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PHOTOHEMOLYTIC ACTIVITY OF TRYPTOPHAN AND PHENYLALANINE METABOLITES

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Abstract. The photohemolytic activity of tryptophan and phenylalanine metabolites has been studied. The experiments were made with both sun-spectrum-like radiation and longwave ultraviolet radiation (UVA). The high photohemolytic activity earlier found by us for kynurenic acid was confirmed in this study. Some other tryptophan metabolites gave a slight degree of hemolysis. The phenylalanine metabolites investigated seemed to be of very minor importance with regard to photohemolytic effect. The photohemolysis of kynurenic acid was proportional to the light dose and increased also linearly with the logarithm of the concentration up to the optimum value. The reaction was oxygen-dependent and inhibited by beta-carotene. When kynurenic acid was irradiated and the erythrocytes added after the exposure, no hemolysis was obtained. The ability of some of the tryptophan metabolites to initiate photochemical reactions in a biological system, here recorded as photohemolysis, may be of importance in diseases with pathological tryptophan metabolism and concomitant light sensitivity where tryptophan metabolites are accumulated in the tissues.

The photochemical properties of tryptophan and several tryptophan metabolites have been extensively studied (1, 2, 6, 8, 9, 12, 17, 18, 27) and some of these compounds may participate in photochemical reactions under physiological conditions. There are some diseases with light sensitivity which have in common an altered tryptophan metabolism, as for instance Hartnup's disease (7, 13), pellagra, carcinoid, hydroxykynureninuria (16) and congenital tryptophanuria (26). Little is known about how these metabolic disturbances may influence the light sensitivity.

The importance of any minor deviation in the tryptophan metabolism has been indicated by Binazzi & Calandra (3, 4, 5) in diseases such as lupus erythematosus and actinic reticuloid.

Swanbeck & Wennersten (23) have presented evidence that kynurenic acid is an endogenous photosensitizer that may be involved in photoallergic reactions.

We have therefore performed a more systematic study of the phototoxic capacity of tryptophan and tryptophan metabolites, but also of some metabolites from the metabolic pathway of phenylalanine.

We have used photohemolytic activity as an established criterion of the phototoxic potential of chemicals with regard to membrane damage. Photohemolysis can be studied in the laboratory under well defined conditions and give reproducible data.

MATERIAL AND METHODS

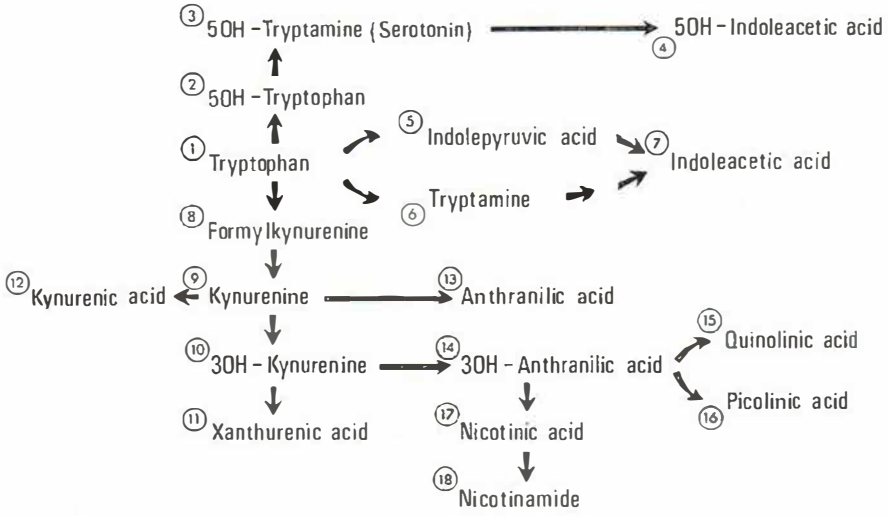
The technique of Kahn & Fleischaker was used (14, 15). Erythrocytes were obtained from healthy human adults and only blood group O Rh+ was used.

Investigated substances were L-tryptophan, 5-hydroxy-L-tryptophan, 5-hydroxytryptamine, 5-hydroxyindole-3-acetic acid, 3-indolepyruvic acid, tryptamine hydrochloride, indole-3-acetic acid, N-formyl-L-kynurenine, DL-kynurenine, 3-hydroxy-DL-kynurenine, xanthurenic acid, kynurenic acid, anthranilic acid, 3-hydroxy-anthranilic acid, quinolinic acid, picolinic acid, nicotinic acid, niacinamid, 3-β-indoleacrylic aid and indoxyl-sulphate (Table I). Also investigated were L-phenylalanine, L-tyrosine, β-phenylpyruvic acid, phenyllactic acid, phenylacetic acid and *o*-hydroxy-phenylacetic acid (Table II). The steric formula for all substances is shown in Table III.

The substances were dissolved in 0.1 M Na-Veronal (barbital) buffer at pH 8.0. Packed human red blood cells were washed three times in physiological saline and 0.1 ml was then added to 10 ml of the buffered solutions of the substances mentioned, and to control solutions which were poured into quartz cuvettes. Controls were incubated in the dark.

The substances were tested at a concentration of 1 mg per 100 ml when sun-spectrum-like radiation was used, which, for several of them, is a physiological concentration. Some potent photosensitizers such as protoporphyrin

Table I. *Main metabolic pathway for tryptophan metabolites*



and chlorpromazin produce an appreciable degree of photohemolysis at this concentration. In the second series of experiments with longwave ultraviolet light (UVA) 100 mg per 100 ml was used. Due to the poor solubility of several of the compounds this would mean a saturated solution with an appreciably lower concentration.

Test solutions were exposed to an Osram High Pressure Xenon Arc Lamp (XBO 150 W) at a distance of 25 cm from the lamp collector aperture in two different ways.

1. The lamp was used with a Schott WG 295 filter, which gives a sun-spectrum-like radiation (SS). Exposure time: 0.6×10^3 sec. Intensity: 28 mW cm^{-2}.

2. The lamp was used with a filter combination that consisted of a Schott WG 295 filter, a KG 1 heat protective filter and a Schott UG 5 filter giving exclusively longwave ultraviolet irradiation (UVA) (Fig. 1). Exposure time: 1.8×10^3 sec. Intensity: 3.4 mW cm^{-2}.

Light doses were measured with a Hewlett-Packard Radiant Flux meter. The irradiated solutions were centrifuged at 2 000 rpm and the optical density of the supernatant fluid was read at 540 nm on a Beckman DB Spectrophotometer and compared with a total hemolysis control and with the dark control (14, 15, 24). Results were expressed in percent relative to the 100% hemolysed solution.

In order to study the oxygen dependence of the photohemolytic reaction the blood was deoxygenated by bubbling nitrogen through the sample. Kynurenic acid at a concentration of 25 mg per 100 ml was used in this experiment and irradiated with UVA.

The effect of beta-carotene at a concentration of 1 mg per 100 ml was investigated under the same experimental conditions but without nitrogen bubbling.

RESULTS

In Table III the steric formula, data of the photohemolytic activity and the absorption peaks in the 300–500 nm region are given. The first column, called SS, corresponding to the experiment with the sun-spectrum-like radiation, and a concentration of test substance of 1 mg per 100 ml, shows that kynurenic acid gives the highest degree of photohemolysis with 17%, followed by anthranilic acid with 12%, 5-hydroxyindoleacetic acid and phenylacetic acid with 7%, and four other substances with 5% photohemolysis.

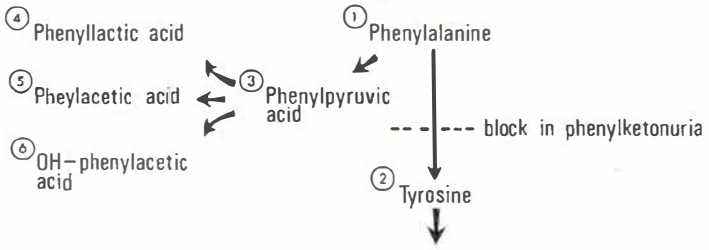
The second column, called UVA, corresponding to the experiment with longwave ultraviolet radiation, and a concentration of test substance of 100 mg per 100 ml or a saturated solution when the solubility was not high enough, shows that kynurenic acid gives about 65% photohemolysis, 3-hydroxykynurenine and 3-hydroxyanthranilic acid 10%, and anthranilic acid 5% photohemolysis.

As shown in the third column, all substances giving photohemolysis with UVA, also have an absorption peak between 300 and 400 nm.

The concentration dependence of the kynurenic acid photohemolysis is illustrated in Fig. 2. A linear relation is obtained up to the maximum level when the degree of hemolysis is plotted against the logarithm of the concentration of kynurenic acid. There is also in all experiments a linear relation between exposure and degree of hemolysis up to the optimum value.

The oxygen dependence of the photochemical reaction was studied by bubbling nitrogen through the sample to deoxygenate the blood. The experi-

Table II. *Main metabolic pathway for phenylalanine metabolites in phenylketonuria, with the metabolic block indicated with a dotted line*



ment was made with 25 mg per 100 ml of kynurenic acid and irradiation with UVA. The degree of photohemolysis was 55 % in the control sample, while only 5 % was found in the deoxygenated blood. By adding 1 mg per 100 ml of betacarotene to the same sample without bubbling nitrogen the degree of photohemolysis was reduced from 55 % to 18 %.

When the kynurenic acid was irradiated and the erythrocytes added after the exposure, no hemolysis was obtained.

DISCUSSION

The experimental conditions chosen in this investigation have been regarded as suitable for screening purposes and it may well be possible to find photohemolytic activity for some of the here inactive compounds under other experimental conditions. However, we find the chosen conditions fairly realistic with regard to what may occur in the human skin under physiological conditions, although the light doses are high.

The high photohemolytic activity earlier found for kynurenic acid by us (23) has been confirmed in the present study. The degree of photohemolysis is proportional to the light dose given but increases linearly with the logarithm of the concentration of the photosensitizer, which is natural as the absorbed amount of light also increases with the logarithm of the concentration.

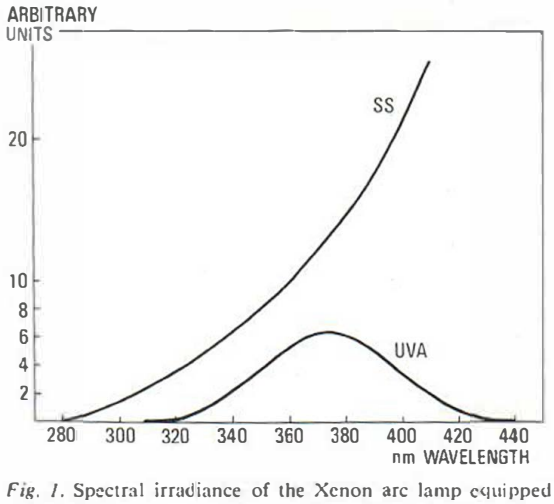


Fig. 1. Spectral irradiance of the Xenon arc lamp equipped with a Schott WG 295 filter which gives a sun-spectrum-like radiation (SS). Exclusively longwave ultraviolet radiation (UVA) is obtained with a filter combination of a Schott WG 295 filter, a KG 1 heat-protecting filter and a Schott UG 5 filter. Relative energy is given in arbitrary units.

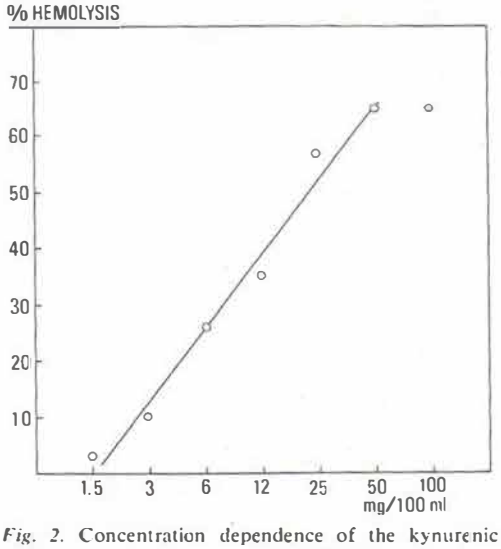


Fig. 2. Concentration dependence of the kynurenic acid photohemolysis. The degree of photohemolysis in percent is plotted against the logarithm of the concentration of kynurenic acid, and a linear relation is obtained up to the maximum level. Exposure with UVA irradiation 30 min.

Table III. *The steric formula for tryptophan and phenylalanine metabolites*

The degree of photohemolysis found is given in percent for two different wavelength ranges called SS, corresponding to sun-spectrum-like radiation, and UVA, corresponding to longwave ultraviolet radiation. The concentration of the test substances, was, in the first column (SS), 1 mg per 100 ml, and in the second column (UVA), 100 mg per 100 ml. In the last column (ABS) is the position of absorption maxima in the 300 to 500 mm range indicated

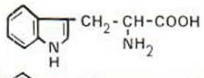
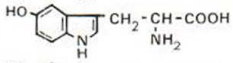
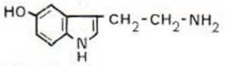
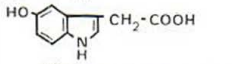
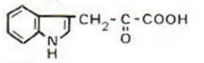
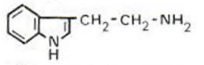
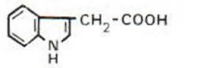
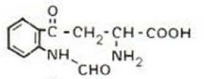
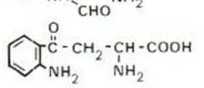
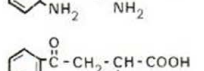
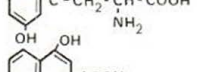
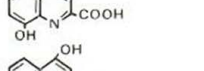
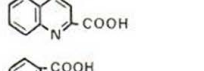
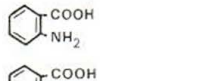
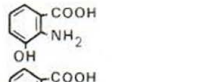
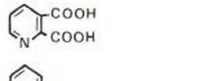
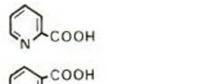
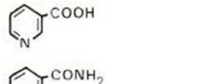
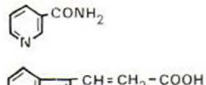
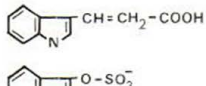
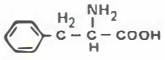
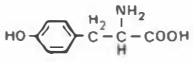
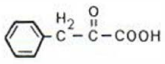
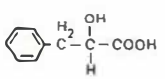
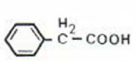
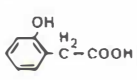
Substance.		% hemolysis		
TRYPTOPHAN METABOLITES		SS	UVA	Abs. max.
① Tryptophan		0	0	
② 5 – Hydroxytryptophan		0	0	
③ 5 – Hydroxytryptamine		0	0	
④ 5 – Hydroxyindoleacetic acid		7	0	
⑤ Indolepyruvic acid		0	0	
⑥ Tryptamine		5	0	
⑦ Indoleacetic acid		0	0	
⑧ Formylkynurenine		5	0	320
⑨ Kynurenine		0	0	355
⑩ 3 – Hydroxykynurenine		0	10	370
⑪ Xanthurenic acid		0	0	330
⑫ Kynurenic acid		17	65	335
⑬ Anthranilic acid		12	5	310
⑭ 3 – Hydroxyanthranilic acid		0	10	320
⑮ Quinolinic acid		0	0	
⑯ Picolinic acid		0	0	
⑰ Nicotinic acid		5	0	
⑱ Nicotinamide		0	0	310
⑲ Indolylacrylic acid		0	0	
⑳ Indoxyl sulfate		0	0	

Table III (*Cont.*)

PHENYLALANINE METABOLITES		% hemolysis		
		SS	UVA	Abs max
① Phenylalanine		0	0	
② Tyrosine		0	0	
③ Phenylpyruvic acid		5	0	
④ Phenyllactic acid		0	0	
⑤ Phenylacetic acid		7	0	
⑥ Hydroxyphenylacetic acid		0	0	

It seems as if the photohemolytic activity of protoporphyrin is dependent on the presence of oxygen (11, 19, 20, 21). In the present study we find that kynurenic acid shows a very low photohemolytic activity in deoxygenated blood and that the activity in oxygenated blood is inhibited appreciably by beta-carotene. The latter fact also indicates that the photohemolytic activity of kynurenic acid is mediated by activated oxygen.

The possibility that by exposing kynurenic acid to UV light a stable photohemolytic substance is formed is ruled out by adding the erythrocytes after the UV exposure, in which case no hemolysis is obtained. It is thus most likely that with kynurenic acid a true photochemical reaction that is mediated by oxygen is injurious to the erythrocyte membrane.

In the last column of Table III the position of absorption maxima in the 300 to 500 nm range is indicated. We regarded absorption maxima outside of this range as being of less importance in photodermatologic problems. As seen in the penultimate column there is no substance that gives photohemolysis with UVA radiation that does not have an absorption maximum in this range. There are, however, some substances that absorb in this range but lack photohemolytic activity with UVA radiation.

The degree of photohemolysis found for some of the other metabolites is so low that it is probably of no importance with regard to photodermatology.

With regard to photohemolytic effect, the phenylalanine metabolites investigated seem to be of very minor importance. They were chosen in consideration of the metabolic block in phenylketonuria.

Many authors have studied the photo-oxidation of tryptophan and the major mechanisms operating seem to be worked out (8, 9, 17, 18, 27).

Upon the irradiation of tryptophan, several products have been identified, and the major reaction products seem to be formylkynurenine, hydroxykynurenine, and kynurenine but many other substances have also been found (1, 2, 6, 12).

Furthermore, it is interesting that excitation of tryptophan and 5-hydroxy-tryptophan at wavelengths longer than 290 and 310 nm respectively, leads to a sensitized splitting of pyrimidine dimers induced in DNA by ultraviolet radiation (10).

The ability of some of the tryptophan metabolites to initiate photochemical reactions in a biological system, here recorded as photohemolysis, may be of importance in diseases where tryptophan metabolites are accumulated in the tissues. An investigation of the effect of beta-carotene on the photosensitivity in such diseases would be of interest. Photochemical reactions may also transfer endogenous proteins to allergens and contribute to autoimmunity as in lupus erythematosus, polymorphous light eruptions and actinic reticuloid. At least in polymorphous light eruptions (22), but probably also for lupus erythematosus treatment with beta-carotene has a beneficial effect (25).

ACKNOWLEDGEMENT

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