

EVIDENCE FOR KYNURENIC ACID AS A POSSIBLE PHOTO-SENSITIZER IN ACTINIC RETICULOID

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Abstract. In the uninvolved skin of a patient having actinic reticuloid lesions, particles with a yellow fluorescence were found in the cytoplasm of the cells of the sweat glands. The patient was practically anhidrotic. The fluorescence and excitation spectra of the particles have been recorded and found to be identical with those of microscopic crystals of kynurenic acid. Kynurenic acid was found to be photochemically active. The action spectrum of the dermatosis and the excitation spectrum of the particles and kynurenic acid seem to be similar. By exposing the uninvolved skin to light from a xenon arc lamp an erythematous infiltrated lesion was obtained. Biopsy 24 hours after exposure showed histologically a lymphocytic infiltrate, mainly around the upper part of the sweat glands. We consider it likely that kynurenic acid is the photosensitizing compound in this patient. It seems most likely, however, that it is predominantly a photoallergic reaction.

Actinic reticuloid, as first defined by Ive et al. (11), is a dermatosis with extreme sensitivity for ultraviolet and visible light. The clinical manifestation is an eczematous and infiltrative dermatitis on light-exposed areas. The infiltrate consists of lymphocytes, lymphoreticular cells and eosinophilic leucocytes. All cases described have been men, where 25 of 28 cases were more than 50 years of age (2, 3, 6, 7, 8, 9, 11, 12, 16, 17).

As it seemed possible to induce actinic reticuloid lesions on any skin region by means of UV light, we found it convenient to study the uninvolved skin of our actinic reticuloid patient in the search for a photosensitizing substance.

METHODS

Microfluorometry

The microfluorometric analysis was carried out at the Institute for Medical Cell Research and Genetics, Karolinska Institutet, Stockholm. It was made on a Zeiss UMSP1 microspectrophotometer converted to microfluor-

rometer (5). The frozen sections were mounted on quartz slides in glycerol.

The spectra were uncorrected for the optical properties of the illuminating and analysing systems but were taken under identical conditions.

Light testing

The light-sensitivity was tested with an Osram high pressure xenon arc lamp XBO 150 W in a Zeiss microscope lamp house with quartz collector. The distance from the patient to the filterholder was 15 cm. The intensity of the beam at this distance without filter is 0.15 W cm^{-2} and with window glass filter 0.11 W cm^{-2} , as measured with a Hewlett & Packard radiant flux meter.

Photochemistry. The technique of Kahn & Fleischaker was used (13, 14). Erythrocytes were obtained from healthy human adults. Kynurenic acid 0.1% and xanthurenic acid 0.1% were soluted 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 mentioned substances, and to control solutions which were poured into quartz cuvettes. Controls were incubated in the dark. Test suspensions of red blood cells were exposed to an Osram high pressure xenon arc lamp (XBO 150 W), unfiltered 10 min ($= 90 \text{ W sec cm}^{-2}$) and through a window glass filter 20 min ($= 132 \text{ W sec cm}^{-2}$). Then the suspensions were centrifuged at 2 000 rpm and the optical density of the supernatant fluid was read at 540 nm on a Beckman DB spectrophotometer. 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 in percent relative to the 100% hemolysed solution.

CASE REPORT

The patient is a 55-year-old, married man with 3 children. He has had atopic dermatitis and asthma from childhood. His atopic dermatitis has always been better in the summer and after sun exposure. In the spring of 1968 he got an acute dermatitis in light-exposed areas on the face and hands, and successively developed an extreme light-sensitivity to sunlight and daylight.

His dermatitis has progressively worsened and when

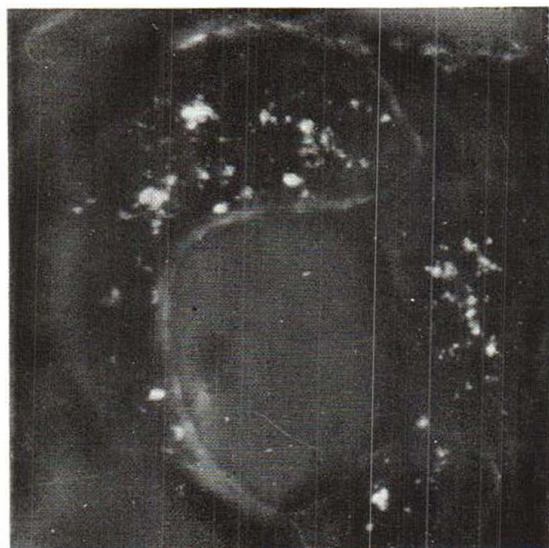


Fig. 1. Fluorescence microscopic picture of a part of a sweat gland. In the centre of the picture the lumen is seen surrounded by the epithelial cells containing the fluorescent granules, appearing white. Approximate magnification, $\times 930$.

seen at the Department of Dermatology he showed an intense redness of the face, capillitium, neck and back of the head, with infiltrated, hard lesions. Similar changes were found on his forearms and on the backs of the hands. There was also a slight redness in two bands over his chest with a pale area over the sternum where his tie had covered. He also experienced light sensitivity through thinner clothes, such as shirts and socks.

He was in good general health and the physical examination revealed no pathological findings. He did not take any medicine orally or parenterally.

Histology (Lagerholm). There was a dense dermal infiltrate, especially in the superficial layer of the dermis with a prevalent localisation around the adnexa and perivascularly. The infiltrate consisted of lymphocytes, lymphoreticular histiocytic cells but eosinophils, plasma cells and fibroblasts were also found. Among the reticulo-histiocytic cells there were a few atypic forms. Histologic examination of biopsy from liver tissue and bone marrow gave a quite normal picture.

Laboratory examinations. Sedimentation rate 20–24 mm. Hemoglobin, leukocyte count, *w.b.c.* differential count, thrombocytes, fibrinogen, coagulation test, serum-iron, serum-creatinine, electrolytes, liver function tests and blood sugar were all within normal values. Wassermann test was negative. Antistreptolysin and antistaphylo-lysin titres were normal, as were autoantibody examinations and protein analysis in serum. Routine urine analysis was normal. Urobilin was found in the urine but not urobilinogen, uro- or coproporphyrins.

Protoporphyrins in red blood cells and porphyrins in faeces were within the normal range. Fluorescence-examinations of capillary red blood cells and bone marrow showed no porphyrin fluorescence.

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Minimal erythema dose (MED) was estimated to $0.15 \text{ W sec cm}^{-2}$ (normal value $2.0 \pm 0.6 \text{ W sec cm}^{-2}$) for unfiltered radiation. When exposed through a window glass filter, a persisting erythema occurred even at an exposure of 7 W sec cm^{-2} (in controls, no reactions are found up to $132 \text{ W sec cm}^{-2}$).

RESULTS

Fluorescence microscopy

In the uninvolved dorsal skin distinctly fluorescent granules were found in the eccrine sweat glands (Fig. 1) and ducts but also on the skin surface in the immediate vicinity of sweat pores. The fluorescence was very strong, and yellow in colour. The size of the granules was estimated to be of the order of a few tenths of a micron in diameter. Similar granules, although fewer and less strongly fluorescent, were also found in the upper part of the dermis, presumably intracellularly, in fibroblasts or histiocytes. The granules in the sweat glands were clearly intracellular and located in the cytoplasm. No fluorescent granules were found in the epidermis. The fluorescence did not show any sign of fading and could be observed weeks later in spite of several minutes' exposure to strong UV-light.

Five minutes' immersion of the sections in ethyl alcohol, water, benzene, pyridine or hexane did not remove the fluorescent material. Even after ordinary formalin fixation and imbedding in paraffin the fluorescent granules could still be seen, although weaker.

In fresh bone marrow smears a considerable number of fluorescent granules were seen here and there in clusters of cells. It was not possible to identify the cell type, since the fluorescence disappears or changes after ordinary staining procedures.

In formalin-fixed paraffin-imbedded liver biopsies, very few granules of this type were seen. They seemed not to be situated in the liver cells, though possibly in Kupffer cells or other cells lining vessels or capillaries. When illuminating the back of the patient with a Wood lamp one can with the help of a lens see a strong yellow fluorescence in the sweat pores.

Microfluorometry

The fluorescence spectrum was registered from a couple of particles and is illustrated in Fig. 3 A. The maximum is about 520 nm. The fluorescence

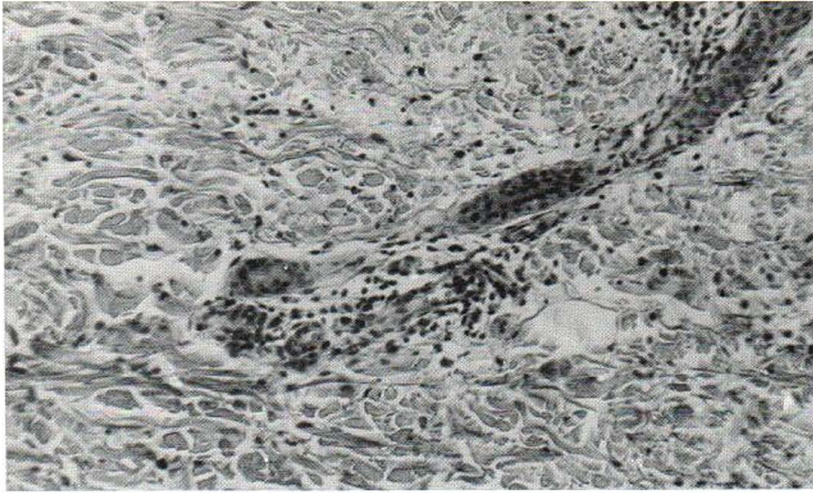


Fig. 2. Hematoxylin-eosin stained section of the upper part of a sweat gland from the previously uninvolved skin 24 hours after xenon arc light exposure. A lymphocytic infiltrate is seen around the sweat gland. Approximate magnification, $\times 90$.

intensity for the background at this wavelength is less than 10% of that of the particles. The excitation spectrum is also illustrated in Fig. 3 A. It shows a maximum at about 370 nm but has not reached the baseline before 450 nm. At the short wave-length end the excitation spectrum has very low values at 300 nm.

The case history of the patient made it unlikely that the fluorescent substance was exogenous. By studying the fluorescence literature and metabolic schemes, we chose xanthurenic acid and kynurenic acid as our candidates for being responsible for the observed fluorescence. By fluorescence microscopy of small crystals from these substances we found that the former did not fluoresce in the visible range when in the solid state. Kynurenic acid crystals, however, had a yellow fluorescence similar to that of the particles in the sections from the patient. A fluorescence spectrum was recorded from one of these microscopic crystals (Fig. 3 B) and was found to be identical with that of the particles found in the sweat glands (Fig. 3). In addition, the excitation spectrum was identical within the errors of the method used.

Photohemolysis

When kynurenic acid 0.1% was exposed, together with erythrocytes, to 90 W sec cm^{-2} we got a photohemolysis of 34%. This experiment was repeated with window glass filtered radiation and the photohemolysis then increased to 56% with an exposure of $132 \text{ W sec cm}^{-2}$, a dose which

normally gives no reactions when applied to the skin of normal controls.

We also investigated blood from our patient who had actinic reticuloid, without adding kynurenic acid but, in contrast to patients with erythropoietic protoporphyria, we obtained no hemolysis.

These experiments were also made with xanthurenic acid but no photohemolytic effect could be demonstrated.

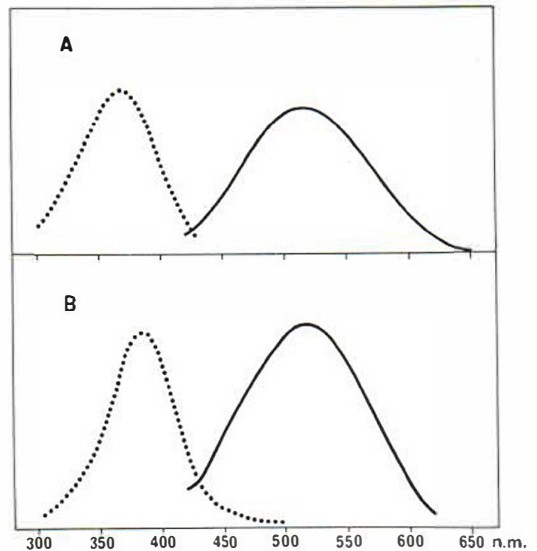


Fig. 3. Fluorescence (—) and excitation (···) spectra of sweat gland granules (A) and microscopic crystals of kynurenic acid (B). The spectra are not corrected for the optical properties of the illuminating and analysing system but are taken under identical conditions.

Studies of sweating

Several attempts were made to collect sweat for chemical analysis. In spite of exercising the patient in a room at 30°C until he fainted, only a few microlitres could be collected. We therefore concluded that the patient was practically unable to sweat. No chemical analysis of sweat could therefore be made.

Provocation of lesions with light

When irradiating the dorsal skin with 3.5 and 6.0 W sec cm⁻² of unfiltered radiation an erythematous infiltrated lesion developed within 24 hours. The lesion thus provoked was biopsied 24 hours after exposure. The infiltrate found was mainly lymphocytic and seemed to be most prominent around the upper half of the sweat glands, as is illustrated in Fig. 2.

DISCUSSION

The finding of abundant, strongly fluorescent granules in the sweat glands in the uninvolved skin of our patient with actinic reticuloid made us suspect that patients with this disease deposit a light-sensitizing substance in the cytoplasm of the sweat gland cells. Some earlier investigators found, fluorescent granules in sweat glands in normal subjects (4) and we have confirmed this in our controls. Whether the fluorescence characteristics are the same for these granules as for those of this actinic reticuloid patient and for kynurenic acid is not known. Further studies will be undertaken, however.

By using a microfluorometer we obtained excitation and fluorescence spectra of a cluster of a few granules in a sweat gland cell. As is seen in Fig. 3, the spectra are distinct and have well-defined maxima. This presented a possibility of checking hypotheses about the nature of the substance in the granules.

On the basis of a study of the biochemical and fluorescence literature, two guesses were made, namely xanthurenic acid and kynurenic acid. Microscopic crystals of the former did not show any fluorescence in the fluorescence microscope. Kynurenic acid, however, showed a strong yellow fluorescence.

As is seen in Fig. 3 the spectra of the microscopic crystals of kynurenic acid very closely cor-

responded to those of the granules. We therefore regard it as likely that the granules are inclusions of kynurenic acid.

In order for kynurenic acid to be a possible photosensitizer in actinic reticuloid it has to be shown that kynurenic acid has phototoxic properties. It is assumed that photoallergenic compounds also have a phototoxic effect (18). The excitation spectrum of kynurenic acid is such that it may well account for the action spectrum of actinic reticuloid.

Photohemolysis study is an established procedure to determine phototoxic potential of chemicals. We found that kynurenic acid is a potent photohemolytically active compound, while xanthurenic acid has no such activity.

The fact that the infiltrate in the light-induced lesions 24 hours after exposure was mainly located around the sweat glands and ducts further supported the hypothesis that the fluorescent granules were implicated in the pathogenesis of actinic reticuloid.

The inability of our patient to sweat may be a manifestation of his old atopic dermatitis but may also be due to a metabolic defect in the sweat gland.

The question whether the photosensitivity in actinic reticuloid is of phototoxic or photoallergic nature is not resolved. The fact that the infiltrate is mainly lymphocytic favours the photoallergic alternative. The low light dose needed to provoke the lesions also points in this direction. The fact that similar fluorescent particles may be found in non-light-sensitive individuals may further support the photoallergic theory. Further studies on this matter are needed, however.

The reason for the deposition of kynurenic acid in the sweat glands has also to be studied. Actinic reticuloid may be caused by a metabolic error similar to that found in other diseases with light sensitivity as for instance in pellagra or in Hartnup's disease (1, 10).

In 1967, Komrower & Westall (15) described one case of hydroxykynurenemia with sensitivity to sunlight. The findings of Binazzi & Calandra (2) may indicate a disturbance in the tryptophan metabolism in actinic reticuloid.

The findings presented in the present paper are based on only one case. However, as the disease is not so common we considered it important to report the present findings.

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Addendum

Since this investigation was made Dr M. Skogh and Dr S. L. Enerbäck in Linköping have been kind enough to send us an embedded biopsy specimen from another case of actinic reticuloid. This specimen was from a lesion and no sweat glands were found in the sections we made. However, in some cells of the dermal infiltrate similar fluorescent granules as described in this paper were found. No spectral analysis of these granules has been made.