

CELLULAR ASPECTS OF PHOTOTOXIC REACTIONS INDUCED BY KYNURENIC ACID

1. Establishment of an Experimental Model Utilizing in Vitro Cultivated Cells

Göran Wennersten and Ulf Brunk

From the Department of Dermatology, Karolinska sjukhuset, Stockholm, and the Department of Pathology, University of Uppsala, Uppsala, Sweden

Abstract. Kynurenic acid is an endogenously occurring tryptophan metabolite, which, under certain conditions, may accumulate in human tissues. In earlier work kynurenic acid showed a pronounced photohemolytic capacity, and conclusive evidence has been presented that the reaction is mediated by singlet oxygen. The aim of the present study was to gain further information about the cellular mechanisms involved in the photo-oxidative damage induced by kynurenic acid. This paper is mainly concerned with the establishment of a proper experimental model utilizing in vitro cultivated cells, but basal cytotoxic effects revealed by the use of cell growth curves, and light- and scanning electron microscopic examinations are also described.

Key words: In vitro cultivated cells, human; Kynurenic acid; Photo-oxidative damage; Electron microscopy, scanning; Cell growth studies

A number of diseases demonstrating light sensitivity have altered tryptophan metabolism in common. Such disorders include Hartnup's disease, pellagra, carcinoid, hydroxykynureninuria (16) and congenital tryptophanuria (27). Little is known of the way in which these metabolic disturbances affect light sensitivity, notwithstanding that the photochemical properties of tryptophan and several tryptophan metabolites have been extensively studied (1, 21). The importance of any minor deviation in tryptophan metabolism has been indicated by diseases such as lupus erythematosus (4), actinic reticuloid (3) and polymorphous light eruptions (15).

Kynurenic acid is an endogenously occurring tryptophan metabolite, which, under certain conditions, may accumulate in human tissues. Evidence was produced in earlier work that kynurenic acid is conceivably an endogenous photosensitizer which

may be involved in photoallergic reactions (24). It is generally assumed that photoallergic compounds also possess phototoxic effects, and further studies proved that kynurenic acid has phototoxic properties as well. These experiments were performed with the photohemolysis technique, an established procedure for the determination of the phototoxic potential of chemicals (14, 19, 22). Kynurenic acid showed a pronounced photohemolytic capacity, and there is conclusive evidence that the reaction is mediated by singlet state excited oxygen (18, 25, 26), an important, active, intermediary in the photo-oxidation of many organic compounds sensitized in various biological systems (17, 23).

Various techniques have been used to evaluate phototoxic reactions in biological systems, including micro-organisms (6), animals (10, 12), erythrocytes (13, 14, 18, 19, 22, 25, 26) and tissue-cultured cells (8, 9, 13). Each of these has specific conditions making its use particularly well suited for selected studies regarding the mechanisms involved in these reactions. The specific cellular target for phototoxic damage may involve various cellular constituents, such as the nucleus, cytoplasmic organelles or the plasma membrane. Photosensitizers may react with any one of these, and often have a biochemical avidity for two or more of these sites. The mechanism of damage may vary with each subcellular site. Red blood cells provide information of value for the study of cell membrane photosensitization because of their anuclear status and lack of cytoplasmic organelles, but this system is a poor screening model for photosensitizing agents with avidity for desoxyribonucleic acid or other cellular

constituents. The use of cultured cells has proved useful for selected aspects of photosensitization and provides a sensitive system for photobiological experiments.

The present work is the first in a planned series of studies intended to elicit further information on the cellular mechanisms involved in the photo-oxidative damage induced by kynurenic acid. This paper is mainly concerned with the establishment of a proper experimental model, and with the description of basal cytotoxic effects revealed by the use of standardized cell culture techniques and by light and scanning electron microscopic examinations.

MATERIAL AND METHODS

Cell lines and culture conditions

All the experiments were performed on normal diploid human glia cells in phase II (11), cultivated *in vitro*. The cells were derived and kept in culture as described by Pontén et al. (20). They were grown in Eagle's minimal essential medium supplemented with 10% calf serum and antibiotics (100 U/ml of penicillin, 50 µg/ml of streptomycin and 1.25 µg/ml of amphotericin B) on the bottom of plastic Petri dishes, or for scanning electron microscopy on 12 mm round glass coverslips no. 0, placed on the bottom of the dishes. The cells were harvested 3 days after subcultivation when they were actively dividing though not yet forming a confluent monolayer. Glia cells were chosen because they have been extensively studied in our laboratories as regards their growth properties and ultrastructural morphology.

Cell growth curves

Growth curves were drawn in order to establish minimal toxic effects of light exposure and of kynurenic acid on the cells *per se*. The cell growth was followed for 4 days after subcultivation. The initial number of cells seeded per 30 mm dish was 7.5×10^4 , counted with an electronic cell counter (Celloscope, AB Lars Ljungberg, Sweden). The following groups were followed: 1) control cells; 2) cells growing in a medium with kynurenic acid added to a concentration of 5.29×10^{-4} M, 1.32×10^{-3} M, or 5.29×10^{-3} M; 3) cells exposed to long-wave ultraviolet light as reported below (exposure times were 0.6×10^3 , 1.2×10^3 and 2.4×10^3 sec); 4) cells exposed to both kynurenic acid and light. Kynurenic acid was added to the medium in a concentration of 5.29×10^{-4} M and the cells were irradiated with long-wave ultraviolet light for 0.6×10^3 sec either immediately following the addition of the acid or after 24 hours.

Ultraviolet light source and conditions of irradiation

The ultraviolet light source was a Black-Ray B-100 a lamp (Ultraviolet Products Inc.) providing long-wave ultraviolet radiation ranging from 350 to 380 nm, intensity maximum at 365 nm. The intensity of the lamp was 12.5

mW/cm², as measured with a Hewlett & Packard Radiant Flux meter. The plastic Petri dishes were placed on a metal plate, 10 cm above the lamp. Thus all irradiation came from below, through the bottom of the plastic dishes. The metal plate was equipped with multiple small holes 5 mm in diameter. This arrangement provides an "internal dark control". Parts of the cell culture are thus shielded from light exposure, and only specific, well demarcated and easily recognized parts are irradiated. The small coverslips used for scanning electron microscopy (SEM) were placed with only half of the cells exposed viz. the central area. In the cell growth studies, however, the whole dish was irradiated. The cultures were cooled by an electric fan in order to avoid heating artefacts and the temperature was thus maintained at 26°C. Before irradiation, the normal medium was replaced with a medium containing kynurenic acid at the final test concentration. The different incubation periods which were used to allow the cells to interact with kynurenic acid are described below. After irradiation, the test medium was immediately replaced by fresh Eagle medium. The cells were observed for 24 hours, or were processed as specified for each experiment. All cells were maintained in a dark incubator except when irradiated. During irradiation, cultures were kept outside the incubator for the same period of time as the irradiated cultures.

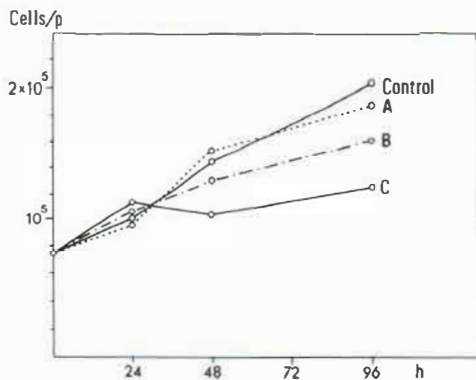
Light microscopy

In these experiments the following concentrations of kynurenic acid were used, 1.32×10^{-3} M, 2.65×10^{-3} M and 5.29×10^{-3} M. The different exposure times to long-wave UV-light applied for each concentration of kynurenic acid were 0.6×10^3 , 1.2×10^3 and 2.4×10^3 sec. Finally different periods of incubation with kynurenic acid were tested before irradiation: in preliminary experiments, 1 min, 2 h, 4 h and 24 h; in final testing, 1 min and 24 h. Cells were harvested for morphologic examination 24 hours post irradiation. The cells were fixed in 5% formaldehyde in 0.15 M cacodylate buffer, pH 7.2 (Histofix special, Histo-Lab AB, Sweden), and were then stained according to Giemsa, air-dried and mounted under coverslips in immersion oil.

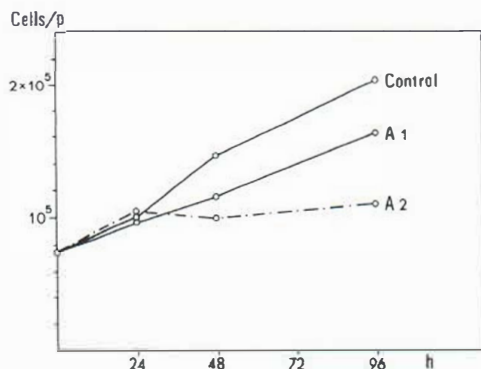
Scanning electron microscopy (SEM)

In the present introductory study only two concentrations of kynurenic acid were used, 2.65×10^{-3} M and 5.29×10^{-3} M. The period of incubation before irradiation was 2 hours. Exposure time was 2.4×10^3 sec.

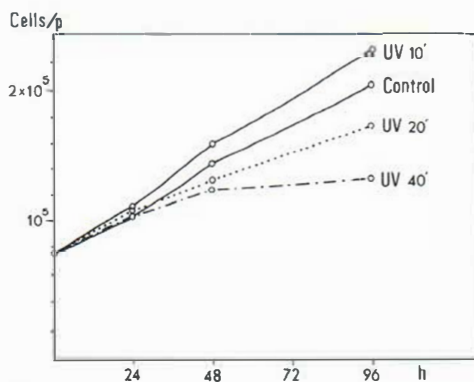
Subsequent to fixation for 60 min at +37°C in 2% glutaraldehyde in 0.1 M Na-cacodylate HCl buffer with 0.1 M sucrose (pH 7.2; total osmolality 510 mOsmol; vehicle osmolality 300 mOsmol) (2), the cells were post-fixed in 2% OsO₄ in *s*-collidine buffer (pH 7.2) for 90 min at 22°C. A short rinse in 0.15 M cac buffer (pH 7.2) at 22°C was interposed between the two fixations. Care was taken not to dry the cells during the replacement of medium with the aforementioned glutaraldehyde fixative, warmed to 37°C. After postfixation, dehydration was performed in a graded series (50%, 70%, 75%, 80%, 85%, 90%, 95%, 100%) of ethanol. The cells were then brought to acetone and critical-point dried from CO₂ in a Polaron E3000 apparatus. After mounting on stubs with silver conductive



1



3



2

Figs. 1–3. Cell growth curves of normal human glia cells. The cells were counted three times after seeding and the result is indicated as number per plate. Fig. 1. Effects of kynurenic acid in different concentrations. No irradiation. A, 5.29×10^{-4} M; B, 1.32×10^{-3} M, and C, 5.29×10^{-3} M. Fig. 2. Effects of long-wave ultraviolet irradiation, no kynurenic acid. UV 10' = 0.6×10^3 sec, UV 20' = 1.2×10^3 sec and UV 40' = 2.4×10^3 sec. Fig. 3. Phototoxic effects of kynurenic acid (5.29×10^{-4} M). The cells were irradiated for 0.6×10^3 sec. Irradiation performed 1 min after the addition of kynurenic acid to the cell culture (A1), or after 24 hours (A2).

paint, the specimens were coated with a 300 Å thick layer of gold in a Polaron sputter apparatus. The specimen were studied in a Jeol JSM-S1 microscope operated at 10 kV.

RESULTS

Cell growth studies

The toxic effect of kynurenic acid *per se* in long-term culture was tested in different concentrations. The highest concentration used, 5.29×10^{-3} M, retarded the growth of the cells. No such significant effects could be demonstrated with the lower concentrations which thus do not seem to be particularly toxic (Fig. 1).

Irradiation with long-wave ultraviolet light caused a certain inhibition of the cellular growth when a long exposure time (2.4×10^3 sec) was used. Shorter exposure times caused no significant growth depression (Fig. 2).

When irradiation was performed immediately after the addition of a very low concentration of kynurenic acid, slight inhibition of cell growth was

registered (Fig. 3, curve A1). When, however, the cells were exposed to the same concentration of kynurenic acid for a period of 24 hours before irradiation the growth inhibition increased (Fig. 3, curve A2).

Light microscopy

The control cultures consisted of glia-like cells with abundant cytoplasm and rounded nuclei containing one or two small nucleoli. The nuclear chromatin was moderately dense and evenly distributed. The cytoplasm contained numerous small granules and had a smooth outline, rounded or polygonal in shape.

No cellular damage was observed following exposure to long-wave ultraviolet light for up to 2.4×10^3 sec when the cells were not treated with kynurenic acid. No effects of kynurenic acid alone in any of the concentrations used was seen in areas shielded from light (Fig. 5).

The increasing cytotoxic effects when cells were exposed to both kynurenic acid and long-wave UV-

Table 1. Phototoxic effects of kynurenic acid on normal human glia cells cultivated *in vitro*

Varying concentrations of kynurenic acid and different light doses were used. Kynurenic acid was added to the cultivating medium 1 min or 24 hours before irradiation

– indicates no changes observable in the light microscope

+++ indicates pronounced changes as illustrated in Fig. 8

Period of preincubation . . .	Concentration of kynurenic acid					
	1.32×10^{-3} M		2.65×10^{-3} M		5.29×10^{-3} M	
	1 min	24 h	1 min	24 h	1 min	24 h
Light exposure time, sec						
0.6×10^3	–	–	–	–	–	–
1.2×10^3	–	–	(+)	+	++	++
2.4×10^3	+	+	++	++	+++	+++

light were clearly noted by gross examination of the cultures (Fig. 4). As only parts of the cultures were exposed, due to the experimental device used during irradiation, an internal dark control was included in every culture exposed.

The effects of different periods of incubation with kynurenic acid in varying concentrations and for different exposure times are compared in Table 1. Threshold conditions for cell damage registered by light microscopy was a concentration of 1.32×10^{-3} M and an exposure time of 2.4×10^3 sec, or a concentration of 2.65×10^{-3} M and an exposure time of 1.2×10^3 sec. Increase in the kynurenic acid concentration and/or light dose resulted in augmented cell damage. The cytotoxic effects observed were cytoplasmic and nuclear condensation, shrinkage of the cell, nuclear pycnosis progressing to cell death, and complete destruction of the irradiated area within 24 hours. Many cells also formed slender cytoplasmic projections, sometimes crossing neighbouring cells. These alterations were increasingly frequent and prominent with higher doses of long-wave ultraviolet light (Figs. 6–8).

No significant differences were registered when the effect of an incubation period of 24 hours with kynurenic acid before irradiation was compared with that of only 1 minute's incubation before irradiation.

Scanning electron microscopy (SEM)

The glia cells in interphase are flattened and thin, with frequent thread-like extrusions from the periphery. The upper cell surfaces are smooth except for a variable number of delicate microvilli and randomly distributed small caveolae and pits, probably representing endocytotic invaginations. The cells

show leading lamella with gracile ruffling membranes, often studded with slender microvilli and possessing occasional longer microspikes. Blebs or blunt extrusions of any kind are never seen on the cells in the extended non-mitotic state (Fig. 9).

Cells incubated with kynurenic acid at the maximum concentration but not irradiated, showed a slight tendency to formation of very small discrete

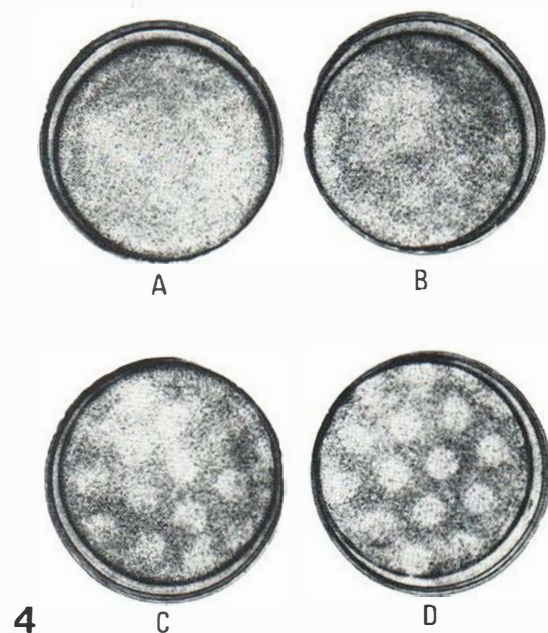
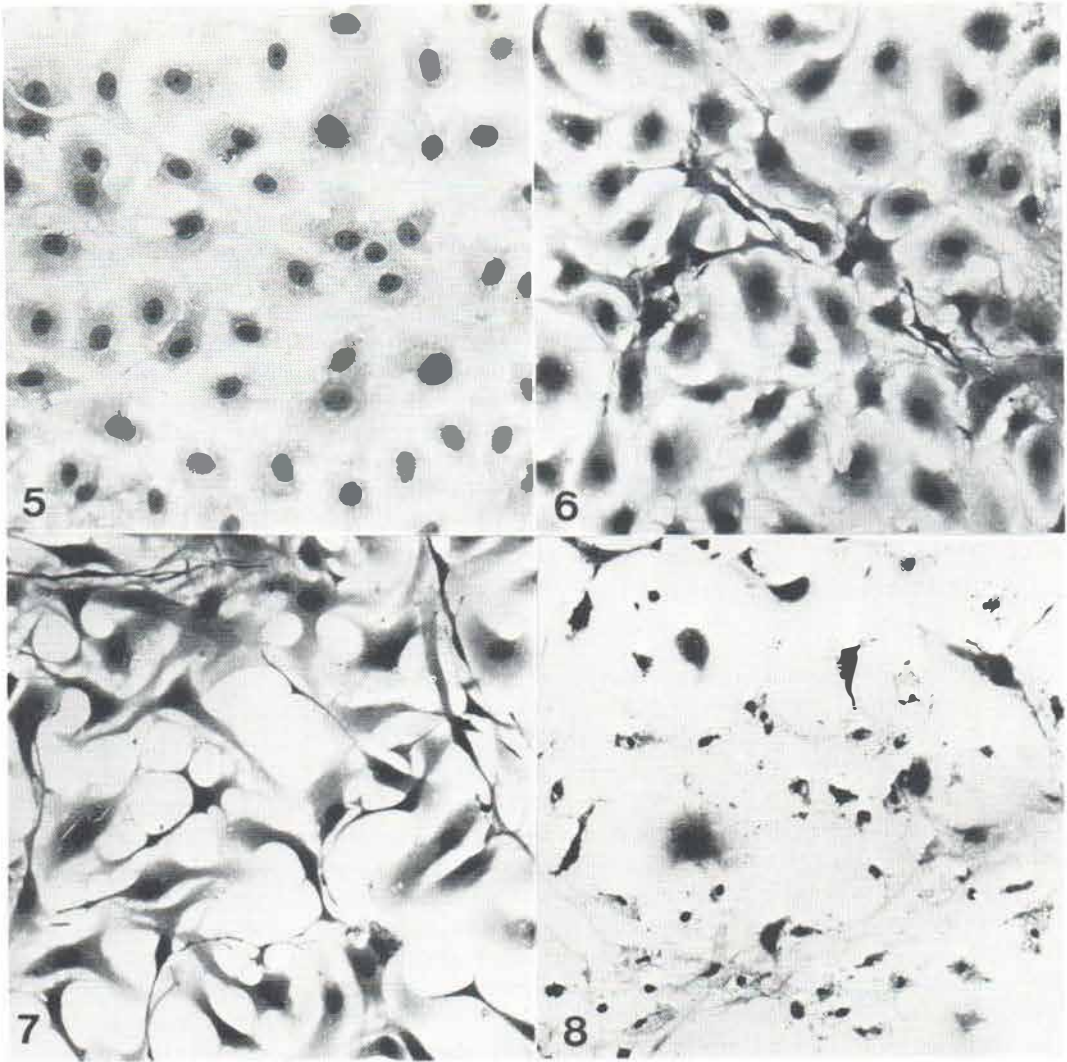


Fig. 4. Petri dishes with irradiated cell cultures (2.4×10^3 sec). Increasing cellular damage is noted with increasing concentrations of kynurenic acid. A clear-cut difference between irradiated and shielded cells is macroscopically visible. A, no kynurenic acid; B, kynurenic acid in a concentration of 1.32×10^{-3} M; C, kynurenic acid in a concentration of 2.65×10^{-3} M; D, kynurenic acid in a concentration of 5.29×10^{-3} M.



Figs. 5-8. Light micrographs of glioma cells treated with kynurenic acid. Figs. 6-8 show cells also exposed to long-wave UV-light with an exposure time of 2.4×10^3 sec. The cultures were irradiated 1 min after addition of kynurenic acid and fixed 24 hours post irradiation. Cellular damage following irradiation is increased with the concentration of kynurenic acid. Obvious cytotoxic effects are cytoplasmic and nuclear condensation, shrinkage of

the cells, slender cytoplasmic projections and nuclear pycnosis progressing to cell death and complete destruction of the irradiated area. $\times 190$.

Fig. 5. Kynurenic acid, 5.29×10^{-3} M, no UV-light.

Fig. 6. Kynurenic acid, 1.32×10^{-3} M, + UV-light.

Fig. 7. Kynurenic acid, 2.54×10^{-3} M, + UV-light.

Fig. 8. Kynurenic acid, 5.29×10^{-3} M, + UV-light.

blebs at the cell surface, but the cells were still thin and flattened and normally attached to the substrate, with microvilli and other structures well preserved. Similar findings were noted after exposure to long-wave ultraviolet light at the highest dose used when the medium contained no kynurenic acid.

Following exposure to both kynurenic acid and long-wave ultraviolet light, cells were uniformly damaged. No convincing differences were found between cells incubated for 24 hours with kynurenic acid before irradiation compared with those exposed to long-wave UV-light already 1 min after the addition of kynurenic acid. Alterations were noted

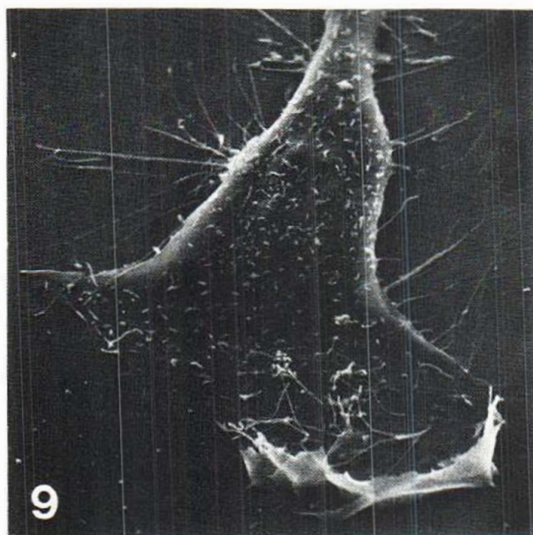


Fig. 9. Scanning electron microscope picture of a control glia cell. Note well developed ruffling membranes. $\times 1\,200$.

in specimens harvested immediately after the irradiation was completed. The damage was more pronounced at higher concentrations of kynurenic acid but it also increased with the time elapsed post irradiation.

Typical changes consisted in shrinkage, rounding up of the cells and formation of long slender cytoplasmic projections. A prominent feature was the formation of cytoplasmic blebs all over the surface of the cell. This was noted in all cells and in all stages of cellular destruction, and was often of enormous extent. The ruffling membranes were severely damaged and converted into a collection of small bubbles. The condensed cells left delicate trailing retraction fibrils in the areas where cell contact with the Petri dish surface was lost. Typical alterations are depicted in Figs. 10–15.

DISCUSSION

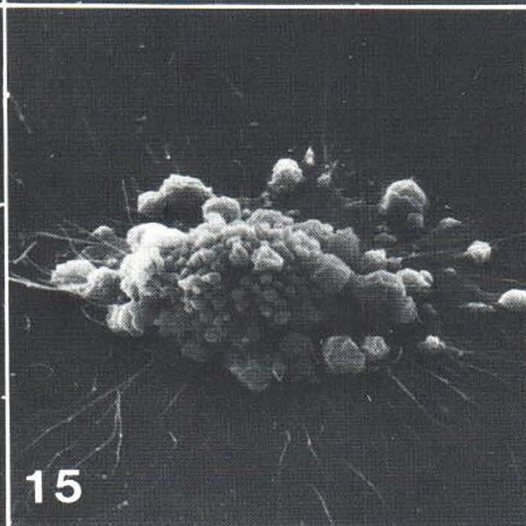
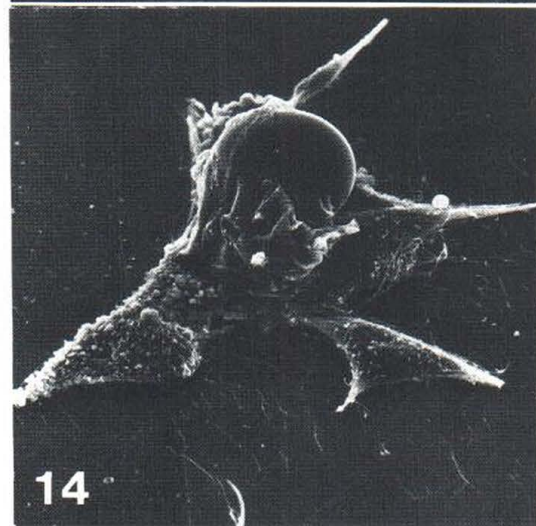
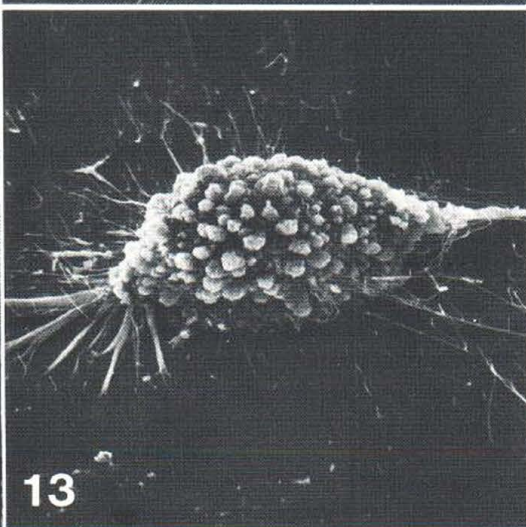
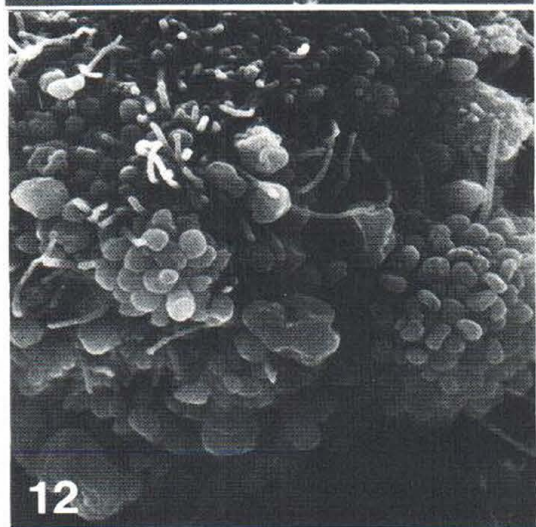
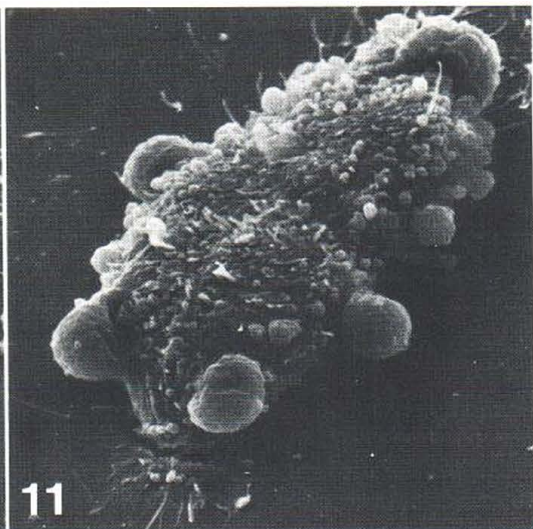
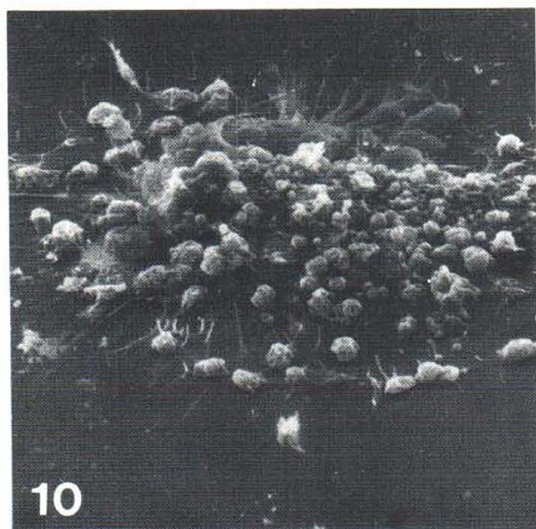
The tryptophan metabolite, kynurenic acid, has been shown to be photohemolytically active (18, 25, 26). In photodynamic action, two major primary mechanisms are found to occur. Direct reaction between sensitizer triplets and the oxidizable substrate, followed eventually by reaction with molecular oxygen or reaction between oxidizable substrate and singlet state excited oxygen, which has been generated by energy transfer from the triplet state

of the photosensitizing substance. Which pathway predominates is mainly dependent on the relative effectiveness of singlet oxygen generation as compared with the rate of direct reaction between sensitizer triplets and oxidizable substrate. Mixed mechanisms are also conceivable (7, 23). Evidence obtained in earlier investigations strongly support the hypothesis that photo-oxidative membrane damage observed in the presence of kynurenic acid is mediated by singlet oxygen (18, 26).

The present work is the first in a planned series of studies intended to elucidate possible specific mechanisms acting during photo-oxidative damage induced by kynurenic acid. This paper reports on the establishment of an experimental mode utilizing cells cultivated *in vitro*, and on basic preliminary findings as regards suitable concentrations of kynurenic acid and doses of light using cell growth curves and light- and scanning electron microscopy.

The use of cells cultivated *in vitro* appeared to be necessary, due to the requirement of a well controlled, easily manipulated system for studies on selected aspects of photosensitization. The use of *in vitro* cultivated cells seems to offer several advantages as compared with experimental animals. Cells in solid tissues are inhomogeneous in respect to stage in the cell cycle, and may also vary in their nutritional state, for instance due to differences in blood supply within different areas. It is also difficult to obtain uniform and reproducible fixation of cells in tissues because of variation in the penetration and distribution of the fixative. The glia cells used in the present report have been extensively studied in our laboratories, especially with regard to their ultrastructural characteristics and growth pattern (5, 30). These cell lines also show

Figs. 10–15. Figs. 10–15 depict surface changes of kynurenic acid and long-wave ultraviolet light treated cells as shown in the scanning electron microscope. The cell surfaces demonstrate varying degrees of blebbing and shrinkage, with well developed retraction fibrils. Most of the smaller vesicles and blebs seem to originate from altered microvilli, best seen in Fig. 12. Figs. 10–12 show alterations following treatment with 2.65×10^{-3} M kynurenic acid and 2.4×10^3 sec irradiation with long-wave ultraviolet light 10 min, 90 min and 6 hours after exposure to light (Fig. 10, $\times 1\,750$; Fig. 11, $\times 2\,000$; Fig. 12, $\times 5\,000$). Figs. 13–15 show alterations following treatment with 5.29×10^{-3} M kynurenic acid and 2.4×10^3 sec irradiation with long-wave ultraviolet light 10 min, 30 min and 3 hours after exposure to light (Fig. 13, $\times 1\,500$; Fig. 14, $\times 1\,000$ and Fig. 15, $\times 2\,000$).



very pronounced topo-inhibition of cell growth, and in a dense monolayer they are almost totally arrested in the G1-phase of the cell cycle.

In the present study pronounced phototoxic damage could be induced by kynurenic acid and irradiation with long-wave ultraviolet light. Minimal threshold conditions were established by the use of cell growth curves. Phototoxic inhibition of cell growth was demonstrated with fairly low concentrations of kynurenic acid and rather low light doses. No noxious influence on cell growth by either kynurenic acid or long-wave ultraviolet light *per se* was noted under these minimal conditions. However, some inhibition of cellular growth, but no killing, by light and kynurenic acid alone in the maximum conditions used in further experiments was noticed.

To induce cellular damage, visible in the light microscope, greatly increased doses of both kynurenic acid and light were demanded.

Scanning electron microscopy revealed various degrees of bleeding of the cell surface as a characteristic finding in the completely exposed cultures. The degree of damage varied with the doses applied. The blebs seemed to originate in microvilli and ruffling membranes. Such structures thus seem to be especially vulnerable, which is also in agreement with findings following hypotonic shock when the same structures first display alterations (2). Such blebbing is a well known non-specific reaction of cells to a wide variety of injuries (29). Exposure to high concentrations of kynurenic acid alone produced slight swellings of the tips of many microvilli. These alterations, however, were readily distinguishable from the often enormous blebs induced in the phototoxic experiments.

Whether or not kynurenic acid acts solely from the outside or following diffusion into the cells or by active uptake, e.g. by way of endocytosis, is not known for the moment. However, the somewhat more pronounced growth retardation in cultures which have been incubated with kynurenic acid for 24 hours before irradiation, compared with those incubated for only 1 minute before irradiation, gives some hint of potentiation following certain uptake of the kynurenic acid with consequent enhancement of the effect.

Lytic change in cell membranes induced by phototoxic substances involves oxidation of either the protein or lipid components of the membrane, or both (22, 23). Inasmuch as membrane lipids are

capable of undergoing peroxidative reactions, one of the effects of singlet oxygen may be the formation of lipid peroxides and subsequent development of free radicals leading to membrane damage (28, 29).

This report is a preliminary one concerned mainly with the establishment of an experimental model. The reported results, however, stress the importance of plasma membrane alterations. Further study including scanning- and transmission electron microscopy is required to elucidate the mechanisms underlying the observed alterations.

ACKNOWLEDGEMENTS

This work was supported by the Finsen Foundation, Research Funds of the Karolinska Institutet, and the Swedish Medical Research Council (project 4481).

REFERENCES

1. Asquith, R. S. & Rivett, D. E.: Studies on the photo-oxidation of tryptophan. *Biochim Biophys Acta* 252: 111, 1971.
2. Bell Jr, P. B., Brunk, U., Collins, P., Forsby, N. & Fredriksson, B.-A.: SEM of *in vitro* cultivated cells, osmotic effects during fixation. *In Proc. 8th Annual Scanning Electron Microscope Symposium* (ed. O. Johari & I. Corvin), pp. 379-386, IIT Research Institute, Chicago, 1975.
3. Binazzi, M. & Calandra, P.: Actinic reticuloid: Pathogenic aspects. *Arch Derm Forsch* 241: 391, 1971.
4. — Tryptophan-niacin pathway in lupus erythematosus. *Acta Vitaminol Enzymol (Milano)* 25: 205, 1971.
5. Brunk, U., Ericsson, J., Pontén, J. & Westermarck, B.: Specialization of cell surfaces in human glia-like cells. *Exp Cell Res* 67: 407, 1971.
6. Daniels, F.: A simple microbiological method for demonstrating phototoxic compounds. *J Invest Dermatol* 44: 259, 1965.
7. Foote, C. S.: Mechanisms of photosensitized oxidation. *Science* 162: 963, 1968.
8. Freeman, R. G., Murtishaw, W. & Knox, J. M.: Tissue culture techniques in the study of cell photobiology and phototoxicity. *J Invest Dermatol* 54: 164, 1970.
9. Fritsch, P., Gschnait, F., Hönigsmann, H. & Wolff, K.: Protective action of beta-carotene against lethal photosensitization of fibroblasts *in vitro*. *Br J Dermatol* 94: 263, 1976.
10. Harber, L. C.: Use of guinea-pigs in photobiologic studies. *In The Biologic Effects of Ultraviolet Radiation* (ed. F. Urbach), pp. 291-293, Pergamon Press, London, 1969.
11. Hayflick, L.: Senescence and cultured cells. *Persp Exp Gerontol* 14: 195, 1966.
12. Ison, A. & Blank, H.: Testing drug phototoxicity in mice. *J Invest Dermatol* 49: 503, 1967.

13. Johnson, B. E.: Cellular mechanisms of chlorpromazine photosensitivity. *Proc Roy Soc Med* 67: 871, 1974.
14. Kahn, G. & Fleischaker, B.: I. Red blood cell hemolysis by photosensitizing compounds. *J Invest Dermatol* 56: 85, 1971.
15. Kobori, T., Toda, K., Araki, H., Ikemura, I., Yoshii, T. & Hasegawa, K.: Polymorphous light eruptions in Japan. *In* Sunlight and Man (ed. T. B. Fitzpatrick, M. A. Pathak, L. C. Harber, M. Seiji & A. Kukita), pp. 695-710, University of Tokyo Press, 1974.
16. Komrower, G. M. & Westall, R.: Hydroxy-kynureninuria. *Am J Dis Child* 113: 77, 1967.
17. Nilsson, R., Merkel, P. B. & Kearns, D. R.: Unambiguous evidence for the participation of singlet oxygen in photodynamic oxidation of amino acids. *Photochem Photobiol* 16: 117, 1972.
18. Nilsson, R., Swanbeck, G. & Wennersten, G.: Primary mechanisms of erythrocyte photolysis induced by biological sensitizers and phototoxic drugs. *Photochem Photobiol* 22: 183, 1975.
19. Oleniacz, W. S., Singer, E. J., Doyle, A. B. & Vinson, L. J.: Induction of photohemolysis by tetrachlorosalicylanilide. *J. Pharm Sci* 57: 2136, 1968.
20. Pontén, J. & MacIntyre, E. H.: Long term culture of normal and neoplastic human glia. *Acta Path Microbiol Scand* 74: 465, 1968.
21. Santus, R. & Grossweiner, L. I.: Primary products in the flash photolysis of tryptophan. *Photochem Photobiol* 15: 101, 1972.
22. Schothorst, A. A., van Steveninck, J., Went, L. N. & Suurmond, D.: Photodynamic damage of the erythrocyte membrane caused by protoporphyrin in protoporphyria and in normal red blood cells. *Clin Chim Acta* 39: 161, 1972.
23. Spikes, J. D. & Livingston, R.: The molecular biology of photodynamic action: Sensitized photoautooxidation in biological systems. *In* Advances in Radiation Biology (ed. L. G. Augenstein, R. Mason & M. Zelle), vol. 3, pp. 29-121. Academic Press Inc., New York, 1969.
24. Swanbeck, G. & Wennersten, G.: Evidence for kynurenic acid as a possible photosensitizer in actinic reticuloid. *Acta Dermatovener (Stockholm)* 53: 109, 1973.
25. — Photohemolytic activity of tryptophan and phenylalanine metabolites. *Acta Dermatovener (Stockholm)* 54: 99, 1974.
26. Swanbeck, G., Wennersten, G. & Nilsson, R.: Participation of singlet state excited oxygen in photohemolysis induced by kynurenic acid. *Acta Dermatovener (Stockholm)* 54: 433, 1974.
27. Tada, K., Ito, H., Wada, Y. & Arakawa, T.: Congenital tryptophanuria with dwarfism. *Tohoku J Exp Med* 80: 118, 1963.
28. Tappel, A. L.: Lipid peroxidation and fluorescent molecular damage to membranes. *In* Pathobiology of Cell Membranes, (ed. B. F. Trump & A. U. Arstila), vol. I, pp. 145-170. Academic Press Inc., New York, 1975.
29. Trump, B. F. & Arstila, A. U.: Cell membranes and disease precesses, *In* Pathobiology of Cell Membranes (ed. B. F. Trump & A. U. Arstila), vol. I, pp. 1-103. Academic Press Inc., New York, 1975.
30. Westermark, B.: Proliferation control of cultivated human glia-like cells under "steady state" conditions. *Exp Cell Res* 69: 259, 1971.

Received March 15, 1976

G. Wennersten, M.D.
Department of Dermatology
Karolinska sjukhuset
S-104 01 Stockholm 60
Sweden