, were considered to be "harmless" from the start. Furthermore, large quantities of the pure pigments needed for such tests were not available until recently.

Several carotenoids, in part synthetically produced and in part isolated from natural products, are technically produced on a large scale today; they are in part also used as food coloring substances. It seems necessary to subject these pigments to strict testing in the course of animal experiments as well, which are required for food additives not found in nature[3].

Present study includes reports on the first results of chronic compatibility (tolerance) tests, performed with various animal species, principally for β-carotene and lycopene; 7,7'-dihydro-β-carotene and bixin were investigated less extensively. The general discussion is focussed thereby on specifics of the general chronic toxicity and A-hypervitaminosis problems. The tests do not yet comply with modern requirements of chronological duration[4] needed to evaluate the carcinogenic effect. During the tests we followed--as far as possible--the guidelines recently established by American and European authorities and commissions[1),2)]. The discussion refers to questions of carotenoid resorption and metabolism which are closely connected with toxicology.

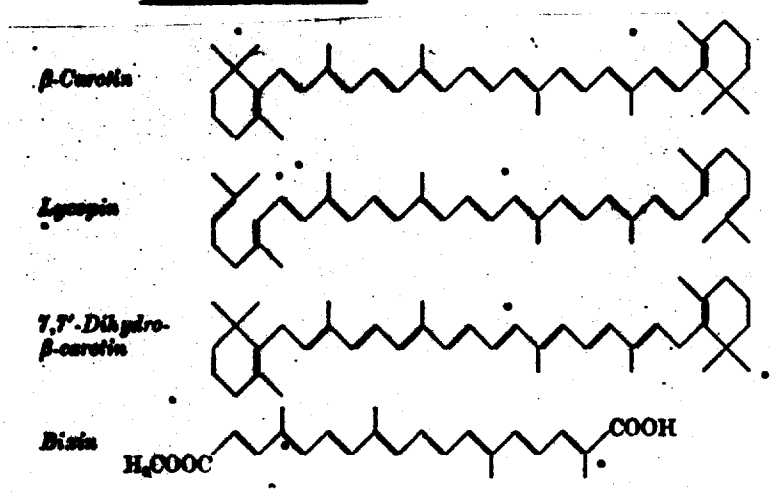
1. Chemistry of the investigated caotenoids.

The four caretenoids, tested for chronic tolerance, occur as natural plant components. The most important A provitamin: β-carotene, is found in all green plant components and in butter as well[3]. Lycopene is principally known as the pigment of the tomato, but it is liekwise present in other fruits[3]. 7,7'-dihydro-β-carotene was verified in the pollen of Cyclamen persicum[4]. Bixin occurs mainly as content of Bixa orellana seeds[5].

Natural bixin[5], isolated from plant material, was investigated. The other preparations were totally synthetized products[6]. But the above circumstance is not significant for the question of chronic compatibility: according to the explicit statement of the "German Commission for the Elaboration of the Food Pigment Problem", the toxicologic evaluation of a pigment must be independent from the question whether it is of natural or synthetic origin[7].

Table 1. includes the chemical formulas of the examined pigments and Table 2 shows the chemical data.

TABLE 1. Structural formulas of the investigated carotenoids.



The chemical changes which occur in the carotenoids during storage with air access, for example: in fodder, are still little-known. The simultaneous testing of the separation products during compatibility tests could not be avoided, despite the fact that pure substances were used. This, however ,should not be evaluated as a disadvantage, since the same decomposition products form and enter the organims when carotenoids are used as food coloring substances.

TABLE 2. Chemical and physical-chemical data of the tested carotenoids

Data	all-trans-β-carotene	lycopene	7,7'-dihydro-β-carotene	bixin
	Data	all-trans-β-Carotin	Lycopin	7,7'-Dihydro-β-carotin	Bixin
1 Bruttoformel	$C_{40}H_{56}$	$C_{40}H_{56}$	$C_{40}H_{58}$	$C_{25}H_{30}O_4$
2 Reinheit	3 mind. 90% mit Beimengungen cis-trans-isomerer Formen, spez. 15,15'-cis-β-Carotin u. Spuren von Dehydro- u. hydriertem β-Carotin	4 nach Chromatographie an Al_2O_3 werden 97—98.5% des einheitl. Farbstoffes gefunden		mind. 99% labiles Bixin 5
6 Gehalt an Schwermetallen	Fe < 5 mg/100 g; Cu u. Mg < 1 mg/100 g			
7 Aschegehalt	< 0,01%	< 0,01%	< 0,01%	< 0,01%
8 Biologische Aktivität	mindestens 9 1 600 000 I.E./g	0	0	0
10 Aussehen der Kristalle	blättchenförmig, violett mit metallischem Glanz 11	nadelförmig, rotbraun bis braun 12	blättchenförmig, gelborange. 13	violett, nadelförmig 14
15 Schmelzpunkt °C	182	172—173	183—184	185°—197
16 Absorptionsdaten $E^{1\%}_{1\,cm}$	in Hexan 17 338 mμ = 200 370 mμ = 250 453 mμ = 2450 481 mμ = 2150	in Petroläth. 18 446 mμ = 2250 472 mμ = 3450 503 mμ = 3150	in Petroläth. 18 382 mμ = 1900 405 mμ = 2900 429 mμ = 2910	in Äthanol 19 485 mμ = 3,6 460 mμ = 3,65
20 Chromatographie	an Al_2O_3 ergibt eine einzige Zone von all-trans-β- 21 Carotin	22 an Al_2O_3	22 an Al_2O_3	23 aus Methylenchlorid an Al_2O_3 ergibt eine Zone von Bixin
24 Beständigkeit	25 zersetzt sich langsam an der Luft wahrsch. durch Oxydation unter Bildung ungef. noch nicht untersuchter Abbauprodukte. Bei Erhitzen teilweise Isomerisierung zu cis-β-Carotin	26 zersetzt sich an Luft etwas schneller als β-Carotin. Bei Erhitzen z. T. Isomerisierung zu Neolycopin	27 etwa wie β-Carotin, eher etwas beständiger	28 verändert sich an Luft b. Zimmertemperatur nicht[1]

Translation of Table 2.

1. Gross formula: . . .
2. Purity: 3. minimum 96%, with the addition of cis-trans-isomeric forms, spec. 15,15β-cis-β-carotene and traces dehydro- and hydrogenated β-carotene
   4. after chromatography at Al_2O_3, 97 to 98.5% of the homogeneous pigment is found
   5. mimimum 99% unstable bixin.

6. Heavy-metal content: . . .

7. Ash content : . . .

8. Biological activity: 9. at least . . .

10. Appearance of the crystals: 11. shape of small lamina, violet with metallic glitter
    12. needle-shaped, reddish-brown to brown
    13. lamina-shaped, yellowish-orange
    14. violet, needle-shaped.

15. Melting point, °C: . . . . .

16. Absorption data $E^{1\%}_{1\,cm}$:
    17. in hexane . . .
    18. in petroleum ether . . .
    19. in ethanol . . .

20. Chromatography:
    21. at Al_2O_3, yields a single zone of all-trans-β- carotene
    22. at Al_2O_3
    23. from methylchloride at Al_2O_3, yields a bixin zone.

24. Chemical Resistance: 25. decomposes slowly in the atmosphere, probably as a result of oxidation, while "ungef." not yet investigated decomposition products form. When heated, partial isomerization into cis-β- carotene
    26. decomposes somewhat faster in the atmosphere as β- carotene. When heated, isomerization into neolycopene
    27. approximately as β- carotene, slightly more resistant
    28. does not change in the atmosphere at room temperature[19].

2. Testing system.

a) Animal material.

White inbred Wistar-rats of our own breed were used; in most cases, their weight was 40 to 50 g (age: 30 to 40 days). Animals from various litters were equally divided into test- and control groups. The rats were kept in single cages with wide-mesh double floors made of wire, at constant room temperature of 24 to 25°C.

Rabbits were tested at the age of 6 to 8 months (weight: 2,5 to 3,5 kg). The dogs were mongrel bitches, aged 4 to 6 years.

b) Feeding of the animals.

The rats received a complete caloric value "synthetic" diet with the following composition:

casein (vitamin-free) . .	20%
coconut oil (Pura[1]) . . .	10%
saccharose . . .	61%
salt mixture . . .	4%
cellulose flour . . .	5%

Vitamin additions per animal and per day supplied in 3 portions per week:

vitamin B_1 . . .	100 µg	vitamin B_2. . .	200 µg
vitamin B_6. . .	100 µg	(+)biotin . . .	0,5 µg
choline . . .	3 mg	nicotinamide. .	1 mg
panthotenic acid Ca . . .	200 µg	2-methyl-1,4-naphtochinone. .	20 µg
DL-α- tocopherol	30 µg	vitamin A	15 i E.

The salt mixture was composed as follows:

sodium chloride . .	5,0	magnesium sulfate . . .	5,5
tricalciumphosphate	15,0	ferrous citrate . . .	3,21
copper sulfate . . .	0,03	di-potassium phosphate, (separated)	26.53
sodium fluoride . .	0,003		
disodiumphosphate (separated) . .	9,6		
calciumlactate . . .	35,0		
potassium iodide. . .	0,09	manganese sulfate . .	0,02.
zinc carbonate . . .	0,02		

The rabbits were fed hay, grass and a mixture of dried sugar beet, oats, bran and boiled potatoes; the dogs were fed oat soup and boiled meat.

c) Administration of the carotenoids.

During the rat tests with a duration of 100 days, each animal received each morning a carotenoid quantity determined according to body weight, mixed into a small amount of fodder and offered in a bowl. Since the rats received no food at night, the pigment was rapidly consumed in most cases. In the course of long-term tests, the carotenoid was added to the fodder. The rabbits received β-carotene (10%) in "glyceride mixture" (a mixture of triglycerides with low-molecular fatty acids (C_8-C_{12}), 5 times per week, administered with the gastric tube. The pigments were administered to the dogs per os in gelatine capsules. For the purpose of intramuscular injections, β- carotene was slightly heated and dissolved in arachis oil.

Fodder mixtures with individual carotenoid doses were freshly prepared each day; the pigments were stored under CO_2 in the refrigerator. Antioxygenes were not used, because such substances not only inhibit the oxidation of the carotenoids, but in vivo could also eventually hinder conversion into vitamin A[2].

d) Laboratory investigations.

The blood tests performed for all animals included hemoglobin, erythrocyte and leukocyte count, differential hemogram, in part also reticulocyte count and thrombocyte count. Hemoglobin was measured with the BECKMAN photometer. We used the customary pipettes and counting chamber for the determination of the erythrocyte and leukocyte count.

Dogs were subjected to the following tests, in addition to the above: blood sedimentation rates, serum bilirubin, " es -. ", TAKATA-reaction, bromsulfalein *) test and urine analyses. The determination of vitamin A, β-carotene and lycopene in the various organs was performed after extraction (modified ROSE-GOTTLIEB method[3]), for carotenoids after saponification with KOH, chromatographic purification and specifically activated Al_2O_3, by measuring the absorbance of the petroleum ether solution at 452 to 454 mμ (β-carotene) or respectively, 472 to 474 mμ (lycopene); for vitamin A the determination was performed according to the MOON method[4] by means of dry saponification with NaOH, chromatography at Al_2O_3 and evaluation according to CARR-PRICE. The chemical determinations were carried out in our vitamin laboratory by Dr. S. NOBILE.

e) Histopathological Technique

Numerous organs were fixed in BOUIN solution, embedded in paraffin and stained with hem-alum-eosin; brain sections were stained according to NISI as well. The fat staining with Sudan III-7 B took place in frozen sections fixed with formol; vitamin A was determined with u.v. fluorencence microscopy.

f) Implementation of reproduction tests in rats.

The mating took place after feeding carotinoid-containing diets for 14 to 16 weeks. For this purpose, a male and a female animal was placed in each case into a cage for 1 night each week. The vagina smear on the next morning made determination of insemination possible. Females with positive sperm findings were weighed each day and subjected to specific observation. When a birth occurred, the litter was weighed and counted.

*) non-protein nitrogen

Animals which showed no positive sperm finding after a test duration of 1 month were no longer mated. It happens quite often when "synthetic" diets are fed that female rats devour or neglect their offspring. This was observed as frequently among animals treated with carotenoids as among those used as controls; as a result, the distribution according to e sex in the 2nd and 3rd generation was partially dissimilar.

g) Number of animals, test duration and dosage.

Table 3 shows a synopsis of all performed tests. It includes--besides the dosage and the type of carotenoid administration--the total pigment volume consumed per animal during the entire test period. For the long-term rat tests in which the carotenoids were administered as a diet additive, said data are based on fodder weights, determined in intervals of 3 to 6 weeks during 7 days in each case.

TABLE 3. Synopsis on chronic compatibility tests with catotenoids.

1 Vers. Nr.	2 Carotinoid	3 Tierart	4 Tierzahl m	4 Tierzahl w	5 Dosierung mg/kg/Tag oder Teile je Million (M), Teile Futter	6 Verabreichungsart	7 Vers.-Dauer Tage	8 Totale pro Tier verabreichte Carotinmenge g	9 Anzahl Kontrolltiere
1	β-Carotin	Ratte 10	5	5	5000/M	Futterzusatz 13	540	etwa 30	0
2	β-Carotin	Ratte	10	10	1000 mg	per os (Schale) 14	100	etwa 10	20
3a	β-Carotin	Ratte	15	15	1000/M	Futterzusatz 14	400	etwa 5,3	30
3b	β-Carotin	Ratte 2. Gen.	16	5	1000/M	Futterzusatz 14	280—280	etwa 3,3	19
3c	β-Carotin	Ratte 3. Gen.	12	10	1000/M	Futterzusatz 14	85—115	etwa 1,3	21
4	β-Carotin	Ratte	—	10	1 mal 1000 mg/Tier	intramuskulär 15	3—48	1	0
5	β-Carotin	Ratte	—	8	9 mg/Tier	intramuskulär 5 mal pro Woche 16	120[1]	etwa 0,6	8[2]
6	β-Carotin	Kaninch. 11	2	2	500 mg.	per os 5 mal pro Woche 17	77	etwa 80	0
7	β-Carotin	Kaninch.	—	1	500 mg	per os 5 mal pro Woche 18	730	etwa 800	0
8	β-Carotin	Hund 12	—	2	100 mg	per os 19	104/200	149/221	0
9	Lycopin	Ratte	10	10	1000 mg	per os (Schale) 20	100	etwa 10	20
10a	Lycopin	Ratte	15	15	1000/M	Futterzusatz 14	288	etwa 2,9	30
10b	Lycopin	Ratte 2. Gen.	13	18	1000/M	Futterzusatz 14	50—90	etwa 1,0	38
11	Lycopin	Hund	—	1	100 mg	per os 19	192	272	0
12	Bixin	Ratte	10	10	1000 mg	per os (Schale) 20	100	etwa 10	20
13	7,7'-Dihydro-β-carotin	Ratte	10	10	1000 mg	per os (Schale) 20	100	etwa 10	

[1] Mit kurzen Unterbrechungen wegen lokaler Reizwirkung.
[2] Behandelt mit 4,5 mg/Tier Vitamin A-Palmitat.

1. Test No: 2. Carotenoid: 3. Animal species:
4. Number of animals (male - female): 5. dosage, mg/kg per day or parts per million (M); fodder parts:
6: Administration method: 7. Test duration, days:
8. Total carotenoid volume supplied per animal:
9. Number of control animals:

10. rat
11. rabbit
12. dog

13. fodder additive
14. per os (bowl)
15. intramuscular
16. " " 5 times per week
17. per os, 5 times per week
18. " " " "
19. per os
20. per os (bowl)

21. approximately

1. With brief interruptions due to local irritating effect
2. Treated with vitamin A palmitate, 4.5 mg per animal.

TABLE 4. Blood tests for rats, rabbits and dogs treated with carotenoids.

1 Vers. Nr.	2 Carotinoid	3 Tierart	4 Dauer der Behandlung Tage	5 Anzahl der Tiere	6 Hämoglobin g/100 ml	7 Erythrocyten 10^6	8 Hb_E µµg
1	BC	Ra	322	9	15,5±0,8	6,3±0,6	25,0±3
2	BC	Ra	56	8	16,9±2,1	7,7±0,7	22,0±2
	BC	Ra	98	8	17,8±1,2	8,2±0,8	21,6±1
	K	Ra	56	8	16,4±1,4	7,5±0,6	21,8±1
	K	Ra	98	8	18,1±1,9	8,6±0,9	21,0±2
2a	BC	Ra	358	28	16,1±0,9	7,4±0,7	21,7±2
	K	Ra	358	27	15,2±1,1	7,1±0,6	21,1±2
3b	BC	Ra	200	21	16,4±0,9	8,4±0,3	19,5±1
	K	Ra	200	18	16,1±0,9	8,3±0,4	19,4±1
3c	BC	Ra	60	20	16,3±1,4	8,5±0,9	19,4±2
	K	Ra	60	20	16,7±2,0	8,7±0,8	19,2±2
6	BC	Kan.	28	5	16,0±1,2	6,1±0,4	25,6±3
	BC	Kan.	77	5	13,2±1,3	4,6±0,8	28,9±3
7	BC	Kan.	175	1	17,4	6,2	27,8
	BC	Kan.	637	1	14,8	5,1	29
8	BC	H1	0	1	15,7	5,6	28
	BC	H1	18	1	16,2	6,1	28
	BC	H1	72	1	19,7	6,1	32
	BC	H1	100	1	18,0	6,5	28
8	BC	H2	0	1	13,4	5,3	25
	BC	H2	15	1	15,7	5,7	28
	BC	H2	74	1	16,7	6,0	28
	BC	H2	142	1	16,3	6,3	26
	BC	H2	200	1	20,0	7,0	28
9	L	Ra	56	8	15,6±1,1	8,1±0,5	19,2±1
	L	Ra	98	8	17,9±1,1	7,9±0,7	22,7±2
	K	Ra	56	8	16,4±1,4	7,5±0,6	21,8±1
	K	Ra	98	9	18,1±1,9	8,6±0,9	21,0±2
10a	L	Ra	190	30	16,1±1,2	8,8±0,6	18,3±2
	K	Ra	190	30	15,0±2	7,7±0,8	20,1±2
10b	L	Ra	40	20	16,1±1,2	7,9±0,6	20,3±2
	K	Ra	40	20	15,6±1,5	8,6±1,0	18,4±2
11	L	H	0	1	19,4	7,9	24
	L	H	19	1	18,1	6,3	29
	L	H	71	1	17,6	6,5	27
	L	H	166	1	19,4	6,4	30
	L	H	188	1	19,7	7,9	25
12	B	Ra	56	8	16,5	7,7	21,5
	B	Ra	98	8	16,8	8,1	20.8
13	7,7'	Ra	56	8	15,9±1,9	7,7±0,5	20,6±2
	7,7'	Ra	98	8	16,6±2,0	8,1±1,3	20,5±1
12+13	K	Ra	56	8	15,7±1,0	7,5±0,6	21,3±1
	K	Ra	98	8	18,1±2,1	8,4±0,6	21,6±2

BC = β-Carotin, L = Lycopin, B = Bixin, 7,7' = 7,7'-Dihydro-β-carotin. — Ra = Ratte.
Kan. = Kaninchen, *H* = Hund. —
K = Kontrolle
µµg = 10^{-12}g

$$SD = \sqrt{\frac{S(x-\bar{x})^2}{n-1}}$$

1. Test No.: 2. Carotenoid: 3. Animal species:
4. Duration of therapy, days: 5. Number of animals:
6. hemoglobin g/100 ml: 7. erythrocytes, 10^6: 8. Hb_E, µµg:
BC=β-carotene; L = lycopene; B = bixin; 7,7' = 7,7'dihydro-β-carotene; Ra = rat, K = control; µµg = 10^{-12}g.

. . . .

3. Results.

It is impossible to specify in detail the extensive observation- and numerical material which resulted from the tests within the framework of present report. The reporting of the same must therefore be limited to the most significant findings and average values.

Average values for rats and rabbits, single values for dogs and rabbits.

Durchschnittswerte für Ratten und Kaninchen, Einzelwerte für Hunde und Kaninchen

1 Leukocyten 10^3	Stab %	2 Segmentk. %	3 Eos. %	4 Baso. %	5 Lymphocyten %	6 Monocyten %	7 Reticulocyten	8 Thrombocyten
9,3±2,6	2,2	[illegible]±5	3,6	—	49,6±8	2,0	20,1	—
16,1±2,3	0,6	[illegible]±8	1,4	0,1	52,4±8	3,9	—	—
18,3±3,4	—	[illegible]±6	1,6	—	71,0±9	3,1	—	—
10,7±2,6	—	[illegible]±7	0,7	—	78,7±9	1,2	—	—
8,4±2,2	0,1	[illegible]±7	0,8	—	67,9±10	1,4	—	—
11,2±2	2,6	[illegible]±5	2,1	—	72,9±8	0,3	—	—
12,5±3	2,5	[illegible]±8	3,1	—	66,6±9	0,3	—	—
12,2±3	0,6	[illegible]±7	2,5	—	70,0±8	1,5	—	—
11,1±2	0,3	[illegible]±6	2,4	0,06	74,1±7	1,7	—	—
12,6±2,5	0,5	[illegible]±14	1,3	—	81,5±7	0,6	—	—
11,2±3,9	1,0	[illegible]±9	1,5	0,02	79,4±19	0,6	—	—
9,9±1,5	0,6	[illegible]±14	0,5	4,7	54,9±13	3,4	—	—
9,9±2,5	0,8	[illegible]±8	1,0	6,0	42,3±8,5	0,7	—	—
8,6	0,5	[illegible]	—	4	41,5	6	—	—
6,7	2,0	[illegible]	1	2	20,5	1	—	—
8,6	1,5	[illegible]	4,0	—	22,0	4,0	—	390000
17,7	3,0	[illegible]	—	—	10,5	3,5	—	270000
7,0	—	[illegible]	3	—	18,5	4,5	—	—
14,1	2	[illegible]	0,5	—	22,5	2,5	—	350000
11,8	21,4	[illegible]	2	—	8,6	8,0	—	—
7,5	10	[illegible]	9	—	12,5	13,0	—	—
5,4	12	[illegible]	5	—	16	9,0	—	—
6,7	5,3	[illegible]	8	—	14	8,0	—	—
7,4	2	[illegible]	4	—	23,5	4,5	—	250000
11,1±3,5	—	[illegible]±6	0,8	—	78,4±6	1,3	—	—
8,7±3	—	[illegible]±6	1,6	—	72,9±10	1,3	—	—
10,7±2,6	—	[illegible]±7	0,7	—	78,7±9	1,2	—	—
8,4±2,2	0,1	[illegible]±7	0,8	—	67,9±10	1,4	—	—
16,1±4	1,9	[illegible]±7	3,4	0,05	71,6±10	0,5	—	—
12,6±5	0,7	[illegible]±9	4,8	—	66,0±9	3,5	—	—
11,8±3,5	1,0	[illegible]±8	2,5	0,13	76,4±9	1	—	—
12,6±3,5	1,5	[illegible]±10	1,5	0,13	75,4±10	1,5	—	—
9,4	1,5	[illegible]	16,5	—	22	4,5	—	—
10,0	1,0	[illegible]	11,5	—	17,5	2,5	—	—
11,9	0,5	[illegible]	6,5	—	27	2,5	—	—
6,5	3,0	[illegible]	12	—	27,5	5,5	—	—
8,7	—	[illegible]	7	—	22	3,5	—	—
10,7	0,9	[illegible]	1	—	63,4	3,8	—	—
15,6	0,4	[illegible]±8	2,0	—	69,2±9	1,4	—	—
10,5±3,2	0,2	[illegible]±10	1,4	—	80,9±10	0,9	—	—
8,9±2,0	0,3	[illegible]±9	2,4	—	58,4±10	5,5	—	—
10,5±2,5	0	[illegible]±6	1,8	—	77,8±10	1,2	—	—
8,4±1,9	0,1	[illegible]±12	2,8	—	66,7±11	1,4	—	—

1. Leucocytes, 10^3: 2. Neutrophils, %:
3. illegible. 4. Eos. %: 5. Baso. %: 6. Lymphocyte %:
7. Monocyte %: 8. Reticulocyte %: Thrombocytes:

Preliminary experiment with β-carotene in rats.

Test 1.was planned as the first preliminary experiment. 10 young rats which had received 0,5% β-carotene (5000 parts per million fodder parts), mixed into the fodder, developed very well for 78 weeks. 3 animals had to be killed prematurely, due to infectious otitis-labyrinthitis or respectively, chronic-recidivist pneumonia with bronchial ectases. The weight of the animals increased normally; the white and red hemograms (47th week) were normal (Table 4).

In one animal the histopathological examination revealed a few broncho-pneumonia foci in the lung; one of the 5 males showed partial testicle atrophy, limited to a few small ducts; a slight decrease of the cell content with edematous saturation of the stroma was found in one of the rats the general condition of which had deteriorated by the end of the testing period. These changes are ascribable to spontaneous diseases which occur frequently in old rats.

Apart from such rare disorders, the liver, kidney, spleen, pancreas, suprarenal gland, lung, heart, stomach, small intestine, colon,testicles, ovary, epidermis, bones and bone marrow proved to be unchanged when subjected to histological examination. No damage whatsoever, determinable as A-hypervitaminosis, was observed. Reference is made in Test 3 to the increased storage of vitamin A.

100-day feeding test with the 4 carotenoids in rats.

Tests 2,9,12 and 13. These tests can be jointly discussed. Young rats received daily during 100 days 1000 mg/kg β- carotene,

or respectively, lycopene, or 7,7'-dihydro-β-carotene, mixed into some fodder and offered in a bowl. In most cases, the preparations were consumed without difficulty; however, the therapy affected the appetite of the animals somewhat after the second half of the testing period; therefore the growth of the males in particular lagged slightly behind the growth rate of the controls (Figure 1.).

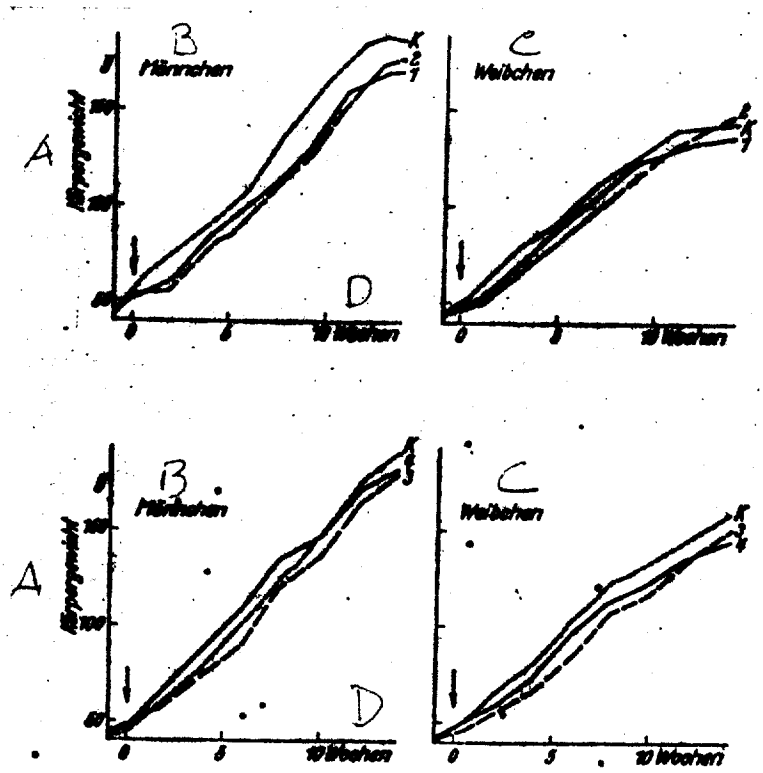
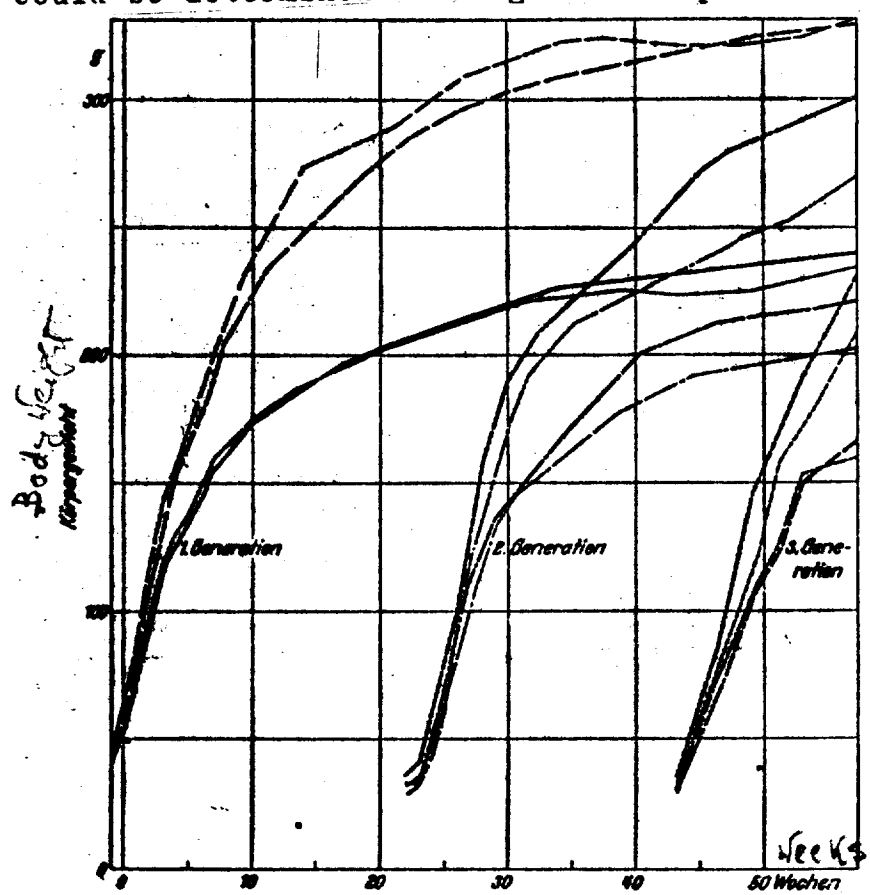


FIGURE 1. Effect of the carotenoids (1000 mg/kg/day) on the body weight of rats (average of 10 animals).
1.: β-carotene; 2.: bixin; 3.: lycopene; 4.: 7,7'-dihydro-β-carotene; K: controls; ↓: start of the 14-week therapy.
A: body weight; B: males; C: females; D: . . weeks.

Apart from single cases of spontaneous death during the first testing weeks (β-caretene: 4/20, lycopene: 2/20, bixin: 3/20, controls: 1/40),all rats progressed very well. No incompatibility symptoms could be verified. The white and red hemograms (8th and 14th week) showed no above-normal deviation measure changes (Table 4). Liver, kidney, suprarenal gland, spleen, pancreas, testicles, epidermis, bones and marrow were histologically intact. No indications of pigment storage could be determined during microscopic examination.



1. generation: males = - with β-carotene ~ — controls; females - - with β-carotene — - controls; 2. generation: males -.. - with β-carotene -·· - controls; females - . - with β-carotene - - controls. 3. generation: males == == with β-carotene .. .. .. controls; females ==== with β-carotene; ···controls.

FIGURE 2. Effect of 0,1% -carotene in the fodder on the body weight of rats (averages of 19 to 30 animals).

Chronic β-carotene feeding test with the 1. and 2. rat generation.

Test 3. A chronic feeding test is at issue here; 0,1% β-carotene (1000 parts/million parts fodder) was mixed into the diet of young rats. The animals were mated after 112 days; the young were separated from their dams 23 to 25 days after birth and likewise received fodder containing β-carotene. The mating of this rat generation took place at the average age of 130 days. 4 animals each from the 1. and 2. generation were killed on the 375th and 230th day respectively, and subjected to histopathological examination.

Table 5 shows the main data on the course of the test. All animals developed excellently. Their growth rate was regular (Figure 2), only sporadic cases of spontaneous death occurred, and only in the control groups; intercurrent diseases were likewise very rare (otitis-labyrinthitis). Fodder consumption was practically identical in case of treated and untreated animals. Hemoglobin, erythrocyte- and leukocyte-counts as well as the differential hemogram showed no deviations either (Table 4).

TABLE 5. Development of rats receiving "synthetic" diet with 0,1% β-carotene during three generations (Test 3).

1 Generation	2 Versuchsdauer Tage	3 Durchschnittlicher Futterkonsum je Tag: 4 Männchen β-Car. g	4 Männchen Kontr. g	5 Weibchen β-Car. g	5 Weibchen Kontr. g	3 Durchschnittliches Körpergewicht je: 4 Männchen β-Car. g	4 Männchen Kontr. g	5 Weibchen β-Car. g	5 Weibchen Kontr. g	6 Spontantodesfälle β-Car.	6 Spontantodesfälle Kontrolle	7 Getötet wegen Krankheit β-Car.	7 Getötet wegen Krankheit Kontrolle
1.	400	11,6	11,6	10,3	9,5	330	332	240	235	0	2	1	1
2.	240—200	10,1	11,9	10,2	10,3	300	270	220	201	0	3	0	0
3.	70—105	12,1	11,9	9,1	9,1	230	208	166	160	0	0	0	0

Generationen (Versuch 3)

1. generation; 2. test duration, days; 3. average fodder consumption per day; 4. males; 5. females; 6. spontaneous cases of death; 7. killed on account of disease; 8. β-carotene; 9. control.

As evident from Figure 2, rats of the 2nd generation treated with β-carotene grew somewhat faster than the controls. No conclusion should however be reached on the basis of said finding concerning the growth-promoting effect of β-carotene, since the animal material of the 2nd generation is of course not homogeneous, and because no similar effect was verified in rats of the 1st generation.

Table 6 offers orientation on the outcome of the reproduction experiment with the 1. and 2. generation. After the mating of the 1. generation 1 case each of pathological pregnancy course occurred among the β-carotene animals and those of the control group (1 abortion, 1 resorption). The weight and number of the young animals showed no significant differences. After the mating of the 2. generation one of the β-carotene-animals gave birth to a single offspring only. A female of the corresponding control group also gave birth to a single offspring only; the dam no.2. aborted. The number and average weight of the young are practically identical in this generation as well. However, it is remarkable that the females of the 2. generation gave birth to less young on the average than their dams. This might be ascribable to seasonal differences (the 1. generation was mated in spring, the second in fall). The duration of pregnancy was abbreviated slightly (20 days) in case of a 1. generation control animal only; otherwise, gestation periods were normal. throughout. Only in a single 3. generation control animal was a malformation found (abnormal posture of the hind paw).

TABLE 6. Reproduction tests with rats receiving "synthetic" diet containing 0.1% β-carotene and lycopene (Tests 3 and 10).

1 Carotinoid	2 Generation	3 Zahl gepaarter Weibchen	4 Zahl besamter Weibchen	5 Zahl der Weibchen mit Partus	6 Tragzeit Tage 7	8 Zahl der Jungen	9 Gewicht der Jungen g	10 Abort	11 Resorption
β-Carotin	1.	15	11	10	22 (22—23)[1]	8,0±2[2]	4,7±0,6[2]	1	—
Kontrolle	1.	15	12	11	22 (20—23)	9,2±3	4,5±0,6	—	1
β-Carotin	2.	5	5	5	22 (21—23)	6,3±3	4,9±0,5	—	—
Kontrolle	2.	13	13	12	22 (21—23)	6,1±2	5,0±0,5	1	—
Lycopin	1.	15	10	9	22 (21—23)	5,5±2	5,7±0,9	1	—
Kontrolle	1.	15	12	10	22 (21—23)	7,5±2	4,9±0,7	2	—

[1] Extremwerte. 12

[2] $SD = \sqrt{\frac{S(x-\bar{x})^2}{n-1}}$

1. Carotinoid: 2. Generation: 3. Number of mated females: 4. Number of inseminated females: 5. Number of females with partus: 6. Gestation period 7. days: 8. Litter size: 9. Weight of young, g: 10: Abortion: 11. Resorption: 12. Extreme values.

Histopathological investigations. In 4 each of 1. and 2. generation rats treated with β- carotene and in 4 animals each from the corresponding control groups no pathological changes resulted in the liver, kidney, suprarenal gland, lung, heart, spleen, pancreas, testicles, epididymis, ovary, small intestine, colon, stomach, epidermis , bones and marrow. In the livers of the β-carotene animals the KUPFFER star cells were amply loaded with fat (Figure 3) and showed intensive yellowish-green fluorescence in u.v. light, which faded completely in the course of 20 minutes. Presumably, stored vitamin A was involved here.

Chemical investigation. The liver and kidney of one each of the 1. generation β-carotene and control rats was investigated after a test duration of 375 days, whereby the following was found:

1 Organ	2 Vitamin A	3 β-Carotin	2 Vitamin A	3 β-Carotin
	4 beim β-Carotin-Tier		5 beim Kontrolltier	
	I.E./g	µg/g	I.E./g	µg/g
Leber 6	7760	2,9	0	0
Niere 7	55	0	0	0

1. Organ: 2. Vitamin A
3. β-carotene
4. in the β-carotene-animal:
5. in the control animal:
6. liver 7. kidney.

The investigation confirms a considerable, but not excessive increase of the vitamin A content in the liver (standard value: approx. 280 I.U./g[1]) of the β-carotene animal. The vitamin A content of the kidney shows a minor increase (standard value approx. 17 I U./g[f]). Carotene storage in the liver is minor. No vitamin A was found in the liver and kidney of the controls, which indicates that the supplied vitamin A additive made normal growth, but no storage in the liver possible.

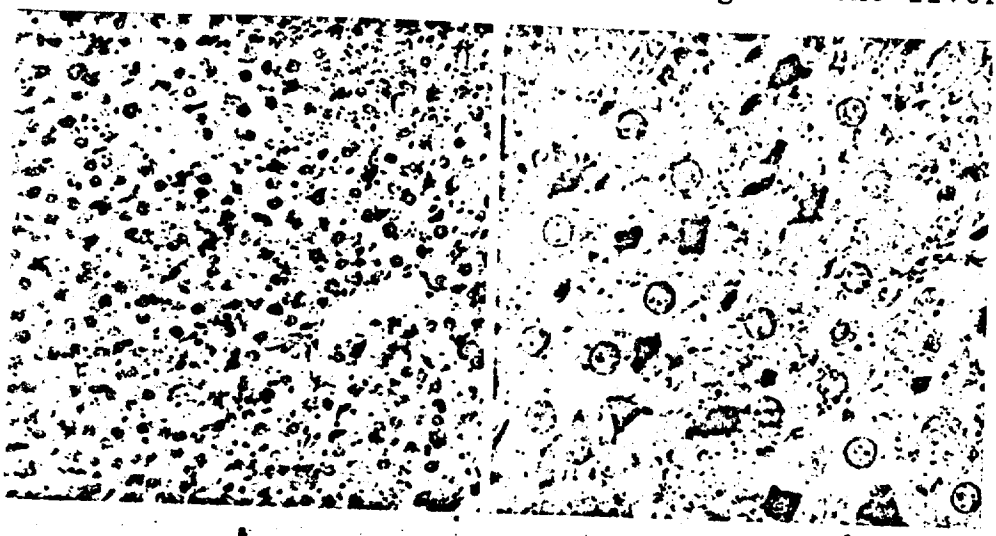


FIGURE 3a and b. Effect of 0.1% β-carotene in the fodder on the histology of the rat liver after 375 days of therapy. (Stained with Sudan III - 7 B-hemo-alum. 200 x magnification). (a) Intensive fatty degeneration of the KUPFFER star cells, indicating vitamin A accumulation. Additional fine-drop fatty degeneration of liver cells. (b) The same animal (500 x magnification). Selective representation of the KUPFFER star cells with fatty degeneration and vitamin A storage.

Parenteral carotenoid administration in rats.

Tests 4 and 5. In case of parenteral administration the metabolism of the carotenoids is different than during the absorption of the pigments from the intestine because in the first-mentioned case the intestinal wall in which the vitamin A-formation occurs in numerous animals, is by-passed. It should therefore be verified whether the intramuscular injection of large β-carotene doses causes any damage.

A single intramuscular administration of 1 g β-carotene per animal in oil, given to rats weighing 145 to 165 g, resulted in no toxic symptoms. The dissection of animals performed between the 3rd and 48th test day showed no organic changes. Microscopically, a proliferating inflammation was verified within the injection area, with a few giant foreign body cells, located at β-carotene crystals (Test 4).

Rats with a weight of 150 to 190 g showed surprisingly good tolerance for intramuscular injections of oily β-carotene solutions (twice daily, 4,5 mg per rat on 5 days of the week), continued for 17 weeks. The treatment, however, had to be interrupted from time to time for a few days on account of local irritation symptoms; but the animals showed normal growth and their general condition was good. One of 8 rats died prematurely. During dissection large cysts containing oil and β-carotene were found in all animals at the site of the injection. Foci of intensively yellow pigmentation were found in the fatty tissue of the large network and in the fatty tissue of the retro-peritoneum; it must therefore be assumed that β-carotene, evacuated through the lymphatic vessels, was stored there. Lungs, liver, spleen, epidermis, bones and marrow proved to be intact in the course of the histopathological examination. The KUPFFER star cells of the liver showed fatty infiltration (storage of vitamin A).

In the kidneys the principal epithelial parts showed frequently cloudy swelling; the epitheliums of the convoluted tubuli, in particular at the transition between marrow and cortex were partially in the process of desquamation.

Secondary symptoms are at issue thereby, as sequelae of chronic inflammation with tissue decomposition at the injection point.

Histopathological investigation. A pronounced, productive inflammation resulted at the injection sites. The granulation tissue of fibroblasts, scarce capillaries, macrophages and sporadic giant foreign body cells was partially necrotic and infiltrated by numerous neutrophile leukocytes and lymphocytes,. Local reactions were definitely less pronounced in the control rats, treated with analogous doses of vitamin A. Accordingly, the kidneys likewise showed minor reactions only in the form of increased tubulus epithelial desquamation at the transition between marrow and cortex (Test 5).

β- caroten feeding tests with rabbits.

Tests 6 and 7. The induction of toxic changes by means of massive oral overloads of β-carotene was tested in rabbits. Despite the fact that the animals received 500 mg/kg β-carotene 5 times per week during 11 weeks, no significant changes became evident in their general condition. 2 rabbits lost 140 and 160 g weight respectively; 3 animals gained 70 to 400 g weight. The hematologic values (4th and 11th week) remained within standard deviation measures (Table 4). Four animals were dissected after a test duration of 11 weeks, whereby no pathologic organ changes were determined. The histopathological investigation indicated that liver, kidney, ovary, testicles, spleen, pancreas, epidermis and bone marrow were intact (Test 6).

Therapy in rabbits with 500 mg/kg β-carotene (in each case, 5 times per week) was extended to 2 years' duration. The animal which had received an approximate total of 800 g β-carotene, developed excellently and gained 420 g weight during the treatment. The blood status remained normal (Table 4). The dissection showed unchanged organs. The liver contained 446 I.U. vitamin A/g, which can be defined as normal. No β-carotene could be verified in the liver. The histopathological investigation of heart, lungs, liver, kidneys, spleen, pancreas, epidermis, stomach, small intestine, colon, bones, cartilage (rib) and bone marrow yielded normal findings in all cases (Test 7).

β-carotene feeding tests with dogs.

Test 8. Since the dog is closer to man regarding carotenoid resorption and utilization than the rat and rabbit[1], 2 dogs were additionally treated per os with 100 mg/kg β-carotene (in gelatine capsules) daily. The most important data of this test are compiled on Table 7; the hematologic investigations are shown on Table 4. It follows from the results that dogs tolerate continued oral overloading with β-carotene without clinically determinable disorders. It is of interest that vitamin A storage was found in the liver (standard value 30 - 100 I.U./g[1]) and in the kidney (standard value approx. 40 I.U./g) of both dogs, while the β-carotene-content did not increase, or increased insignificantly . The histopathological examination therefore showed certain modifications in liver and kidneys which can possibly be interpreted as sequelae of increased vitamin A storage:

TABLE 7. Results for dogs treated with β-carotene (100 mg/kg β-carotene in gelatine capsules per day, per os. Test 8).

Feststellung	Hund Nr. 1	Hund Nr. 2
Behandlungszeit . . . . . Tage	104	200
Total verabreichte Menge β-Carotin . . . g	149	221
Körpergewicht bei Beginn des Versuches kg	14	12
Körpergewicht bei Schluß des Versuches kg	15	11
Allgemeinzustand	sehr gut	sehr gut
Blutstaten (vgl. Tab. 4).	Vorübergehende Leukocytose mit relat. Lymphopenie	Vorbestehende Linksverschiebung normalisiert sich im Laufe des Versuches
Laboratoriumsuntersuchungen bei Versuchsabschluß		
Takata-Reaktion	negativ	negativ
Serumbilirubin . . . . . mg/100 ml	0,33	0,21
Bromsulphaleinprobe	negativ	negativ
Rest-N . . . . . mg/100 ml	35	38
Blutsenkungsreaktion (1 u. 2 Std) . . . mm	1/2/1	1/2/1
Urin (Zucker, Eiweiß, Urobilinogen, Sediment)	normal	normal
Sektionsbefund	Organe o. B., Ascaridiasis	Organe o. B.
Vitamin A-Gehalt der Leber . . . . I.E./g	2025	5062
β-Carotin-Gehalt der Leber . . . . . µg/g	5	8
Vitamin A-Gehalt der Nieren . . . . I.E./g	—	419
β-Carotin-Gehalt der Nieren . . . . µg/g	—	0

1. Finding:	2. Dog No.1	3. Dog No. 2.
Treatment period . . . days		
Total administered β-carotene quantity . . . g		
Body weight at the start of the test, kg . . .		
General condition . . .	very good	very good
Blood counts (see Table 4) . .	temporary leucocytosis with relat.lymphopenia	preexistant shift to the left returns to normal during the test
Laboratory examinations at the termination of the test.		
TAKATA-reaction . . .	negative	negative
Serum bilirubin, mg/100 ml . . .		
Bromosulfalein-test	negative	negative
Non-protein nitrogen. . . .		
Blood sedimentation reaction (1 and 2 hrs) , mm . .		
Urine(sugar, albumen, urobiligonen, sediment) . . .	normal	normal
Dissection findings . . .	no organic findings, ascaridiasis	no organic findings

TABLE 7 continued:

1. Finding:	2. Dog No. 1.	3. Dog No. 2.
Vitamin A content of the liver I.U./g . . .		
β-carotene content of the liver, µg/g . µg/g . . .		
Vitamin A content of the kidneys I.U./g . . .		
β-carotene content of the kidneys, I.U./g . . .		

The liver cells are so what enlarged; the protoplasm is very light and shows pronounced granulation, in Dog No. 1 without stainable fat; in Dog No. 2 diffuse fine-drop fatty infiltration. The KUPFFER star cell count is slightly higher; they are partially somewhat swollen and show an ample fatty load; the capillaries are narrow; the GLISSON sheaths are delicate. In the kidney, within the area of the tubuli recti, a few nuclear pykoses of the epitheliums and a slightly intensified epithelial desquamation are verified.

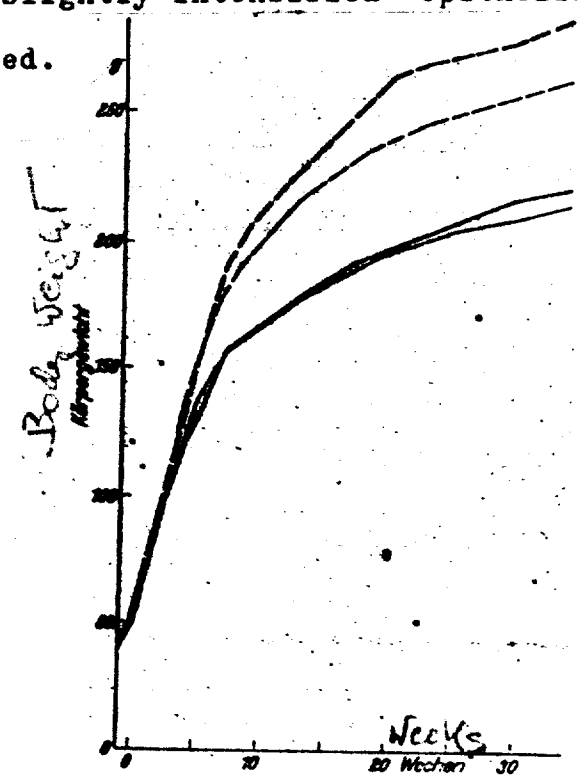


FIGURE 4. Effect of 0,1% lycopene in the diet on the body weight of rats (averages for 30 to 38 animals).
Males: * - with lycopene, - - — controls;
females: ---* with lycopene, — — controls.

The other histologically examined organs, such as spleen, pancreas, stomach, colon, small intestine, suprarenal gland, hypophysis, cerebrum, cerebellum and trunk ganglia were unchanged. Numerous primordial follicles were found in the ovaries, as well as maturing follicles and corpora lutea in course of regression.

Chronic lycopene feeding test with rats

Test 10. A chronic feeding test with rats was performed with lycopene as well (0,1% diet additive = 1000 parts/million parts fodder); the test included the 2. generation. The course of the test results from Table 8 and from weight curves as indicated in Figure 4. All rats treated with lycopene developed well; no cases of spontaneous death occurred.

While the growth rate of the 1. generation females was practically identical with the growth rate of the controls, the male animals gained more weight as compared to those in the corresponding control group (Figure 4). When the weight gains of individual animals are compared, the above difference is also statistically significant ($p < 0,01$, calculated according to STUDENT's t-distribution). Reference is made to the discussion of the results concerning the evaluation of the above-mentioned finding.

The hematologic findings show no specific aspects (Table 4). No substantial difference between treated and untreated animals is evident either in the reproduction tests with the first rat generation (results on Table 6).

One of the females treated with lycopene aborted; the litter count was somewhat, but not significantly less than the control litter size. 2 controls aborted. Pregnancy duration was normal throughout, no malformations were detected.

TABLE 8. Development of rats fed "synthetic" diet with 1% lycopene (Test 10).

1 Generation	2 Versuchsdauer Tage	3 Durchschn. Futterkonsum je Tag				4 Durchschn. Körpergewicht je				5 Spontantodesfälle		6 Getötet wegen Krankheit	
		7 Männchen		8 Weibchen		7 Männchen		8 Weibchen					
		9 Lycopin	10 Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle
1.	200	11,2	10,5	10,3	9,9	286	263	221	213	0	0	0	0
2.	50—80	11,5	8,6	8,8	9,4	199[1]	187[1]	153[1]	148[1]	0	1	0	0

[1] Nur mindestens 60 Tage alte Tiere berücksichtigt.

1. Generation: 2. Test duration, days: 3. Average fodder consumption per day: 4. Average body weight, each: 5. Spontaneous cases od death: 6. Killed on account of disease: 7. males; 8. females; 9. lycopene; 10 control.

1. Only animals at least 60 days old were considered.

Histopathological investigation. After 200 testing days 4 treated animals and 4 controls of the 1st generation were killed. Among the lycopene animals, the females showed pronounced and the males only slight storage of a brown rather rough-grained pigment in the liver cells (Figure 5), which is completely dissolved and vanished, when embedded in paraffin was eliminated. Lycopene is undoubtedly at issue here, while the liver structure is very well preserved. The normal large liver cells show a quite light, granular protoplasm, the capillaries are average-wide, the KUPFFER star cell count is not increased, nor are said cells infiltrated by fat or swollen. Fine GLISSON sheath.

Other histologically examined organs, such as kidney, suprarenal gland, spleen, pancreas, testicles, epididymis, ovary, lung, heart, epidermis, bones and marrow yielded no findings at variance from those of the controls. No pigment accumulation outside of the liver could be microscopically determined.

Lycopene feeding test with a dog.

Test 11. A dog was treated daily with 100 mg/kg lycopene(in gelatine capsules), per os. The animal received a total of 272 g lycopene in 192 doses without showing any incompatibility symptoms thereby. The body weight increased from 13 to 15 kg; the blood count, checked on 4 occasions, was always normal (Table 4). The following laboratory findings resulted at the end of the test: TAKATA- reaction negative; serum bilirubin 0,1 mg/100 ml; bromosulfalein test negative; non-protein nitrogen 35 mg/100 ml; blood sedimentation reaction after 1 and 2 hours: ½ and 1 mm respectively; no sugar and no albumen in the urine, uribiligonen normal, sediment: no finding.

Accordingly, a quite pronounced lycopene storage occured in the liver. The pigment could be histologically determined in the liver cells as well and was readily distinguishable from lipofuchsin, frequently present in the dog, when the paraffin embedding vanished. Apart from the above, the liver showed a completely regular structure under the microscope. Kidney, suprarenal gland, heart, lung, spleen, epidermis, stomach, small intestine, pancreas, cerebrum, cerebellum, trunk ganglia, hypophysis , ovary and bone marrow were likewise intact.

Carotenoid	Liver	Kidney
Vitamin A, I.U./g:	75	57
Lycopene, µg/g:	22.6	0,6
Carotene " "	0	0

4. Discussion of the results.

As stated in the introduction: no compatibility(tolerance) investigations proper are as yet available on the carotenoids. Admittedly, Nature demonstrates a large-scale experiment to us daily, since in vegetable nutrients of man and animal enormous quantities of carotene are consumed.

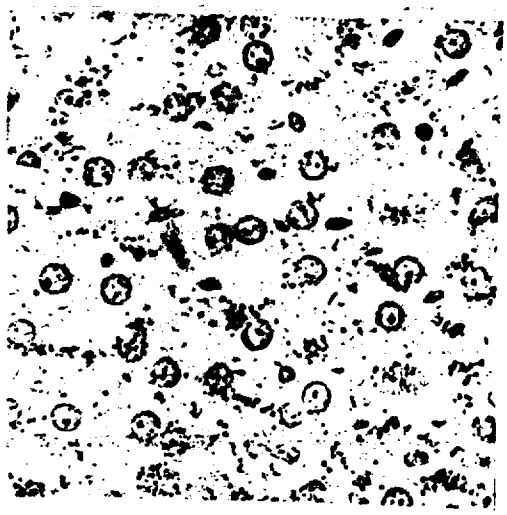


FIGURE 5. Effect of 0,1% lycopene in the fodder on the histology of the rat liver after 200 days of treatment. (Stained with Sudan III - 7-B hem- alum. 500 x magnificantion). Storage of lycopene in the liver cells. No fatty infiltration of the KUPFFER star cells.

Reference is made here only to the milch cow, the life-long carotenemia of which is responsible for the yellow color of butter as well as for the yellow coloring of a considerable part of our vitamin A supply. Literature includes several reports on not quite as natural "compatibility experiments" with carotene in man; they concern persons who consumed several pounds of raw carrots daily for several months; this resulted in carotenemia with yellow skin pigmentation(1), 2), among others); the latter was even transmitted to a child with the mother's milk[2], without damaging the child in any way.

Despite this natural evidence proving that carotenoids are harmless, compatibility investigations seem to be justified because conditions are quite different when pure carotenoids are added to foods; in particular, resorption in that case is quite different as compared to consumption of carotenoids in vegetable products when the pigments are mostly enclosed in cells and are accessible for resorption in part only.

Numerous new facts became known during recent years on the resorption and metabolism of the carotenoids. Not all related problems have as yet been clarified. Neverthless, a synopsis of up-to-date knowledge, in conjunction with the toxicologic question, on the utilization, conversion and decomposition of the carotenoids in the human and animal organism is needed.

The fact that resorption, storage and metabolism of carotenoids in man and in various animal species show considerable differences[3] has long been considered significant. Accordingly, mammals can be classified in three groups[3-5] concerning their storage capacity of carotene (α, β, γ) and xantophylls (oxycarotenoids, carotenols):

Group	Stores principally		
	carotene	xantophylls	Example
A	+	+	man
B	+	0	horse
C	0	0	hog
D	0	+	chicken

Chickens are listed in the 4th group; they store mainly xantophylls[3,4,6].

These differences of carotenoid storage led to the conclusion that the metabolism of the pigments varies; it is assumed that the (hypothetical) enzyme systems (carotinase, xantophyll-oxidase) are not homogeneous in various animal species[5,7]. Other authors discuss differences in the formation of specific albumen receptors in the blood and in the tissues[4].

More specific information on the metabolism of the catoneoids was provided by the results of experiments performed during recent years with complex technology. We are probably most knowledgeable today on the courses of carotene decomposition in the rat; this example will therefore be discussed somewhat more specifically.

The diagrammatic representation of the fate of carotene consumed with food (Figure 6) shows that in the rat, a large part of the pigment passes through the intestine unchanged and is excreted with the feces. The percentage of carotene in the feces is very variable and depends largely on secondary factors. It could amount to approximately 70%, as indicated by investigations with ^{14}C-labelled carotene under physiologic conditions. Pigment resorption from vegetable material is generally poor; it is better from oily solutions, and optimal from watery emulsions.[9] The same applies to man as well, in whose stool--depending on the application method-- 30 to 99% of the carotenoids consumed per os is recovered[1]. Resorption is inhibited by lack of bile[2], low albumen content of the food[3] and feeding of mineral oils which cannot be absorbed;

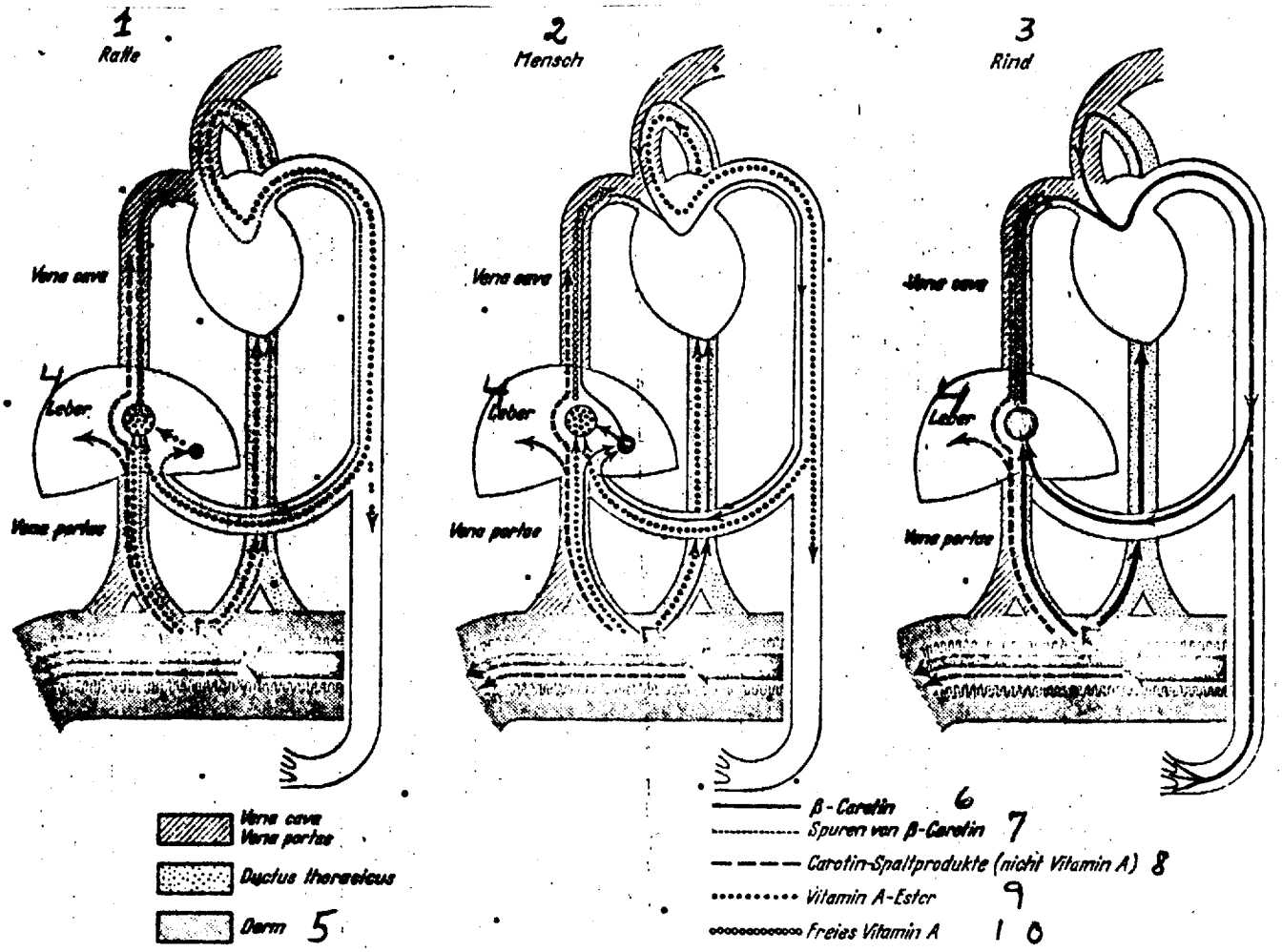
the resorption is promoted by the simultaneous oral administration of auromycine[4], vitamin B_{12}[5] and chloromycetin[4,6]. The vitamin A-content of the fodder and the method of the vitamin B-application likewise affect carotene utilization.[7]

Part of the carotene is destroyed during contact with stomach acid or in the intestine by auto-oxidation and bacterial effects; it is a fact which should not be overlooked in the course of analytical investigations[6],[8]. However, various tests indicate that the quota of carotene destroyed in the intestine is frequently numerically insignificant[9].

The major part of the resorbed carotene is separated in the intestinal wall of the rat[10], but little is known as yet on the process involved thereby, probably progressing under the effect of enzymes[11]. The most important separation product is vitamin A; it reaches the liver in esterified form through the lymphatic system and probably also through the portal artery; it is stored in the liver and is once more released, as needed, into the blood as free vitamin A (vitamin A-alcohol). Only a minimal part of the carotene passes unchanged through the intestinal wall and probably reaches the liver through the lymphatic system [12]. The carotene quota separated as vitamin A is quantitatively insignificant, while it is of foremost importance from the viewpoint of <u>metabolism</u>. For example: only 1,5% of the resorption activity of orally administered ^{14}C-labelled carotene is determinable in the liver-vitamin A;

FIGURE 6. Diagrammatic comparison of β-carotene and vitamin A metabolism in man, rat and cattle.

1. rat; 2. man; 3. cattle; 4. liver; 5. intestine;
6. β-carotene; 7. traces of β- carotene;
8. carotene separation products (not vitamin A)
9. vitamin A-esters; 10. free vitamin A.



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on the other hand, 5% is present in the CO_2 of the exhaled air, 15% in the fatty acids and approximately 40% is present in the carotenoid-free non-saponifiable quota of the total animal[13), 14)].

The above insignificant conversion of carotene into vitamin A alone makes it near-impossible to induce A-hypervitaminosis in the rat by means of overloading with β-carotene. Another fact, important for compatibility tests, is: the resorption of carotenes and their separation into vitamin A depends largely on the saturation of the organism with vitamin A[14] and also on the magnitude of the available carotene volume. An animal saturated with vitamin A utilizes a much smaller part of the available carotene than an animal deficient in vitamin A; in case of oral overloading with carotene the percentage of the resorbed pigment decreases considerably. Reference could be made here to "natural protection" against the development of A-hypervitaminosis; without such protection, good chronic β-carotene tolerance would be unthinkable.

A few more recent investigations have to be mentioned, to complete the aspect of the carotene metabolism in the rat; in the course of these investigations the fate of the <u>parenterally</u> applied pigment was observed. Intravenously injected carotene, or carotene injected into the spleen is mainly stored in the liver and lung within the reticulo-endothelial system. It is retained there in such a way that an animal deficient in vitamin A dies as a result of A-avitaminosis, despite its carotene-reserve which --if orally administered--would suffice for 1 year[1)].

More recent tests, in the course of which carotene was intravenously applied in finely emulsified form, showed however that vitamin A can be formed from carotenes in the rat outside of the intestine as well[2], not only in the liver, but also in other tissues[3]. At least some correlations with the carotene metabolism in man result from the above important finding. Therefore the initially mentioned differences between species regarding the storing of carotenoids seem to be quantitative rather than of a fundamental nature. The circumstances of the carotene metabolism in man are less well known. We must rely here mainly on blood value determination[4] and on the determination of carotene excretion in the stool. The diagram of the carotene metabolism in man can be prepared on the basis of such investigations and on the basis of carotene-storage in the organs (Figure 6). The difference, as compared with the rat is: in man, as in the dog, quite large quantities of carotene pass the intenstinal wall unchanged and enter the liver circulation through the portal artery; only a minor part reaches the liver circulation <u>via ductus thoraicus</u>. It is partially stored in the liver and partially separates as vitamin A[5].

When a very large volume of carotene is available, serum carotene increase will result, and larger quantities of carotene settle in the organs, among others in the skin as well[6].

The carotine utilization is similar in the sheep and goat to utilization of the same in the rat, i.e. they form their Vitamin A mainly in the intestinal tract and store only traces of carotene[7]; under specific conditions, however, they are neverthless able to form vitamin A from carotene outside of the intestine. The same applies to the rabbit and to the guinea pig[10].

The carotene metabolism in cattle differs in essential points from the carotene metabolism of man and of the rat (diagram in Figure 6). A large part of the resorbed carotene passes unchanged through the intestinal wall, enters the blood stream and is partially released into the milk as well. Ample supplies of carotene are also stored in the tissues[7]. Transformation into vitamin A occurs to a minor extent only. The principal location of carotine separation is the liver. Intravenously injected carotene is not stored as by other animals, nor does it immediately separate; instead, it is determinable in the blood for a prolonged period of time[11].

In said comparative carotine metabolism diagram, the following factors are significant for evaluation tests with β-carotene:

a) Under specific circumstances rats, rabbits and other animals as well, which normally separate carotene in the intestinal wall, can form vitamin A^0 in the liver and in other organs. Therefore no fundamental carotene utilization differences exist as compared to man who is able to utilize part of the carotene outside of the intestine.

b) The carotine metabolism of the dog is presumably similar to the carotene metabolism of man; the dog is therefore especially suitable as test animal.

c) Carotene added to food is resorbed in considerable quantities. If it is nerevthless impossible to induce A-hypervitaminosis, this is due to the fact that when the organism is saturated, the normally very small conversion quota of carotene into vitamin A is additionally reduced.

d) The storage of carotene in the cells of various organs is a natural and reversible process.

e) A large part of the resorbed carotene enters the metabolism and is decomposed and converted into products most of which have not been identified so far. The study of carotene metabolism should include a study of these metabolites as well and should not be limited to carotene storage and vitamin A-formation only.

The present investigations with β-carotene shows that even the long-term administration of the pigment caused no clinically or pathologic-anatomically determinable damage, nor any symptoms of A-hypervitaminosis. However, it is still to be verified whether safety margins required for the testing of food additives were observed concerning the dosages. The above can be carried out with the aid of a few diagrammatic examples and comparisons.

A rabbit fed dandelion leaves consumes about 10 mg carotene per kg of body weight each day. The rabbits for Tests 6 and 7 received 500 mg/kg β-carotene per day. This corresponds approximately to the 50-fold daily dose.

For coloring margarine, 2 to 4 g β-carotene per ton are used. The dog No. 2, which consumed a total of 221 g β-carotene in 200 days, accordingly received a volume of β-carotene sufficient for coloring 55000 to 110000 kg of margarine. Assuming that 50 to 100 g margarine is the "standard daily ration" per dog, the compatibility test was performed with a 5000 to 10000-fold overdosage ..

Rats with an average weight of 200 g consumed, with the 0,1% - .. β-carotene fodder additive, approximately 50 mg/kg of the pigment per day; translated into body weight, this corresponds, for a man weighing 60 kg, to a daily carotene consumption of 3 g, i.e. once more sufficient for coloring 1 ton of margarine. An approximate 10000-fold overdosage must be assumed in this case as well.

These examples indicate that the tests were performed with very large doses of β-carotene and that the safety margins customary for toxicity investigations were largely exceeded.

The only finding, certainly ascribable to chronic overloading with β-carotene, was determined in rats and dogs when subjecting the liver to histological examination. Both animal species showed strong fatty infiltration of the KUPFFER star cells with intensive fluorescence in u.v. light. These changes are sequelae of A-vitamin storage which was chemically verifed as well. Minor liver cell changes also found in dogs (swelling, adiposis in one animal) as well as minor reactions of the small kidney ducts are probably likewise sequelae of increased vitamin A-storage; no A-hypervitaminosis was at issue thereby.

It is obvious that when β-carotene is used as food coloring agent, any substantial increase of the vitamin- A storage is excluded, also for reasons of dosage. Furthermore, no case of human alimentary carotenemia with A-hypervitaminosis is known (even the carotenemia case described by HENSCHEN[1] showed no A-hypervitaminosis symptoms). This differs from the therapy with cod liver oil or vitamin A-preparations during which symptoms of said syndrome were occasionally observed. The finding that in man a normal vitamin A-blood level is present despite much increased serum-carotene values[2] makes it appear unlikely that damage could results, even with extreme carotene overdosages, due to A-hypervitaminosis.

Among carotenoids, lycopene holds a specific position: it is chemically an isomer of carotene, but follows a largely independent path in metabolism. No vitamin A[1),2)] forms from lycopene ; the pigment(2 to 6% or more) is nerevthless stored in the rat liver [2)3)]. It normally also occurs in the liver, fatty tissues and serum of man[4]; some of it is stored in the chicken[5] and in the course of present investigations, it was determinable in the dog liver as well. However, the entire liver contained only 0,0025% of the total administered lycopene volume. Lycopene is connected with the metabolism of carotene and vitamin A insofar as small doses of lycopene promote the resorption and storage of the vitamin as well as of the provitamin[3].

During compatibility tests with lycopene , storage of the pigment in the liver cells was microscopically determinable as well. However, it results from the above-mentioned chemical verification and from the histological investigations that a negligibly small percentage of the lycopene was stored in the liver, and that the deposit rate of 2 to 6% or more, cited in literature in case of chronic over-loading, was not attained by far. Despite the neverthless quite considerable pigment storage in the liver, disorders of the liver function and liver structure could not be verified either clinically or by means of histopathological investigations. The male animals of the first rat generation treated with lycopene fodder additive even developed significantly better than the untreated control animals; this is a single finding, evaluated by us merely as additional proof for the atoxicity of lycopene; no conclusions should be reached from said case concerning a specific, growth-stimulating effect.

A few dosage comparisons are useful here as well for the evaluation of present compatibility tests with lycopene:

A dog consumed a total of 272 g lycopene in 192 days, which corresponds approximately to a volume of 10-to 15000 kg pigment, extracted from fresh tomatoes. Rats with an average weight of 200 g consumed, with a 0,1% lycopene diet additive daily, consumed appro 50 mg/kg of the pigment. Translated into body weight, the above is equal--for a man weighing 60 kg--to a daily consumption of 3 g lycopene, contained in approx. 20 kg concentrated tomato paste.

Accordingly, the safety factor is very high for lycopene tests as well. Toxic damage when using said carotenoid as food color is unthinkable.

The tests with 7,7'-dihydro-β- carotene and bixin were performed on a small scale only. Rats tolerated these pigments in large doses of 1000 mg/kg/day per os without difficulty. Therefore both substances can likewise be defined as atoxic.

The carotenoids investigated by us are very well tolerated substances which do not cause organic damage, not even when administered for a prolonged period of time in large doses per os or parenterally, regardless of the question whether or not storage in organs occurs. A carcinogenic effect of these natural pigments is highly unlikely. So far, not a single spontaneous tumor was detected in the course of animal tests with carotenoids. However, as mentioned earlier, for chronological reasons no final conclusions can as yet be reached.

Numerous State authorities and international professional commissions recognize the carotenoids by now as harmless.

For example: carotenoids were included in the list of non-objectionable pigments[1)] by the "Commission for the Study of the Food Color Problem, German Research Community". Bixin, for which additional testing was requested in 1952, figures in the 1957 list with other carotenoids as authorized pigment. The list was compiled by the "European Research Committee for Protection of Peoples Against Chronic Toxic Environmental Damage[2)]; said list was officially accepted in Switzerland a short while ago.

It must be pointed out that pigm nts of the large carotenoid class show no homogeneous behavior rega ding resorption, storage and decomposition; it must therefore be assumed that chronic toxicity differences exist.

The clarification of these differences and the prevention of organ damage is the principal purpose of animal tests in the course of the compatibility investigations requested today. Only additional research will show whether similar uniform standardization is admissible when characterizing the well-defined conception "carotenoids" from the standpoint of toxicology.

Remark during proof-reading: Since the termination of the manuscript, Tests No. 3a and 3c(β- carotene, 0,1%, rats, 1st and 3rd generation) and No. 10a and 10b (lycopene, 0,1%, rats, 1st and 2nd generation) were continued for 200 days; no damage whatsoever nor any spontaneous tumors became evident. The course of the reproduction test with 2nd generation rats, fed lycopene-containing diet, was normal.

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Tierexperimentelle Untersuchungen über die chronische Verträglichkeit von β-Carotin, Lycopin, 7,7'-Dihydro-β-carotin und Bixin

Von

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Mitteilung aus der Forschungsabteilung der F. Hoffmann-La Roche & Co. A.-G., Basel

Mit 6 Textabbildungen

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Carotinoide sind natürliche Farbstoffe, die im Pflanzenreich außerordentlich weit verbreitet sind[1]. Mit der Nahrung gelangen sie in den menschlichen und tierischen Organismus, wo sie teilweise resorbiert und abgebaut, z. T. auch zu Vitamin A gespalten werden. Verschiedene Tierarten speichern Carotinoide entweder als Farbe sichtbarer Körperteile (z. B. Gefieder des Kanarienvogels, Schnabel und Haut des Flamingo, Chitinpanzer vieler Crustaceen[2]) oder in inneren Organen (Leber, Ovar, Nebenniere, Fettgewebe). Der Stoffwechsel der wichtigsten Carotinoide wurde in den vergangenen 20—30 Jahren besonders im Hinblick auf ihre Bedeutung als Provitamine A eingehend erforscht, doch sind unseres Wissens toxicologische Untersuchungen bisher unterblieben, wohl weil man die Carotinoide als natürliche Bestandteile gebräuchlicher Nahrungsmittel von vornherein für „unschädlich" hielt. Außerdem waren die für solche Prüfungen erforderlichen großen Mengen der reinen Pigmente bis vor kurzer Zeit nicht verfügbar. Mehrere z. T. synthetisch hergestellte, z. T. aus Naturprodukten isolierte Carotinoide werden heute jedoch in großtechnischem Maßstab hergestellt und u. a. auch zur Färbung von Lebensmitteln verwendet. Es erscheint notwendig, daß auch diese Farbstoffe die strengen tierexperimentellen Prüfungen durchlaufen, wie sie für die nicht in der Natur vorkommenden Lebensmittelzusätze verlangt werden[3].

In der vorliegenden Arbeit wird über erste Resultate chronischer Verträglichkeitsversuche berichtet, die an verschiedenen Tierarten vor allem mit *β-Carotin* und *Lycopin*, in kleinerem Umfang auch mit *7,7'-Dihydro-β-carotin* und *Bixin* durchgeführt wurden. Hierbei wird vor allem auf die Frage der allgemeinen chronischen Toxicität und der A-Hypervitaminose eingegangen. Zur Beurteilung einer carcinogenen Wirkung erfüllen die Versuche die modernen Forderungen bezüglich zeitlicher Dauer[4] noch nicht. Bei der Durchführung der Prüfungen hielten wir uns nach Möglichkeit an die in letzter Zeit von amerikanischen und europäischen Behörden

[1] Karrer, P., u. E. Jucker: *Carotinoide*. Basel: Birkhäuser 1948.
[2] Fox, H. M.: Endeavour 14, 40 (1950).
[3] Lehman, A. J., W. I. Patterson, B. Davidow, E. C. Hagan, G. Woodard, E. P. Lang, J. P. Frawley, O. G. Fitzhugh, A. R. Bourke, J. H. Draize, A. A. Nelson u. B. J. Vos: Food Drug Cosmetic Law J. 10, 679 (1955). — Druckrey, H.: Dtsch. med. Wschr. 1957, 1310.
[4] Lehman, A. J., W. I. Patterson, B. Davidow, E. C. Hagan, G. Woodard, E. P. Lang, J. P. Frawley, O. G. Fitzhugh, A. R. Bourke, J. H. Draize, A. A. Nelson u. B. J. Vos: Zit. diese S., Anm. 3. — Druckrey, H.: Zit. diese S., Anm. 3. — Barnes, J. M., u. F. A. Denz: Pharm. Rev. 6, 191 (1954).

und Kommissionen aufgestellten Richtlinien[1, 2]. In der Diskussion werden auch die mit der Toxikologie eng verbundenen Fragen betreffs Resorption und Stoffwechsel der Carotinoide berührt.

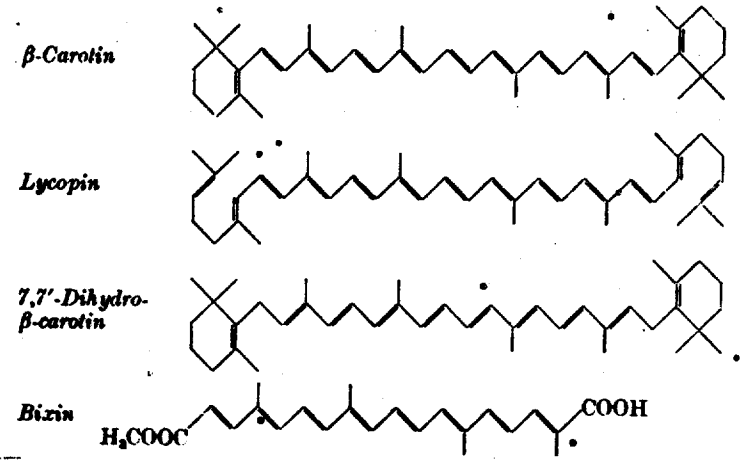
1. Chemie der geprüften Carotinoide

Die vier auf chronische Verträglichkeit geprüften Carotinoide kommen als natürliche Bestandteile in Pflanzen vor. β-Carotin ist das wichtigste Provitamin A und findet sich in allen grünen Pflanzenteilen und in der Butter[3]. Lycopin ist vor allem als Farbstoff der Tomate bekannt, findet sich aber auch in vielen anderen Früchten[3]. 7,7′-Dihydro-β-carotin wurde in Pollen von *Cyclamen persicum* nachgewiesen[4]. Bixin ist hauptsächlich in den Samen von *Bixa orellana* enthalten[5].

Untersucht wurde natürliches, aus pflanzlichem Material isoliertes Bixin[5]. Bei den übrigen Präparaten handelt es sich um total synthetisierte Produkte[6]. Für die Frage der chronischen Verträglichkeit spielt dieser Unterschied jedoch keine wesentliche Rolle, da nach der ausdrücklichen Feststellung der „Deutschen Kommission zur Bearbeitung des Lebensmittelfarbstoffproblems" die toxicologische Beurteilung eines Naturfarbstoffes unabhängig davon sein muß, ob er natürlicher oder synthetischer Herkunft ist[7].

Die chemischen Formeln der untersuchten Farbstoffe sind in Tab. 1, weitere physikalisch-chemische und chemische Angaben in Tab. 2 zusammengestellt. Über chemische Veränderungen, welche die Carotinoide bei der Lagerung an der Luft,

Tabelle 1. *Strukturformeln der geprüften Carotinoide*



[1] Zit. S. 113, Anm. 4.

[2] DUNN, C. W.: Food Drug Cosmetic Law J. 10, 637 (1955). — LANG, K.: Dtsch. med. Wschr. 1957, 137. — Europäisches Forschungskomitee für den Schutz der Bevölkerung vor chronisch-toxischen Umweltschädigungen. Sitzung am 10.—12. IV. 1957 in Ascona [vgl. diese Z. 108, 82 (1958).]

[3] KARRER, P., u. E. JUCKER: Zit. S. 113, Anm. 1.

[4] KARRER, P., u. E. LEUMANN: Helvet. chim. Acta 34, 1412 (1951).

[5] In unseren chemischen Laboratorien dargestellt von Herrn PD. Dr. A. WINTERSTEIN.

[6] ISLER, O., H. LINDLAR, M. MONTAVON, R. RÜEGG u. P. ZELLER: Helvet. chim. Acta 39, 249 (1956). — ISLER, O., M. MONTAVON, R. RÜEGG u. P. ZELLER: Helvet. chim. Acta 39, 451 (1956). — ISLER, O., H. GUTMANN, H. LINDLAR, M. MONTAVON, R. RÜEGG, G. RYSER u. P. ZELLER: Helvet. chim. Acta 39, 463 (1956).

[7] Deutsche Forschungsgemeinschaft, Wissenschaftliche Kommission zur Bearbeitung des Lebensmittelfarbstoffproblems. Mitteilung 2 vom 30. Mai 1952: [Diese Z. 96, 264 (1953)]. Mitteilung 5 vom 15. Juli 1954: [Diese Z. 102, 129 (1955)].

z. B. im Futter, erleiden, ist noch wenig Sicheres bekannt. Daß Spaltprodukte bei den Verträglichkeitsprüfungen mituntersucht wurden, ließ sich trotz Verwendung der Reinsubstanzen nicht vermeiden. Dies ist jedoch nicht als Nachteil zu werten, da beim Gebrauch der Carotinoide als Lebensmittelfarbstoffe dieselben Abbauprodukte entstehen und in den Organismus gelangen.

Tabelle 2. *Chemische und physikalisch-chemische Daten der geprüften Carotinoide*

Daten	all-trans-β-Carotin	Lycopin	7,7'-Dihydro-β-carotin	Bixin
Bruttoformel	$C_{40}H_{56}$	$C_{40}H_{56}$	$C_{40}H_{58}$	$C_{25}H_{30}O_4$
Reinheit	mind. 96% mit Beimengungen cis-trans-isomerer Formen, spez. 15,15'-cis-β-Carotin u. Spuren von Dehydro- u. hydriertem β-Carotin	nach Chromatographie an Al_2O_3 werden 97—98,5% des einheitl. Farbstoffes gefunden		mind. 99% labiles Bixin
Gehalt an Schwermetallen	Fe < 5 mg/100 g; Cu u. Mg < 1 mg/100 g			
Aschegehalt	< 0,01%	< 0,01%	< 0,01%	< 0,01%
Biologische Aktivität	mindestens 1600000 I. E./g	0	0	0
Aussehen der Kristalle	blättchenförmig, violett mit metallischem Glanz	nadelförmig, rotbraun bis braun	blättchenförmig, gelborange	violett, nadelförmig
Schmelzpunkt °C	182	172—173	133—184	195—197
Absorptionsdaten $E^{1\%}_{1\,cm}$	in Hexan 338 mμ = 200 370 mμ = 250 453 mμ = 2450 481 mμ = 2150	in Petroläth. 446 mμ = 2250 472 mμ = 3450 505 mμ = 3150	in Petroläth. 382 mμ = 1300 405 mμ = 2990 429 mμ = 2910	in Äthanol 485 mμ = 3,6 460 mμ = 3,65
Chromatographie	an Al_2O_3 ergibt eine einzige Zone von all-trans-β-Carotin	an Al_2O_3	an Al_2O_3	aus Methylenchlorid an Al_2O_3 ergibt eine Zone von Bixin
Beständigkeit	zersetzt sich langsam an der Luft wahrsch. durch Oxydation unter Bildung ungef. noch nicht untersuchter Abbauprodukte. Bei Erhitzen teilweise Isomerisierung zu cis-β-Carotin	zersetzt sich an Luft etwas schneller als β-Carotin. Bei Erhitzen z. T. Isomerisierung zu Neolycopin	etwa wie β-Carotin, eher etwas beständiger	verändert sich an Luft b. Zimmertemperatur nicht[1]

2. Versuchsanordnung

a) *Tiermaterial*

Verwendet wurden weiße Inzuchtratten vom Wistarstamm eigener Zucht, meist im Gewicht von 40—45 g (30—40 Tage alt). Tiere verschiedener Würfe wurden gleichmäßig auf Versuchs-

[1] Kon, S. K., W. A. McGillivray u. S. Y. Thompson: Brit. J. Nutrit. 9, 244 (1955).

und Kontrollgruppen verteilt. Die Ratten wurden in Einzelkäfigen mit weitmaschigen Doppelböden aus Draht bei konstanter Raumtemperatur von 24—25° C gehalten.

Kaninchen kamen im Alter von 6—8 Monaten (Gewicht 2,5—3,5 kg) in den Versuch. Bei den Hunden handelte es sich um 4—6jährige weibliche Bastarde.

b) *Ernährung der Tiere*

Die Ratten erhielten eine calorisch vollwertige, „synthetische" Diät folgender Zusammensetzung:

Casein (vitaminfrei)	20%,
Cocosfett (Pura[1])	10%,
Saccharose	61%,
Salzmischung	4%,
Cellulosemehl	5%.

Vitaminzulagen pro Tier und Tag, verabreicht in 3 Portionen pro Woche:

Vitamin B_1	100 µg,	Vitamin B_2	200 µg,
Vitamin B_6	100 µg,	(+)-Biotin	0,5 µg,
Cholin	3 mg,	Nicotinamid	1 mg,
Pantothensaures Ca	200 µg,	2-Methyl-1,4-naphthochinon	20 µg,
DL-α-Tokopherol	30 µg,	Vitamin A	15 iE,
Vitamin D_3	5 iE,		

Die Salzmischung hatte folgende Zusammensetzung (in%):

Natriumchlorid	5,0,	Magnesiumsulfat	5,5,	Calciumlactat	35,0,
Tricalciumphosphat	15,0,	Eisencitrat	3,21,	Kaliumjodid	0,09,
Kupfersulfat	0,03,	Dikaliumphosphat (getr.)	26,53,	Zinkcarbonat	0,02,
Natriumfluorid	0,003,	Dinatriumphosphat (getr.)	9,6,	Mangansulfat	0,02.

Die *Kaninchen* wurden mit Heu, Gras und einem Gemisch aus trockenen Zuckerrüben, Hafer, Kleie und gekochten Kartoffeln. *Hunde* mit Hafersuppe und gekochtem Fleisch gefüttert.

c) *Verabreichung der Carotinoide*

Bei den 100 Tage dauernden Versuchen an *Ratten* erhielt jedes Tier jeden Morgen seine entsprechend dem Körpergewicht abgewogene Carotinoid-Menge unter eine kleine Menge Futter gemischt in einer Schale vorgesetzt. Da die Ratten nachts kein Futter erhielten, wurde der Farbstoff meistens rasch aufgefressen. Bei den langfristigen Versuchen wurde das Carotinoid dem Futter zugesetzt. Die *Kaninchen* erhielten β-Carotin 10%ig in „Mischglycerid" [Mischung von Triglyceriden mit niedermolekularen Fettsäuren (C_8—C_{12})] 5mal wöchentlich mit der Schlundsonde. Den *Hunden* wurden die Farbstoffe täglich per os in Gelatinekapseln verabreicht. Zur intramuskulären Injektion wurde β-Carotin unter leichtem Erwärmen in Arachisöl gelöst.

Futtermischungen und Carotinoid-Einzeldosen wurden täglich frisch zubereitet, die Farbstoff-Vorräte unter CO_2 im Kühlschrank aufbewahrt. Antioxydantien kamen nicht zur Anwendung, da solche Substanzen nicht nur die Oxydation der Carotinoide hemmen, sondern in vivo unter Umständen auch die Umwandlung zu Vitamin A beeinträchtigen[2].

d) *Laboratoriumsuntersuchungen*

Die bei allen Tieren durchgeführten Blutuntersuchungen umfaßten Hämoglobin, Erythrocyten- und Leukocytenzahl, Differentialblutbild, teilweise auch Zahl der Reticulocyten und der Thrombocyten. Die Messung des Hämoglobins erfolgte im Beckman-Photometer. Zur Bestimmung der Erythrocyten- und Leukocytenzahlen bedienten wir uns der üblichen Pipetten und Zählkammern. Bei *Hunden* wurden außerdem folgende Untersuchungen vorgenommen: Blutsenkungsgeschwindigkeit, Serumbilirubin, Rest-N, *Takata*-Reaktion, Bromsulphaleinprobe und Urinanalysen. Die Bestimmung von Vitamin A, β-Carotin und Lycopin in verschiedenen Organen erfolgte nach Extraktion [modifizierte Röse-Gottlieb-Methode[3]], für Carotinoide nach Verseifung mit KOH, chromatographischer Reinigung an speziell aktiviertem Al_2O_3 durch Messung der Extinktion der Petrolätherlösung bei 452—454 mµ (β-Carotin) bzw. 472—474 mµ (Lycopin), für Vitamin A nach der Methode MOOR[4] durch trockene Verseifung mit NaOH.

[1] Walz und Eschle A.-G., Basel.

[2] HIGH, E. G., L. A. WOODS u. S. S. WILSON: J. biol. Chemistry 195, 787 (1952). — HIGH, E. G., S. S. WILSON, H. C. SMITH u. H. H. TAYLOR: Fed. Proc. 13, 229 (1954).

[3] Schweiz. Lebensmittelbuch. 4. Aufl., S. 53. Bern: Zimmermann 1937.

[4] MOOR, H.: Mitt. Lebensmittelunters. Hyg. 44, 257 (1953).

Chromatographie an Al_2O_3 und Auswertung nach CARR-PRICE. Die chemischen Bestimmungen erfolgten in unserem Vitaminlaboratorium durch Frl. Dr. S. NOBILE.

e) *Histopathologische Technik*

Zahlreiche Organe wurden in Bouinscher Lösung fixiert, in Paraffin eingebettet und mit Hämalaun-Eosin, Gehirnschnitte auch nach NISSL gefärbt. An Formol-fixierten Gefrierschnitten erfolgte die Fettfärbung mit Fettrot 7 B und der Nachweis von Vitamin A durch UV-Fluorescenzmikroskopie.

f) *Durchführung der Reproduktionsversuche an Ratten*

Die Paarung erfolgte nach 14—16wöchiger Fütterung carotinoid-haltiger Diäten. Hierzu wurden je ein männliches und ein weibliches Tier 4mal wöchentlich für eine Nacht in einen Käfig gesetzt. Der Vaginalabstrich am folgenden Morgen ermöglichte die Feststellung einer Besamung. Weibchen mit positivem Spermienbefund wurden täglich gewogen und speziell beobachtet. Kam es zur Geburt, dann wurden die Jungen gewogen und gezählt. Tiere, die nach 1monatiger Versuchsdauer keinen positiven Spermienbefund zeigten, wurden nicht mehr gepaart. Bei Fütterung „synthetischer" Diäten kommt es recht oft vor, daß Rattenmütter ihre Jungen auffressen oder vernachlässigen. Dies wurde ebenso oft bei den mit Carotinoiden behandelten Tieren wie bei den Kontrollen beobachtet und hatte zur Folge, daß die Geschlechtsverteilung in der 2. und 3. Generation z. T. ungleich war.

g) *Tierzahlen, Versuchsdauer und Dosierung*

Tab. 3 gibt eine Übersicht über alle durchgeführten Versuche. Neben der Dosierung und der Verabreichungsart der Carotinoide ist auch die totale, pro Tier während der ganzen Versuchsdauer aufgenommene, Farbstoffmenge angegeben. Für die langfristigen Rattenversuche, bei welchen die Carotinoide als Diätzusätze verabreicht wurden, basieren diese Angaben auf Futtermessungen, die im Abstand von 3—6 Wochen während je 7 Tagen erfolgten.

Tabelle 3. *Übersicht über die chronischen Verträglichkeitsversuche mit Carotinoiden*

Vers. Nr.	Carotinoid	Tierart	Tierzahl m	Tierzahl w	Dosierung mg/kg/Tag oder Teile je Million (M), Teile Futter	Verabreichungsart	Vers.-Dauer Tage	Totale pro Tier verabreichte Carotinoidmenge g	Anzahl Kontrolltiere
1	β-Carotin	Ratte	5	5	5000/M	Futterzusatz	540	etwa 30	0
2	β-Carotin	Ratte	10	10	1000 mg	per os (Schale)	100	etwa 10	20
3a	β-Carotin	Ratte	15	15	1000/M	Futterzusatz	400	etwa 5,3	30
3b	β-Carotin	Ratte 2. Gen.	16	5	1000/M	Futterzusatz	240—260	etwa 3,3	19
3c	β-Carotin	Ratte 3. Gen.	12	10	1000/M	Futterzusatz	85—115	etwa 1,3	21
4	β-Carotin	Ratte	—	10	1mal 1000 mg/Tier	intramuskulär	3—48	1	0
5	β-Carotin	Ratte	—	8	9 mg/Tier	intramuskulär 5mal proWoche	120[1]	etwa 0,6	8[2]
6	β-Carotin	Kaninch.	2	2	500 mg	per os 5mal pro Woche	77	etwa 80	0
7	β-Carotin	Kaninch.	—	1	500 mg	per os 5mal pro Woche	730	etwa 800	0
8	β-Carotin	Hund	—	2	100 mg	per os	104/200	149/221	0
9	Lycopin	Ratte	10	10	1000 mg	per os (Schale)	100	etwa 10	20
10a	Lycopin	Ratte	15	15	1000/M	Futterzusatz	238	etwa 2,9	30
10b	Lycopin	Ratte 2. Gen.	13	18	1000/M	Futterzusatz	50—80	etwa 1,0	38
11	Lycopin	Hund	—	1	100 mg	per os	192	272	0
12	Bixin	Ratte	10	10	1000 mg	per os (Schale)	100	etwa 10	20
13	7,7'-Dihydro-β-carotin	Ratte	10	10	1000 mg	per os (Schale)	100	etwa 10	

[1] Mit kurzen Unterbrechungen wegen lokaler Reizwirkung.
[2] Behandelt mit 4,5 mg/Tier Vitamin A-Palmitat.

Tabelle 4. *Blutuntersuchungen bei mit Carotinoiden behandelten Ratten, Kaninchen und Hunden.*

Vers. Nr.	Carotinoid	Tierart	Dauer der Behandlung Tage	Anzahl der Tiere	Hämoglobin g/100 ml	Erythrocyten 10^{12}	Hb_E $\mu\mu g$
1	BC	Ra	322	9	15,5±0,8	6,3±0,6	25,0±3
2	BC	Ra	56	8	16,9±2,1	7,7±0,7	22,0±2
	BC	Ra	98	8	17,8±1,2	8,2±0,8	21,6±1
	K	Ra	56	8	16,4±1,4	7,5±0,6	21,8±1
	K	Ra	98	8	18,1±1,9	8,6±0,9	21,0±2
3a	BC	Ra	358	28	16,1±0,9	7,4±0,7	21,7±2
	K	Ra	358	27	15,2±1,1	7,1±0,6	21,1±2
3b	BC	Ra	200	21	16,4±0,9	8,4±0,3	19,5±1
	K	Ra	200	18	16,1±0,9	8,3±0,4	19,4±1
3c	BC	Ra	60	20	16,3±1,4	8,5±0,9	19,4±2
	K	Ra	60	20	16,7±2,0	8,7±0,8	19,2±2
6	BC	Kan.	28	5	16,0±1,2	6,1±0,4	25,6±3
	BC	Kan.	77	5	13,2±1,3	4,6±0,8	28,9±3
7	BC	Kan.	175	1	17,4	6,2	27,8
	BC	Kan.	637	1	14,8	5,1	29
8	BC	H1	0	1	15,7	5,6	28
	BC	H1	18	1	16,2	6,1	28
	BC	H1	72	1	19,7	6,1	32
	BC	H1	100	1	18,0	6,5	28
8	BC	H2	0	1	13,4	5,3	25
	BC	H2	15	1	15,7	5,7	28
	BC	H2	74	1	16,7	6,0	28
	BC	H2	142	1	16,3	6,3	26
	BC	H2	200	1	20,0	7,0	28
9	L	Ra	56	8	15,6±1,1	8,1±0,5	19,2±1
	L	Ra	98	8	17,9±1,1	7,9±0,7	22,7±2
	K	Ra	56	8	16,4±1,4	7,5±0,6	21,8±1
	K	Ra	98	9	18,1±1,9	8,6±0,9	21,0±2
10a	L	Ra	190	30	16,1±1,2	8,8±0,6	18,3±2
	K	Ra	190	30	15,6±2	7,7±0,8	20,1±2
10b	L	Ra	40	20	16,1±1,2	7,9±0,6	20,3±2
	K	Ra	40	20	15,6±1,5	8,6±1,0	18,4±2
11	L	H	0	1	19,4	7,9	24
	L	H	19	1	18,1	6,3	29
	L	H	71	1	17,6	6,5	27
	L	H	166	1	19,4	6,4	30
	L	H	188	1	19,7	7,9	25
12	B	Ra	56	8	16,5	7,7	21,5
	B	Ra	98	8	16,8	8,1	20,8
13	7,7′	Ra	56	8	15,9±1,9	7,7±0,5	20,6±2
	7,7′	Ra	98	8	16,6±2,0	8,1±1,3	20,5±1
12+13	K	Ra	56	8	15,7±1,0	7,5±0,6	21,3±1
	K	Ra	98	8	18,1±2,1	8,4±0,6	21,6±2

BC = β-Carotin, L = Lycopin, B = Bixin, 7,7′ = 7,7′-Dihydro-β-carotin. — Ra = Ratte, Kan. = Kaninchen, *H* = Hund. —
K = Kontrolle
$\mu\mu g = 10^{-12} g$

$$SD = \sqrt{\frac{S(x-\bar{x})^2}{n-1}}$$

3. Ergebnisse

Es ist im Rahmen dieses Berichtes unmöglich, das große Beobachtungs- und Zahlenmaterial, das aus den Versuchen resultierte, im Detail darzustellen. Eine Beschränkung auf die wichtigsten Befunde und die Mittelwerte ist deshalb unumgänglich.

Durchschnittswerte für Ratten und Kaninchen, Einzelwerte für Hunde und Kaninchen

Leukocyten 10^3	Stab %	Segmentk.	Eos. %	Baso. %	Lymphocyten %	Monocyten %	Reticulocyten	Thrombocyten
9,3±2,6	2.2	[illegible] 5	3,6	—	49,6± 8	2,0	20,1	—
16,1±2.3	0.6	[illegible] 8	1,4	0.1	52,4± 8	3,9	—	—
18,3±3.4	—	[illegible] 6	1,6	—	71,0± 9	3,1	—	—
10,7±2.6	—	[illegible] 7	0,7	—	78,7± 9	1,2	—	—
8,4±2,2	0.1	[illegible] 7	0,8	—	67,9±10	1,4	—	—
11,2±2	2.6	[illegible] 5	2,1	—	72,9± 8	0,3	—	—
12,5±3	2.5	[illegible] 8	3,1	—	66,6± 9	0,3	—	—
12,2±3	0.6	[illegible] 7	2,5	—	70,0± 8	1,5	—	—
11,1±2	0.3	[illegible] 6	2,4	0.06	74,1± 7	1,7	—	—
12,6±2,5	0.5	[illegible] 14	1.3	—	81,5± 7	0,6	—	—
11.2±3.9	1.0	[illegible] 9	1.5	0.02	79,4±19	0,6	—	—
9,9±1.5	0.6	[illegible] 14	0,5	4,7	54,9±13	3,4	—	—
9,9±2,5	0.8	[illegible] 8	1.0	6.0	42.3± 8,5	0,7	—	—
8,6	0.5	[illegible]	—	4	41,5	6	—	—
6,7	2.0	[illegible]	1	2	20.5	1	—	—
8,6	1.5	[illegible]	4.0	—	22,0	4,0	—	390000
17.7	3.0	[illegible]	—	—	10.5	3,5	—	270000
7,0	—	[illegible]	3	—	18,5	4,5	—	—
14,1	2	[illegible]	0,5	—	22,5	2,5	—	350000
11,8	21.4	[illegible]	2	—	8,6	8,0	—	—
7.5	10	[illegible]	9	—	12,5	13.0	—	—
5,4	12	[illegible]	5	—	16	9,0	—	—
6,7	5.5	[illegible]	8	—	14	8,0	—	—
7,4	2	[illegible]	4	—	23,5	4,5	—	250000
11.1±3,5	—	[illegible] 6	0,8	—	78,4± 6	1.3	—	—
8,7±3	—	[illegible] 6	1.6	—	72,9±10	1,3	—	—
10,7±2,6	—	[illegible] 7	0.7	—	78,7± 9	1,2	—	—
8,4±2,2	0.1	[illegible] 7	0,8	—	67,9±10	1,4	—	—
16,1±4	1.9	[illegible] 7	3,4	0,05	71,6±10	0,5	—	—
12,6±5	0.7	[illegible] 9	4,8	—	66,0± 9	3,5	—	—
11,8±3,5	1.0	[illegible] 8	2,5	0.13	76,4± 9	1	—	—
12,6±3.5	1.5	[illegible] 10	1,5	0.13	75,4±10	1,5	—	—
9,4	1.5	[illegible]	16,5	—	22	4,5	—	—
10.0	1.0	[illegible]	11,5	—	17,5	2,5	—	—
11,9	0.5	[illegible]	6,5	—	27	2,5	—	—
6,5	3.0	[illegible]	12	—	27,5	5,5	—	—
8,7	—	[illegible]	7	—	22	3,5	—	—
10,7	0.9	[illegible]	1	—	63,4	3,8	—	—
15,6	0.4	[illegible] 8	2,0	—	69,2± 9	1.4	—	—
10.5±3.2	0.2	[illegible] 10	1,4	—	80,9±10	0.9	—	—
8,9±2,0	0.3	[illegible] 9	2,4	—	58,4±10	5.5	—	—
10,5±2,5	0	[illegible] 6	1,8	—	77,8±10	1,2	—	—
8.4±1,9	0.1	[illegible] 12	2,8	—	66,7±11	1,4	—	—

Tastversuch mit β-Carotin an Ratten

Versuch 1 war als erster Tastversuch gedacht. 10 junge Ratten, denen 0,5% β-Carotin (5000 Teile Million Teile Futter) unter das Futter gemischt wurden, gediehen während 78 Wochen sehr gut. 3 Tiere mußten wegen infektiöser Otitis-Labyrinthitis bzw. chronisch-rezidivierender Pneumonie mit Bronchiektasen vorzeitig getötet werden. Die Tiere nahmen regelmäßig an Gewicht zu, weißes und rotes Blutbild (47. Woche) waren normal (Tab. 4).

Die histopathologische Untersuchung ergab bei einem Tier einige bronchopneumonische Herde der Lunge, bei einem der 5 Männchen eine partielle, auf einige Kanälchen beschränkte

Hodenatrophie und bei einer Ratte, die gegen Versuchsende in einen schlechten Allgemeinzustand geriet, im Knochenmark eine leichte Verminderung des Zellgehaltes mit ödematöser Durchtränkung des Stromas. Es handelt sich bei diesen Veränderungen um spontane Krankheiten, wie sie bei alten Ratten gehäuft vorkommen.

Abgesehen von diesen seltenen Störungen erwiesen sich Leber, Niere, Milz, Pankreas, Nebenniere, Lunge, Herz, Magen, Dünn- und Dickdarm, Hoden, Ovar,

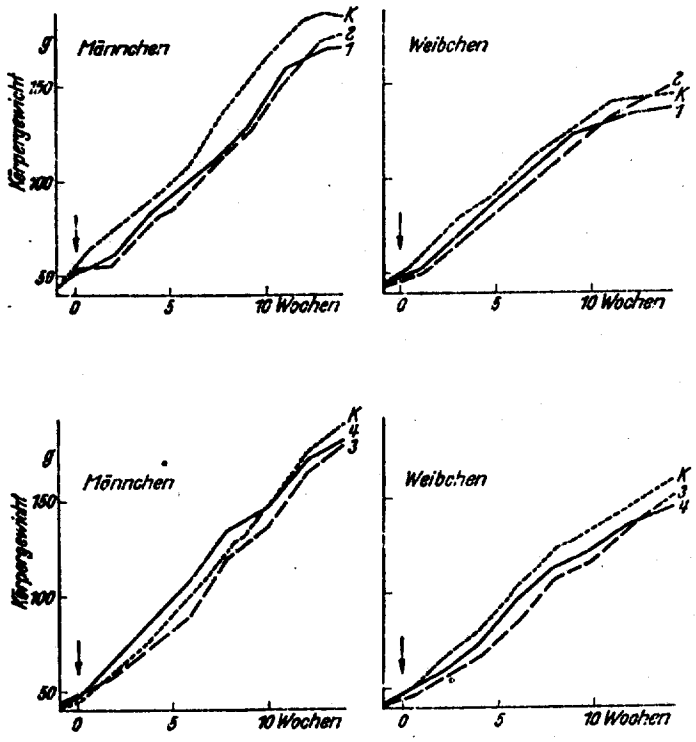


Abb. 1. *Einfluß von Carotinoiden (1000 mg/kg/Tag) auf das Körpergewicht von Ratten* (Durchschnitt von 10 Tieren). 1: β-Carotin, 2: Bixin, 3: Lycopin, 4: 7,7'-Dihydro-β-carotin, K: Kontrollen; ↓: Beginn der 14wöchigen Behandlung

Haut, Knochen und Knochenmark bei der histologischen Untersuchung als unversehrt. Irgendwelche Schädigungen im Sinne einer A-Hypervitaminose wurden nicht beobachtet. Auf die vermehrte Speicherung von Vitamin A wird bei Versuch 3 hingewiesen.

100 Tage-Fütterungsversuch mit den 4 Carotinoiden bei Ratten

Versuche 2, 9, 12 und 13. Diese Versuche können gemeinsam besprochen werden. Junge Ratten erhielten täglich während 100 Tagen 1000 mg/kg β-Carotin, bzw. Lycopin, bzw. 7,7'-Dihydro-β-carotin unter etwas Futter gemischt in einer Schale vorgesetzt. Die Präparate wurden zwar meist anstandslos gefressen, doch wirkte sich die Behandlung besonders in der 2. Versuchshälfte etwas auf den Appetit der Tiere aus, so daß vor allem die Männchen im Wachstum leicht hinter den unbehandelten Kontrollen zurückblieben (Abb. 1).

Abgesehen von einzelnen Spontantodesfällen in den ersten Versuchswochen (β-Carotin 4/20, Lycopin 2/20, Bixin 3/20, Kontrollen 1/40) gediehen alle Ratten sehr gut. Äußerliche Unverträglichkeitssymptome konnten nicht festgestellt werden. Weißes und rotes Blutbild (8. und 14. Woche) zeigten keine über die normalen

Schwankungsbreiten hinausgehenden Veränderungen (Tab. 4). Leber, Niere, Nebenniere, Milz, Pankreas, Hoden, Ovar, Haut, Knochen und Knochenmark waren histologisch intakt. Zeichen einer Speicherung der Farbstoffe konnten bei der mikroskopischen Untersuchung nicht nachgewiesen werden.

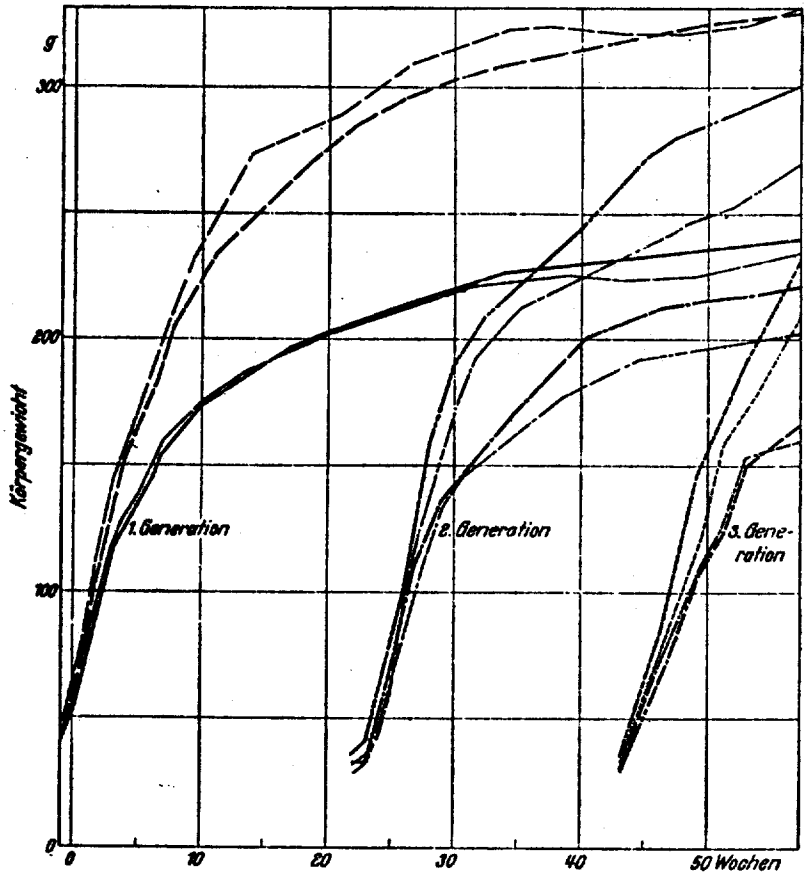


Abb. 2. *Einfluß von 0,1% β-Carotin im Futter auf das Körpergewicht von Ratten* (Durchschnitte von 19—30 Tieren). *1. Generation:* Männchen ——— mit β-Carotin - - - Kontrollen. Weibchen ——— mit β-Carotin - - - Kontrollen. *2. Generation:* Männchen —·— mit β-Carotin —··— Kontrollen. Weibchen —·— mit β-Carotin —·— Kontrollen. *3. Generation:* Männchen ═══ mit β-Carotin ····· Kontrollen. Weibchen ═══ mit β-Carotin ····· Kontrollen

Chronischer β-Carotin-Fütterungsversuch mit der 1. und 2. Rattengeneration

Versuch 3. Hier handelt es sich um einen chronischen Fütterungsversuch, bei welchem jungen Ratten 0,1% β-Carotin (1000 Teile/Million Teile Futter) unter die Diät gemischt wurde. Nach 112 Tagen wurden die Tiere gepaart, die Jungen 23—25 Tage nach der Geburt von ihren Müttern getrennt und ebenfalls auf β-carotin-haltiges Futter gesetzt. Die Paarung dieser 2. Rattengeneration erfolgte im Alter von durchschnittlich 130 Tagen. Je 4 Tiere der 1. und 2. Generation wurden nach 375 bzw. 230 Tagen getötet und histopathologisch untersucht.

In Tab. 5 sind die hauptsächlichsten Angaben über den Versuchsverlauf wiedergegeben. Sämtliche Tiere gediehen vorzüglich. Das Wachstum war regelmäßig (Abb. 2), Spontantodesfälle kamen nur vereinzelt und nur bei Kontrollgruppen vor, intercurrente Erkrankungen *(Otitis-Labyrinthitis)* waren ebenfalls sehr selten.

Der Futterkonsum war bei behandelten und unbehandelten Tieren praktisch gleich. Hämoglobin, Erythrocyten- und Leukocytenzahlen und Differentialblutbild zeigten ebenfalls keine Unterschiede (Tab. 4).

Tabelle 5. *Entwicklung von Ratten bei „synthetischer" Diät mit 0,1% β-Carotin während dreier Generationen (Versuch 3)*

Generation	Vers.-Dauer	Durchschnittlicher Futterkonsum je Tag				Durchschnittliches Körpergewicht je				Spontantodesfälle		Getötet wegen Krankheit	
		Männchen		Weibchen		Männchen		Weibchen					
	Tage	β-Car. g	Kontr. g	β-Car. g	Kontr. g	β-Car. g	Kontr. g	β-Car. g	Kontr. g	β-Car.	Kontrolle	β-Car.	Kontrolle
1.	400	11,6	11,6	10,3	9,5	330	332	240	235	0	2	1	1
2.	240—260	10,1	11,9	10,2	10,3	300	270	220	201	0	3	0	0
3.	70—105	12,1	11,9	9,1	9,1	230	208	166	160	0	0	0	0

Wie aus Abb. 2 hervorgeht, wachsen die mit β-Carotin behandelten Ratten der 2. Generation etwas rascher als die Kontrollen. Man darf jedoch aus diesem Befund nicht auf eine wachstumsfördernde Wirkung des β-Carotins schließen, da es sich bei der 2. Generation naturgemäß nicht um ein homogenes Tiermaterial handelt und da ein ähnlicher Effekt bei den Ratten der 1. Generation nicht festzustellen ist.

Über den Erfolg des Reproduktionsexperimentes mit der 1. und 2. Generation orientiert Tab. 6. Nach der Paarung der 1. Generation war bei den β-Carotin-Tieren und der Kontrollgruppe je 1 pathologischer Trächtigkeitsverlauf (1 Abort, 1 Resorption) festzustellen. Zahl und Gewicht der Jungtiere waren nicht signifikant verschieden. Nach der Paarung der 2. Generation warf ein β-Carotin-Tier nur 1 Junges. Von der entsprechenden Kontrollgruppe warf ein Weibchen ebenfalls nur ein Junges, ein 2. Muttertier abortierte. Zahl und Durchschnittsgewicht der Jungen sind auch in dieser Generation praktisch identisch, doch fällt auf, daß die Weibchen der 2. Generation durchschnittlich weniger Junge warfen als ihre Mütter, was wohl auf jahreszeitliche Unterschiede (1. Generation im Frühling, 2. Generation im Herbst gepaart) zurückzuführen ist. Die Tragzeit war lediglich bei einem Kontrolltier der 1. Generation mit 20 Tagen leicht verkürzt, sonst durchwegs normal. Mißbildungen wurden nur bei einem Kontrolltier der 3. Generation (abnorme Stellung einer Hinterpfote) beobachtet.

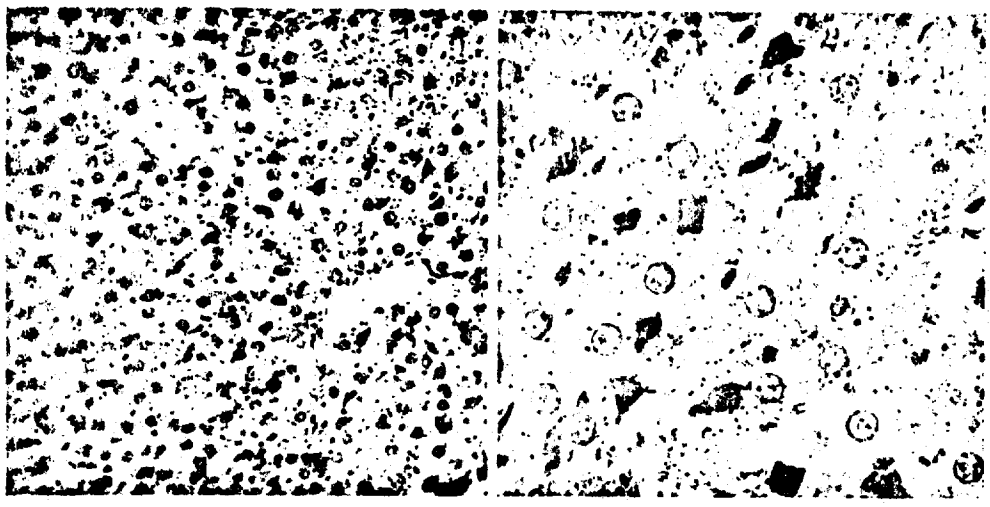
Tabelle 6. *Reproduktionsversuche mit Ratten bei „synthetischer" Diät mit 0,1% β-Carotin und Lycopin (Versuch 3 und 10)*

Carotinoid	Generation	Zahl gepaarter Weibchen	Zahl besamter Weibchen	Zahl der Weibchen mit Partus	Tragzeit Tage	Zahl der Jungen	Gewicht der Jungen g	Abort	Resorption
β-Carotin	1.	15	11	10	22 (22—23)[1]	8,6 ± 2[2]	4,7 ± 0,6[2]	1	—
Kontrolle	1.	15	12	11	22 (20—23)	9,2 ± 3	4,5 ± 0,6	—	1
β-Carotin	2.	5	5	5	22 (21—23)	6,3 ± 3	4,9 ± 0,5	—	—
Kontrolle	2.	13	13	12	22 (21—23)	6,1 ± 2	5,0 ± 0,5	1	—
Lycopin	1.	15	10	9	22 (21—23)	5,5 ± 2	5,7 ± 0,9	1	—
Kontrolle	1.	15	12	10	22 (21—23)	7,5 ± 2	4,9 ± 0,7	2	—

[1] Extremwerte.

[2] $SD = \sqrt{\frac{S(x-\bar{x})^2}{n-1}}$

Histopathologische Untersuchungen. Es ergaben sich bei je 4 mit β-Carotin behandelten Ratten der 1. und 2. Generation und von je 4 Tieren aus den entsprechenden Kontrollgruppen keine pathologischen Veränderungen von Leber, Niere, Nebenniere, Lunge, Herz, Milz, Pankreas, Hoden, Nebenhoden, Ovar, Dünn- und Dickdarm, Magen, Haut, Knochen und Knochenmark. In der Leber der β-Carotin-Tiere waren die Kupfferschen Sternzellen mit reichlich Fett beladen (Abb. 3) und zeigten im UV-Licht eine intensive gelb-grüne Fluorescenz, die im Lauf von 20 min völlig abblaßte. Es handelte sich somit wohl um gespeichertes Vitamin A.



a b

Abb. 3a u. b. *Einfluß von 0,1% β-Carotin im Futter auf die Histologie der Rattenleber nach 375 Tagen Behandlung* (gefärbt mit Fettrot 7 B-Hämalaun. Vergr. 200mal). (a) Starke Verfettung der Kupfferschen Sternzellen als Ausdruck der Vitamin A-Speicherung. Daneben spärlich feintropfige Verfettung der Leberzellen. (b) Gleiches Tier (Vergr. 500mal). Selektive Darstellung der verfetteten, Vitamin A speichernden Kupfferschen Sternzellen

Chemische Untersuchung. Leber und Niere von je einer β-Carotin- und Kontrollratte der 1. Generation wurden nach 375tägiger Versuchsdauer aufgearbeitet. Dabei wurde folgendes festgestellt:

Organ	Vitamin A beim β-Carotin-Tier	β-Carotin beim β-Carotin-Tier	Vitamin A beim Kontrolltier	β-Carotin beim Kontrolltier
	I.E./g	µg/g	I.E./g	µg/g
Leber	7700	2,9	0	0
Niere	55	0	0	0

Die Untersuchung bestätigt die beträchtliche, wenn auch nicht excessive Vermehrung des Leber-Vitamin A (Normalwert um 280 I.E./g[1]) beim β-Carotin-Tier. Der Gehalt an Vitamin A in der Niere ist leicht erhöht (normal um 17 I.E./g[1]). Die Carotinspeicherung in der Leber ist gering. Beim Kontrolltier wurde kein Vitamin A in Leber und Niere gefunden, was darauf hinweist, daß die verabreichte Vitamin A-Zulage zwar ein normales Wachstum, jedoch keine Leberspeicherung ermöglichte.

Parenterale Carotinoid-Zufuhr bei Ratten

Versuch 4 und 5. Bei parenteraler Verabreichung ist der Stoffwechsel der Carotinoide anders als bei der Aufnahme der Pigmente aus dem Darm, da hierbei die

[1] EDEN, E., u. T. MOORE: Biochemic. J. 49, 77 (1951).

Darmwand, in welcher bei vielen Tieren die Vitamin A-Bildung erfolgt, umgangen wird. Es sollte deshalb geprüft werden, ob die intramuskuläre Injektion großer Dosen von β-Carotin irgendwelche Schädigungen verursacht.

Die einmalige intramuskuläre Verabreichung von 1 g β-Carotin pro Tier in Öl an 145—165 g schwere Ratten führte nicht zu toxischen Symptomen. Bei den vom 3. bis zum 48. Versuchstag vorgenommenen Sektionen der Tiere zeigten die Organe keine Veränderungen. Mikroskopisch fand sich im Bereich der Injektionsstellen eine proliferative Entzündung mit einigen an β-Carotin-Kristalle gelagerten Fremdkörperriesenzellen (Versuch 4).

Auch die fortgesetzte intramuskuläre Injektion öliger β-Carotin-Lösungen (2 mal täglich 4,5 mg pro Ratte an 5 Tagen der Woche) wurde von 150—190 g schweren Ratten während 17 Wochen erstaunlich gut vertragen. Die Behandlung mußte zwar von Zeit zu Zeit wegen lokaler Reizerscheinungen im Injektionsgebiet für einige Tage unterbrochen werden, doch zeigten die Tiere normales Wachstum und guten Allgemeinzustand. Eine von 8 Ratten starb vorzeitig. Bei der Sektion fanden sich an den Injektionsstellen bei allen Tieren große öl- und β-carotin-haltige Cysten. Auch das Fettgewebe des großen Netzes und des Retroperitoneums war herdweise intensiv gelb gefärbt, so daß anzunehmen ist, daß hier auf dem Lymphweg abgeführtes β-Carotin gespeichert wurde. Lungen, Leber, Milz, Haut, Knochen und Knochenmark erwiesen sich bei der histopathologischen Untersuchung als intakt. Zeichen einer A-Hypervitaminose fehlten. Die Kupfferschen Sternzellen der Leber waren verfettet (Speicherung von Vitamin A).

In den Nieren waren die Hauptstückepithelien oft etwas trübe geschwollen, die Epithelien der gestreckten Tubuli besonders am Mark-Rindenübergang teilweise in Desquamation begriffen. Es handelt sich hierbei um sekundäre Erscheinungen im Gefolge der chronischen Entzündung mit Gewebszerfall am Injektionsort.

Histopathologische Untersuchung. Bei den Injektionsstellen ergab sich eine ausgeprägte produktive Entzündung. Das Granulationsgewebe aus Fibroblasten, spärlichen Capillaren, Makrophagen und vereinzelten Fremdkörperriesenzellen war teilweise nekrotisch und von vielen neutrophilen Leukocyten und Lymphocyten durchsetzt. Bei den mit analogen Dosen Vitamin A behandelten Kontrollratten waren die lokalen Reaktionen deutlich weniger stark ausgebildet. Entsprechend zeigten auch die Nieren nur geringe Reaktionen in Form einer vermehrten Desquamation von Tubulusepithelien am Mark-Rindenübergang (Versuch 5).

β-Carotin-Fütterungsversuche mit Kaninchen

Versuch 6 und 7. Am Kaninchen wurde versucht, durch massive orale Überbelastung mit β-Carotin toxische Veränderungen zu erzeugen. Trotzdem die Tiere während 11 Wochen 5mal wöchentlich 500 mg/kg β-Carotin erhielten, zeigten sich keine wesentlichen Schädigungen des Allgemeinzustandes. 2 Kaninchen nahmen um 140 bzw. 160 g ab, 3 Tiere um 70—400 g zu. Die hämatologischen Werte (4. und 11. Woche) hielten sich in den normalen Schwankungsbreiten (Tab. 4). 4 Tiere wurden nach 11wöchiger Versuchsdauer seziert, wobei keine pathologischen Organveränderungen festzustellen waren. Leber, Niere, Ovar, Hoden, Milz, Pankreas, Haut und Knochenmark waren bei der histopathologischen Untersuchung intakt (Versuch 6).

Bei einem Kaninchen wurde die Behandlung mit 500 mg/kg β-Carotin (je 5mal pro Woche) auf 2 Jahre ausgedehnt. Das Tier, das insgesamt etwa 800 g β-Carotin erhielt, gedieh vorzüglich und nahm während der Behandlung um 420 g an Gewicht zu. Der Blutstatus blieb normal (Tab. 4). Bei der Sektion waren die Organe unverändert.

Die Leber enthielt 446 I. E. Vitamin A/g, was als normal zu bezeichnen ist. β-Carotin konnte in der Leber nicht nachgewiesen werden. Die histopathologische Untersuchung von Herz, Lungen, Leber, Nieren, Nebennieren, Milz, Pankreas, Haut, Magen, Dünn- und Dickdarm, Knochen, Knorpel (Rippe) und Knochenmark ergab durchwegs normale Befunde (Versuch 7).

β-Carotin-Fütterungsversuche mit Hunden

Versuch 8. Da der Hund bezüglich Carotinoid-Resorption und -Verwertung dem Menschen näher steht als Ratte und Kaninchen[1], wurden zusätzlich 2 Hunde mit täglich 100 mg/kg β-Carotin (in Gelatinekapseln) per os behandelt. Die wichtigsten Daten aus diesem Versuch sind in Tab. 7, die hämatologischen Untersuchungen in Tab. 4 zusammengefaßt. Es geht aus diesen Resultaten hervor, daß die fortgesetzte orale Überbelastung mit β-Carotin ohne klinisch nachweisbare Störungen von Hunden vertragen wird. Von Interesse ist die bei beiden Tieren gefundene Speicherung von Vitamin A in Leber (Normalwert 30—100 I. E./g[1, 2]) und Niere (Normalwert um 40 I. E./g), während der β-Carotin-Gehalt nicht oder nur unwesentlich erhöht ist. Bei der histopathologischen Untersuchung zeigten denn auch Leber und Nieren gewisse Veränderungen, die möglicherweise als Folgen einer vermehrten Vitamin A-Speicherung aufzufassen sind:

Tabelle 7. *Ergebnisse von mit β-Carotin behandelten Hunden (100 mg/kg β-Carotin in Gelatinekapseln pro Tag per os. Versuch 8)*

Feststellung	Hund Nr. 1	Hund Nr. 2
Behandlungszeit . . . . . Tage	104	200
Total verabreichte Menge β-Carotin . . . g	149	221
Körpergewicht bei Beginn des Versuches kg	14	12
Körpergewicht bei Schluß des Versuches kg	15	11
Allgemeinzustand	sehr gut	sehr gut
Blutstaten (vgl. Tab. 4).	Vorübergehende Leukocytose mit relat. Lymphopenie	Vorbestehende Linksverschiebung normalisiert sich im Laufe des Versuches
Laboratoriumsuntersuchungen bei Versuchsabschluß		
Takata-Reaktion	negativ	negativ
Serumbilirubin . . . . . mg/100 ml	0,33	0,21
Bromsulphaleinprobe	negativ	negativ
Rest-N . . . . . mg/100 ml	35	38
Blutsenkungsreaktion (1 u. 2 Std) . . . mm	1/2/1	1/2/1
Urin (Zucker, Eiweiß, Urobilinogen, Sediment)	normal	normal
Sektionsbefund	Organe o. B., Ascaridiasis	Organe o. B.
Vitamin A-Gehalt der Leber . . . . I. E./g	2025	5062
β-Carotin-Gehalt der Leber . . . . . μg/g	5	8
Vitamin A-Gehalt der Nieren . . . . I. E./g	—	419
β-Carotin-Gehalt der Nieren . . . . μg/g	—	0

Die Leberzellen sind etwas vergrößert, das Protoplasma ist sehr hell und deutlich gekörnt, bei Hund Nr. 1 frei von färbbarem Fett, bei Hund Nr. 2 diffus feintropfig verfettet. Die Kupfferschen Sternzellen sind etwas vermehrt, teilweise leicht geschwollen und mit reichlich Fett beladen:

[1] Kowalewski, K., E. Henrotin u. J. Van Geertruyden: Acta gastroent. belg. 14, 7 (1951).

[2] Chevallier, A.: Ann. Physiol. 14, 109 (1938).

Capillaren eng. Glissonsche Scheiden zart. In der Niere finden sich im Bereiche der *Tubuli recti* einige Kernpyknosen der Epithelien und eine etwas verstärkte Epitheldesquamation.

Die übrigen histologisch untersuchten Organe wie Milz, Pankreas, Haut, Knochenmark, Magen, Dick- und Dünndarm, Nebenniere, Hypophyse, Groß- und Kleinhirn und Stammganglien waren unverändert. In den Ovarien fanden sich zahlreiche Primordialfollikel, reifende Follikel und in Regression befindliche *Corpora lutea*.

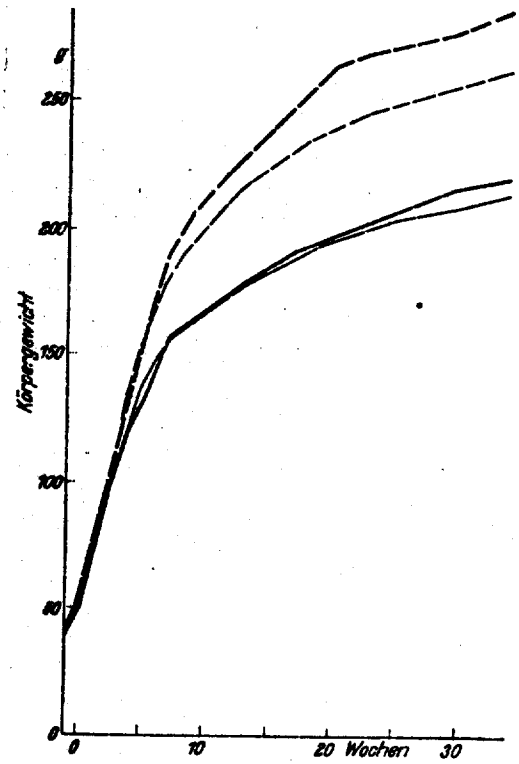


Abb. 4. *Einfluß von 0,1% Lycopin im Futter auf das Körpergewicht von Ratten* (Durchschnitte von 30—38 Tieren). Männchen: — — — mit Lycopin, ——— Kontrollen; Weibchen: ——— mit Lycopin, ——— Kontrollen

Chronischer Lycopin-Fütterungsversuch mit Ratten

Versuch 10. Ein chronischer Fütterungsversuch an Ratten wurde auch mit Lycopin (0,1% Diätzusatz = 1000 Teile/Million Teile Futter) durchgeführt und auf die 2. Generation ausgedehnt. Der Versuchsverlauf ergibt sich aus Tab. 8 und den Gewichtskurven (Abb. 4). Alle mit Lycopin behandelten Ratten entwickelten sich gut, Spontantodesfälle traten nicht auf.

Während die Weibchen der 1. Generation praktisch gleich schnell wuchsen wie die Kontrollen, war die Gewichtszunahme der männlichen Tiere im Vergleich zur entsprechenden Kontrollgruppe deutlich besser (Abb. 4). Beim Vergleich der Gewichtszunahme der einzelnen Tiere ist dieser Unterschied auch statistisch signifikant ($p < 0.01$ berechnet nach der t-Verteilung von STUDENT). Für die Bewertung dieses Befundes sei auf die Diskussion der Ergebnisse verwiesen.

Die hämatologischen Befunde zeigten keine Besonderheiten (Tab. 4). Auch beim Reproduktionsexperiment mit der 1. Rattengeneration zeigten sich keine wesentlichen Unterschiede zwischen behandelten und unbehandelten Tieren (Ergebnisse in Tab. 6). Von den mit Lycopin behandelten Weibchen verwarf eines; die Zahl der Jungen war etwas, jedoch nicht signifikant, geringer als bei den Kontrollen, von denen 2 Tiere abortierten. Die Tragzeit war durchwegs normal, Mißbildungen wurden nicht festgestellt.

Tabelle 8. *Entwicklung von Ratten bei „synthetischer" Diät mit 0,1% Lycopin (Versuch 10)*

Generation	Versuchsdauer	Durchschn. Futterkonsum je Tag				Durchschn. Körpergewicht je				Spontantodesfälle		Getötet wegen Krankheit	
		Männchen		Weibchen		Männchen		Weibchen					
	Tage	Lycopin	Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle	Lycopin	Kontrolle
1.	200	11,2	10,5	10,3	9,9	286	263	221	213	0	0	0	0
2.	50—80	11,5	8,6	8,8	9,4	199[1]	187[1]	153[1]	148[1]	0	1	0	0

[1] Nur mindestens 60 Tage alte Tiere berücksichtigt.

Histopathologische Untersuchung. Nach 200 Versuchstagen wurden 4 behandelte und 4 Kontrolltiere der 1. Generation getötet. Von den Lycopin-Tieren zeigen die Weibchen in den Leberzellen eine deutliche, die Männchen nur eine schwache Speicherung eines braunen, ziemlich grobkörnigen Pigmentes (Abb. 5), welches bei Paraffineinbettung und Entparaffinierung völlig weggelöst wird. Es handelt sich hierbei zweifellos um Lycopin. Daneben ist die Leberstruktur sehr gut erhalten. Die normal großen Leberzellen zeigen ein recht helles, körniges Protoplasma, die Capillaren sind mittelweit, die Kupfferschen Sternzellen weder vermehrt noch geschwollen oder verfettet. Glissonsche Scheiden zart.

Die übrigen histologisch untersuchten Organe, wie Niere, Nebenniere, Milz, Pankreas, Hoden, Nebenhoden, Ovar, Lunge, Herz, Haut, Knochen und Knochenmark zeigten keine von den Kontrolltieren abweichende Befunde. Eine Farbstoffspeicherung konnte mikroskopisch außerhalb der Leber nicht nachgewiesen werden.

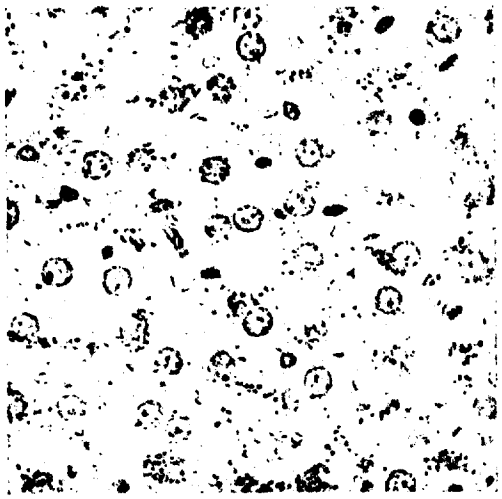


Abb. 5. *Einfluß von 0,1% Lycopin in Futter auf die Histologie der Rattenleber nach 200 Tagen Behandlung* (gefärbt mit Fettrot 7 B-Hämalaun. Vergr. 500 mal). Speicherung von Lycopin in den Leberzellen. Keine Verfettung der Kupfferschen Sternzellen

Lycopin-Fütterungsversuch mit einem Hund

Versuch 11. Ein Hund wurde täglich mit 100 mg/kg Lycopin (in Gelatinekapseln) per os behandelt. In 192 Dosen erhielt das Tier insgesamt 272 g Lycopin, ohne dabei irgendwelche Unverträglichkeitssymptome zu zeigen. Das Körpergewicht stieg von 13 auf 15 kg, der Blutstatus war bei 4maliger Kontrolle stets normal (Tab. 4). Bei Versuchsabschluß ergaben sich folgende Laboratoriumsbefunde: Takata-Reaktion negativ, Serumbilirubin 0,1 mg/100 ml, Bromsulphaleinprobe negativ, Rest-N 35 mg/100 ml, Blutsenkungsreaktion nach 1 und 2 Std $^1/_2$ bzw. 1 mm, Urin frei von Zucker und Eiweiß, Urobilinogen normal, Sediment ohne Befund.

Bei der Sektion waren die Organe makroskopisch unverändert. Die chemische Untersuchung ergab (s. nebenstehende Tabelle):

Carotinoid		Leber	Niere
Vitamin A	I.E./g	75	57
Lycopin	µg/g	22,6	0,6
Carotin	µg/g	0	6

Es kam somit zu einer recht deutlichen Speicherung von Lycopin in der Leber. Das Pigment war auch histologisch in den Leberzellen nachzuweisen und von dem beim Hund häufig vorhandenen Lipofuscin durch das Verschwinden nach Paraffineinbettung leicht zu unterscheiden. Im übrigen zeigte die Leber mikroskopisch einen völlig regelmäßigen Bau. Auch Niere, Nebenniere, Herz, Lunge, Milz, Knochenmark, Pankreas, Groß- und Kleinhirn, Stammganglien, Hypophyse, Ovar, Haut, Magen, Dünn- und Dickdarm waren intakt.

4. Besprechung der Ergebnisse

Wie in der Einleitung erwähnt, liegen über Carotinoide noch keine eigentlichen Verträglichkeitsuntersuchungen vor. Die Natur allerdings demonstriert uns täglich ein Großexperiment, da ja in pflanzlicher Nahrung von Mensch und Tier Riesenmengen von Carotinoiden aufgenommen werden.

Es sei hier nur auf die Milchkuh hingewiesen, deren lebenslänglicher Carotinämie wir nicht nur die gelbe Farbe der Butter, sondern auch einen guten Teil unserer Vitamin A-Versorgung verdanken. Über nicht ganz so natürliche „Verträglichkeitsexperimente" mit Carotin am Menschen ist in der Literatur mehrfach berichtet: es handelt sich um Leute, die durch mehrmonatige Einnahme von täglich mehreren Pfund roher Karotten eine Carotinämie mit Gelbfärbung der Haut erwarben ([1, 2] u. a), diese sogar mit der Muttermilch auf das eigene Kind übertrugen[2], ohne dabei irgendwelchen Schaden zu nehmen.

Trotz dieser natürlichen Beweise für die Unschädlichkeit der Carotinoide erscheinen Verträglichkeitsversuche gerechtfertigt, liegen doch die Verhältnisse bei Zusatz der reinen Carotinoide zu Lebensmitteln, vor allem bezüglich der Resorption wesentlich anders, als bei der Aufnahme in pflanzlichen Produkten, wo die Pigmente meistens in Zellen eingeschlossen und der Resorption nur teilweise zugänglich sind.

Über Resorption und Stoffwechsel der Carotinoide sind in den letzten Jahren viele neue Tatsachen bekannt geworden. Noch sind zwar längst nicht alle diesbezüglichen Probleme gelöst, doch scheint im Zusammenhang mit der Frage der Toxikologie eine Zusammenfassung der heutigen Kenntnisse über Verwertung, Um- und Abbau der Carotinoide im menschlichen und tierischen Organismus notwendig.

Es gilt seit langem als wichtige Tatsache, daß Resorption, Speicherung und Stoffwechsel der Carotinoide beim Menschen und verschiedenen Tierarten wesentliche Unterschiede aufweisen[3]. So können die Säuger bezüglich ihrer Speicherungsfähigkeit von Carotin (α, β, γ) und Xanthophyllen (Oxycarotinoide, Carotinole) in 3 Gruppen eingeteilt werden [3–5]:

Gruppe	Speichern hauptsächlich		Beispiel
	Carotine	Xanthophylle	
A	+	+	Mensch
B	+	0	Pferd
C	0	0	Schwein
D	0	+	Huhn

Als 4. Gruppe sind die Hühner zu erwähnen, welche hauptsächlich Xanthophylle speichern[3, 4, 6]. Aus dieser Verschiedenheit in der Ablagerung von Carotinoiden wurde auf einen unterschiedlichen Stoffwechsel der Pigmente geschlossen und vermutet, daß die (hypothetischen) Fermentsysteme (Carotinase, Xanthophyll-Oxydase) bei den verschiedenen Tierarten nicht gleichmäßig ausgebildet seien[6, 7]. Andere Autoren erwägen Unterschiede in der Ausbildung spezifischer Eiweißreceptoren in Blut und Geweben[4].

Nähere Aufschlüsse über den Stoffwechsel der Carotinoide gaben die Ergebnisse zahlreicher, mit anspruchsvoller Technik durchgeführter Experimente der letzten Jahre. Am besten ist man wohl heute über den Abbauweg der Carotine an der *Ratte* unterrichtet, weshalb auf dieses Beispiel etwas näher eingegangen wird.

Aus der schematischen Darstellung über das Schicksal des mit der Nahrung aufgenommenen Carotins (Abb. 6) geht hervor, daß bei der Ratte ein großer Teil des Farbstoffes den Darm unverändert passiert und mit den Faeces ausgeschieden wird. Der Prozentsatz des im Kot vorhandenen Carotins ist sehr variabel und stark abhängig von sekundären Faktoren. Er dürfte, wie Untersuchungen mit ^{14}C-markiertem Carotin ergaben[8] unter physiologischen Bedingungen etwa 70% betragen. Die Resorption der Pigmente ist im allgemeinen aus pflanzlichem Material schlecht, aus öligen Lösungen besser und am besten aus wäßrigen Emulsionen[9]. Dasselbe trifft auch für den Menschen zu, bei welchem je nach Art und Form der Applikation 30–99% der per os

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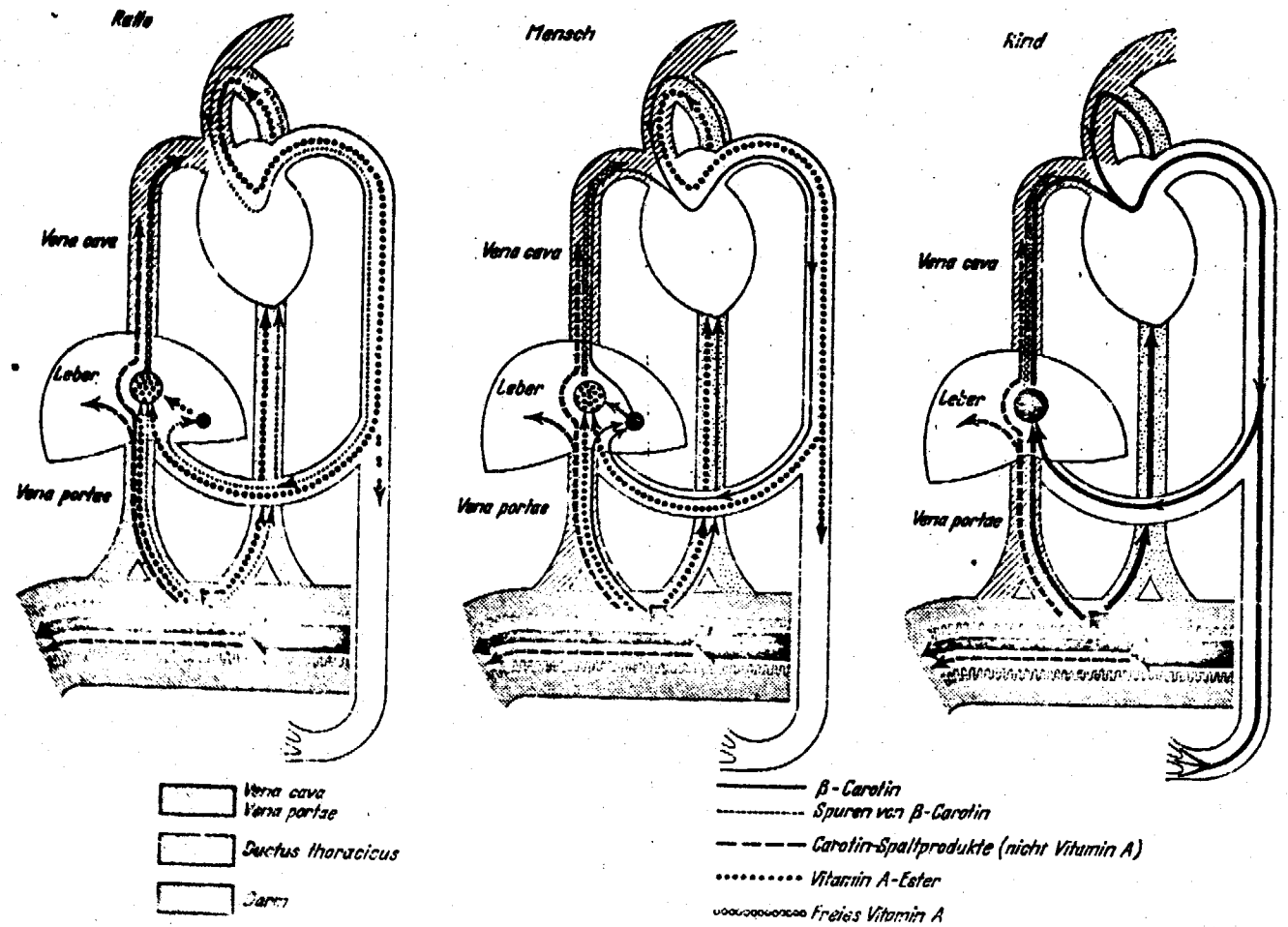

Abb. 6. *Schematischer Vergleich des β-Carotin- und Vitamin A-Stoffwechsels bei Mensch, Ratte und Rind*

aufgenommenen Carotinoide im Stuhl wiedergefunden werden[1]. Hemmend auf die Resorption wirken Mangel an Galle[2], geringer Eiweißgehalt der Nahrung[3] und Fütterung nicht resorbierbarer Mineralöle[3], fördernd gleichzeitig orale Verabreichung von Aureomycin[4], Vitamin B_{12}[5] und Chloromycetin[4,6]. Auch der Vitamin C-Gehalt des Futters und die Art der Vitamin B-Darreichung beeinflussen die Carotin-Verwertung[7].

Ein Teil der Carotine wird beim Kontakt mit der Magensäure oder im Darm durch Autooxydation und bakterielle Einflüsse zerstört, ein Umstand, der bei der Bewertung von Bilanzuntersuchungen nicht vergessen werden darf[6,8], doch zeigten verschiedene Versuche, bei denen Carotin mit Faeces oder im excidierten Gastrointestinaltrakt inkubiert wurde, daß der Anteil des im Darm zerstörten Carotins häufig zahlenmäßig nicht wesentlich ins Gewicht fällt[9].

In der Darmwand der Ratte wird der weitaus größte Teil des resorbierten Carotins gespalten[10], doch ist über den dabei beteiligten, wohl durch Fermente gesteuerten Prozeß noch wenig bekannt[11]. Das wichtigste Spaltprodukt ist das Vitamin A, welches in veresterter Form über den Lymph- und wohl auch den Pfortaderweg in die Leber gelangt, hier gespeichert wird und bei Bedarf als freies Vitamin A (Vitamin A-Alkohol) wieder ins Blut abgegeben wird. Nur ein verschwindend kleiner Teil des Carotins passiert die Darmwand unverändert und gelangt wohl auf dem Lymphweg in die Leber[12]. Der zu Vitamin A gespaltene Anteil des Carotins ist *stoffwechselmäßig* zwar der wichtigste, quantitativ jedoch nur unbedeutend. So läßt sich beispielsweise nur 1,5% der resorbierten Aktivität von oral verabreichtem, ^{14}C-markiertem Carotin im Leber-Vitamin A nachweisen, während 5% im CO_2 der Ausatmungsluft, 15% in Fettsäuren und etwa 40% im carotinoid-freien unverseifbaren Anteil des Gesamttieres vorhanden sind[13, 14]. Schon wegen dieser geringen Umwandlung des Carotins zu Vitamin A erscheint es kaum möglich, bei der Ratte durch Überbelastung mit β-Carotin eine A-Hypervitaminose zu erzeugen. Eine weitere für die chronischen Verträglichkeitsuntersuchungen wichtige Tatsache besteht darin, daß die Resorption der Carotine und ihre Spaltung zu Vitamin A in starkem Maß von der Sättigung des Organismus mit Vitamin A[14] und auch von der Größe des Carotin-Angebots abhängig sind. Das mit Vitamin A gesättigte Tier verwertet einen viel kleineren Teil des zur Verfügung stehenden Carotins als ein Vitamin A-Mangeltier, und bei oraler Überbelastung mit Carotin nimmt der Prozentsatz des resorbierten Pigments stark ab. Man könnte hier von einem „natürlichen Schutz" gegen die Entstehung einer A-Hypervitaminose sprechen, ohne den die gute chronische Verträglichkeit des β-Carotins gar nicht denkbar wäre.

Um das Bild des Carotinstoffwechsels der Ratte abzurunden, müssen noch einige neuere Untersuchungen erwähnt werden, bei welchen das Schicksal des *parenteral* in den Organismus gelangenden Farbstoffs verfolgt wurde. Intravenös oder in die Milz injiziertes Carotin wird im reticuloendothelialen System hauptsächlich der Leber und der Lunge gespeichert und dort so festgehalten, daß ein Vitamin A-Mangeltier trotz einer Carotinreserve, die bei oraler Verabreichung

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[13] KRAUSE, R. F., M. O. COOVER u. L. T. POWELL: Zit. S. 128, Anm. 8.

[14] KRAUSE, R. F., u. P. L. SANDERS: Zit S. 128, Anm. 8.

für 1 Jahr genügen würde, an A-Avitaminose zugrunde geht[1]. Neuere Untersuchungen, bei denen Carotin in feinemulgierter Form intravenös appliziert wurde, haben nun aber ergeben, daß bei der Ratte auch außerhalb des Darms aus Carotinen Vitamin A gebildet werden kann und das nicht nur in der Leber, sondern auch in anderen Geweben[2]. Durch diesen wichtigen Befund ergeben sich wenigstens gewisse Beziehungen zum Carotinoidstoffwechsel des Menschen, und die eingangs erwähnten Speciesunterschiede bei der Speicherung der Carotinoide scheinen somit eher quantitativer als prinzipieller Natur. Beim Menschen sind die Stoffwechselverhältnisse des Carotins weniger bekannt. Man ist hier in der Hauptsache auf die Bestimmung der Blutwerte[4] und der Carotinausscheidung im Stuhl angewiesen. Auf Grund solcher Untersuchungen und der Carotin-Speicherung in den Organen läßt sich ein Schema des Carotinstoffwechsels beim Menschen aufstellen (Abb. 6). Der Unterschied gegenüber der Ratte besteht darin, daß beim Menschen und in ähnlicher Weise auch beim Hund Carotin in recht beträchtlichen Mengen unverändert die Darmwand passiert, hauptsächlich auf dem Pfortaderwege und nur zu einem geringen Teil *via Ductus thoracicus* in den Kreislauf und die Leber gelangt, hier teilweise gespeichert und teilweise zu Vitamin A gespalten wird[5].

Bei sehr großem Carotinangebot kommt es zu einer Erhöhung des Serumcarotins und zu einer vermehrten Ablagerung des Farbstoffes in den Organen u. a. auch in der Haut[6].

Schaf und Ziege verhalten sich bezüglich der Carotinverwertung ähnlich wie die Ratte, d. h. sie bilden ihr Vitamin A hauptsächlich in der Darmwand, speichern unverändertes Carotin nur in Spuren[7], sind jedoch unter besonderen Bedingungen (parenterale Verabreichung) trotzdem in der Lage, auch außerhalb des Darmes aus Carotin Vitamin A zu bilden[8]. Dasselbe gilt für das Kaninchen[9] und das Meerschweinchen[10].

Beim Rind unterscheidet sich der Carotinstoffwechsel in wesentlichen Punkten von demjenigen des Menschen und der Ratte (Schema in Abb. 6). Ein großer Teil der resorbierten Carotine passiert die Darmwand unverändert, gelangt ins Blut und wird von hier teilweise auch in die Milch abgegeben. Reichlich Carotine werden auch in den Geweben gespeichert[7]. Eine Umwandlung zu Vitamin A findet nur in geringem Maß statt. Der hauptsächlichste Sitz der Carotinspaltung ist die Leber. Intravenös injiziert, wird Carotin nicht, wie bei anderen Tieren, gespeichert oder sofort gespalten, sondern bleibt lange im Blut nachweisbar[11].

Für die Beurteilung der chronischen Verträglichkeitsversuche mit β-Carotin sind aus dieser vergleichenden Darstellung des Carotinstoffwechsels folgende Punkte von Wichtigkeit:

a) Unter besonderen Bedingungen können auch Ratte, Kaninchen und andere Tiere, die normalerweise Carotin in der Darmwand spalten, in der Leber und in anderen Organen Vitamin A bilden. Somit bestehen gegenüber dem Menschen, der einen Teil des Carotins außerhalb des Darmes verwendet, bezüglich Carotinverwertung keine prinzipiellen Unterschiede.

b) Der Hund hat wahrscheinlich einen ähnlichen Carotinstoffwechsel wie der Mensch und eignet sich deshalb als Versuchstier besonders gut.

c) Das der Nahrung zugesetzte Carotin wird in beträchtlicher Menge resorbiert. Wenn es trotzdem nicht möglich ist, durch Verabreichung von Carotin eine A-Hypervitaminose zu erzeugen, so liegt dies darin, daß die schon normalerweise sehr kleine Umwandlungsquote von Carotin zu Vitamin A bei Sättigung des Organismus noch weiter verkleinert wird.

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[10] Woytkiw, L., u. N. C. Esselbaugh: J. Nutrit. 43, 451 (1951).

[11] Kon, S. K., W. A. McGilivray u. S. Y. Thompson: Zit. S. 115, Anm. 1

d) Speicherung von Carotin in Zellen verschiedener Organe ist ein natürlicher und reversibler Prozeß.

e) Ein großer Teil des resorbierten Carotins gelangt in den allgemeinen Stoffwechsel und wird zu bisher größtenteils noch nicht identifizierten Produkten ab- und umgebaut. Die Bearbeitung des Carotinstoffwechsels hat sich auch mit diesen Metaboliten zu beschäftigen und darf sich nicht auf Carotinspeicherung und Vitamin A-Bildung beschränken.

Aus den vorliegenden Untersuchungen mit β-Carotin geht hervor, daß selbst bei langdauernder Verabreichung des Farbstoffes keine klinisch oder pathologisch-anatomisch nachweisbaren Schädigungen, noch sichere Zeichen einer A-Hypervitaminose erzeugt wurden. Es ist jedoch noch festzustellen, ob bei der Dosierung die für die Prüfung von Lebensmittelzusätzen zu fordernden Sicherheitsmargen eingehalten wurden. Dies läßt sich anhand einiger schematischer Beispiele und Vergleiche durchführen.

Ein mit Löwenzahnblättern ernährtes Kaninchen nimmt pro Tag etwa 10 mg Carotin pro kg Körpergewicht auf. Die Kaninchen der Versuche 6 und 7 erhielten täglich 500 mg/kg β-Carotin. Dies entspricht ungefähr der 50fachen Tagesdosis.

Zur Färbung von Margarine werden 2—4 g β-Carotin pro Tonne verwendet. Hund Nr. 2, der in 200 Tagen total 221 g β-Carotin gefressen hat, nahm somit die gleiche β-Carotin-Menge auf, wie sie zur Färbung von 55000—110000 kg Margarine benötigt wird. Wenn als „normale Tagesportion" 50—100 g Margarine pro Hund angenommen wird, erfolgte die Verträglichkeitsprüfung mit einer 5000—10000fachen Überdosierung.

Ratten von durchschnittlich 200 g Gewicht nahmen bei 0,1%igem β-Carotin-Futterzusatz täglich etwa 50 mg/kg des Farbstoffes auf. Umgerechnet auf das Körpergewicht entspricht dies für einen 60 kg schweren Menschen einer täglichen β-Carotin-Aufnahme von 3 g, d. h. wiederum so viel, wie für die Färbung einer Tonne Margarine verwendet wird. Auch hier ist eine etwa 10000fache Überdosierung anzunehmen.

Aus diesen Beispielen geht hervor, daß die Versuche mit sehr großen Dosen β-Carotin durchgeführt wurden und daß dabei die für Toxicitätsuntersuchungen üblichen Sicherheitsmargen weit überschritten wurden.

Der einzige, mit Sicherheit auf die chronische Überbelastung mit β-Carotin zurückzuführende Befund wurde an Ratten und Hunden bei der histologischen Untersuchung der Leber erhoben. Bei beiden Tierarten war hier eine starke Verfettung der Kupfferschen Sternzellen mit intensiver Fluorescenz im UV-Licht festzustellen. Diese Veränderungen sind Folgen einer auch chemisch nachgewiesenen Speicherung von Vitamin A. Wahrscheinlich sind auch die bei den Hunden erkennbaren leichten Veränderungen der Leberzellen (Schwellung; bei einem Tier Verfettung) sowie die geringgradigen Reaktionen der Nierenkanälchen Folgen der vermehrten Vitamin A-Speicherung, ohne daß dabei von einer A-Hypervitaminose gesprochen werden könnte.

Es ist klar, daß bei Verwendung von β-Carotin als Lebensmittelfarbstoff eine im Gewicht fallende Vermehrung der Vitamin A-Speicherung beim Menschen schon aus Gründen der Dosierung ausgeschlossen ist. Außerdem ist kein Fall von menschlicher alimentärer Carotinämie bekannt, bei welchem A-Hypervitaminosesymptome aufgetreten wären (auch der von HENSCHEN[1] beschriebene Fall von Carotinämie zeigt nicht die Symptome der A-Hypervitaminose), dies im Gegensatz zur Therapie mit Lebertran oder Vitamin A-Präparaten, bei welcher Anzeichen dieses Syndroms gelegentlich beobachtet werden. Auch die Feststellung, daß beim Menschen trotz stark erhöhter Serum-Carotinwerte ein normaler Vitamin A-Blutspiegel vorhanden ist[2], läßt es als unwahrscheinlich erscheinen, daß selbst bei schwerster Carotinüberdosierung Schädigungen durch A-Hypervitaminose entstehen könnten.

[1] HENSCHEN, C.: Schweiz. med. Wschr. 1941, 331.
[2] McCONAGHEY, R. M. S.: Zit. S. 128, Anm. 1.

Unter den Carotinoiden nimmt das Lycopin eine gewisse Sonderstellung ein, da es zwar chemisch ein Isomeres von Carotin ist, im Stoffwechsel jedoch weitgehend eigene Wege geht: aus Lycopin entsteht kein Vitamin A[1, 2], der Farbstoff wird jedoch von der Ratte zu 2—6% oder mehr in der Leber gespeichert[2, 3]. Er kommt normalerweise auch in Leber, Fettgewebe und Serum des Menschen vor[4], wird beim Huhn etwas gespeichert[5] und konnte in den vorliegenden Versuchen auch in der Hundeleber festgestellt werden; die ganze Leber enthielt jedoch nur 0,0025% der total verabreichten Lycopinmenge. Mit dem Carotin- und Vitamin A-Stoffwechsel steht das Lycopin insofern in Beziehung, als es in kleinen Dosen die Resorption und Speicherung sowohl des Vitamins wie des Provitamins fördert[3].

Bei den mit Lycopin durchgeführten Verträglichkeitsversuchen konnte eine Speicherung des Pigments in den Leberzellen auch mikroskopisch nachgewiesen werden. Es geht jedoch aus der oben erwähnten chemischen Bestimmung wie aus den histologischen Untersuchungen hervor, daß nur ein verschwindend kleiner Prozentsatz des Lycopins in der Leber abgelagert wurde und daß die aus der Literatur zitierte Ablagerungsrate von 2—6% oder mehr bei chronischer Überbelastung bei weitem nicht erreicht wird. Trotz der immerhin doch recht beträchtlichen Farbstoffspeicherung in der Leber konnten weder durch klinische noch durch histopathologische Untersuchungen Störungen der Leberfunktion und -struktur nachgewiesen werden. Die männlichen Tiere der ersten mit Lycopin-Futterzusatz behandelten Rattengeneration entwickelten sich sogar signifikant besser als die unbehandelten Vergleichstiere, ein Einzelbefund, den wir nur als weiteren Beweis für die Atoxicität des Lycopins bewerten und aus dem nicht auf einen spezifischen wachstumsfördernden Effekt geschlossen werden darf.

Zur Bewertung der vorliegenden Verträglichkeitsversuche mit Lycopin sind auch hier einige Dosierungsvergleiche angebracht:

Ein Hund fraß in 192 Tagen total 272 g Lycopin, was ungefähr der aus 10—15000 kg frischen Tomaten extrahierbaren Farbstoffmenge entspricht. Ratten von durchschnittlich 200 g Gewicht nahmen bei einem 0,1%igen Diätzusatz von Lycopin pro Tag etwa 50 mg/kg des Farbstoffes auf. Umgerechnet auf das Körpergewicht ergibt dies für einen 60 kg schweren Menschen eine tägliche Aufnahme von 3 g Lycopin, eine Menge, die in etwa 20 kg konzentriertem Tomatenpuree enthalten ist.

Somit liegt auch in den Versuchen mit Lycopin der Sicherheitsfaktor sehr hoch. Eine toxische Schädigung bei Verwendung dieses Carotinoids als Lebensmittelfarbstoff ist undenkbar.

Die Versuche mit *7,7'-Dihydro-β-carotin* und *Bixin* sind nur in kleinem Rahmen durchgeführt worden. Ratten vertrugen diese Farbstoffe in der sehr großen Dosis von 1000 mg/kg/Tag per os ohne weiteres, so daß auch diese beiden Carotinoide als atoxisch bezeichnet werden dürfen.

Die von uns untersuchten Carotinoide sind sehr gut verträgliche Substanzen, die selbst dann keine Organschädigungen verursachen, wenn sie über lange Zeit, in sehr großen Dosen, per os oder parenteral verabreicht werden, unabhängig davon, ob eine Speicherung in den Organen eintritt oder nicht. Eine carcinogene Wirkung dieser natürlichen Farbstoffe ist sehr unwahrscheinlich. Während der bisherigen Tierversuche mit Carotinoiden wurde in unserem Material kein einziger Spontantumor

[1] McGillivray, W. A., S. Y. Thompson u. N. A. Worker: Zit. S. 131, Anm. 3.
[2] Painter, R. H., u. J. Glover: Biochem. J. 60, 16 (1955).
[3] High, E. G., u. G. H. Day: J. Nutrit. 48, 369 (1952).
[4] Zechmeister, L., u. P. Tuzson: Z. physiol. Chem. 231, 259 (1935); 234, 241 (1935).
[5] Ganguly, J., J. W. Mehl u. H. J. Deuel: Zit. S. 128, Anm. 6.

beobachtet, doch können, wie bereits erwähnt, aus zeitlichen Gründen noch keine definitiven Schlüsse gezogen werden.

Die Unschädlichkeit der Carotinoide wird bereits von vielen staatlichen Behörden und internationalen Fachkommissionen anerkannt.

Carotinoide wurden beispielsweise von der „Kommission zur Bearbeitung des Lebensmittelfarbstoff-Problems der Deutschen Forschungsgemeinschaft" in die Liste der unbedenklichen Farbstoffe eingereiht[1]. Das 1952 noch zu weiterer Prüfung zurückgestellte Bixin erschien 1957 mit den anderen Carotinoiden in der Liste der gestatteten Farbstoffe, welche vom „Europäischen Forschungskomitee für den Schutz der Bevölkerung vor chronisch-toxischen Umweltschädigungen" aufgestellt wurde[2] und die seit kurzer Zeit in der Schweiz offiziell anerkannt ist.

Es muß aber darauf hingewiesen werden, daß sich die Farbstoffe aus der großen Klasse der Carotinoide in bezug auf Resorption, Speicherung und Abbau nicht einheitlich verhalten, so daß auch Unterschiede in der chronischen Toxicität anzunehmen sind. Diese Verschiedenheiten abzuklären und chronische Organschädigungen auszuschließen, ist der wichtigste Zweck der heute verlangten tierexperimentellen Verträglichkeitsprüfungen. Erst weitere Untersuchungen mit anderen Carotinoiden werden zeigen, ob es gestattet ist, den wohldefinierten chemischen Begriff der „Carotinoide" vom toxicologischen Standpunkt aus ebenso einheitlich zu charakterisieren.

Zusammenfassung

β-Carotin, Lycopin, 7,7'-Dihydro-β-carotin und *Bixin* wurden in großen Dosen an Ratten, teilweise auch an Kaninchen und Hunden per os (β-Carotin bei Ratten auch intramusculär) verabreicht. Die Versuchsdauer betrug bei Ratten bis 540 Tage, bei Kaninchen bis 730 Tage und bei Hunden bis 200 Tage. Insgesamt wurden an 210 Tiere total etwa 2250 g β-Carotin, 580 g Lycopin, 200 g 7,7'-Dihydro-β-carotin und 200 g Bixin verabfolgt. Irgendwelche toxischen Schädigungen konnten weder durch klinische noch durch hämatologische und histopathologische Untersuchungen festgestellt werden. Die Fertilität blieb bei Ratten, denen 0,1% β-Carotin oder Lycopin ins Futter gemischt wurde, unbeeinflußt. Spontantumoren traten nicht auf. Intramuskulär injiziert, führte β-Carotin zu ziemlich starker Gewebsreizung. Durch chemische und histologische Untersuchungen konnte bei β-carotin-behandelten Ratten und Hunden, nicht aber bei Kaninchen eine Speicherung von Vitamin A hauptsächlich in der Leber nachgewiesen werden; doch kam es nie zu Zeichen einer A-Hypervitaminose. Auch Lycopin und β-Carotin waren in der Leber von Ratten und Hunden, die mit den entsprechenden Pigmenten behandelt wurden, in geringer Menge vorhanden.

Die Versuche bestätigen, daß es sich bei den 4 untersuchten Carotinoiden um atoxische Substanzen handelt, deren Verwendung als Lebensmittelfarbstoffe bedenkenlos gestattet werden darf.

Bemerkungen bei der Korrektur: Seit Abschluß des Manuskriptes wurden die Versuche Nr. 3a und 3c (β-Carotin, 0,1%, Ratten, 1. und 3. Generation) und Nr. 10a und 10b (Lycopin, 0,1%, Ratten, 1. und 2. Generation) während 200 Tagen weitergeführt, ohne daß sich irgendwelche Schädigungen oder Spontantumoren zeigten. Der Reproduktionsversuch mit der 2. Rattengeneration auf lycopin-haltiger Diät verlief normal.

[1] Vgl. Zit. S. 114, Anm. 7.
[2] Vgl. Zit. S. 114, Anm. 2.