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# TREATMENT OF LIGHT SENSITIVITY WITH CAROTENOIDS

## *Serum Concentrations and Light Protection*

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*Abstract.* When beta-carotene was administered to 24 volunteers as single oral doses varying from 2 to 8 mg per kg bodyweight, the average maximal increase of carotenoid concentration in serum was about 30 $\mu$g per 100 ml, though with great individual variations. Maximal serum concentrations were obtained mostly within 4 hours after the administration. The binding of beta-carotene to human serum proteins was estimated to about 75%, by equilibrium dialysis. Carotenoids were then given as a combination of beta-carotene and canthaxanthin, in multiple oral doses of 50 mg every twelfth hour, to patients with light-sensitive psoriasis, polymorphous light eruptions and erythropoietic protoporphyria, but also to a control group consisting of not particularly light-sensitive patients with vitiligo. Maximal serum concentrations of carotenoids were reached after treatment for 20 to 40 days by most patients, with the fastest increase during the first 15 days. The maximal serum concentrations ranged from 338 to 1 219 $\mu$g per 100 ml, and the average for these two groups of patients was about the same. The maximal light protection factor for the group of patients with light sensitivity varied between a factor of 4 and 8, with an average value of 5.4. In the control group of patients with vitiligo the average light protection factor was 1.6 for normal, and 1.9 for vitiliginous skin. According to results obtained by light testing at various time points during the carotenoid treatment, the maximal protection factor was never estimated before about 2 to 4 months of treatment. For patients with polymorphous light eruptions there is a positive correlation between maximal light protection and maximal serum concentration ($r = 0.74$), but none for the patients in the control group. In this study a pronounced difference in the light protection factor could be distinguished for light-sensitive patients vis-a-vis a control group, in spite of the fact that the minimal erythema dose before treatment, whereas the serum concentration obtained during treatment was approximately the same for both these groups.

There are several earlier reports concerning the absorption and metabolism of beta-carotene; most of the them emphasize the relationship with vitamin A (5, 12, 14, 20, 25, 26). There has also been a great interest in carotenoids, stressing their light-protective mechanisms, in particular in microorganisms and plants (17, 23, 24, 29, 30, 31, 39).

Good therapeutic effects have been obtained with carotenoids in patients with pronounced light sensitivity, such as in erythropoietic protoporphyria (2, 7, 27, 32, 33) and polymorphous light eruptions (35, 40). Work has also been done to elucidate the mechanisms underlying the light-protective effect (1, 4, 6, 8, 9, 10, 11, 23, 24, 41).

Light protection obtained by beta-carotene slowly increases with time after the inception of therapy, and the maximal effect is reached after several weeks of treatment. The purpose of the present study is to give more information on the time course of carotenoid concentrations in serum, when given to fasting subjects as single or multiple oral doses, and to see whether or not there is any correlation between serum concentration and light protection.

As shown by earlier experiments, the absorption of carotenoids is dependent on test meal regimens (5, 12, 14, 20, 25, 26). Therefore, the present study with single oral doses was confined to fasting individuals but with different formulations, capsule and water suspensions, and with varying doses of beta-carotene.

In a group of patients treated with carotenoids for light sensitivity, the serum concentrations and the increase of light tolerance was observed. As a control group, patients with vitiligo but otherwise normal light sensitivity were used, as they offered both normally pigmented skin, and unpigmented spots. It is known that carotenoids combine with proteins to form carotenoproteins (4, 13, 43). This phenomenon has been extensively studied by various authors in human serum too (5, 22). The concentration dependence of the binding of beta-carotene to human serum proteins was investigated in the present study by equilibrium dialysis.

## MATERIAL AND METHODS

*Determination of total carotenoids in human serum*

The serum concentrations of carotenoids were analysed by colorimetric measurement (3, 5). Freshly drawn blood from patients or volunteers was centrifuged at 3 000 rpm for 15 min. 1.0 ml serum was then transferred to another tube and 1.0 ml of absolute ethanol added drop by drop and mixed thoroughly for denaturation. Carotenoids were then extracted by adding 2.0 ml of *n*-hexane and shaken for 10 min.

The solution was centrifuged and the *n*-hexane layer immediately used for the colorimetric measurement. 1.3 ml of the *n*-hexane layer was pipetted into a semi-micro cell of 1 cm light path and the absorbance at 454 nm was a read on a Beckman DB spectrophotometer against *n*-hexane as a blank.

A calibration factor was determined by measuring the absorbance of crystalline beta-carotene dissolved in hexane in known concentrations. The concentration of carotenoids in serum was calculated from the following formula:

Absorbance × 750 = $\mu$g of carotene per 100 ml of serum. The serum samples were processed regularly in duplicates and, when possible, in triplicates.

The reproducibility of the method was determined for 20 analyses of a pooled serum from patients on long-term treatment with carotenoids, and the mean was 994 $\mu$g per 100 ml and the standard deviation 12 $\mu$g per 100 ml.

This method measures the total carotenoid concentration in human serum. The normal range without carotenoid treatment is 60 to 200 $\mu$g per 100 ml (28).

*Protein binding*

The degree of protein binding was determined by equilibrium dialysis using cellophane dialysis tubing. Each bag contained 10.0 ml pooled serum with known concentrations of carotenoids. The dialysis was performed in a bath of slowly running water. The equilibrium concentrations of carotenoids were determined spectrophotometrically as described above. Several different dialysis periods were used, viz. 1, 2, 4, 6, 8, 16 and 24 hours. The initial serum concentrations of beta-carotene before dialysis were varied from 50 to 1 125 $\mu$g per 100 ml in 5 samples.

*Administration of carotenoids*

Pure beta-carotene, obtained from Hoffman-La Roche Ltd., was administered orally in gelatin capsules to 20 healthy volunteers, in a single dose of 2, 3, 4 or 6 mg per kg bodyweight. To 4 further volunteers beta-carotene was dispensed as a water solution in a dose of 8 mg per kg body weight. The single dose was always taken in the morning and the subjects were fasting. Two subjects (G. W. and H. S.) were given a single oral dose on two different occasions, first dispensed as a water solution and secondly as gelatin capsules.

Multiple oral doses of carotenoids were given as capsules to 12 patients with various light dermatoses (Table II). 8 patients were treated for polymorphous light eruptions (PMLE), 3 patients for psoriasis with light sensitivity and 1 patient for erythropoietic protoporphyria (EPP). It should be noted that the patients H. K., B. M., R. B., V. H., M. S. V. and M. B. B. in Table II showed a pronounced sensitivity to longwave ultraviolet light (UVA) as revealed by light testing.

As a control group of not particularly light-sensitive patients, 10 healthy individuals with vitiligo were given multiple oral doses of carotenoids. One of these subjects (E. B.) had a total confluent vitiligo.

Two of the patients with vitiligo (E. B. and B. E. M.) were given pure beta-carotene in a dose of 50 mg every 12th hour. Every second patient on multiple oral dose received capsules which contained a combination of 10 mg beta-carotene and 15 mg canthaxanthin each, in a total dose of 50 mg every 12th hour. The treatment was continued for 2 to about 4 months; each single dose corresponded to about 0.5 to 1.0 mg per kg body weight.

*Serum specimen collections*

Before the administration of carotenoids a blood specimen was drawn and the initial fasting value of carotenoids in serum was estimated (Table I). Blood samples were then drawn at different time points, viz. 0.5, 1, 2, 4, 8, 24, 48, 72 and 96 hours, after the administration of the single oral dose, in order to chart the increase and decline of beta-carotene in serum.

During the multiple-dose administration, the accumulation of carotenoids in serum and the apparent steady-state concentration were estimated from blood specimens drawn at repeated intervals.

*Light testing procedure*

The light sensitivity was tested with an Osram High Pressure Xenon arc lamp, XBO 150 W, in a Zeiss microscope lamp house with a quartz collector. The distance from the patient to the filterholder was 15 cm. The intensity of the beam at this distance when equipped with a Schott WG 295 filter to give a sun-spectrum-like radiation, was 130 mW $cm^{-2}$. In order to test sensitivity to UVA the lamp was furthermore used with a 3 mm glass filter which excluded short ultraviolet radiation below 320 nm. The intensity with this filter combination was 90 mW $cm^{-2}$. The intensity was measured with a Hewlett & Packard radiant flux meter.

The minimal erythema dose was established for all patients given multiple dose administration, and performed on an area usually protected by clothes, viz. on the skin of the back (40). Patients with vitiligo were light tested on both normally pigmented skin and unpigmented vitiligo spots.

## RESULTS

*Single-dose experiments*

The initial fasting values of serum carotenoids, before administration of beta-carotene, ranged from 23 to 184 $\mu$g per 100 ml for the 24 investigated healthy volunteers. The mean value was 80 $\mu$g, with a standard deviation of 37 $\mu$g.

Table I summarizes data for the different dose groups. As there were great individual variations of the carotenoid fasting values in serum, the increase in carotenoids were not expressed in percentages but in absolute values. The increment in absolute units, expressed as $\mu$g per 100 ml serum, was about the same for the different groups. This

Table I. *Results of single-dose administration of pure beta-carotene in doses between 2 and 8 mg/kg bodyweight*

Patients 21 to 24 were given beta-carotene as a water solution

| Patients | Sex | Body-weight | Dose (mg/kg) | Concentrations of carotenoids/serum (μg/100 ml) | | Hours for maximal increase | Maximal carotenoid increment (μg/ml) |
|---|---|---|---|---|---|---|---|
| | | | | Before treatment | Maximal value | | |
| 1. EVA | ♀ | 80 | 2 | 79 | 131 | 2 | 52 |
| 2. AL | ♂ | 84 | 2 | 75 | 90 | 4 | 15 |
| 3. RH | ♂ | 75 | 2 | 184 | 218 | 1 | 34 |
| 4. SN | ♂ | 105 | 2 | 45 | 75 | 4 | 30 |
| 5. HS | ♂ | 74 | 2 | 47 | 61 | 4 | 14 |
| Mean | | | | | | | 29 |
| 6. AB | ♀ | 64 | 3 | 68 | 80 | 4 | 12 |
| 7. MF | ♀ | 62 | 3 | 23 | 45 | 4 | 22 |
| 8. OL | ♂ | 70 | 3 | 139 | 180 | 4 | 41 |
| 9. JC | ♂ | 71 | 3 | 79 | 113 | 4 | 34 |
| 10. PB | ♂ | 67 | 3 | 38 | 64 | 2 | 26 |
| Mean | | | | | | | 27 |
| 11. KL | ♀ | 53 | 4 | 71 | 79 | 4 | 8 |
| 12. NJ | ♂ | 73 | 4 | 107 | 127 | 8 | 20 |
| 13. TL | ♂ | 83 | 4 | 23 | 64 | 8 | 41 |
| 14. SL | ♂ | 80 | 4 | 103 | 144 | 8 | 41 |
| 15. GW | ♂ | 72 | 4 | 110 | 146 | 8 | 36 |
| Mean | | | | | | | 29 |
| 16. IN | ♀ | 53 | 6 | 125 | 144 | 2 | 19 |
| 17. AE | ♀ | 63 | 6 | 67 | 94 | 2 | 27 |
| 18. BJ | ♀ | 55 | 6 | 110 | 134 | 2 | 24 |
| 19. KK | ♂ | 63 | 6 | 70 | 107 | 4 | 37 |
| 20. SE | ♂ | 72 | 6 | 41 | 70 | 4 | 29 |
| Mean | | | | | | | 27 |
| 21. UL | ♀ | 60 | 8 | 94 | 169 | 4 | 75 |
| 22. IE | ♀ | 60 | 8 | 75 | 94 | 1 | 20 |
| 23. GW | ♂ | 72 | 8 | 94 | 113 | 2 | 19 |
| 24. HS | ♂ | 74 | 8 | 51 | 60 | 8 | 9 |
| Mean | | | | | | | 31 |

was also true for the patients given the oral beta-carotene dose as a water solution. Thus in spite of the great variations in the doses given, the increment of the serum carotenoids was about the same. The serum concentration peak was reached after about 2 to 8 hours (Table I). The average maximal increment was then calculated for all individuals together, since no differences could be seen between the different dose groups, and was estimated to 28.5 ± 15 μg per 100 ml serum. The mean value for females and males was 28.8 and 28.4 μg/100 ml respectively; thus no sex difference was found. Fig. 1 shows the average increment of carotenoids in serum for the various dose groups. The time it takes for these increments to decrease 50% from their maximal values, can be estimated from Fig. 1, and ranged from 26 to more than 96 hours without any apparent correlation to the dose, but when beta-carotene was given as a water solution it appeared to be only 7 hours.

*Multiple-dose experiments*

Multiple oral doses of carotenoids were given to patients with light sensitivity, of the type polymorphous light eruptions, light-sensitive psoriasis and erythropoietic protoporphyria, and to a control group of patients with vitiligo. Two subjects with vitiligo (E. B. and B. E. M.) were given beta-carotene alone, and all other patients the above-described combination of beta-carotene and canthaxanthin.

The increases of the carotenoids in serum from 10 of the patients are seen in Fig. 2.

Table II. *Light-sensitive patients treated with multiple oral doses of carotenoids*

Maximal values of carotenoids in serum and maximal light protection factor, expressed as increase of the minimal erythema dose in seconds. 8 patients had polymorphous light eruptions (PMLE), 1 patient erythropoietic protoporphyria (EPP) and 3 patients light-sensitive psoriasis (Psor). UVA indicates light testing with longwave ultraviolet light alone

| Patients | Diagnosis | Maximal value carotenoids/ serum ($\mu$g/100 ml) | Minimal erythema dose, in seconds | | Maximal light protection factor |
|---|---|---|---|---|---|
| | | | Before treatment | After treatment | |
| 1. BMO | PMLE | 731 | 54 | 324 | 6 |
| 2. HL | PMLE | 468 | 24 | 96 | 4 |
| 3. IP | PMLE | 900 | 30 | 120 | 4 |
| 4. MS | PMLE | 1 219 | 10 | 80 | 8 |
| 5. EJ | PMLE | 581 | 20 | 100 | 5 |
| 6. HK | PMLE | 788 | 1[a] | 6 | 6 |
| 7. BM | PMLE | 900 | 4[a] | 28 | 7 |
| 8. KS | PMLE | 544 | 6 | 30 | 5 |
| 9. RB | EPP | 1 219 | 120 (UVA) | 480 (UVA) | 4 |
| 10. VH | Psor | 656 | 6[a] | 36 | 6 |
| 11. MSV | Psor | 806 | 6[a] | 30 | 5 |
| 12. MBB | Psor | 506 | 10[a] | 50 | 5 |
| Mean | | 776.5 | | | 5.4 |

[a] These patients were also sensitive for longwave ultraviolet light but the light testing was done with unfiltered Xenon light.

The maximal serum concentrations were reached after treatment for 20 to 40 days by 7 patients, and remaining patients showed a further (slight) progressive increase. There was a faster increase of the serum concentrations during the first 15 days, whereas the subsequent increase was usually much slower. When the maximal serum concentration was reached, a slight but successive decrease in the serum concentrations of 7 patients seemed to occur.

The maximal serum concentration obtained ranged from 338 to 1 219 $\mu$g per 100 ml, and the initial carotenoid values before treatment ranged from 19 to 131 $\mu$g per 100 ml, with an average of 74.9 $\mu$g, and with no apparent correlation to the maximal serum concentration obtained.

The patients given pure beta-carotene started with a serum concentration within the above-mentioned range and reached a maximal value of 731 and 1 106 $\mu$g per 100 ml, respectively.

*Protein binding*

When the protein binding of pure beta-carotene to human serum proteins was determined, the equilibrium state was reached within 8 hours. For

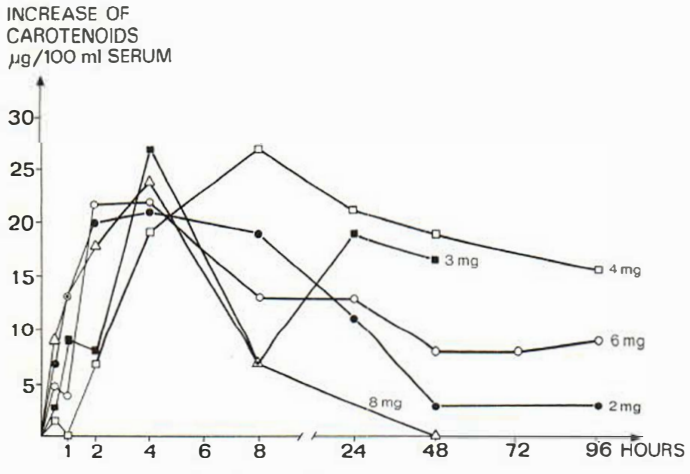


*Fig. 1.* The increment of carotenoids in serum (in $\mu$g per 100 ml) obtained by single oral doses of beta-carotene, varying from 2 to 8 mg per kg bodyweight. The dose of 8 mg was given as a water solution. The increase was calculated as the mean value for all individuals in the respective dose groups at each time point for serum specimen collection.

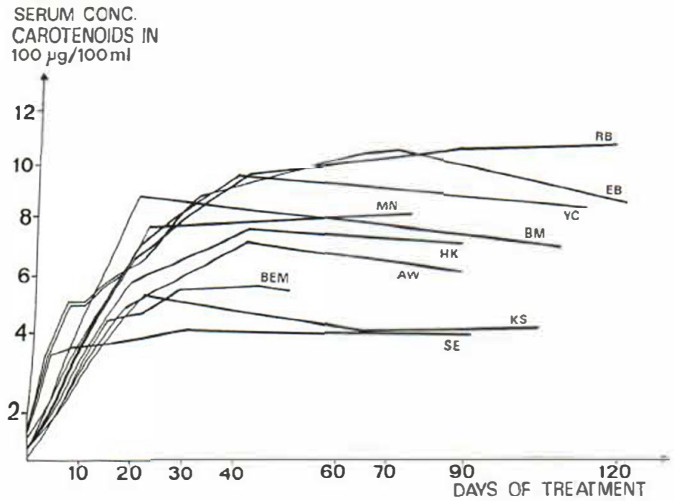


*Fig. 2.* Increase of carotenoids in serum (in µg per 100 ml) obtained by multiple oral doses of carotenoids, as a function of the treatment period. Subjects E. B. and B. E. M. were given pure beta-carotene in daily doses of 100 mg, and otherwise the same dose was administered as a combination of beta-carotene/canthaxanthin.

all 5 samples with different initial concentrations of beta-carotene, ranging from 50 to 1 125 µg per 100 ml, a protein binding of about 75 % was found. There seemed to be no correlation between initial carotenoid concentration and the degree of protein binding.

*Serum concentration and light tolerance*

The maximal serum concentrations of carotenoids were most regularly obtained after treatment for 20 to 40 days (Fig. 2). To obtain information on the most suitable time for light testing of the patients after commencement of the carotenoid treatment, a series of 6 patients were light tested at various time intervals. Fig. 3 gives the protection factor and serum concentration of carotenoids obtained by these patients, as a function of the duration of treatment. On the basis of these results we decided to light test the patients not earlier than after 2 months of treatment. In the following, the maximal protection factor has been determined after about 2 to 4 months of treatment.

In the group of light-sensitive patients (Table II), the maximal serum concentrations obtained varied from 468 to 1 219 µg per 100 ml, with a mean value of 776.5 µg. The light protection factor for these patients varied between 4 and 8, with an average value of 5.4. In Fig. 4 the maximal light protection for these patients is plotted against the maximal serum concentration obtained, and for the patients with polymorphous light eruptions there seems to be a moderate correlation, with a correlation factor of 0.74.

In the control group of patients with normal light

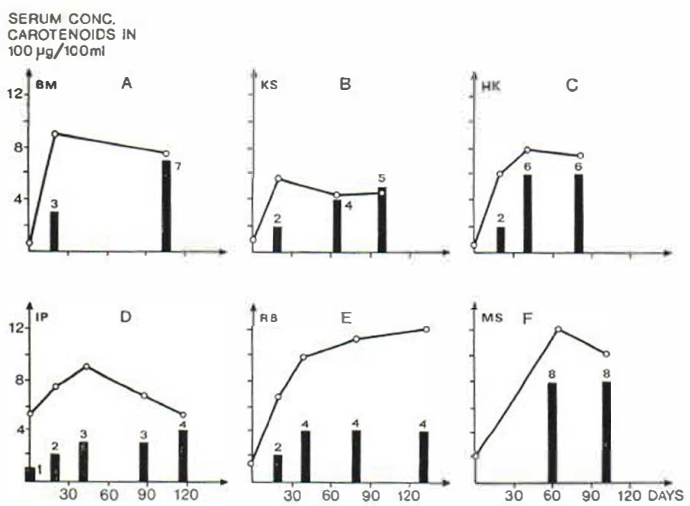


*Fig. 3.* Light protection factor and serum concentration of carotenoids in 100 µg per 100 ml, as a function of the duration of the treatment period. Solid columns indicate light protection factor obtained, expressed in multiples of minimal erythema dose.

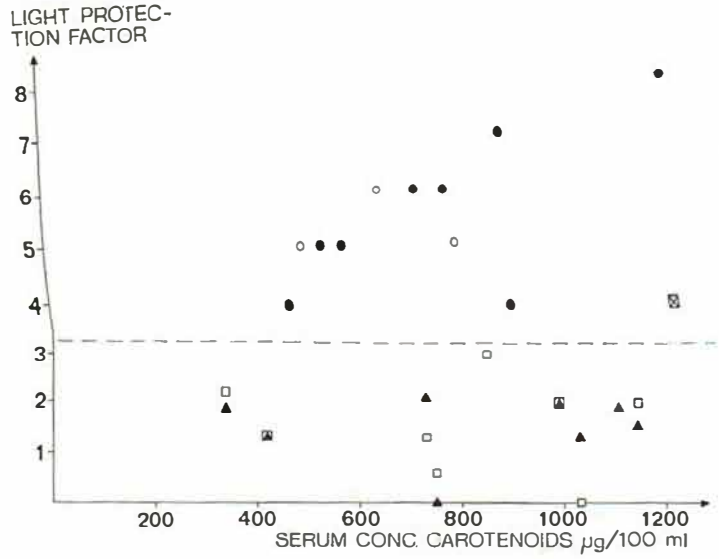


*Fig. 4.* Maximal light protection factor plotted against the maximal serum concentration of carotenoids obtained. Light protection expressed as increase of minimal erythema dose, in multiples, and serum concentration in µg per 100 ml. ●, 8 patients with polymorphous light eruptions—correlations coefficient 0.74; ○, 3 patients with light-sensitive psoriasis; ⊠, 1 patient with erythropoietic protoporphyria; □, normally pigmented skin of 10 patients with vitiligo; ▲, vitiligo spots of 10 patients with vitiligo. The light protection factor of the control group of patients with vitiligo never exceeded factor 3; all of them are located below the dotted line in the figure. Results from the patients with light sensitivity are seen above the dotted line.

sensitivity of non-vitiliginous skin (Table III), the maximal serum concentrations ranged from 338 to 1 144 µg per 100 ml, with a mean value of 818 µg. The increase of light tolerance on normal pigmented skin and on vitiligo spots is given in Table III. The average light protection factor in this group was 1.6 for normal, and 1.9 for vitiliginous skin and never exceeded a factor of 3. The increase in minimal erythema dose after long-term administration of carotenoids in this group was thus slight, but just statistically significant ($p < 0.05$). As seen in Fig. 4, there seems to be no correlation between the maximal serum concentration and the protection factor obtained for these patients.

Table III. *Maximal serum concentrations of carotenoids (in µg/100 ml), and light protection obtained on normal skin and on vitiligo spots in 10 patients with vitiligo, treated with multiple oral doses of carotenoids*

Subjects EB and BEM were given pure beta-carotene and otherwise the combination of beta-carotene/canthaxanthin was administered. Subject EB had a total confluent vitiligo, and offered no normal skin suitable for light testing

| Patients | Maximal value carotenoids/ serum (µg/100 ml) | Minimal erythema dose (sec) before and after treatment | |
|---|---|---|---|
| | | Normal skin | Vitiligo spots |
| 1. EB | 1 106 | – | 18–34 |
| 2. BEM | 731 | 6– 8 | 16–34 |
| 3. YC | 990 | 10–20 | 8–16 |
| 4. MN | 853 | 6–18 | 2–30 |
| 5. AW | 750 | 10– 6 | 6– 6 |
| 6. SE | 422 | 16–20 | 14–18 |
| 7. YB | 1 144 | 8–16 | 24–36 |
| 8. JEB | 1 031 | 6– 6 | 6– 8 |
| 9. TR | 338 | 18–40 | 16–30 |
| 10. BO | — | 10– 8 | 12–16 |
| Mean | 818.3 | 10.0–15.8 | 12.2–22.8 |
| Factor | | 1.6 | 1.9 |

Thus the increase of carotenoids in serum was about the same for both these groups, but a pronounced difference in their light protection factor could be established (Fig. 4), in spite of the fact that their minimal erythema dose values before treatment were approximately the same.

All patients obtained a moderate yellow pigmentation, most pronounced on light-exposed areas and on the palms and soles, but also seen on vitiligo spots. Whereas normally pigmented skin also became darker, the contrasts remained and for the patients with vitiligo a cosmetic success was achieved only in the patient with total, confluent vitiligo.

## DISCUSSION

It is a clinical fact that in patients treated with carotenoids the degree of light protection increases slowly (2, 7, 32, 33, 40). The purpose of the present study was to obtain more information about the dependence of the light protection on the serum concentration of the carotenoid and on the length of the treatment period.

Single oral doses of beta-carotene gave a small increase of the serum concentration, which seemed

to be limited to about 30 μg per 100 ml for all the different doses given. This is in agreement with earlier findings where a limited absorption capacity of the small intestine for carotene has been found (5, 12, 14, 20).

The time taken for the serum concentration of carotenoids to decrease from its maximal value to 50% of the maximal increment value, ranged from 26 to more than 96 hours. There seemed to be no correlation with the dose given. The variations seen were probably partly dependent on uncontrolled food intake, even though the subjects participating were asked to keep a diet as free from carotenoids as possible (3, 15, 16, 18, 44). However, the elimination of carotenoids from the blood seems to be so slow that a cumulative effect will occur if single doses of sufficient size are given daily.

Multiple-dose administrations of carotenoids gave fairly high serum concentrations, but maximal values were obtained first after 20 to 40 days of treatment. The increase in light protection was developed at the same time or perhaps somewhat later than the increase in serum concentration of carotenoids, as is seen in Fig. 3. There might be a lag between the increase in serum concentration and tissue concentration of carotenoids. From these results we therefore conclude that the light-protective effect of carotenoids on a photodermatosis should be tested after more than 2 months of daily oral intake. As there is normally a certain carotenoid concentration in human serum, there was no sense in estimating the degree of protein binding after single-dose experiments, but when measured after long-term treatment the protein binding was about 75%, irrespective of which level the serum concentration had reached.

Earlier findings indicate that patients with some types of light-sensitive diseases (2, 7, 19, 32, 33, 35, 40) have a much better light protection from beta-carotene than have patients with other photodermatoses (21) or normal individuals (34). In the present investigation this has been confirmed. In a group of vitiligo patients beta-carotene gave a light protection factor of 3 or less and, interestingly enough, there seemed to be no difference between vitiligo spots and normal skin with regard to light sensitivity, either before or after carotenoid treatment in a slightly pigmented Swedish population. These patients obtained the same serum concentration of carotenoids as did the light-sensitive group.

The light protection afforded by carotenoid treatment in 8 patients with PMLE, in 3 light-sensitive patients with psoriasis, and in 1 patient with EPP, was considerably better than in the control group. In these cases the light protection factor was between 4 and 8. For the patients with PMLE there seems to be a correlation between serum concentration of carotenoids and light protection (correlation coefficient = 0.74).

Beta-carotene functions as a singlet oxygen quencher under certain circumstances (1, 6, 8, 9, 10, 11, 23, 24). We have reason to believe that this may be the case in biological systems too (41). It is tempting to speculate that the low protective effect of beta-carotene in normal indivuduals in spite of a high serum concentration indicates that singlet oxygen is not involved in the UV-erythema reaction of normal individuals but that in the case of PMLE and EPP, singlet oxygen may be involved. With regard to the PMLE patients there is definitely a correlation between the serum concentration of carotenoids and the light protection factor, though for normal and vitiliginous skin there is no evident correlation ($r = 0.01$ and 0.13 respectively).

For EPP it is likely that the photo-oxidation is sensitized by protoporphyrin and mediated by singlet oxygen (36, 37, 38). For PMLE we do not know which substance is the sensitizer. There is no indication that porphyrins are involved in the light reaction in PMLE. Another type of endogenous metabolite that has photohemolytic activity mediated by singlet oxygen is kynurenic acid (42). As yet it remains to be investigated whether kynurenic acid is the most important photosensitizer in PMLE.

In our photohemolysis studies we have found that for protoporphyrin-sensitized photohemolysis, the main inhibitory effect of beta-carotene is due to a quenching effect and not to a light filter effect (41).

What is puzzling is that some light-sensitive patients, such as those with PMLE, have a normal MED and get a considerably higher MED than normal individuals after carotenoid treatment (34, 40). We believe that the normal MED for the PMLE patients may be a question of dose rate, a factor that has been insufficiently investigated.

On the basis of our studies we are inclined to believe that the normal solar erythema reaction is not mediated by singlet oxygen and is therefore only slightly affected by carotenoids while the reaction in some photodermatoses, such as in EPP and PMLE, singlet oxygen is involved. It is even possible that when a photosensitizing metabolite is present

in high concentrations another type of photochemical reaction is competing with the normal erythema-producing mechanism and therefore, under certain circumstances, a higher MED may be the effect while the patient develops cutaneous lesion by a relatively small repeated light dose.

Concomitant with the erythema reaction there may be damage to other cellular structures in the case of some photodermatoses. For diagnostic purposes, repeated light provocation is a better method than simply measuring MED.

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## REFERENCES

1. Anderson, S. M. & Krinsky, N. I.: Protective action of carotenoid pigments against photodynamic damage to liposomes. Photochem Photobiol *18:* 403, 1973.
2. Baart de la Faille, H., Suurmond, D., Went, L. N., van Steveninck, J. & Schothorst, A. A.: Beta-carotene as a treatment for photohypersensitivity due to erythropoietic protoporphyria. Dermatologica *145:* 389, 1972.
3. Borenstein, B. & Bunnell, R. H.: Carotenoids: Properties, occurrence, and utilization in foods. Adv Food Res *15:* 195, 1966.
4. Chessman, D. F., Lee W. L. & Zagalsky, P. F.: Caroteno-proteins in invertebrates. Biol Rev *42:* 132, 1967.
5. Cornwell, D. G., Kruger, F. A. & Robinson, H. B.: Studies on the absorption of beta-carotene and the distribution of total carotenoid in human serum lipoproteins after oral administration. J Lipid Res *3:* 65, 1962.
6. Farmilo, A. & Wilkinson, F.: On the mechanism of quenching of singlet oxygen in solution. Photochem Photobiol *18:* 447, 1973.
7. Fitzpatrick, T. B., Pathak, M. A., Parrish, J. A. & Mathews-Roth, M.: Topical and systemic approaches to photoprotection. Proc R Soc Med *64:* 861, 1971.
8. Foote, C. S.: Mechanisms of photosensitized oxidation. Science *162:* 963, 1968.
9. Foote, C. S. & Denny, R. W.: Chemistry of singlet oxygen. VII. Quenching by beta-carotene. J. Am Chem Soc *90:* 6233, 1968.
10. Foote, C. S., Chang, Y. C. & Denny, R. W.: Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection. J Am Chem Soc *92:* 5216, 1970.
11. Fujimori, E. & Tavla, M.: Light-induced electron transfer between chlorophyll and hydroquinone and the effect of oxygen and beta-carotene. Photochem Photobiol *5:* 877, 1966.
12. Fujita, A. & Morimoto, H.: Absorption of carotene in man as observed from the increase of serum vitamin A and carotene levels. J Vitaminol *6:* 278, 1960.
13. Ganguly, J., Krinsky, N. I., Mehl, J. W. & Deuel, H. J., Jr: Studies of the distribution of vitamin A as ester and alcohol and of carotenoids in plasma proteins of several species. Arch Biochem Biophys *38:* 275, 1952.
14. Goodman, D. S., Blomstrand, R., Werner, B., Huang, H. S. & Shiratori, T.: The intestinal absorption and metabolism of vitamin A and beta-carotene in man. J Clin Invest *45:* 1615, 1966.
15. Goodwin, T. W.: Mammalian carotenoids. *In* Comparative Biochemistry of the Carotenoids, pp. 229–253. Chapman & Hall, London, 1952.
16. — Distribution of carotenoids. *In* Chemistry and Biochemistry of Plant Pigments, pp. 136–139. Academic Press, London, 1965.
17. Griffiths, M., Sistrom, W. R., Cohen-Bazire, G. & Stanier, R. Y.: Function of carotenoid in photosynthesis. Nature *176:* 1211, 1955.
18. Isler, O.: Introduction *In* Carotenoids (ed. O. Isler), pp. 11–27. Birkhäuser Verlag, Basel, 1971.
19. Kesten, B. M.: Urticaria solare. Arch Derm (Chicago) *65:* 221, 1951.
20. Kimura, K.: Changes in serum carotene and vitamin A levels after ingesting carotene. Vitamins *14:* 21, 1958.
21. Kobza, A., Ramsay, C. A. & Magnus, I. A.: Oral beta-carotene therapy in actinic reticuloid and solar urticaria. Br J Dermatol *88:* 157, 1973.
22. Krinsky, N. I., Cornwell, D. G. & Oncley, J. L.: The transport of vitamin A and carotenoids in human plasma. Arch Biochem Biophys *73:* 233, 1958.
23. Krinsky, N. I.: Protective function of carotenoid pigments. *In* Photophysiology, vol. III (ed. A. C. Giese), pp. 123–191. Academic Press, New York, 1968.
24. — Function. *In* Carotenoids (ed. O. Isler), pp. 669–716. Birkäuser Verlag, Basel, 1971.
25. Kübler, W.: Carotine in der Säuglingsernährung. Wissenschaftliche Veröffentlichungen der Deutschen Gesellschaft für Ernährung, vol. 9 (ed. K. Lang), pp. 222–234. Steinkopff, Darmstadt, 1963.
26. — Mechanisms regulating blood levels of vitamin A and carotenoids in man. Vitamine 2, pp. 21–30. 1970. F. Hoffman-La Roche & Co AG, Basel.
27. Lewis, M. B.: The effect of beta-carotene on serum vitamin A levels in erythropoietic protoporphyria. Aust J Dermatol *13:* 75, 1972.
28. Mandel, G. H. & Weiss, W. P.: Vitamin A and Vitamin D. *In* The Pharmacological Basis of Therapeutics (ed. L. S. Goodman & A. Gilman), 3rd ed., pp. 1681–1696. Macmillan Co., New York, 1966.
29. Mathews, M. M. & Sistrom, W. R.: Function of carotenoid pigment in non-photosynthetic bacteria. Nature *184:* 1892, 1959.
30. Mathews, M. M.: Protective effect of beta-carotene against lethal photosensitization by haematoporphyrin. Nature *203:* 1092, 1964.
31. Mathews-Roth, M.: Carotenoid pigments and photokilling by acridine orange. J Bacteriol *93:* 506, 1967.
32. Mathews-Roth, M. M., Pathak, M. A., Fitzpatrick, T. B., Harber, L. C. & Kass, E. H.: Beta-carotene as a photoprotective agent in erythropoietic protoporphyria. New Engl J Med *282:* 1231, 1970.
33. Mathews-Roth, M. M., Pathak, M. A., Fitzpatrick, T. B. & Kass, E. H.: Beta-carotene as a photoprotective agent in erythropoietic protoporphyria—report of 30

treated cases. VI. Intern Congr Photobiol, Bochum, Aug 21–25, 1972.
34. Mathews-Roth, M. M., Pathak, M. A., Parrish, J., Fitzpatrick, T. B., Kass, E. H., Toda, K. & Clemens, W.: A clinical trial of the effects of oral beta-carotene on the responses of human skin to solar radiation. J Invest Dermatol *59:* 349, 1972.
35. Nordlund, J. J., Klaus, S. N., Mathews-Roth, M. M. & Pathak, M. A.: New therapy for polymorphous light eruption. Arch Dermatol *108:* 710, 1973.
36. Schothorst, A. A., van Steveninck, J., Went, L. N. & Suurmond, D.: Protoporphyrin-induced photohemolysis in protoporphyria and in normal red blood cells. Clin Chim Acta *28:* 41, 1970.
37. — Metabolic aspects of the photodynamic effect of protoporphyrin in protoporphyria and in normal red blood cells. Clin Chim Acta *33:* 207, 1971.
38. — Photodynamic damage of the erythrocyte membrane caused by protoporphyrin in protoporphyria and in normal red blood cells. Clin Chim Acta *39:* 161, 1972.
39. Sistrom, W. R., Griffiths, M. & Stainer, R. Y.: The biology of a photosynthetic bacterium which lacks colored carotenoids. J Cell Physiol *48:* 473, 1956.
40. Swanbeck, G. & Wennersten, G.: Treatment of polymorphous light eruptions with beta-carotene. Acta Dermatovener (Stockholm) *52:* 462, 1972.
41. — Effect of beta-carotene on photohemolysis. Acta Dermatovener (Stockholm) *53:* 283, 1973.
42. — Photohemolytic activity of tryptophan and phenylalanine metabolites. Acta Dermatovener (Stockholm) *54:* 99, 1974.
43. Thommen, H.: Metabolism. *In* Carotenoids (ed. O. Isler), pp. 637–668. Birkhäuser Verlag, Basel, 1971.
44. Ullrey, D. E.: Biological availability of fat-soluble vitamins: Vitamin A and carotene. J Anim Sci *35:* 648, 1972.

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