Acta Dermatovener (Stockholm) 58: 383–389, 1978

ULTRASTRUCTURAL CHANGES IN NUCLEI AND NUCLEOLI IN PSORIATIC EPIDERMIS DURING AND AFTER PUVA THERAPY

Takuo Tsuji and Alvin J. Cox

Department of Dermatology, Stanford University School of Medicine, Stanford, California, USA

Abstract. Quantitative and qualitative changes of the nuclei and nucleoli in psoriatic epidermis at different intervals during therapy with psoralen and long-wave ultraviolet light (PUVA) were revealed with the electron microscope. Early in the therapy (24 to 96 hr after commencing treatment) changes occur, including irregularity of the nuclear envelope, enlargement of the nuclei and nucleoli, temporary disappearance of fibrillar centres, followed by temporary increase in nuclear bodies and increasing amounts of heterochromatin. After clearing of lesions, the nuclear and nucleolar structures were identical with those of normal keratinocytes, except that the relative amount of heterochromatin was greater. It is suggested that PUVA therapy brings about inhibition of abnormal keratinization, possibly due to gene repression, and that this may be related to changes in the fibrillar centres of psoriatic keratinocyte nucleoli.

Key words: Psoriasis; PUVA therapy; Nuclear changes; Gene repression; Fibrillar centres

There are several published reports of successful therpy of psoriasis with oral psoralens and long-wave ultraviolet light (UVA), as well as a wide variety of studies on the effects of these agents. There is evidence to suggest that the therapy acts by a direct effect on DNA. An increased frequency of chromosome aberrations in human lymphocytes (10, 11) and the induction of DNA repair synthesis in human fibroblasts (1) have been reported after 8-methoxypsoralen (8-MOP) plus UVA therapy (PUVA) in vitro.

In view of this knowledge, it is important that studies should be conducted on nuclear changes in connection with PUVA therapy of psoriasis patients.

The purpose of the present study was to define the ultrastructural alterations in psoriatic keratinocyte nuclei during and after PUVA therapy. As far as we know, there is no other published detailed description of these changes.

MATERIALS AND METHODS

The material consisted of biopsy specimens from 17 patients who had had extensive and long-standing psoriasis for several years. Patients with pustular psoriasis were not admitted to the study. The ages of the patients ranged from 17 to 57 years. Prior to this investigation, the lesions had not been treated for at least 1 month.

Between 30 and 60 mg of 8-methoxypsoralen (8-MOP) was given 2 hr prior to the UVA exposure, depending upon the patient's body weight. The lesions were exposed to long-wave ultraviolet light with a peak range of 360–370 nm, from General Electric high output blacklight lamps (no. F72T12/BL/HO). The initial treatment was from 0.5 to 2.5 Joules/cm^2, depending upon the skin type. The patients were treated 2 or 3 times per week. Three specimens were taken from each patient: 1) before treatment, 2) 24, 48, 72 or 96 hr after the first treatment, and 3) one week after the lesions had cleared clinically. The tissue specimens were fixed in 2% osmium tetroxide solution in 0.1 M phosphate buffer at 4°C for 2 hr. Following dehydration in a graded series of ethanol dilutions, the blocks were embedded in Epon 812. Ultrathin sections were cut on a Porter-Blum MT-2 ultramicrotome. Sections were stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop 1 A electron microscope.

Thirty spinous cell nuclei, each containing one or more nucleoli, were selected at random from each specimen. Each nucleus was photographed at magnifications of 4 000 and 10 000. Our attention was concentrated upon the spinous cell nuclei, since basal cells are germinative cells whose nuclei show various aspects, depending on their cell cycle phase, and because granular cells show distinct changes in nuclei during keratinization. The spinous cells studied were always at least three cells away from the basal lamina. The area of each nucleus and nucleolus was measured with a planimeter. The relative amounts of heterochromatin were estimated by a photographic method by which the image of a fine-meshed grid was superimposed on the nuclear image and the structures beneath the points of intersection of grid lines were

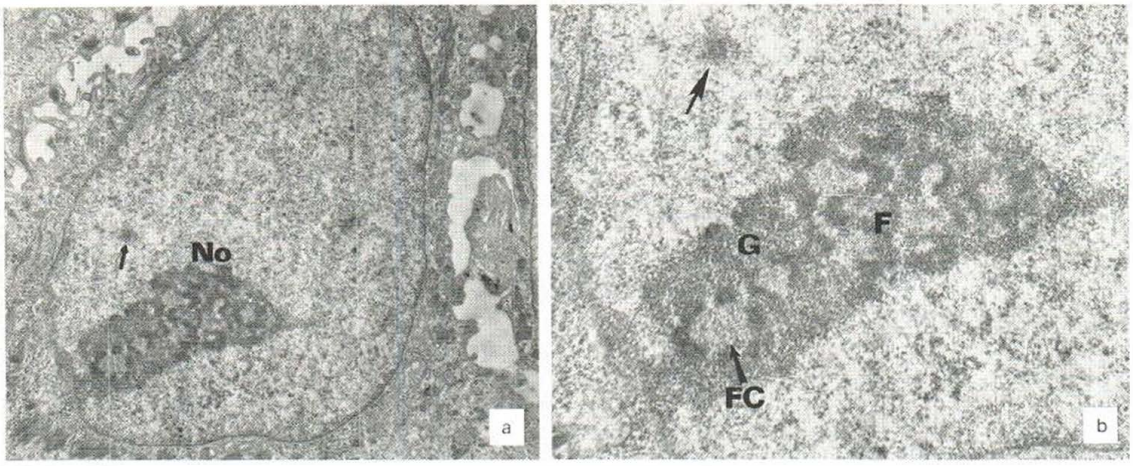

Fig. 1. A psoriatic keratinocyte nucleus and nucleolus before therapy. (*a*) The nuclear envelope is smooth, heterochromatin is sparse and a nuclear body (→) can be seen. *No*, nucleolus. (×3400.) (*b*) The nucleolus shows distinct nucleolonema which consist of a granular component (*G*) and fibrillar centres (*FC*) surrounded by a fibrillar component (*F*). →, nuclear body. (×11000.)

identified to provide an expression of the proportion of dense chromatin in each nucleus. The number of nuclear bodies in these nuclei was also counted.

RESULTS

Qualitative study

Spinous cell nuclei in psoriatic lesions before PUVA therapy are characterized as reported previously (13) as follows: (*a*) the nuclei and nucleoli are much larger than those of the normal keratinocytes, respectively, (*b*) the nuclear envelope is smooth, (*c*) less heterochromatin can be seen than in normal keratinocytes, (*d*) nuclear bodies can often be observed in the nucleoplasm, and (*e*) the nucleoli show a distinct nucleolonema which consists of a granular component, a fibrillar component and fibrillar centres surrounded by the fibrillar component (Figs. 1 *a* and *b*).

By 24 hr after the first treatment, the nuclear

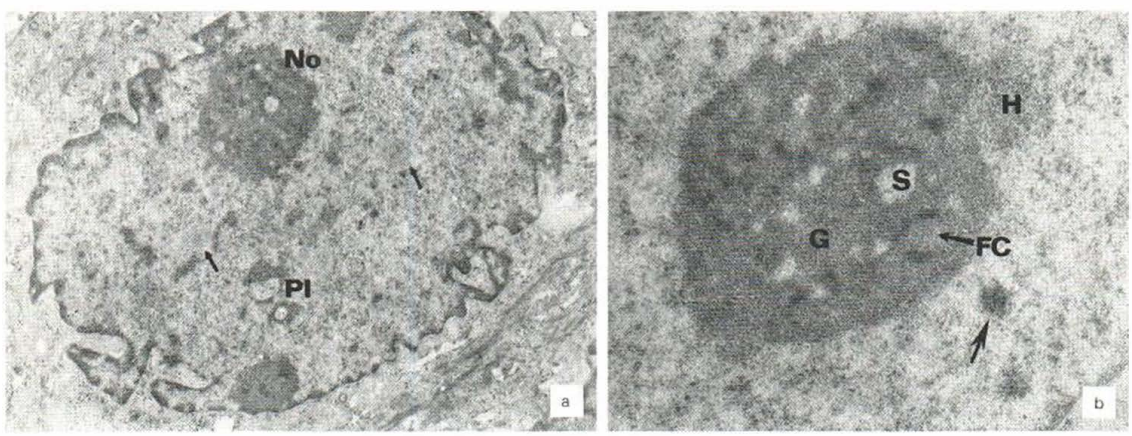

Fig. 2. A nucleus and nucleolus at 24 hr after 1st treatment. (*a*) The nuclear envelope is irregular, showing zigzag contours and nuclear pseudoinclusions (*Pl*). Heterochromatin granules are aggregated along the periphery of the nucleus, around the nucleolus (*No*) and in some central parts of the nucleus. →, nuclear bodies showing edematous change. (×3000.) (*b*) The nucleolus retains a nucleolonema with fibrillar centres (*FC*), some of which are smaller than those before therapy. *G*, granular component; *H*, heterochromatin granules; →, nuclear body; *S*, internucleolonemal spaces. (×10000.)

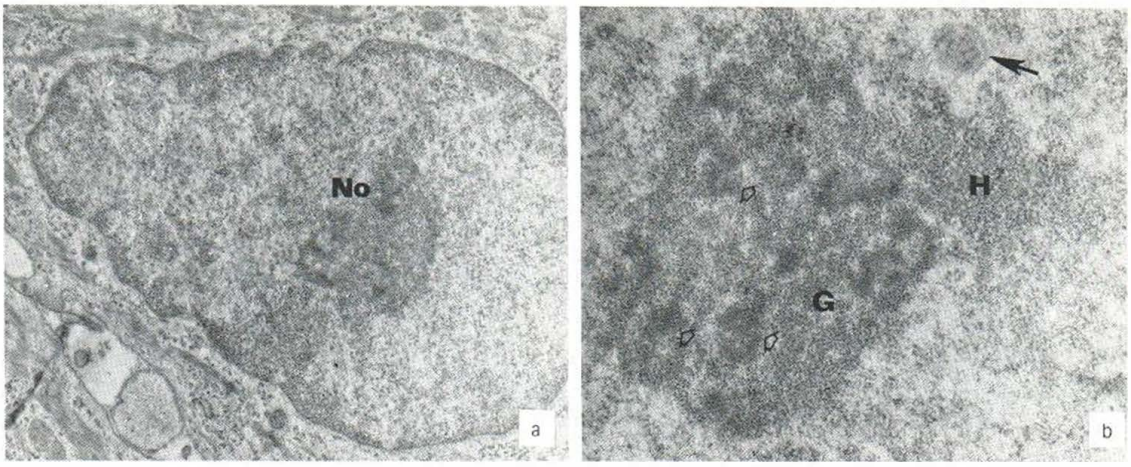

Fig. 3. A nucleus and nucleolus at 48 hr after 1st treatment. (*a*) The nuclear envelope is still irregular. Heterochromatin granules appear to be increased in all parts of the nucleus. *No*, nucleus. (×3 000.) (*b*) The nucleolus consists of granualr (*G*), and fibrillar components showing a globular form (white arrows). Few fibrillar centres can be seen. H, heterochromatin granules; →, nuclear body. (×20 000.)

structure had altered distinctly. The size of the nuclei was increased. The nuclear envelope had become irregular, exhibiting zigzag contours along the entire periphery of the nucleus. Cross sections of surface invaginations sometimes revealed nuclear pseudoinclusions (Fig. 2*a*). Heterochromatin granules were aggregated along the periphery, around the nucleolus and in some central parts of the nucleus. Perichromatin granules were increased in number, compared with those before therapy. Nuclear bodies were often seen. Most of these had a homogeneous or fine filamentous structure, showing little edematous change. Nucleoli retained a distinct nucleolonema with fibrillar centres, although some fibrillar centres were smaller than those before therapy. Enlargement of internucleolonemal

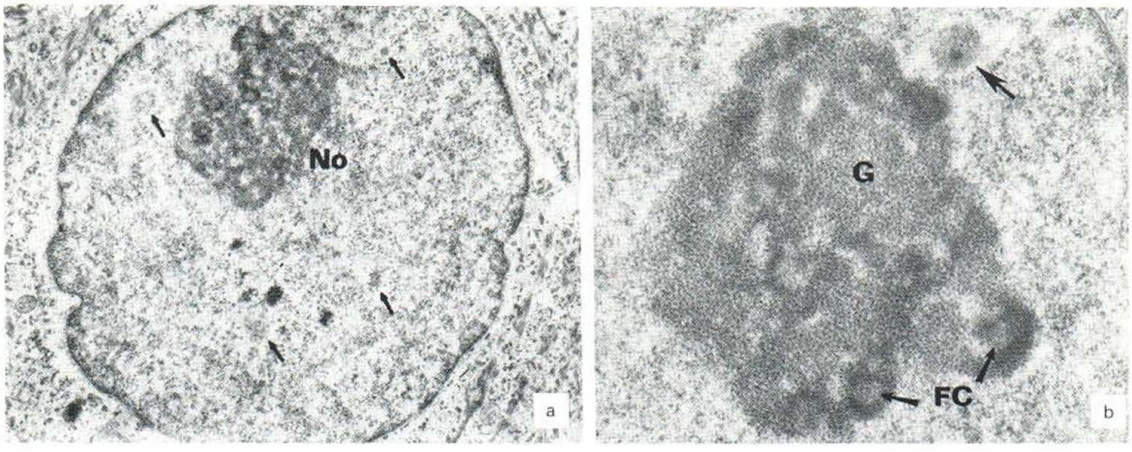

Fig. 4. A nucleus and nucleolus at 72 hr after 1st treatment. (*a*) The nuclear envelope is fairly regular. Heterochromatin granules are increased throughout the nucleus. Multiple nuclear bodies (→) are present. *No*, nucleolus. (×3 000.) (*b*) Fibrillar centres (FC) are present, mainly at the periphery of the nucleolus, although they are smaller than those seen before therapy. A nuclear body (→) close to the nucleolus can be seen. *G*, granular component. (×10 000.)

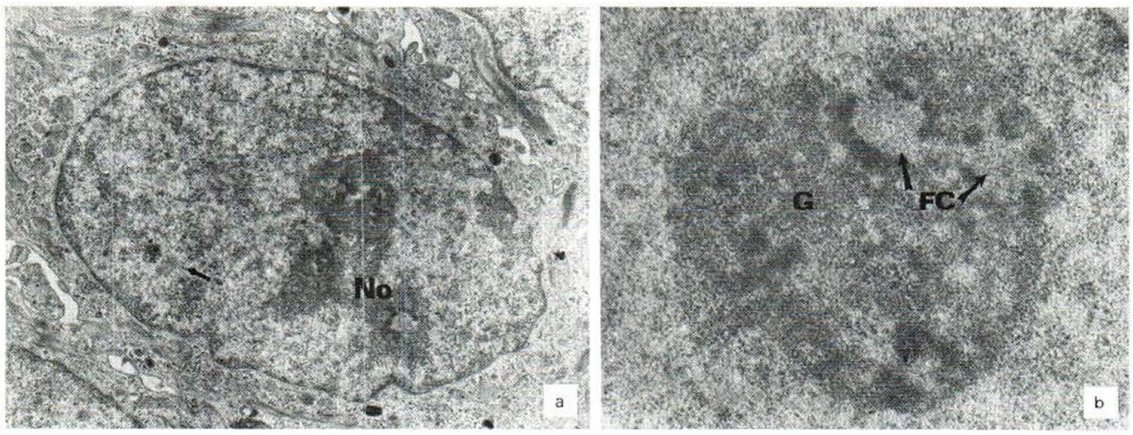

Fig. 5. A nucleus and nucleolus at 96 hr after 1st treatment. (*a*) The nuclear envelope is mostly smooth. Heterochromatin granules seem to be decreased, as compared with those at 72 hr after 1st treatment. *No*, nucleolus; →, nuclear body. (×3 000.) (*b*) Two large fibrillar centres (*FC*) can be seen. *G*, granular component. (×10 000.)

spaces was also seen. However, no changes were found in the fibrillar and granular components of the nucleolus (Fig. 2*b*).

At 48 hr after the first treatment, the nuclear envelope was still irregular, but nuclear pseudoinclusions, as seen at 24 hr after the first treatment, were no longer observed. Heterochromatin granules appeared to be increased, not only along the periphery and around the nucleoli, but also in the central areas of the nucleus (Fig. 3*a*). In most of the nucleoli, no fibrillar centres could be found and the nucleoli consisted of a fibrillar and a granular component. The fibrillar component was seen in a globular form (Fig. 3*b*). Nuclear bodies seemed to be increased in number at this time.

At 72 hr after the first treatment, the nuclear envelope became relatively regular. Heterochromatin granules were increased throughout the nucleus, together with an increase in perichromatin granules. Nuclear bodies were seen more often than at 48 hr

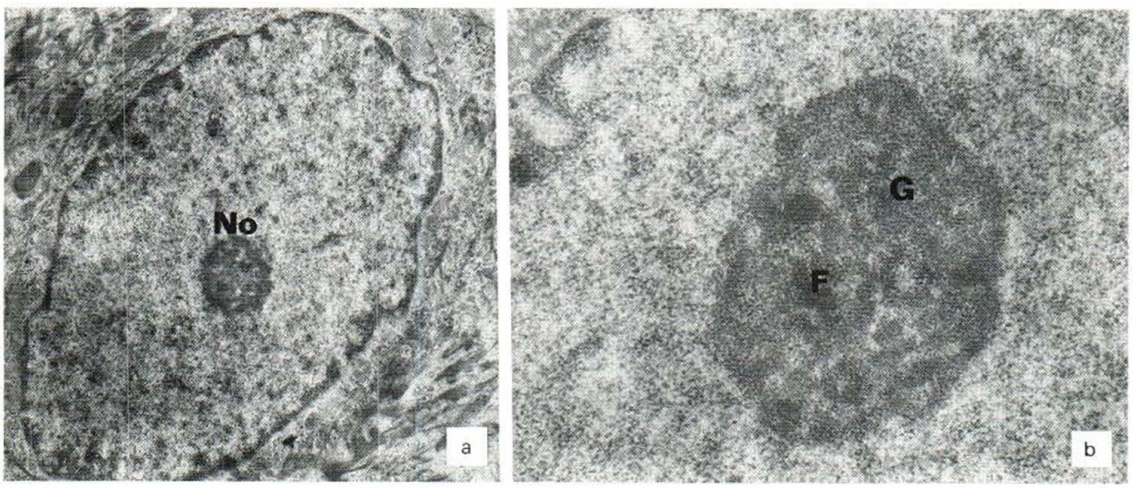

Fig. 6. A nucleus and nucleolus one week after clearing of a psoriatic lesion. (*a*) Heterochromatin granules are more prominent than before therapy. Nuclear bodies are not seen. The nucleolus (*No*) is compact. (×4 000.) (*b*) The nucleolus shows neither distinct nucleolonema, nor fibrillar centres. *F*, fibrillar component; *G*, granular component. (×14 000.)

Table 1. *The effect of PUVA therapy on nuclear and nucleolar size, nuclear bodies and heterochromatin*
Area=μm^2 (mean±S.D.). Number=number per nucleus (mean±S.E.).
Relative amount= $\frac{\text{Heterochromatin}}{\text{Total nuclear substance}} \times 100$ (mean±S.D.).

	Nuclear size (n=30) Area	Nucleolar size (n=30) Area	Nuclear bodies (n=30) Number	Heterochromatin (n=10) Relative amount
Before therapy	139±21	14.7±2.2	1.71±0.05	12.6±3.0
24 hrs after therapy	173±38[a]	15.9±2.9	1.42±0.08	34.8±4.1[a]
48 hrs after therapy	162±27	18.8±6.0	2.33±0.10[a]	40.5±7.8[a]
72 hrs after therapy	141±20	28.1±8.3[a]	4.25±0.13[a]	61.3±8.8[a]
96 hrs after therapy	137±15	20.3±4.1[a]	1.50±0.09	42.9±19.6[a]
After clearing	93±19[b]	6.3±1.9[b]	0.31±0.05[b]	36.1±2.9[a]

[a] Significant increase as compared with corresponding value before therapy at $p<0.05$ or <0.01.
[b] Significant decrease as compared with corresponding value before therapy at $p<0.05$ or <0.01.

after the first treatment (Fig. 4*a*). Nucleoli were larger and increased in volume relative to that of the nucleus. Fibrillar centres appeared again, mainly at the periphery of the nucleoli, although they were smaller than those before therapy. At this time we could often find nuclear bodies close to the nucleolus (Fig. 4*b*).

At 96 hr after the first treatment, the nuclear envelope was mostly smooth, as seen in the nuclei before therapy. Heterochromatin granules seemed to be decreased as compared with those at 72 hr after the first treatment (Fig. 5*a*). Nucleolar fibrillar centres had returned to the size existing before therapy. They were surrounded by a fibrillar component (Fig. 5*b*). Nuclear bodies and perichromatin granules were also similar in number to those before therapy.

One week after clearing of the lesions, the nuclear envelope was mostly smooth. Heterochromatin granules were still incraeased as compared with those before therapy and were dispersed throughout the nucleus. Nuclear bodies were not seen in most nuclei (Fig. 6*a*). The nucleoli were compact and did not show a distinct nucleolonema. Nucleolar fibrillar and granular components were intermingled. No or few fibrillar centres could be observed (Fig. 6*b*).

Quantitative study

At 24 hr after the first treatment, the size of the nuclei was significantly increased and, after clearing, it decreased significantly compared with the size before therapy (Table I). After clearing, the nuclear size was similar to that of normal keratinocytes (13).

The size of nucleoli was significantly increased at 72 and 96 hr after the first treatment and it decrased after clearing (Table I). The size of the nucleoli after clearing was similar to that of normal keratinocyte nucleoli (13).

The frequency of nuclear bodies was significantly increased at 48 and 72 hr after the first treatment and decreased after clearing (Table I). The frequency of nuclear bodies after clearing was similar to that in normal keratinocytes (13).

The relative amount of heterochromatin was significantly increased at 24 to 94 hr after clearing (Table I), but it did not decrease to the amount found in normal keratinocyte nuclei, even after clearing (13).

DISCUSSION

The present study indicates that the nuclei and nucleoli of psoriatic keratinocytes are markedly affected by 8-MOP and UVA irradiation (PUVA therapy). A series of early changes occurs, including irregularity of the nuclear envelope, changes in size of the nuclei and nucleoli, temporary disappearance of fibrillar centres followed by temporary increase in nuclear bodies and condensation of heterochromatin along the nuclear envelope and around the nucleolus as well as in the central part of the nucleus. After clearing of the lesions, nuclear and nucleolar structures were similar to those of normal keratinocytes except that the relative

amounts of heterochromatin were significantly greater.

There are some differences between the early changes and changes after clearing of the lesions. Compared with those before treatment, nuclei and nucleoli became larger after the first treatment, while they became smaller after clearing of the lesions. Likewise, the number of nuclear bodies was increased after the first treatment, whereas it was decreased after clearing. In addition, nuclear envelopes became irregular after the first treatment, but regular after clearing. On the other hand, increase in heterochromatin granules and disappearance of fibrillar centres were observed, both after the first treatment and after clearing of the lesions. This suggests that there are two different types of changes reflecting: 1) regression of psoriatic lesions, and 2) processes triggered by PUVA treatment. Of the various changes in nuclei and nucleoli, the disappearance of fibrillar centers and decrease in nuclear bodies, as seen after clearing of the lesions, may reflect regression of psoriatic lesions. On the other hand, irregular nuclear envelopes, enlargement of nuclei and nucleoli, and increase in the number of nuclear bodies, as seen after the first treatment, may reflect processes triggered by PUVA treatment. The increase in heterochromatin granules may represent both regression of psoriatic lesions and processes triggered by PUVA treatment. In fact, some of the previous reports prove this hypothesis. Toda et al. (12), using normal human skin, found that 6 hr after trimethyl-psoralen (TMP) application plus UVA irradiation, the keratinocytes next to the melanocytes showed indented nuclear envelopes. Konrad et al. (7) found enlargement of nucleoli of keratinocytes of normal and psoriatic skin peaking at 72 hr after 8-MOP application plus UVA irradiation. Moreover, Jimbo et al. (6) reported an indented nuclear envelope, an increase in the size of nuclei as well as nucleoli, and an increase in the number of heterochromatin granules in the nuclei of human melanocytes during immediate and delayed tanning reaction. In addition Wilborn & Montes (14) reported that an increase in heterochromatin granules and a decrease in nuclear bodies were found in psoriatic lesions showing excellent clinical response after 1 and 3 months of anthralin treatment.

It is also important to describe the relationship between regression of psoriatic lesions and changes in the nuclei and nucleoli after PUVA treatment. In general, fibrillar centres become prominent when there is some abnormality of RNA synthesis or some disturbance in maturation or transport of ribosomal precursors (3, 4, 8). Consequently, it is possible that the presence of multiple fibrillar centres in psoriatic keratinocyte nucleoli may be associated with abnormal keratinization and that the disappearance of fibrillar centres after clearing of lesions may be associated with normal keratinization. The significance of the appearance of nuclear bodies is not known. Bouteille et al. (2) suggested that they are related to cellular hyperactivity. Since it is known that psoriatic keratinocytes show hyperactivity, the decrease in nuclear bodies after clearing of lesions may reflect suppression of hyperactivity. The partition of chromatin into euchromatin and heterochromatin is modified by PUVA therapy. The concentration of aggregated heterochromatin is only about half as great in epidermal nuclei of psoriatic plaques as in normal nuclei (13). This value is rapidly increased by therapy to exceed the normal by 24 hr. The relative amount of heterochromatin remains high during all stages of the therapy period that have been examined, even after clearing. The implications of this finding with respect to the possibility of gene repression by the therapy are still being considered. Since heterochromatin represents repressed segments of chromosomal RNA (5, 9), it is suggested that psoriatic keratinocyte nuclei may have undergone gene repression by the therapy. The persistence of relatively large amounts of heterochromatin after clearing suggests the possibility of long-term gene repression, possibly due to photoaddition of psoralen to the DNA.

REFERENCES

1. Baden, H. P., Parrington, J. M., Delhaty, J. D. A. & Pathak, M. A.: DNA synthesis in normal and xeroderma pigmentosum fibroblasts following treatment with 8-methoxypsoralen and long wave ultraviolet light. Biochim Biophys Acta *262:* 247, 1972.
2. Bouteille, M., Kalifat, S. R. & Delarue, J.: Ultrastructural variations of nuclear bodies in human diseases. J Ultrastruct Res *19:* 474, 1967.
3. Daskal, Y., Crooke, S. T., Smetana, K. & Busch, H.: Ultrastructural study of the effect of Bleomycin A_2 on the nucleolus and its possibly related cytoplasmic constituents in Novikoff hepatoma cells. Cancer Res *35:* 374, 1975.
4. Daskal, Y., Györkey, F., Györkey, P. & Busch, H.: Ultrastructural study of pulmonary Bleomycin toxicity. Cancer Res *36:* 1267, 1976.

5. Frenster, H. J. & Herstein, P. R.: Gene de-repession. N Engl J Med *288:* 1224, 1973.
6. Jimbow, K., Pathak, M. A., Szabo, G. & Fitzpatrick, T. B.: Ultrastructural changes in human melanocytes after ultraviolet radiation. *In* Sunlight and Man (ed. T. B. Fitzpatrick, M. A. Pathak, L. C. Harber, M. Seiji & A. Kukita), p. 195. University of Tokyo Press, Tokyo, 1974.
7. Konrad, K., Gschnait, F., Honigsmann, H. & Wolff, K.: UVA-mediated psoralen–tissue interactions: Subcellular events. J Invest Dermatol *66:* 258, 1976 (Abstr).
8. Love, R. & Sporiano, R. Z.: Correlation of nucleolini with fine structural nucleolar constituents of cultured normal and neoplastic cells. Cancer Res *31:* 1030, 1971.
9. Nakatsu, S. L., Landrum, S. & Frenster, J. H.: Quantitative ultrastructural probes of de-repressed DNA templates in human bone marrow cells. Blood *40:* 944, 1972.
10. Sasaki, M. S. & Tonomura, A.: A high susceptibility of Fanconi's anemia to chromosome breakage by DNA crosslinking agents. Cancer Res *33:* 1829, 1973.
11. Swanbeck, G., Thyresson-Hök, M., Bredberg, A. & Lambert, B.: Treatment of psoriasis with oral psoralens and longwave ultraviolet light. Acta Dermatovener (Stockholm) *55:* 367, 1975.
12. Toda, K., Nakayama, Y. & Morikawa, F.: Electron microscopic observations in human skin after psoralen photosensitization. *In* Sunlight and Man (ed. T. B. Fitzpatrick, M. A. Pathak, L. C. Harberg, M. Seiji & A. Kukita), p. 419. University of Tokyo Press, Tokyo, 1974.
13. Tsuji, T. & Cox, A. J.: Ultrastructural studies of nuclei and nucleoli in psoriatic epidermis, compared with those in normal epidermis. J Invest Dermatol *69:* 205, 1977.
14. Wilborn, W. H. & Montes, L. F.: Ultrastructural changes in psoriatic epidermis following anthralin treatment. J Cutaneous Pathol *1:* 132, 1974.

Received February 14, 1978

Takuo Tsuji, M.D., Ph.D.
Department of Dermatology
Osaka City University
Medical School
1-5-7 Asahimachi, Abeno
Osaka, 545 Japan