

suprabasal LI values remained almost unchanged. This may indicate an incipient normalization of the proliferative activity of the epithelium in both atrophic and hyperplastic l.p.m.

Further insight into the subsequent development of the epithelial proliferation pattern during retinoid therapy could not be obtained as the patients, being satisfied with the success of retinoid therapy, refused additional mucosa biopsies. Thus the question remains unsolved as yet, whether and to what extent the dosage influences the degree as well as the velocity of the drug-induced epithelial restitution, as recently shown in epidermal lesions of l.p. (10). Moreover, the small number of biopsies so far available does not allow definite conclusions to be drawn with regard to the basic cellular mechanisms involved in the benefit of aromatic retinoid therapy in oral l.p.

#### ACKNOWLEDGEMENT

This study was supported by Deutsche Forschungsgemeinschaft (SFB 118/C1). Mrs G. Ritter is gratefully acknowledged for technical assistance.

We are indebted to Hoffmann-La Roche for providing test preparations of Ro 10-9359, out now as Tigason®.

#### REFERENCES

1. Ebner, H.: Erfahrungen mit der systemischen Retinoidbehandlung (Ro 10-9359) bei Lichen ruber planus. *Wien Klin Wochenschr* **91**: 161-164, 1979.
2. Farber, E. M. & Nall, L.: The use of oral retinoids in psoriasis therapy: an overview of past, present, and future developments. *In Retinoids* (ed. Orfanos et al.), pp. 515-520. Springer, 1981.
3. Fritsch, P.: Oral retinoids in dermatology. *Int J Dermatol* **20**: 314-329, 1981.
4. Lassus, A.: Systemic treatment of psoriasis with an oral acid derivate (Ro 10-9359). *Br J Dermatol* **102**: 195-202, 1980.
5. Maidhof, R., Hornstein, O. P., Rosenberger, H., Schell, H. & Prantl, F.: Vergleichende histologische und histoautoradiographische Untersuchungen an benignen oralen Leukoplakien. *Arch Dermatol Res* **259**: 71-82, 1977.
6. Maidhof, R. & Hornstein, O. P.: Autoradiographic study on some proliferative properties of human buccal mucosa. *Arch Dermatol Res* **265**: 165-172, 1979.
7. Maidhof, R.: Zur systemischen Behandlung des oralen Lichen planus mit einem aromatischen Retinoid (Ro 10-9359). *Z Hautkr* **54**: 873-876, 1979.
8. Maidhof, R., Hornstein, O. P., Bauer, G., Schönberger, A. & Bäurle, G.: Cell proliferation in lichen planus of the buccal mucosa. *Acta Dermatovener (Stockholm)* **61**: 17-22, 1981.

9. Marks, R., Finlay, A., Nicholls, S. & Barton, S.: The effects of retinoids on stratum corneum. Structure and function. *In Retinoids* (ed. Orfanos et al.), pp. 77-83. Springer, 1981.
10. Pohl, J. & Christophers, E.: Mitogenic properties of aromatic retinoids: in vivo and in vitro effects on epidermal cells. *In Retinoids* (ed. Orfanos et al.), pp. 133-138. Springer, 1981.
11. Scheiber, W. & Plewig, G.: Behandlung des Lichen ruber mucosae mit Vitamin-A-Säure-Derivaten. *Dermatologica* **157**: 171-180, 1978.
12. Schultz-Ehrenburg, U. & Orfanos, C. E.: Light and electron microscopic changes of human epidermis under oral retinoid treatment. *In Retinoids* (ed. Orfanos et al.), pp. 85-92. Springer, 1981.
13. Viglioglia, P. A.: Therapeutic evaluation of the oral retinoid Ro 10-9359 in several non-psoriatic dermatoses. *Br J Dermatol* **103**: 483-487, 1980.
14. Walker, D. M. & Dolby, A. E.: Labelling index in the mucosal lesions of lichen planus. *Br J Dermatol* **91**: 549-556, 1974.

#### Assay of Comedolytic Activity in Acne Patients

Otto H. Mills, Jr. and Albert M. Kligman

*Department of Dermatology, University of Pennsylvania,  
and the Simon Greenberg Foundation  
Philadelphia, PA 19104, USA*

Received April 16, 1982

**Abstract.** Comedolytic activity was assessed in acne patients by determining the reduction in facial microcomedones using the cyanoacrylate follicular biopsy technique. Microcomedones were counted in five-cm squares after 8 to 12 weeks of treatment.

Only salicylic acid and tretinoin among seven conventional anti-acne medications were found to possess comedolytic activity. The latter was the more effective.

**Key words:** Comedolytic activity; Acne population; Microcomedone; Tretinoin; Salicylic acid

The initial event in the pathogenesis of acne is the formation of the comedo, an impaction of horny cells in sebaceous follicles. Agents which can prevent this event are effective in moderating the disease.

We have described models for assaying comedolytic drugs. The first utilized comedones in-

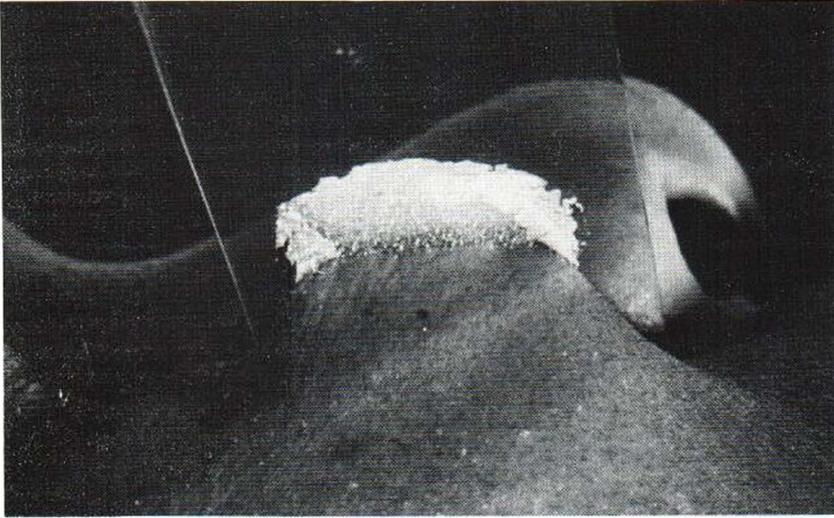


Fig. 1. Follicular biopsy. Removal of glass slide from the skin with sheet of adhesive and microcomedones.

duced in the external ear canal of rabbits by coal tar (5). However, animal comedones are not entirely analogous to human ones. The horn is less tightly packed and they are more easily exfoliated. We then developed a human model (3, 4) applying coal tar occlusively to the back for 2 weeks. Finding a parallelism between comedolytic assays in humans and rabbits, we argued that the results of rabbit testing were relevant. Critics could still argue that

coal tar comedones are not entirely analogous to those which arise spontaneously.

Accordingly, we have appraised comedolytic agents in humans. The method entails treating patients with large numbers of microcomedones.

#### MATERIALS AND METHODS

##### *Acne patients*

These were young male and female college students with moderately severe inflammatory acne vulgaris. We ac-

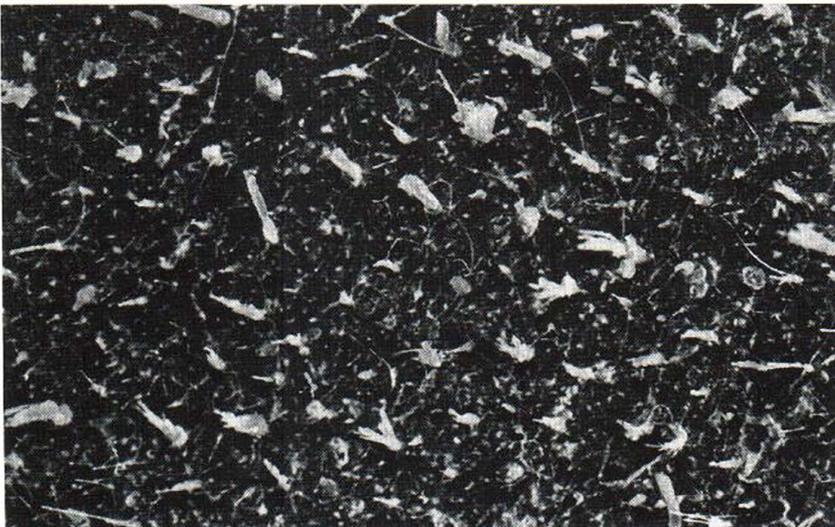


Fig. 2. Density of microcomedones before treatment.

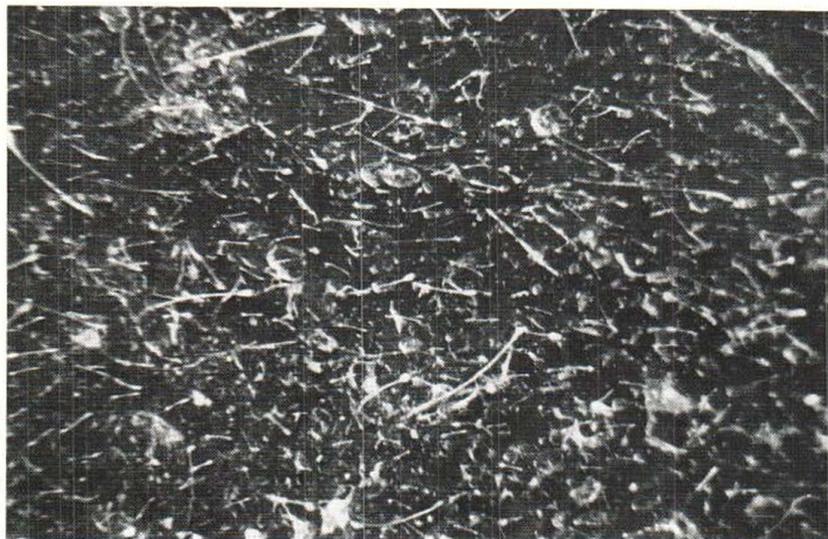


Fig. 3. Density of microcomedones after 12 weeks of treatment with tretinoin.

cepted only those who, by the follicular biopsy technique (2, 4) had many microcomedones on facial skin. We excluded patients with so many papulo-pustules that a relatively lesion-free skin area could not be found. Informed consent was obtained.

#### *Assessment of microcomedones*

The cyanoacrylate "follicular biopsy" technique was used to sample the follicular contents. This is an extension of the Marks & Dawber (1) method. A coating of the cyanoacrylate adhesive (Aron Alpha, Toagosei Chemical Industri Co., Ltd., Tokyo, Japan. Distributed by Vigor Company, Division of B. Jadov and Sons, Inc.) was laid down and allowed to polymerize for one minute. A second coating was applied and a glass slide pressed down for another 60 seconds to spread the glue evenly. The slide was then pulled off with a rocking motion, lifting off a thin sheet of the outer horny layer to which the microcomedones are attached as horny cylinders (Fig. 1).

The density of microcomedones was determined by counting under the stereoscope all the casts within five fields, 1 sq cm each (Fig. 2). The mean was then calculated. Each subject initially had to have a minimum of 15 microcomedones per cm<sup>2</sup>. The cheeks and foreheads were then treated twice daily. After 8 weeks, the opposite side of the forehead was sampled and, after 12 weeks, the opposite side of the cheeks.

Ten to twelve subjects comprised a test panel. The results were expressed as the percentage reduction in microcomedones.

#### *Test agents*

We studied seven drugs that are commonly used in anti-acne medications benzoyl peroxide 10% (Persa-Gel®; Dermatology Division, Ortho Pharmaceuticals), clindamycin phosphate 1% (Cleocin®; Upjohn), lotio alba

(U.S.P.), salicylic acid 5%, sulfur 5%, tretinoin 0.05% (Retin A®; Dermatology Division, Ortho Pharmaceuticals) and resorcinol 10%.

## RESULTS

We deemed substances to be inactive when there was less than a 25% reduction in microcomedones. Only two of the seven preparations used in this assay were comedolytic: tretinoin and salicylic acid. The former caused 75% and 87% reductions

Table I

	% Microcomedone reduction	
	8 weeks	12 weeks
Benzoyl peroxide 10% (Persa-Gel®; Dermatology Division, Ortho Pharmaceuticals)	10	19
Clindamycin phosphate 1% (Cleocin®; Upjohn Company)	9	8
Lotio alba (U.S.P.)	5	7
Salicylic acid 5% (ETOH 85%, propylene glycol 15%)	40	48
Sulfur 5% (in shake lotion)	7	9
Tretinoin 0.05% (Retin-A®, liquid; Dermatology Division, Ortho Pharmaceuticals)	75	20
Resorcinol 10% (ETOH 85%, propylene glycol 15%)	13	20

at 8 and 12 weeks respectively; the latter, 40% and 48% decreases over the same time periods (Table I). Tretinoin was capable of dramatically reducing microcomedone counts by 12 weeks as seen by comparing Fig. 2 (pre) and Fig. 3 (12 weeks).

## DISCUSSION

This limited study bears out what we have previously reported in the human coal tar model. Few chemicals possess comedolytic activity. Human comedones, natural or coal tar induced, are firmly anchored and are dislodged only with great difficulty. Most classic 'peeling' agents such as resorcinol and phenol are ineffective: they are merely irritants which cause scaling. While salicylic acid and tretinoin are also irritants, their efficacy is dependent on specific pharmacologic effects. The former seems to detach horny cells from each other by weakening the intercellular cement. As a result, the comedones tend to undergo disorganization. Salicylic acid penetrates skin readily and increases turnover which also favors exfoliation of the comedo. Tretinoin, on the other hand, does not cause dehiscence of pre-existing horny cells. Rather it affects the keratinization process itself so that horny cells cannot cohere strongly to each other. Consequently, comedones are no longer anchored and eventually fall out.

Not surprisingly, the antibiotic clindamycin did not possess comedolytic activity. Its probable mode of action is to suppress *P. acnes* and this must hold also for benzoyl peroxide.

In a previous study, using the rabbit ear model, we reported that benzoyl peroxide was moderately comedolytic. Assays with various commercial formulations since that time have led us to revise this assessment. The rabbit comedolytic activity of most benzoyl peroxides is insignificant. We found this to be the case in the present study, as well as in the human coal tar model. The softer comedones of the rabbit are much more easily exfoliated; hence, comedolytic activity tends to be overrated. The vehicle itself also becomes a factor. Not only percentage reduction but speed of action can be used to characterize comedolytic drugs. For example, at 8 weeks, tretinoin had produced a 75% reduction, compared with 40% for salicylic acid.

The follicular biopsy technique constitutes a practical method of assessing comedolytic activity.

It takes into account all the microcomedones within five-cm<sup>2</sup> fields, the large as well as the smaller ones. The accuracy of this method is far greater than clinical counting of comedones. Except for the conspicuous open comedones, many of the closed comedones are barely visible, a source of great variability.

Of course, knowledgeable persons will appreciate that there are agents besides tretinoin and salicylic acid that are effective in acne vulgaris. This assay measures *only* comedolytic activity. Agents whose mode of action is different must be assessed differently.

## REFERENCES

1. Marks, R. & Dawber, R. P. R.: Skin surface biopsy: An improved technique for the examination of the horny layer. *Br J Dermatol* 84: 177, 1971.
2. Mills, O. H.: The follicular biopsy. *Dermatologica* (in press).
3. Mills, O. H. & Kligman, A. M.: A human model for assaying comedolytic substances. *Br J Dermatol* (in press).
4. — The follicular biopsy: Methodology and application. *Clin Res* 23: 251A, April, 1980.
5. — Assay of comedolytic agents in the rabbit ear. *In Animal Models in Dermatology* (ed. Howard I. Maibach), p. 176, Churchill Livingstone, New York, 1975.

## Age at Menopause of Females with Systemic Sclerosis

Jørgen Serup and Hans Kenneth Hagdrup

*Department of Dermatology, University of Copenhagen, Rigshospitalet, Copenhagen, Denmark*

Received June 5, 1982

**Abstract.** Clinical symptoms of menopause were recorded in 27 females with systemic sclerosis, mean age 53.5 years (range 22–68). Five had regular menstruation, and 22 were in the postmenopause, 2 with climacterium praecox. Mean age at menopause was 47.8 years (range 30–59) among the 22 females, and 49.3 years (range 40–59) among the 20 females with a normal climacterium. Eleven had physiological subjective symptoms of menopause. The 22 females had 34 living children, and 13 abortions. It is concluded that the menopause is normal in females with systemic sclerosis. This indicates that the ovaries are normally not involved in the disease.