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REPORTS

A Method for Measuring Desquamation and its Use for Assessing the Effects of Some Common Exfoliants

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Desquamation has been measured in the past by a counting chamber technique after corneocytes are removed from the skin surface and disaggregated in a dilute surfactant solution. However, we have found that complete corneocyte disaggregation is not always possible when these aggregates are recovered from sites where patent peeling is induced. Corneocyte counting in such instances is difficult or impossible. We have devised a method of measuring desquamation wherein the desquamating cells are determined as the total alkali-soluble protein after they are removed from the skin surface with an inert, self-hardening gel. Highly reproducible desquamation rates are obtained, characteristic of the individual subject. Using some common exfoliants, we show that pharmacologic response, observed as an increase in desquamation rate, is also an individual characteristic.

There is an obvious need for more practical methodology to measure cutaneous exfoliation. Such highly technical procedures as scanning electron microscopy (SEM) are adequate but far too sophisticated for routine use. McGinley, Marples, and Plewig [1] have described a counting chamber technique which utilizes a surfactant solution as a collection and dispersion medium. However, the size of the corneocyte aggregates released by scrubbing the skin surface is far from uniform when obvious peeling is observed and some such aggregates are too large to permit accurate counting. We describe herein a practical, reliable, and accurate method to measure desquamation. This method is based on our finding that desquamating cells can be entrapped in an inert gel applied to the skin. When the gel hardens to form a discrete film, this film, along with its attached cells, is easily peeled from the skin. The captured cells then are recovered from the film and determined as total alkali-soluble protein. This protein, as we show, is proportional to the number of cells recovered.

MATERIALS AND METHODS

Gel Composition and Preparation

The gel consists of:

Methocel F-50 Premium*	10% w/v
Ethanol	35% v/v
Glycerin	5% v/v
Propylene Glycol	15% v/v
Water q.s. to	100% v/v

It is prepared by dispersing the Methocel F-50 Premium in the ethanol and stirring vigorously to assure complete wetting of the polymer. The glycerin and propylene glycol are then added. Finally, the mixture is brought to volume with distilled water.

The gel fulfills 3 basic requirements: (1) It hardens after application to the skin surface and is readily removed as an intact film without discomfort to the patient; (2) it is minimally adhesive and therefore captures only loosely attached corneocytes; and (3) the captured corneocytes are easily recovered and analyzed because the gel components are soluble in water or dilute surfactant.

Gel Application

The gel is conveniently applied to the skin by means of a template which defines the test area and permits the use of surface coordinates; thus, the same site can be sampled on successive days. We have used a template made from a 5 × 11 cm cellulose acetate sheet in which there has been cut 2 symmetrically-spaced square openings, each 4 × 4 cm with a 1 cm strip between openings. These openings define the test areas on the forehead. The template is positioned on the forehead with the midline just over the bridge of the nose and the lower edge just above the eyebrows. Then, 0.4 ml of gel is applied to each of the 2 areas defined by the template and spread uniformly over the skin. The time that the gel is left in contact with the skin is not critical; however, it must be thoroughly dry when peeled off. We have tested contact times from 1 to 6 hr and found no increase in corneocytes recovered after 1 hr. Apparently, only those cells are removed that become entrapped as the gel hardens. Unless otherwise specified, in the studies reported here the gel was applied at 9:00 AM daily and was left on for 3 hr as a matter of convenience to the subjects so they could pursue their normal activities. The gel is peeled off as a discrete film at the end of the collection period and stored in individual glassine envelopes for subsequent chemical analysis.

Exfoliants

In all instances, exfoliants were applied to one side of the forehead; the other side served as a no-treatment control. Applications were scheduled to avoid conflict with the sampling period, which as stated was 9:00 AM to noon.

For one of the test exfoliants we used salicylic acid at 5% w/v concentration in ethanol-water (70:30) containing 0.1% sodium dodecyl sulfate and 1% glycerol†. The solution was applied twice a day, once before retiring and once at about mid-day, right after the Methocel film had been removed. About 0.2 ml was applied to the treated side of the forehead with a Dacron‡ swab. Treatment was stopped 3 days after peeling first became evident.

The other test exfoliant was a resorcinol-sulfur cream—a commercial preparation containing 2% w/w resorcinol and 8% w/w sulfur in a bentonite-containing base§. This same cream also was tested without resorcinol, without sulfur, and without both. In all cases, a 1 cm-long

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Abbreviation:
SEM: scanning electron microscopy

* Dow Chemical Company, Midland, Michigan.

† Note: This vehicle was selected for delivery of salicylic acid to the skin since all 5% solutions are not similarly effective. We found that 1 and 2% solutions of salicylic acid in this vehicle caused a much lower incidence of peeling and thus are confident that the vehicle contributes little to the observed effect.

‡ DuPont, Wilmington, Delaware.

§ Clearasil Regular Tinted Cream Medication, Vick Chemical Co., Division of Richardson-Merrell, Inc., Wilton, Connecticut.

ribbon of cream was spread on one-half the forehead. Dosage regimens for the individual studies are given in Table IV.

Corneocyte Recovery and Protein Determination

Once removed from the subject, the dried Methocel films—one each from the treated and control sites—with their entrapped corneocytes are each placed in a 15 ml Corex¶ tube containing 5 ml of a 0.2% Tween 80** solution. The tubes are shaken vigorously for 30 seconds with a Vortex mixer. An additional 5 ml of Tween solution then is added to each tube with mixing, and the corneocytes are separated from the dissolved film components by centrifugation for 10 min at 10,000 ×g in a Sorvall†† centrifuge, Model RC2-B, at 5°C. The film is readily solubilized in this solution and the detergent reduces the surface tension so that there is good sedimentation and packing of the corneocytes. After centrifugation, the supernatant is aspirated and the corneocytes are resuspended and washed a second time as before.

After the second washing, the corneocytes are suspended in 1.5 ml of distilled water plus 0.75 ml of 3 N NaOH and analyzed for alkali-soluble protein by a modification of the biuret procedure of Herbert, Phipps, and Strange [2]. The samples are heated in a boiling water-bath for 10 min and then cooled in running tap water. Next, 0.75 ml of a 0.5% $CuSO_4$ solution is added to the samples. We have found that with the generally low protein concentrations found in these specimens, 0.5% $CuSO_4$ gives greater reproducibility than the 2.5% used by Herbert, Phipps, and Strange [2]. Typical values obtained in our studies were in the range 0.1 to 0.5 mg of protein, determined as bovine serum albumin. At this level, replicates vary ± 10%, and even a fivefold increase to 2.5 mg of protein is well within the Beer's Law portion of the standard curve. Heating beyond the stated 10-min period results in no additional release of chromogenic material, even when large clumps of corneocytes are analyzed, indicating that extraction of the alkali-soluble protein is complete.

Determination of Desquamation Rates

Data in this paper pertain to sites on the forehead, which were sampled once daily on successive days. It should be emphasized that the template permits sampling of the same site each time. In any given experiment, the sample was always taken at the same time each day. Cumulative amounts of protein were plotted as a function of the number of days of sampling. From these plots it was possible to determine: (1) a desquamation "rate", (i.e., the slope of the curve in micrograms of protein removed from the site per sq cm per day); (2) reproducibility, as estimated by the correlation coefficient obtained from regression analysis; and (3) an increase in the slope from the baseline value, which reflects an increase in desquamation at the sampling site.

RESULTS

Material Removed by Methocel Films

The corneocytes can be examined after the Methocel film has been dissolved and the washed corneum sample has been resuspended for protein analysis. There are no obvious histological differences between the corneocyte aggregates washed with the dilute Tween solution (Fig 1*A*), and aggregates obtained by scrubbing the skin surface with plain water (Fig 1*B*).

If a site is sampled serially at 1 hr intervals (Table I), the second sample yields only about 40% of that of the first, and the third sample is below the level of detectability of the method. Thus, unlike cellulose-tape-stripping procedures, which remove progressively more material until the "glistening layer" is exposed, the Methocel film captures only loosely-attached cells. Compared to the scrub technique [1] the film removes five- to tenfold fewer cells (Table II).

Corneocyte protein correlates well with the number of corneocytes removed from the Methocel film. The yield of protein was about 2.5×10^{-4} micrograms per cell in 5 of 6 subjects tested (Table II).

Variation and Reproducibility

Replicate sampling of the skin on a given day yields information on the reproducibility of the sampling technique, but does not reveal if the values obtained are characteristic of the individual subject; many common environmental hazards (e.g. sunburn, friction, chemical irritants) can cause transient changes in the shedding process. We have circumvented this difficulty by doing replicates on successive days; our values thus also reflect the normal daily variation of the skin surface.

When the data are presented as plots of cumulative amounts of protein removed from a given site as a function of time, the curves typically deviate very little from linearity, with correlation coefficients in the range 0.97 to 0.99 (Figs 2 and 3). Although the slope is constant in any given individual, its value differs from subject to subject and in our work varied from 5 to 20 $\mu g/cm^2/day$. This indicates that these rates reflect an individual feature and not a sampling artifact. It is also clear that each individual must serve as his own control if changes in desquamation rates are to be correctly determined. Differences in the desquamation rate between one side of the forehead and the other side in the same subject (Fig 2) are much smaller than differences between comparable sites in different subjects (Fig 3). This similarity in contralateral sites in the same individual has been observed by other workers [1]; it forms the basis for the half-forehead studies described in this paper.

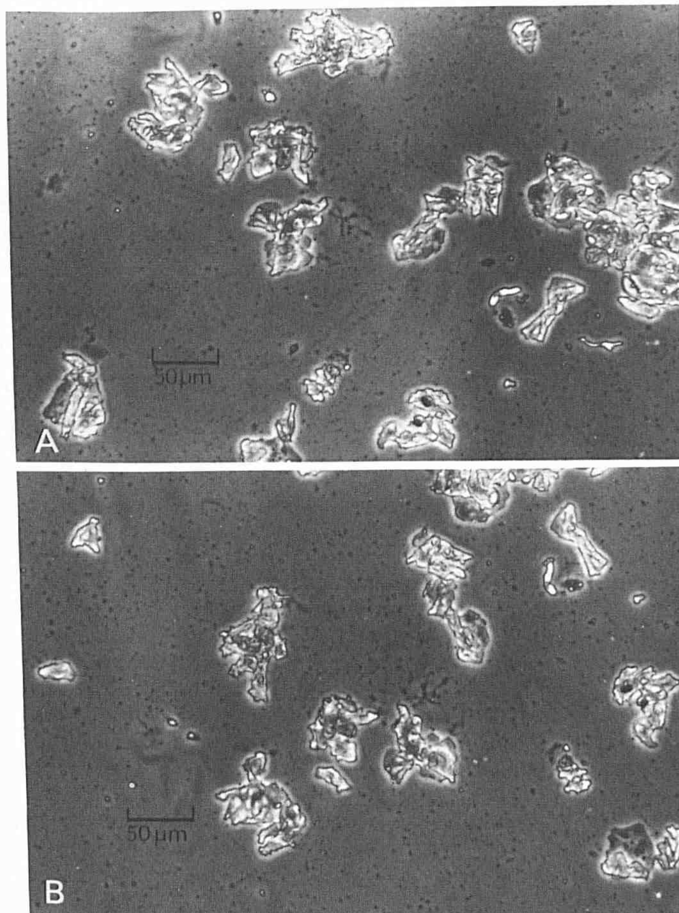

FIG 1. Corneocyte suspensions (unstained cells, photographed with phase contrast) *A*, Cell suspension after washing with dilute (0.2%) Tween 80. *B*, Cells isolated and suspended in distilled water.

TABLE I. *Decreasing yield of corneocyte protein with serial sampling*

	Corneocyte protein (mg/36 cm²)	
Subject	1	2
Site	Forehead	Forehead
1st Sampling (hour 1)	0.41	0.33
2nd Sampling (hour 2)	0.19	0.10
3rd Sampling (hour 3)	<0.05[a]	<0.05

[a] The limit of detectability of the biuret method.

¶ Corning Glass Works, Corning, New York.
** ICI, United States, Wilmington, Delaware.
†† DuPont-Sorvall, Newtown, Connecticut.

TABLE II. *Corneocyte recovery (scrub vs film) and protein/cell number correlation*

Subject	1	2	3	4	5	6
A. Cell number[a]						
Scrub	430	290	430	300	580	240
Film	49	51	58	30	64	48
B. Corneocyte protein[b]						
Film	14	13	14	14	16	12
C. Protein per cell[c]						
Film	2.9×10^{-4}	2.5×10^{-4}	2.4×10^{-4}	4.7×10^{-4}	2.5×10^{-4}	2.5×10^{-4}

[a] In thousands of corneocytes/cm^2.
[b] In μg/cm^2.
[c] In μg/corneocyte.
Note: Subjects were females, ages 20 to 25 yr.

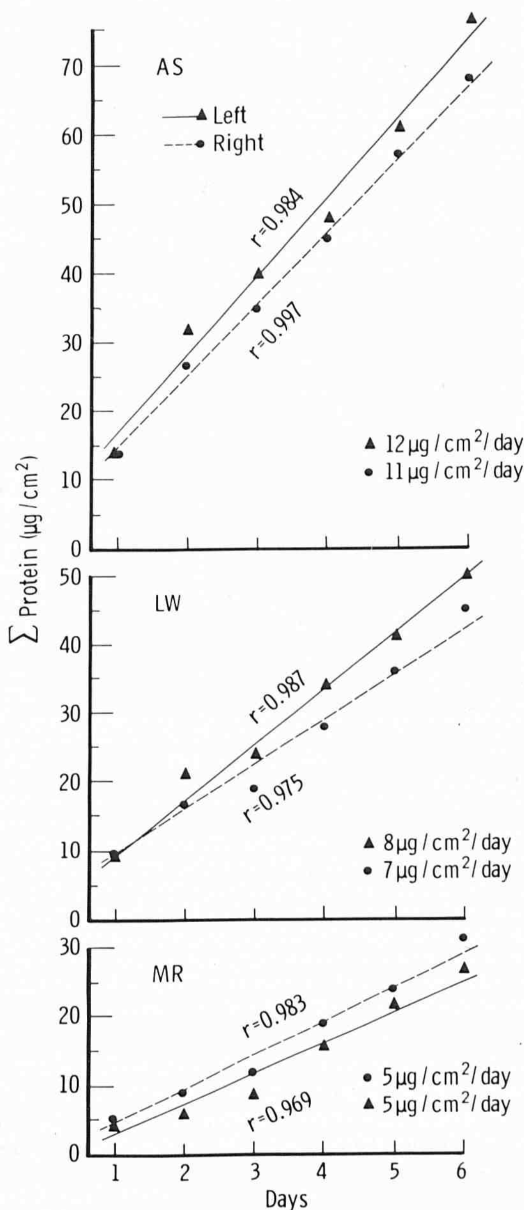

FIG 2. Baseline desquamation rates from contralateral sites. Individual rates (in μg protein/cm^{2}skin/day) are the slopes of the respective "cumulative protein vs. time" curve, obtained by linear regression analysis. Correlation coefficients are indicated on each *line*. Note the close agreement between opposite sides of the forehead on the same subject, but the difference between comparable sites on different subjects.

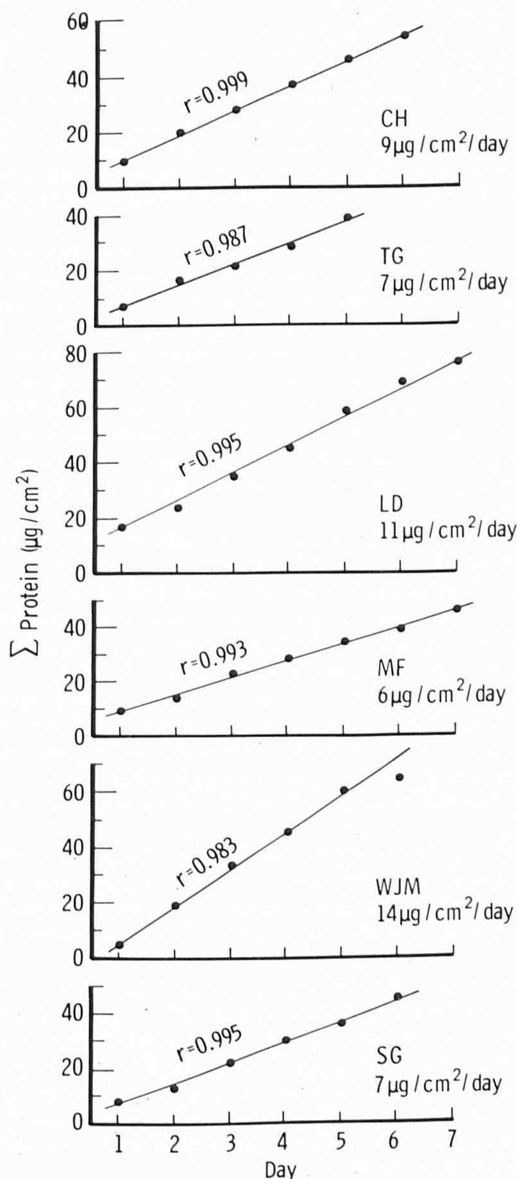

FIG 3. Individual variation in baseline desquamation rates. Rates and correlation coefficients are as in Fig 2. Subjects: male and female, ages 20 to 30 yr.

Effect of Topical Exfoliant Preparations Upon Desquamation

With salicylic acid treatment, large sheets of cells are entrapped by the Methocel films (Fig 4). Dozens of corneocytes are present in just one focal plane in a typical aggregate, which may be several cell layers thick. Quantification by counting is difficult because these aggregates resist mechanical disaggregation both in 0.2% Tween 80 and in 0.1% Triton X-100§§, a problem overcome by determination of extractable cell protein.

The effect of an exfoliant upon desquamation rate is evident from the study in which salicylic acid was applied to one-half the forehead, the other half serving as a no-treatment control.

§§ Rohm & Haas, Philadelphia, Pennsylvania.

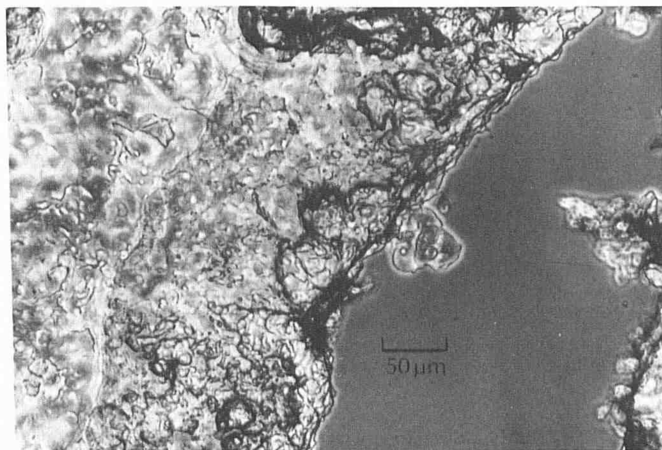

FIG 4. Corneocyte aggregate obtained by salicylic acid-induced desquamation. Typical cell aggregate from a site undergoing patent peeling after treatment with salicylic acid. The cell aggregate is suspended in 0.2% Tween 80 (unstained cells, photographed with phase contrast).

Treatment was stopped 3 days after the appearance of any visible signs of peeling to estimate the recovery or return to baseline. Figure 5 illustrates the changes typically observed. There was an increase in desquamation rate, generally starting a day or so after visible signs of peeling, and a return to baseline was seen 3 to 4 days after cessation of treatment. Both the time of onset and the extent of the change in desquamation differed in individual subjects. In Table III we show the onset of desquamation as well as the increase in total protein recovered at the end of treatment from the treated site over that from the control.

Also noteworthy is the fact that no inflections in the no-treatment (control) curves (Fig 5) appear where the collection regimen was interrupted for the weekend. This shows that the Methocel film does not remove more corneocytes than would be normally shed in one day; otherwise, the value on the first collection day after the interruption would be higher.

The results obtained with the salicylic acid treatment suggest that the pharmacologic potency of exfoliants can be assessed by: (1) the number of responders within the test group; (2) the magnitude of the peeling response in individual subjects; and (3) the time elapsed from initiation of treatment to the onset of peeling.

The results from 3 separate studies in which the exfoliant was a commercial resorcinol-sulfur cream are summarized in Table IV(A). Groups I and II were studied in the authors' laboratory; group III was studied by Richard B. Stoughton, M.D.¶¶

The exfoliant preparation used in these 3 studies—2% resorcinol in combination with 8% elemental sulfur—induced peeling in a majority of the subjects tested. Eight of these subjects also participated in the salicylic acid study (Table III). The rank order or response of each of these 8 individuals within the group was identical for both salicylic acid and resorcinol-sulfur cream. However, the magnitude of the individual response was greater with the salicylic acid solution.

The sensitivity of the method is demonstrated in a study where we attempted to assess the contribution of each of the active exfoliants in the resorcinol-sulfur cream tested before. Figure 6 depicts the factorial design used in the study and a typical response in a single subject. Response in a group of 6 subjects is summarized in Table IV(B). As may be seen, treatment with the cream base alone did not induce an increase in desquamation. When either resorcinol or elemental sulfur was added to the cream base, modest increases in desquamation

¶¶ Head, Division of Dermatology, Scripps Clinic and Research Foundation, LaJolla, California.

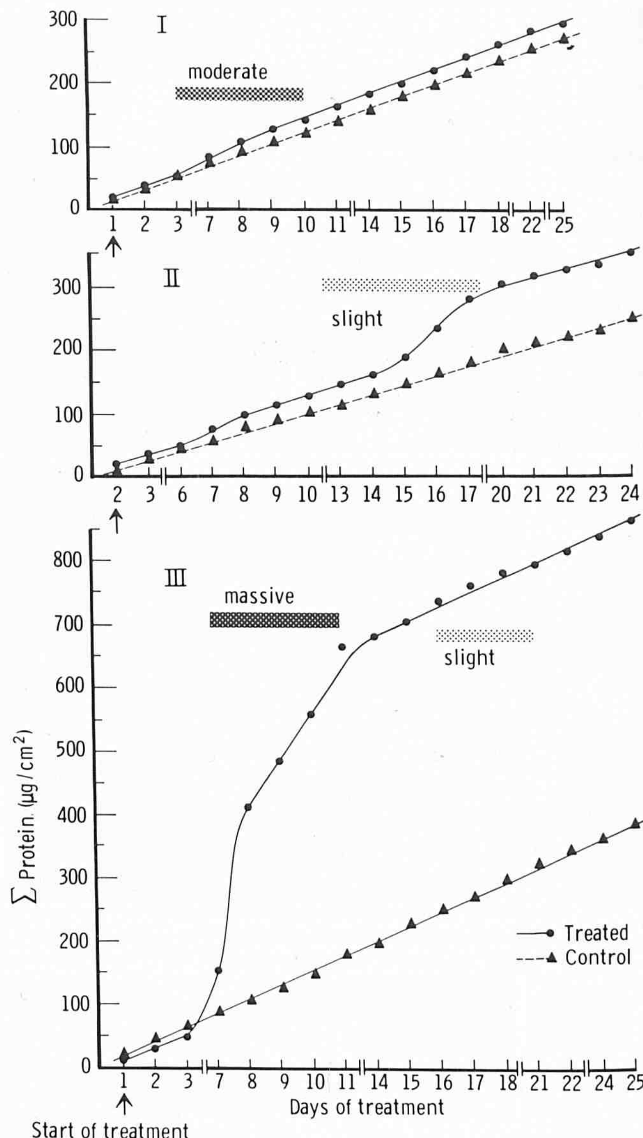

FIG 5. Effect of a topical exfoliant upon desquamation rates. A 5% salicylic acid solution was applied twice a day. Treatment was stopped 3 days after initial signs of patent peeling. *Cross-hatched bars* define the onset and end of patent peeling. Note that the visual estimate of peeling did not agree with corneocyte protein removed. *Arrows on abscissae* indicate when treatment was started. *Gaps on the abscissae* indicate an interruption of the sampling collection for the weekend (2 days), without interruption of the treatment regimen.

(generally late in the study) were observed. However, the combination of both these substances caused substantially increased rates of desquamation (Table IVA, Group III), generally occurring within one week after the application regimen was started.

DISCUSSION

Our technique for measuring desquamation and assessing exfoliants circumvents 3 aspects of an earlier technique [1]. These are: (1) rubbing or scrubbing the surface; (2) the use of a detergent, which may influence the number of corneocytes released from the surface; and (3) the necessity to disaggregate removed corneocytes so that they can be accurately counted.

As an alternative to scrubbing, corneocytes are collected by a Methocel film which forms upon the test site after the polymer is applied in the form of a wet gel. Corneocyte removal is effected by simply peeling off this film, and the cells are

determined as alkali soluble protein, the extraction of which does not require a prior disaggregation of clumped cells.

With the described procedure, baseline rates of desquamation are constant in the absence of treatment or environmental insults such as sunburn. This is demonstrated by the linearity of the corneocyte protein curves, which shows that a constant amount of protein is collected from each sampling site every day. Desquamation, as measured by this technique, is an individual characteristic, since rates varied from subject to subject.

As seen in Table II, a rather constant amount of protein per corneocyte was found when baseline figures were compared. Thus, the individual variation observed is due to differences in the number of corneocytes shed daily by the subjects. It is clear that the desquamation rate, as measured by this technique, can also vary among subjects if the amount of protein per corneocyte differs. This situation may also occur when peeling is induced. Thus, this method will yield an increase in the desquamation rate either if the amount of alkali-soluble protein per cell increases, or if the number of cells shed increases, and both events are recognized as an alteration of the desquamation process.

We have shown (Table I) that 2 or 3 successive hourly samplings yield decreasing amounts of corneocyte protein. In contrast to this (Figs 2, 3, and 5), successive daily samplings yield a constant amount. These observations indicate that a significant part of the shedding portion of the stratum corneum is removed by the film, but that this fraction can be restored within 24 hr. That there is no further buildup of this shedding portion is demonstrated by control curves such as those in Fig 5. These curves confirm that no increment in removable stratum corneum occurs when the sampling schedule is interrupted for 2 or 3 days. Hence, the Methocel film collects about what can normally be shed in a day under ordinary conditions.

A useful application of the method as an indicator of pharmacologic effect is illustrated by the comparison of the response to a combination of resorcinol and sulfur, to resorcinol or sulfur separately, and to a vehicle without these ingredients. Increased corneocyte shedding shows both that exfoliant is being delivered into the skin and that its putative exfoliant effect is occurring. In this particular case, it is evident that resorcinol and elemental sulfur act cooperatively; that is, the combination is demonstrably more effective than either substance used singly or than the vehicle base to which they are added.

TABLE III. *Effect of salicylic acid upon desquamation*

Subject	Day of onset[a]	Cumulative increase in corneocyte protein in response to treatment[b]
GM[c]	13	53
HS[c]	7	32
MT[c]	7	18
RM[c]	6	318
DT[c]	7	280
DF[c]	7	67
FT[c]	0	—
PG	8	8
EJ	8	17
PB	9	17
BV	15	34
KW	7	102
LW[c]	7	18

[a] Onset is defined as an inflection in the peeling curve derived from the treated site.
[b] The increase in desquamation is shown here as the percent difference between treated and control sites at the end of treatment.
[c] These subjects also participated in the study summarized in Table IV—Group III.
Note: Subjects were males and females, ages 20 to 30 yr.

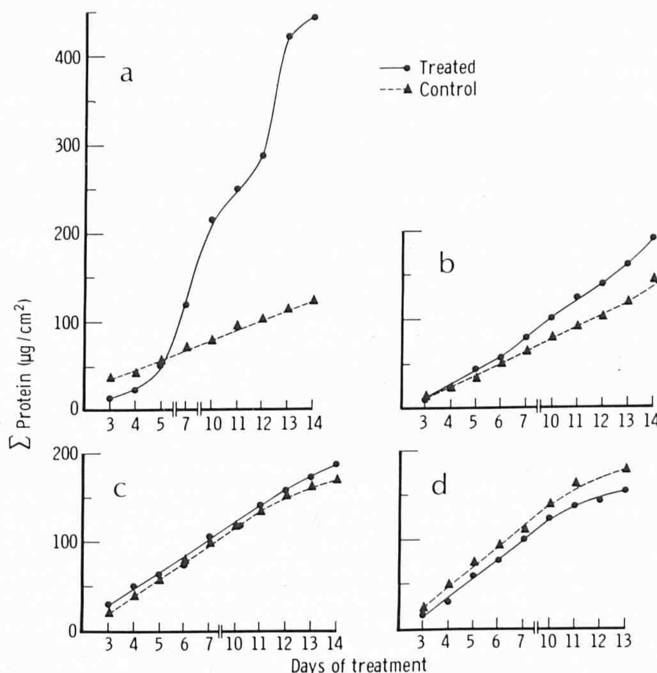

FIG 6. Increased desquamation induced by a sulfur-resorcinol cream and the contribution of each of the active exfoliants. *a*, Sulfur-resorcinol cream; *b*, sulfur cream (no resorcinol); *c*, resorcinol cream (no sulfur); and *d*, cream base. Data from a single subject, female, age 24 yr.

TABLE IV. *A. Effects of resorcinol-sulfur cream upon desquamation*

	Number of Responders[a]	Average Onset of Response[b]	Slope[c] Treated	Slope[c] Untreated	Slope Difference[c]	
Single daily application						
Group I	7/7	3	18	11	7	$.1 > p > .05$[d]
Twice daily applications						
Group II	4/6	6	29	12	17	$.1 > p > .05$
Group III	4/8	5	31	16	15	($p < .001$)
B. Contribution of individual ingredients to the exfoliant effect						
Cream w/o Resorcinol	3/6	9	17	13	4	
Cream w/o Sulfur	4/6	12	16	14	2	
Cream base	0/6	—	14	17	−3	

[a] Slope of treated side significantly higher than that of untreated side.
[b] Days after instituting treatment.
[c] μg protein/cm²/day.
[d] Significance of the slope difference as determined by the Student *t*-test.
Note: subjects were males and females, ages 20 to 30 yr.

These studies also affirm that pharmacologic response to exfoliants can be easily measured by using contralateral sites, one of which is not treated; thus, the subject serves as his own control. Such a design is essential, since baseline rates of desquamation are an individual characteristic. Our experiments further indicate that pharmacologic response to exfoliants also is an individual characteristic.

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Announcement

Dermatology Meeting in Conjunction with the American College of Physicians Meeting March 25, 1979, San Francisco

The American Academy of Dermatology, American Dermatological Association, and the AMA Section Council for Dermatology are sponsoring a Dermatology program on Sunday, March 25, 1979, in conjunction with the American College of Physicians Annual Meeting in San Francisco (March 26–29). The format will consist of 12-minute presentations with 3 minutes for discussion. If you wish to participate, please submit abstract(s) of no more than 200 words, double-spaced and in duplicate by December 1, 1978, to Dr. George W. Hambrick, Jr., Department of Dermatology, College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267.