Acnegenicity Testing in Rabbits

An Objective Quantification Method

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An objective method for the quantification of follicular hyperkeratosis is proposed for the rabbit ear assay of acnegenicity. The area (*area%*) of hyperkeratinized follicles, covering a stereomicroscopic photo of the epidermis, is proposed for the quantification of the response. The uniformity of two observers' quantification was evaluated in six tested formulations. The quantification of the response in *area%* was found to be a suitable and objective alternative to visual scoring on a graded scale. The median relative difference between two observers' *area%*-quantification was found to be 26 %, and there was no statistically significant difference between the two observers' quantification ($p > 0.10$). An *area%* of 2, 7, and 15 is preliminarily suggested to be equivalent to the lower limit for scores 1, 2 and 3 respectively. *Key words: Acne; Predictive testing; Rabbits; Topical preparations; Isopropyl myristate.* (Received February 4, 1985.)

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"The rabbit ear model"—for detection of acnegenic materials has been developed by Kligman & Mills (3). The one ear of two rabbits is used for application of the test material, and the contralateral untreated ears serve as control. Follicular hyperkeratosis is estimated by visual scoring on a 4-point rating scale (score 0–3) with the aid of a stereomicroscope (4).

In our use of the scoring system (4) we have realized the need for an objective measurement of the test response instead of visual rating (score 0–3, according to no, slight, moderate, and strong follicular hyperkeratosis). We expressed the responses as the area (*area%*) of hyperkeratonized follicles covering a stereomicroscopic photo of the epidermis. The agreement between two observers' quantification is evaluated using 6 formulations.

MATERIAL AND METHODS

Rabbits: Conventional, young adult, albino male rabbits (of about 4 months and weighing 2 kg) of New Zealand White breed (Ssc: CPH). The rabbits were kept on a standardized diet. *Microscope*: Technival stereomicroscope (magnification × 12) equipped with a Technival polaroid camera. Polacolar 2 type 108 film (size 8.3 × 10.8 mm). *Test-material*: Mineral oil (Ph. Nord. 63), isopropyl myristate (DAK 63), and six topical formulations. *Assay*: The method described by Kligman & Kwong (4) was used. Instead of the visual stereomicroscopic rating (score 0–3), the responses were quantified as follows: A stereomicroscopic (× 12) picture of the follicles was taken. After an exposure of about 10 sec the area of the photo (*area%*) covered by hyperkeratinized follicles was calculated as shown (Fig. 1).

With the photo in a fixed position the horizontal diameter ($d \pm 0.25$ mm) of the follicles (n_1) not touching the edge of the photo was measured. The mean diameter ($\bar{d}$) was calculated, and the number of follicles (n_2) touching the upper and right edges of the photo was counted. $n = n_1 + n_2$ was the total sum of the follicles. The *area%* was calculated by means of the following formula, where $\pi/4 = 78.54$:

$$\text{Area\%} = \frac{78.54 \cdot \bar{d}^2 \cdot n}{\text{Area of photo}} \%$$
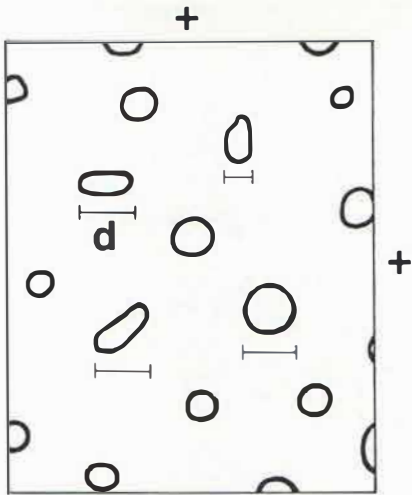
Fig. 1. Illustration of the method used for quantification of the test response. The response is expressed as the area (area%) of follicles covering the stereomicroscopic photo. The area% is calculated on the basis of the amount and the mean diameter of the follicles (see "materials and methods"). ⊢——⊣, Vertical diameter (d) of a follicle. +, Follicles (n_2) touching the upper and right edges are also used in the calculations.

RESULTS

Fig. 2 shows photos of different area%, 1.2, 6.5, and 14.7 for a, b, and c respectively. Table I shows two observers' area% quantifications for six tested, topical formultions. The relative median difference between the two observers was 26%. By mean of Pratts' test for paired data (8) there was found no statistically significant difference between the two observers' quantification ($p > 0.10$).

DISCUSSION

The proposed method for quantification (area%) is found suitable as an objective assessment. As found (Table I) there may be differences between observers up to about 100% in quantifications, but these differences are primarily found for an area% smaller than 2, which is close to the control value level.

The photos (Fig. 2) are suggested by A. M. Kligman (personal comunication 1982) to represent the limit between his scores 0–1, 1–2, and 2–3. Therefore the lower limit for scores 1, 2, and 3 (representing slight, moderate, and strong comedogenicity) may be placed at 2, 7, and 15 area% respectively. The clinical relevance, however, of the assay and scoring (3, 4) has to be further evaluated (1, 4, 6). Fulton et al. (2) find that the method is extremely sensitive and think that mild or moderate offenders may have no clinical significance. Further, the comedones formed in the rabbit ear differ in some respects from the human comedones of acne vulgaris (1, 5), and substances known to be comedogenic in humans may not respond in rabbits, such as e.g. corticosteroids (7). Until the correlation between the rabbit ear model and the clinical situation has been further documented, a fixed level for comedogenicity, such as area% ≥ 7 and ≥ 15 for scores 2 and 3 respectively, must be made with a reservation. At present a fixed limit (e.g. area% <7 as for scores 1 and 0) might be suggested as a "safety limit" not wise to exceed. The continuous scale (area%) has the advantage that it reduces the need for fixed limits, e.g. when different formulations are compared. Further, the comedogenicity may be exclusively expressed as area%, and the area% may be more objective and reproducible among laboratories. Finally the photo serves as documentation for the test.

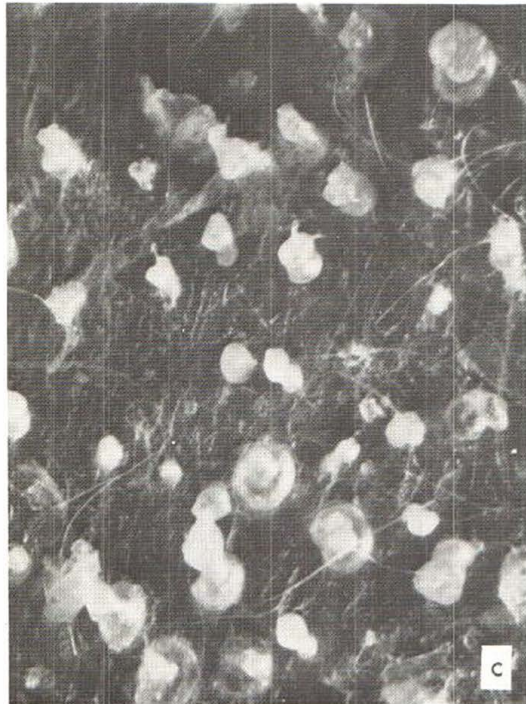Like Fulton et al. (2) we have found undiluted isopropyl myristate to exhibit a pro-
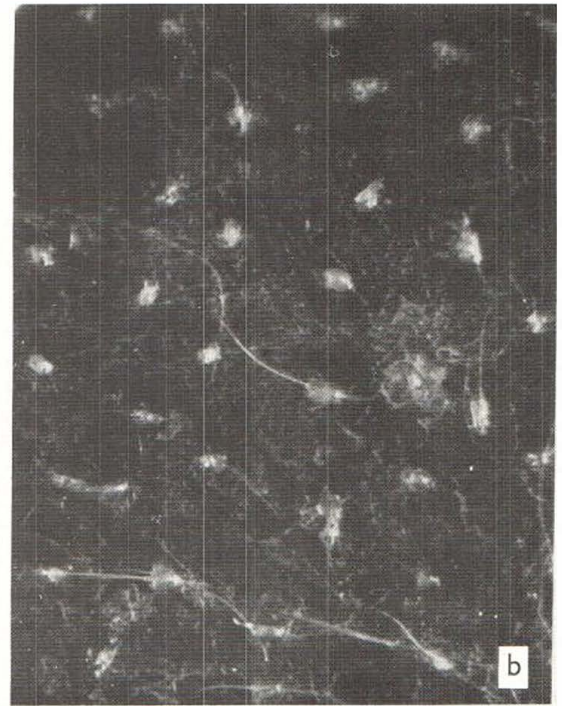
Fig. 2. Photos (×12) showing rabbit ear hyperkeratin-
ized follicles covering an area (area%) of:
(*a*) 1.2 % (mean diameter 2.1 mm (SD 0.6), *n*=24),
(*b*) 6.5 % (mean diameter 4.0 mm (SD 1.3), *n*=35),
(*c*) 14.7% (mean diameter 5.9 mm (SD 1.8), *n*=38).

Table I. *Comparison of two observers' (A and B) area% quantification for six tested formulations (N=2). Control values are not subtracted*

Difference $\% = \dfrac{(A-B)\cdot 100}{(A+B)\cdot 0.5}\%$

Formulation	Rabbit	Area %			
		Observer A	Observer B	Difference	Difference %
I	1	6.4	6.3	0.1	2
	2	6.1	6.7	0.6	9
	Mean	6.3	6.5	0.2	3
II[a]	1	6.7	5.1	1.6	27
	2	3.8	3.0	0.8	24
	Mean	5.3	4.1	1.2	26
III[a]	1	6.7	5.3	1.4	23
	2	3.7	2.7	1.0	31
	Mean	5.2	4.0	1.2	26
IV[b]	1	1.2	2.0	0.8	50
	2	2.0	3.2	1.2	46
	Mean	1.6	2.6	1.0	48
V	1	1.5	1.5	0.0	0
	2	2.0	0.6	1.4	108
	Mean	1.8	1.1	0.7	47
VI[c]	1	0.2	0.2	0.0	0
	2	0.2	0.4	0.2	67
	Mean	0.2	0.3	0.1	33

Difference % (means not included):
Median   26
Range   0–108

[a] Containing 8% isopropyl myristate.
[b] Formulation II without isopropyl myristate.
[c] Formulation II containing 0.05% retinoic acid.

nounced response (13 area%) in the rabbit ear. The response was found to depend on the concentration in the vehicle, observed for formulations II and III (Table 1) containing 8% of isopropyl myristate. Withdrawal of isopropyl myristate (formulation IV) from formulation II decreased the response further, and addition of retinoic acid (0.05%, formulation IV) to formulation II completely eliminated the response.

The frequency of follicular hyperkeratosis for "no treatment" is reported (3) to be about 15%. On the basis of 10 observations we found no response (mean response < ½ area%) for "no treatment".

It is concluded that the quantification of test results in area% is a suitable and objective alternative to visual scoring on a graded scale. An area% of 2, 7, and 15 may preliminarily be suggested to represent the lower limit for scores 1, 2 and 3 respectively.

ACKNOWLEDGEMENTS

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