Acta Dermatovener (Stockholm) 53: 283–289, 1973

EFFECT OF BETA-CAROTENE ON PHOTOHEMOLYSIS[1]

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Abstract. In the present investigation the effect of beta-carotene on chemically induced photohemolysis has been studied. It is shown that chlorpromazine, demethylchlortetracycline, and protoporphyrin readily induce photohemolysis under appropriate experimental conditions. Furthermore, an inverse photohemolytic effect was demonstrated where the red blood cells are irradiated first and the photosensitizer is added subsequently. When the red blood cells were irradiated with unfiltered Xenon arc radiation together with one of the above-mentioned photosensitizers only a small, insignificant inhibition of the photohemolysis was obtained if beta-carotene was placed in a cuvette in front of that containing the red blood cells, thereby acting as a filter only. A much stronger inhibition was obtained if the same amount of beta-carotene was put in the same cuvette as the red blood cells. Under these circumstances the inhibitory effect of beta-carotene on photohemolysis is thus not mainly a filter effect but is more specific. With window glass filtered radiation, however, only a filter effect of beta-carotene could be demonstrated when chlorpromazine and demethylchlortetracycline were used as photosensitizer but not for the photohemolysis induced by protoporphyrin. The mode of action of beta-carotene inhibition of photohemolysis thus varies with the spectral distribution of the radiation and the photosensitizer used. The inverse photohemolysis induced by unfiltered radiation and chlorpromazine was also inhibited by beta-carotene specifically and not mainly through a filter effect.

Photosensitization is a medical problem and its increasing importance is due largely to the more frequent use of photosensitizing pharmacologic agents.

Photohemolysis is an effect of many photosensitizing compounds and is now an established in vitro procedure to determine the phototoxic potential of chemicals.

According to Willis & Kligman (28), all photoallergenic compounds are also phototoxic.

Since some photosensitizing agents act by way of union with DNA and others have a membrane effect, the advantage of using the photohemolysis technique is that a pure membrane effect is studied.

The technique of photohemolysis is well known and has been successfully used by several authors earlier (1, 2, 4, 6, 8, 9, 10, 14, 15, 22).

Each photohemolyser reacts with red blood cells under certain conditions, which are well described by Kahn & Fleishaker (14, 15). These factors, which have to be considered and kept at special optima, are: the concentration of the photosensitizing agent and the buffer used, the nature of the light source, the exposure, and the incubation time after exposure.

Chlorpromazine gave photohemolysis promptly in the system of Kahn & Fleishaker and of Freeman et al. (14, 6). The latter could also induce photohemolysis with demethylchlortetracycline. Protoporphyrin also gives a very good photohemolysis, according to Kahn & Fleishaker (14).

Schwarz (23, 24) and Jung (11, 12, 13) have experimentally produced a so-called inverse photoallergy with sulfanilamides and chlorpromazine, whereby the skin is irradiated immediately before application of the photosensitizing agent. Thus, the irradiation energy may be stored in the skin and could be supplied afterwards to the photosensitizing agent.

Since beta-carotene has been shown to have a light-protective function in microorganisms, plants, animals (17, 18, 19, 26) and also in man concerning diseases with light sensitivity such as urticaria solare (16), erythropoietic protoporphyria (20, 21), and polymorphous light eruptions (27), it seemed to be of interest to investigate its protective function on photohemolysis. To our knowledge, no in vitro study on a system with

[1] The present investigation was presented at the Third Meeting of the European Society for Dermatological Research in Amsterdam, April 1973.

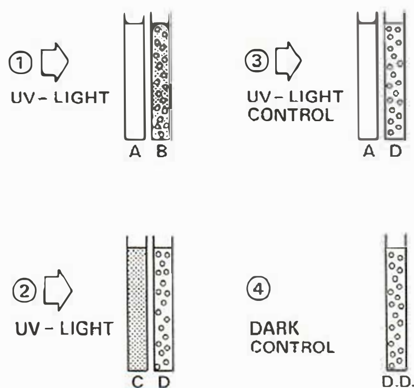

Fig. 1. Schematic model over photohemolysis experiments. *Model 1* represents investigations when beta-carotene was added to the drug-solution. *A*, Buffer solution only; *B*, RBC-drug-beta-carotene-buffer-suspension. *Model 2* shows investigations of the filter effect of beta-carotene. *C*, Buffer solution with beta-carotene added; *D*, RBC-drug-buffer suspension. *Model 3* is the control drug photohemolysis. *A*, Buffer solution only; *D*, RBC-drug-buffer suspension. *Model 4* is the dark control reference. *DD*, RBC-drug-buffer suspension incubated in darkness without light exposure. Models 1–3 are exposed to either unfiltered radiation or to window glass filter (*WGF*) radiation.

clinical connection has been made of the protective effect of beta-carotene. Our primary interest was to see whether beta-carotene functions mainly as a systemically administered sunscreen or whether it has some other, more specific effect.

We have used chlorpromazine, demethylchlortetracycline and protoporphyrin as photosensitizers, with emphasis on chlorpromazine.

MATERIAL AND METHODS

The technique of Kahn & Fleishaker (14, 15) was used. Erythrocytes were obtained from healthy human adults by venipuncture. Only blood group O Rh+ was used. The blood was stored no longer than 10 days at +4°C.

Chlorpromazine (CPZ), demethylchlortetracycline or protoporphyrin was dissolved in 0.1 M Na-Veronal (barbital) buffer at pH 8.0.

Human red blood cells (RBC) were washed three times in physiological saline and then 0.1 ml of the packed RBC was added to 10 ml of the buffered drug solution, and to control buffered solutions without drug. These experiments were made with and without addition of beta-carotene to the solutions, and also with beta-carotene as a filter in front of the test-solution (Fig. 1). This arrangement enables us to separate a filter effect of beta-carotene from an effect where beta-carotene has to be in contact with the photosensitizer or the cell membranes. All solutions were kept in the dark when not exposed to the light used.

The solutions were poured into 2 mm quartz glass cuvettes. Control cuvettes were incubated in the dark at 37°C, while test solutions were exposed to the Xenon lamp radiation at various light dosages and afterwards also incubated in the dark. The degree of photohemolysis was estimated after various intervals of incubation up to 3 hours (Fig. 2). After ultraviolet exposure and/or incubation in the dark, the solutions were centrifuged at 2 000 rpm. Optical density of the supernatant fluid was read at 540 nm on a Beckman DB Spectrophotometer after dilution in buffer solution 1 : 10. A total hemolysis control of 100% was prepared by adding 0.02 ml of the washed red blood cells to 10 ml of a 0.04% NH_4OH solution. Results were expressed as per cent relative to the 100% hemolysed solution, using the following formula

$$\frac{E-D}{T} \times 100$$

E = Optical density of exposed solution
D = Optical density of dark control solution
T = Optical density of total hemolysis control solution.

The ultraviolet light source was an Osram High Pressure Xenon Arc Lamp (XBO 150 W). The solutions were irradiated in their quartz cuvette at a distance of 15 cm from the lamp aperture, for various exposure times. The lamp was used unfiltered or with a window glass filter of 3 mm (WGF) to exclude short ultraviolet radiation below 320 nm. Light doses were measured with a Hewlett & Packard Radiant Flux meter.

No spontaneous hemolysis occurred when beta-carotene was added to the RBC-suspension in concentrations of 1 mg or 2 mg per 100 ml in the dark or when exposed to unfiltered radiation for 10 min.

The protective effect of these two concentrations was studied on chlorpromazine-induced photohemolysis. In the other experiments we used 1 mg of beta-carotene per 100 ml which can easily be achieved in the serum of patients receiving beta-carotene capsules per os as a treatment for light sensitivity. According to Fitzpatrick (3) it is necessary to reach a serum concentration of more than 0.4 mg/100 ml to obtain a good light-protective effect in patients with erythropoietic protoporphyria. Swanbeck & Wennersten (27) treated patients with polymorphous light eruptions with beta-carotene and these patients obtained serum concentrations of beta-carotene varying between 0.5 and 4.1 mg/100 ml.

Thus, the beta-carotene concentration used in our photohemolysis experiments corresponds well to that which can be expected in clinical use.

A blood sample was also taken from a 26-year-old male who had erythropoietic protoporphyria. He was extremely light-sensitive. On light testing through the window glass filter with our Xenon arc lamp he showed a marked erythema even after an exposure time of 80 sec (in normals no reactions up to 20 min). When treated with beta-carotene capsules he could tolerate sunshine up to 2–3 hours and 6–8 min when light-tested as above. The blood sample from this patient was treated exactly in the same way as described earlier. When the blood was

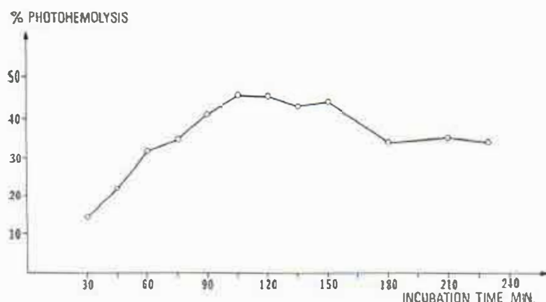

Fig. 2. Photohemolysis by chlorpromazine for varying periods of incubation in the dark after irradiation. Chlorpromazine 1.0 mg per 100 ml in 0.1 M Veronal buffer, pH 8.0. Exposure time 5 min with unfiltered radiation.

taken from this patient he had not been on beta-carotene for 5 months.

Exposure time and doses. With the unfiltered radiation of the Xenon lamp we used exposure times of 5, 10 and 20 min and with the window glass filter 10, 20 and 40 min. This corresponds to 45, 90 and 180 W sec/cm² respectively 66, 132 and 264 W sec/cm². This may be compared with clinically used doses in testing procedures. With our Xenon Arc XBO 150 W we have a MED range on normal subjects of 1.5–3 W sec/cm² and with the window glass filter no reactions up to 132 W sec/cm².

RESULTS

There was no spontaneous hemolysis of red blood cells in 0.1 M Veronal buffer at pH 8.0 incubated in the dark at 37°C up to 3 hours and without previous exposure to light.

When the RBC-buffer suspension was exposed to unfiltered ultraviolet light a slight hemolysis occurred, depending on exposure time and length of incubation. With unfiltered ultraviolet light of 10 min or 20 min exposure there was in both instances 5% photohemolysis at an incubation time of 2 hours and 10% resp. 20% after 3 hours.

This was repeated with window glass filtered radiation and no photohemolysis was obtained with exposure for 20 min or 40 min, and measured after 2 and 3 hours of incubation in the dark.

Photohemolysis by chlorpromazine

When chlorpromazine was added in a concentration of 1 mg per 100 ml to the buffered erythrocyte suspension and irradiated, a different degree of hemolysis occurred depending on the exposure time, the wavelength range used and the time of incubation in the dark (Table I). Fig. 2 illustrates the degree of photohemolysis caused by irradiation with unfiltered UV light for 5 min, estimated after various periods of incubation in the dark, as shown in column 1 of Table I. There is a peak of hemolysis between 105 to 150 min of incubation but extending from 75 to 180 min.

The reproducibility of the photohemolytic method has been determined for 1 mg CPZ per 100 ml, 10 min unfiltered radiation, and 90 min incubation. For 15 determinations the mean was 44.7% and the standard deviation 3.9%.

Inverse photohemolysis by chlorpromazine

When chlorpromazine was added at a concentration of 1 mg per 100 ml to previous irradiated red blood cells in buffer, photohemolysis also occurred though to a lesser degree than when CPZ had been present during the irradiation. A photohemolysis of 30% was obtained when CPZ was added to the irradiated RBC-buffer suspension directly after its exposure. This value was reached with an exposure time of 10 min and with unfiltered radiation. The corresponding value with the same light dose by the classical photohemolysis procedure was 53% and in controls with exposure of only the RBC-buffer-suspension 10%.

Inverse photohemolysis also occurred with irradiation of the erythrocytes through the WGF for 20 min with a hemolysis of 27% compared with 47% in the classical way and with 0% in controls.

Photohemolysis by demethylchlortetracycline

Demethylchlortetracycline (DMCT) was added to the RBC-buffer suspension at a concentration of

Table I. *Degree of photohemolysis (in percent) with chlorpromazine at a concentration of 1 mg per 100 ml for various exposure periods and periods of incubation in the dark after exposure to unfiltered radiation and with window glass filter*

Time of incubation in the dark (min)	Unfiltered radiation, Exposure time, min			Window glass filtered radiation, Exposure time, min			
	5	10	20	5	10	20	40
30	14	26	47				
60	32	38	55	4	4	3	44
90	41	45	57	7	7	10	61
120	45	53	58	7	10	20	70
180	34	53	47	10	24	47	89

Table II. *Degree of photohemolysis (in percent) with demethylchlortetracycline at the concentration of 5 mg per 100 ml for various exposure periods and periods of incubation in the dark after exposure to unfiltered radiation and with window glass filter*

Time of incubation in the dark (min)	Unfiltered radiation, Exposure time, min				Window glass, filtered radiation, Exposure time, min		
	5	10	20	40	10	20	40
60	2	5	17	36	2	5	29
90	2	7	27	46	2	7	40
120	5	12	36	54	2	24	45
180	10	20	39	63	2	26	43
240	12	24	49	66	2	36	40

5 mg per 100 ml, and Table II shows the different degrees of photohemolysis obtained with different light doses, different periods of incubation in the dark, and with different wavelength ranges. With the unfiltered radiation, 49% hemolysis was obtained with an exposure time of 20 min, and 66% with exposure time of 40 min.

With window glass filtered radiation the corresponding value were, with the same exposure times, 36% resp. 40%.

In an experiment with DMCT at a concentration of 1 mg per 100 ml we still found a hemolysis of 33% with unfiltered radiation and exposure time 20 min, in contrast to 49% when DMCT at a concentration of 5 mg per 100 ml was used.

Photohemolysis of erythrocytes from patient with erythropoietic protoporphyria

A blood sample taken from the patient with erythropoietic protoporphyria gave 75% photohemolysis when exposed with the window glass filter for 20 min compared with 0% in blood from normal control persons.

Photohemolysis of normal erythrocytes with protoporphyrin added

When protoporphyrin was added to the RBC-buffer suspension and irradiated, varying degrees of photohemolysis occurred depending on the concentration of protoporphyrin, wavelength range used and exposure time. We reached a value of 55% hemolysis when protoporphyrin was added at a concentration of 0.5 mg per 100 ml and irradiated with the window glass filter for 20 min, and 76% hemolysis with unfiltered radiation for 10 min.

PROTECTIVE EFFECT OF BETA-CAROTENE ON PHOTOHEMOLYSIS

Chlorpromazine

When beta-carotene, at a concentration of 1 mg per 100 ml, was added to the suspension containing chlorpromazine and red blood cells, and then irradiated with unfiltered radiation, the photohemolysis decreased significantly to less than half the initial value (Fig. 3). When beta-carotene was added to a buffer solution and was placed as a filter in front of the CPZ-RBC suspension a very small and insignificant decrease of photohemolysis occurred (Fig. 3).

When the beta-carotene concentration in the CPZ-RBC suspension was doubled the hemolysis was further reduced to 1/4 of the initial value (Fig. 4). Fig. 5 shows the filter protective effect of beta-carotene in two different concentrations. When beta-carotene was used in other concentrations the inhibitory effect on the photohemolysis also varied proportionally. With beta-carotene concentrations in the CPZ-RBC suspension of 0.1 mg, 1.0 mg and 2.0 mg per 100 ml at exposure time 5 min, and unfiltered radiation, there was a decrease in the photohemolysis from the initial value of 40% to 30%, 21% and 11% respectively.

When the procedure was repeated with the window glass filtered radiation the most effective inhibition of the photohemolysis was seen when beta-carotene was used as a filter in front of the CPZ-RBC suspension and only slight inhibition when added to the suspension—thus the reverse of what was found with unfiltered radiation. The results can be compared in Table III.

Chlorpromazine and inverse photohemolysis

We also investigated whether the so-called inverse photohemolysis induced by chlorpromazine could be prevented by beta-carotene at a concentration of 1 mg per 100 ml. The initial hemolysis of 30% with an exposure time of 10 min of unfiltered radiation and CPZ added directly after the exposure, decreased to 23% when beta-carotene was used as a filter in front of the RBC-buffer suspension during exposure and to 10% when it

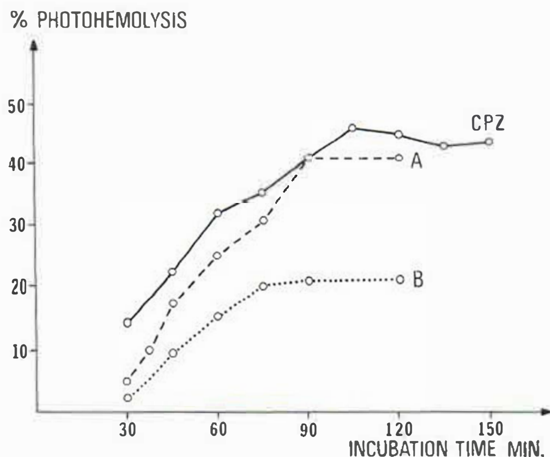

Fig. 3. Inhibition of chlorpromazine-induced photohemolysis by beta-carotene. Beta-carotene used as a filter compared with when added to the erythrocyte suspension. Chlorpromazine 1.0 mg per 100 ml. Beta-carotene 1.0 mg per 100 ml. Unfiltered radiation. Exposure time 5 min. *CPZ*, Chlorpromazine-RBC-buffer-suspension. *A*, Chlorpromazine-RBC-buffer-suspension with beta-carotene used as a filter in front of the suspension. *B*, Chlorpromazine-RBC-buffer-suspension with beta-carotene added to the suspension.

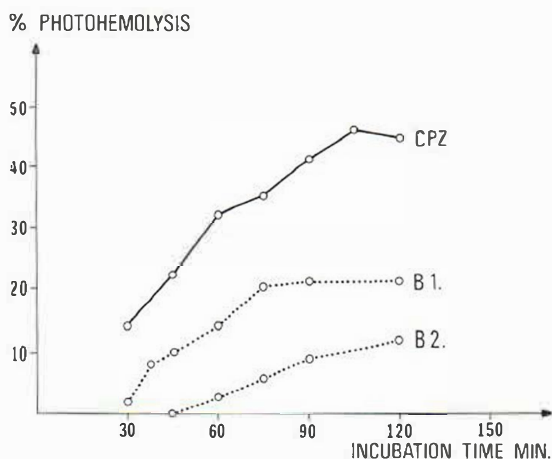

Fig. 4. Inhibition of chlorpromazine-induced photohemolysis by beta-carotene added to the solution, in different concentrations. Chlorpromazine 1.0 mg per 100 ml. Unfiltered radiation. Exposure time 5 min. *CPZ*, Control. Chlorpromazine-hemolysis without beta-carotene inhibition. *B* 1, Beta-carotene added to the CPZ-RBC-buffer suspension in a concentration of 1 mg per 100 ml. *B* 2, Same as B 1, but a beta-carotene concentration of 2 mg per 100 ml.

was added to this suspension before exposure (Table IV).

Demethylchlortetracycline

Photohemolysis induced by DMCT was inhibited by beta-carotene in a similar manner. When the RBC-DMCT suspension was exposed to unfiltered radiation for 20 min, the initial value of 39% hemolysis decreased to 25% when beta-carotene was used as a filter in front of the test suspension and to 10% when it was added to this suspension.

This experiment was repeated using window glass filtered radiation and an exposure time of 40 min. The initial value of 43% hemolysis decreased to 30% when beta-carotene was used as a filter and to 35% when it was added to the test-suspension—thus here too the reverse of what was found with unfiltered radiation.

Erythrocytes from patient with erythropoietic protoporphyria

The blood sample taken from the patient with erythropoietic protoporphyria showed an initial value of 75% photohemolysis which decreased to 70% when beta-carotene was used as a filter at a concentration of 1 mg per 100 ml and exposure with window glass filtered radiation for 20 min, but to 10% when beta-carotene was added to his red blood cells at the same concentration and with the same exposure time.

Erythrocytes with protoporphyrin added

With pure protoporphyrin at a concentration of 0.1 mg per 100 ml added to our RBC-suspension and exposed to unfiltered radiation for 10 min, beta-carotene at a concentration of 1 mg per 100 ml showed no significant protection when

Table III. *Illustration of the inhibitory effect of beta-carotene on photohemolysis induced by chlorpromazine (CPZ)*

Concentration of both CPZ and beta-carotene, 1 mg per 100 ml. Exposure time 20 min. Incubation time 3 hours. Degree of hemolysis in percent

	RBC–CPZ + irradiation	RBC–CPZ beta-carotene + irradiation	RBC–CPZ + beta-carotene (filter) irradiation
Unfiltered radiation	47	14	50
Window glass filtered radiation	47	38	26

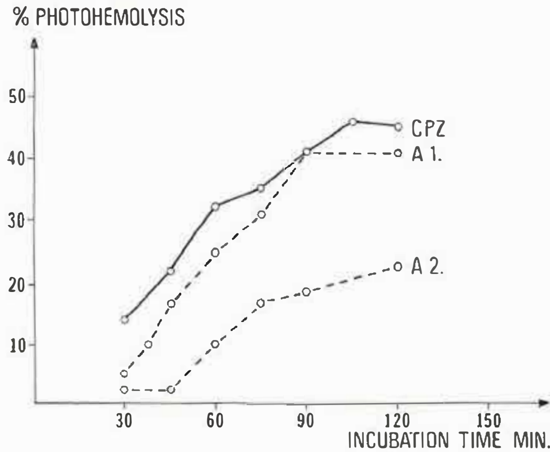

Fig. 5. Inhibition of chlorpromazine-induced photohemolysis by beta-carotene used as a filter in different concentrations. Chlorpromazine 1.0 mg per 100 ml. Unfiltered radiation. Exposure time 5 min. *CPZ*, Control. Chlorpromazine-hemolysis without beta-carotene inhibition. *A 1*, Beta-carotene as a filter in front of the CPZ-RBC-buffer suspension. Concentration of beta-carotene 1 mg per 100 ml. *A 2*, Same as A 1 but beta-carotene-concentration 2 mg per 100 ml.

used as a filter but when added to the suspension the initial photohemolysis of 33% decreased to 16%. This was repeated with window glass filtered radiation and an exposure time of 20 min and here too beta-carotene in the same concentration gave no protection when used as a filter but when added to the RBC-protoporphyrin suspension the photohemolysis decreased to 21% as compared with 47% when beta-carotene was used as a filter.

Table IV. *Illustration of the inhibitory effect of beta-carotene on inverse photohemolysis induced by unfiltered radiation and subsequent addition of chlorpromazine (CPZ)*

The values show the degree of hemolysis in percent. Concentration of both CPZ and beta-carotene 1 mg per 100 ml. Exposure time 10 min. Incubation time 3 hours

RBC + irradiation + CPZ	RBC + beta-carotene (filter) irradiation + CPZ	RBC beta-carotene + irradiation + CPZ
30	23	10

DISCUSSION

Red blood cells constitute an ideal system for the study of photo-induced membrane damage caused by chemicals, since an effect can be detected and perhaps also quantifiable by simple laboratory techniques. Many phototoxic substances have been shown to act photohemolytically (6, 14, 15, 22). This has been confirmed in the present study for chlorpromazine, demethylchlortetracycline and protoporphyrin. The endogenous protoporphyrin of the erythrocytes from patients with erythropoietic protoporphyria also hemolyse when exposed to UV-light as found earlier (4, 8, 9, 10, 25) and confirmed in the present study.

Beta-carotene is an efficient orally administered drug against light sensitivity in erythropoietic protoporphyria. The mechanism of its action is not known. It has been suggested that it may act as an internally given sunscreen. In the present investigation we have found that when beta-carotene is put in a cuvette in front of that containing the protoporphyric blood, no significant protective effect is obtained, whereas good protection is found when beta-carotene is added to the same cuvette as the blood when both unfiltered and WGF radiation were used. This clearly shows that with respect to the protective effect of beta-carotene on the light-induced hemolysis of protoporphyric blood, beta-carotene does not act as a filter or sunscreen but in some other way.

It has been shown (25) that in protoporphyrin-induced photohemolysis there is an appreciable oxidation of some amino acid residues in the membranes. The ability of beta-carotene to inhibit photo-oxidation in in vitro, acellular systems (5, 7) makes it seem possible that beta-carotene acts in this way also in its protective effect on a photohemolytic system.

When other photosensitizing substances such as chloropromazin and demethylchlortetracycline were added to normal blood the same effect of beta-carotene was observed when unfiltered radiation but not when window glass filtered radiation was used. In the latter case a pure filter or absorbing effect was noticed. This difference in effect of different types of radiation may mean that beta-carotene is not a universal light protection agent but is effective only under certain circumstances.

To obtain photohemolysis with WGF-radiation (wavelength > 320 nm) a larger amount of radiation is needed than when unfiltered radiation is used, including wavelengths shorter than 320 nm.

The hitherto unexplained so-called inverse photosensitizing effect (11, 12, 13, 23, 24) could be demonstrated also in our photohemolytic system and beta-carotene was found even here to have a protective effect other than as a filter or light absorber.

The concentrations of beta-carotene used in this study have been between 1 and 2 mg per 100 ml, which is within the range found in blood from patients treated with beta-carotene. The protective effect was concentration-dependent in this concentration range.

ACKNOWLEDGEMENT

The present investigation has been supported by the Finsen Foundation.

REFERENCES

1. Cook, J. S. & Blum, H. F.: Dose relationships and oxygen dependence in ultraviolet and photodynamic hemolysis. J Cell Comp Physiol *53:* 41, 1959.
2. Cook, J. S.: Some characteristics of hemolysis by ultraviolet light. J Cell Comp Physiol *47:* 55, 1956.
3. Fitzpatrick, T. B., Pathak, M. A., Parrish, J. A. & Mathews-Roth, M.: Topical and systemic approaches to photoprotection. Proc Roy Soc Med *64:* 861, 1971.
4. Fleischer, A. S., Harber, L. C., Cook, J. S. & Baer, R. L.: Mechanism of in vitro photohemolysis in erythropoietic protoporphyria. J Invest Derm *46:* 505, 1966.
5. Foote, C. S. & Denny, R. W.: Chemistry of singlet oxygen. VII. Quenching by beta-carotene. J Amer Chem Soc *90:* 6233, 1968.
6. Freeman, R. G.: Interaction of phototoxic compounds with cells in tissue culture. Arch Derm (Chicago) *102:* 521, 1970.
7. 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.
8. Harber, L. C., Fleischer, A. & Baer, R. L.: Photohemolysis associated with protoporphyrinaemia. J Invest Derm *42:* 483, 1964.
9. — Erythropoietic protoporphyria and photohemolysis. JAMA *189:* 191, 1964.
10. Hsu, J., Goldstein, B. D. & Harber, L. C.: Photoreactions associated with in vitro hemolysis in erythropoietic protoporphyria. Photochem Photobiol *13:* 67, 1971.
11. Jung, E. G.: In Vitro Untersuchungen zur Chlorpromazine-Photoallergie. Arch Klin Exp Derm *237:* 501, 1970.
12. Jung, E. G.: Inverse chlorpromazine photosensitization and persistent light reaction in guinea-pig. Ref. SID and ESDR joint meeting, May 17–19, 1972, Amsterdam.
13. — Basic mechanism of photoallergic action by phenothiazines and other compounds. VI Intern. Congr. Photobiol. Bochum, Aug. 21–25, 1972.
14. Kahn, G. & Fleischaker, B.: I. Red blood cell hemolysis by photosensitizing compounds. J Invest Derm *56:* 85, 1971.
15. — II. Evaluation of phototoxicity of salicylanilides and similar compounds by photohemolysis. J Invest Derm *56:* 91, 1971.
16. Kesten, B. M.: Urticaria solare. Arch Derm (Chicago) *65:* 221, 1951.
17. Mathews, M. M. & Sistrom, W. R.: Function of carotenoid pigment in non-photosynthetic bacteria. Nature *184:* 1892, 1959.
18. Mathews, M. M.: Protective effect of beta-carotene against lethal photosensitization by haematoporphyrin. Nature *203:* 1092, 1964.
19. Mathews Roth, M.: Carotenoid pigments and photokilling by acridine orange. J Bact *93:* 506, 1967.
20. Mathews-Roth, M. M., Pathak, M. A., Fitzpatrick, T. B., Harber, L. C. & Kass, E. H.: Beta-carotene as a photoprotective agent in erytropoietic protoporphyria. New Engl J Med *282:* 1231, 1970.
21. Mathews-Roth, M. M., Pathak, M. A., Fitzpatrick, T. B. & Kasse, H.: Beta-carotene as a photoprotective agent in erythropoietic protoporphyria—report of 30 treated cases. VI Intern. Congr. Photobiol., Bochum, Aug. 21–25, 1972.
22. Oleniacz, W. S., Singer, E. J., Doyle, A. B. & Vinson, L. J.: Induction of photohemolysis by tetrachlorosalicylanilide. J Pharm Sci *57:* 2136, 1968.
23. Schwarz, K. J.: Experimentelle Untersuchungen zur Photoallergie gegen Sulfanilamid und Chlorpromazin. Dermatologica *139:* Suppl. I, 1969.
24. Schwarz, K.: Basic mechanism of photoallergic actions by sulfanilamides. VI Intern. Congr. Photobiol., Bochum, Aug. 21–25, 1972.
25. Schothorst, A. A., Van Steveninck, J., Went, L. N. & Suurmond, D.: Photodynamic damage of the erytrocyte membrane caused by protoporphyrin in protoporphyria and in normal red blood cells. Clin Chim Acta *39:* 161, 1972.
26. Sistrom, W. R., Griffiths, M. & Stainer, R. Y.: The biology of a photosynthetic bacterium which lacks colored carotenoids. J Cell Comp Physiol *48:* 473, 1956.
27. Swanbeck, G. & Wennersten, G.: Treatment of polymorphous light eruptions with beta-carotene. Acta Dermatovener (Stockholm) *52:* 462, 1972.
28. Willis, I. & Kligman, A. M.: The mechanism of photoallergic contact dermatitis. J Invest Derm *51:* 378, 1968.

Received November 1, 1972

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