DERMATOLOGICAL EVALUATION OF COSMETIC PRODUCTS FOR SKIN DETERGENCY


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Received: 10 January, 1993

Key words: Safety, Efficacy, Cleansing.

Synopsis

In this study the Authors evaluated, on the dermatological and cosmetological point of view, a panel of sixteen products of cutaneous cleansing, through the utilization of standardized methods. Today is still necessary to execute codified tests of high reliability to establish the safety and efficacy use of cosmetic products.

These tests were conducted both by Dermatologists and Cosmetologists.

Riassunto

In questo studio gli Autori valutano sotto l’aspetto cosmetologico e dermatologico un gruppo di sedici prodotti detergenti utilizzando metodiche standard. È necessario, infatti, utilizzare test codificanti che stabiliscono la sicurezza e l’efficacia nell’uso dei prodotti cosmetici.

Questi test sono stati condotti da Dermatologi e Cosmetologi.
Introduction

Cleansing of the skin without altering its physiological aspects is the goal of all those who work in the field of cosmetology. The habits of modern-day life have resulted in an increased use of skin cleansers, but, fortunately, advances in technology have rendered these products less aggressive to the skin. Dermatologists know that cleansing of the skin is extremely important to keep it in good condition. It is also essential to the health of the skin. When the skin is not cleaned regularly, microorganisms and tissue debris accumulate, and infection can occur. On the other hand, overly aggressive or frequent cleansing will inevitably lead to alterations in the cutaneous defense system.

Although water is the most common means of cleansing the skin, its use is not without risks. Water removes the hydrolipid film from the skin, as well as the dirt that is attached to it. When surfactants are added, complete delipidization of the skin and alterations of the horny layer cells can occur. It is important, then, to understand the structures and mechanisms at the base of the cutaneous defense system and to utilize products that not only provide satisfactory cleansing, but also respect the physiological needs of the skin.

Dermatological problems caused by skin cleansing

The principal effects of skin cleansing can be summarized as follows:
- delipidization
- modification of the pH
- alteration of the microbial flora
- alteration of the stratum corneum
- dehydration and increased transepidermal water loss
- irritation
- sensitization

These effects are all inter-related. The alterations touched off by cleansing initiate a chain of reactions which produce structural damage with multiple effects. For simplicity’s sake, however, we will discuss each effect independently. (1-15,50)

Delipidization

Use of water alone for cleansing can cause a 25% reduction in the lipidic film that protects the skin. The reduction is obviously greater when soaps or other surface-active substances are used.

Delipidization can become almost total if repeated cleansing occurs. This risk is especially high for persons who are forced to wash their hands often for professional reasons (health care workers or those employed in the chemical industry) but the same situation often occurs with housewives.

Delipidization results in the removal of substances that are dispersed within the hydrolipid film of the skin barrier and alteration of the functions performed by these substances. Reduction of the skin's protective barrier increases the risk of damage to the underlying stratum corneum and the removal of the chemical substances contained in both the epidermal and sebaceous lipids also leads to changes in the skin's pH. The effect that these lipids, especially those of the epidermis, have in keeping the stratum corneum pliant, is thus lost.

It is important to remember that loss of the lipid film also leads to loss of water and can facilitate drying. Tactile sensitivity is also reduced when this microfilm is removed.

Since certain fatty acids, unsaturated (C12, C22) and saturated (C9, C15), have been shown to exert bacteriostatic and antymycotic actions in the skin, it is clear that modification of the skin's normal flora will also occur with removal of skin lipids. This situation can, at least in theory, predispose the skin to attack by pathogens. (2-20)

Modification of pH

Changes in skin pH are caused by loss of the lipid layer, as we have already seen, as well as by the use of products with pHs that are different from that of normal skin. The effects on pH exerted by traditional soaps and synthetic deter-
gents are, in this latter respect, quite different. Soaps traditionally have alkaline pHs while synthetic detergents do not. The most important factors involved in the modification of cutaneous pH are the amount of time the detergent substance remains in contact with the skin, the amount of delipidization that occurs and the frequency with which the substance is used. Although soaps are often better tolerated than synthetic detergents, the former undoubtedly cause greater changes in skin pH. Recent studies have shown that the time needed for normalization of the cutaneous pH is significantly longer when alkaline products are used. The pH has a marked effect on the bacterial flora of the skin, and modification of the latter, especially in areas subject to maceration or pseudoanaerobiosis, can lead to fungal or bacterial infections. (2-20)

**Changes in the microbial flora of the skin**

We have already mentioned that certain fatty acids exert a powerful fungicidal effect. These are the saturated fatty acids C9 and C15. Bactericidal effects are produced by the unsaturated fatty acids C12 and C22. Delipidization, then, along with pH alterations, is the principal cause of alterations in the skin's microbial flora. (21) Ronchi and others have shown that, after cleansing with Marseille soap, skin pH does not return to normal for at least two hours. Regions of the skin that are rich in sebaceous and apocrine glands (e.g. the axillae or pubic zone) and which consequently host a potentially pathogenic flora are particularly threatened by this effect of cleansing. (4)

**Alteration of the stratum corneum**

These changes are caused by a) keratin swelling, b) the direct action of hard water in the presence of soaps, c) delipidization, d) dehydration and e) absorption of tensioactive substances. Each of these mechanisms has effects on the cells of the horny layer. (3,25,26)

Deposition on the skin of fatty acid Ca and Mg salts, which are insoluble in water, causes occlusion of the openings of the hair follicles and sweat glands, which leads to irritation. The phenomenon of dehydration causes the stratum corneum to lose its flexibility. At their isoelectric points, the proteins of the stratum corneum absorb water poorly. The isoelectric point of keratin is 4.1. During washing, the alkalinity of the soap and the actions of the anionic surface-active substances cause breakage of the hydrogen bonds of the interchain peptides and the stratum corneum thus becomes more absorbent. Absorption of the surfactants that are left on the skin also alters the skin barrier allowing increased absorption of water and an influx of ions and other substances. These surfactants also dissolve the hydrolipid film. Some surface-active substances, such as the alkyl sulfates and the alkyl-benzene-sulfates, bind to the proteins of the horny layer where they damage the keratin by transforming the alpha helixes into beta helixes. (27)

**Dehydration and increased trans-epidermal water loss**

Delipidization, alterations in the stratum corneum, breakdown of the keratin alpha helixes and removal of the natural moisturizing factor all contribute to a reduction in the water-binding capacity of the stratum corneum and an increase in the trans-epidermal water loss (TEWL). Delipidization alone increases normal TEWL by 10%. (4,5,11,23)

**Irritation**

All of the mechanisms that we have mentioned thus far ultimately lead to a breakdown of the horny layer, which allows the surface-active
Dermatological evaluation of cosmetic products for skin detergency

substances themselves to penetrate through to the vital layers of the epidermis. Short-chain fatty acids contained in detergents are more irritating than long-chain saturated fatty acids. The irritating effect of the fatty acids increase up to C12 and then diminish as the length of the chain increases. Sodium laurate is, thus, quite irritating to the skin while sodium stearate, even though it has a higher pH, is better tolerated. For these reasons, protein hydrolysates are often added to soaps or synthetic detergents to bind the free short-chain fatty acids and decrease the irritant potential of the product. It is important to remember that, in addition to the irritating effects of the surfactants, the other negative effects of cleansing work together to bring about severe irritation and even irritative contact dermatitis. (3,5,11-13,25,28,29)

**Sensitization**

As the cutaneous barrier weakens, haptons and allergens penetrate the skin and reach the vital layers of the epidermis. Here immunocompetent cells initiate a process of defense which, after repeated contact, results in allergic contact dermatitis. (30-33)

**Experimental work**

**Introduction**

We attempted to evaluate several skin cleansing products currently on the market to determine how well they were tolerated and how acceptable they were from cosmetic points of view. These products included both traditional soaps and synthetic detergents. The term “soap” is hereafter used to refer not only to traditional, alkaline soaps but also to those synthetic detergents sold in bar form. Sixteen soaps, either traditional or synthetic, were selected for this study and each was assigned a number. The choice of products was based on sales data and reflects the common belief that all products used for cleansing of the skin are soaps.

The products were tested on a total of 150 healthy volunteers. The subjects were randomly assigned to groups which were similar in make-up. Informed consent was obtained from all participants. The study lasted six months.

Evaluation of patient tolerance and cosmetic acceptability involved the difficult task of standardizing our methods and parameters of evaluation. The first parameters that had to be considered were the aggressivity of the product and the patient’s cutaneous tolerance of it. In addition, the products were also evaluated from cosmetic points of view, i.e. moisturizing, smoothing and softening effects, etc. For this reason we decided to subject the products to a battery of tests to compare their effects and identify the characteristics of each. The tests included:

- Draize test for cutaneous irritation
- Soap chamber test
- Measurement of cutaneous pH
- Measurement of sebum levels
- Measurement of stratum corneum water content
- Scotch tape test
- Half-face test
- Flex wash test
- Cutaneous imprints

**Cutaneous irritation test**

The purpose of this test was to evaluate the irritative potential of the product when applied to the skin. The most effective method for determining the incidence of irritative contact dermatitis due to use of topical drugs or cosmetics is patch testing, which was first introduced by Jadassohn and Block. Since its introduction, it has been standardized according to the indications of numerous investigators. (31-35)

**Materials**

The products must remain in contact with the skin for a certain period of time if its irritative potential is to be evaluated. The “patches” used to maintain this contact must be made from ma-
Materials that are, themselves, non-sensitizing. They must also be able to absorb the test substance and, finally, to isolate the test area from the rest of the skin. According to specific needs the following products can be used:

1. Dermotest diagent: this patch consists of a small sheet of aluminium foil covering a layer of polyethylene. In the center of the polyethylene sheet is a small cellulose disk upon which the test substance is applied.

2. Finn Chambers: A hypoallergenic adhesive strip with two rows of aluminium chambers attached to one side. Cellulose disks can be inserted into the chambers if liquid substances are to be tested.

3. Covertest diagent: this adhesive strip does not contain any of the haptens that are present in normal tapes. It can be used to secure either the Dermotest patches or Finn chambers.

4. Dermographic ink: used to mark the skin areas being tested so that they can be checked after the patches have been removed.

5. Micropore tape: used to attach strips containing test substances to the skin.

6. Whatmann paper filter disks (nos. 1 and 3): useful for absorbing liquid substances to be tested.

7. Non-occlusive patch tests: a porous, non-occlusive adhesive strip with a Whatmann paper disk (no. 1) attached. These strips are designed for use with products that might cause skin damage if applied with an occlusive cover.

**Study methods**

The method used to determine the irritant potential of the cleansing products tested was a modification of the Draize test. (34) This test was originally designed for use in animals but over the years minor modifications have been made, mostly in the interpretation of the results, and it has now been used for many years to test both strong and weak irritants in man.

The method involves direct application of the test substance on the skin of the subject. In order to safeguard the subject, all substances have previously been tested in animals. Although these preliminary studies do not permit us to predict the human subject's reaction to a given substance, the investigator at least has an idea of the substance's irritative potential: strong, moderate or weak. All of the substances examined in the present study had been classified as weak irritants on the basis of animal studies.

**Modified Draize test**

**Preparation of test substances**

Samples that contain surfactants are generally tested at concentrations ranging from 1% to 10%. All of our samples were tested at a 2% dilution. Products must be diluted on the day of testing.

**Application**

The substances may be secured to the skin using either occlusive or non-occlusive adhesive strips. The former are generally used unless the test substance is known to be a strong irritant.

**Preparation of Finn Chambers**

In the present study aluminium Finn chambers containing product samples were attached to the subject's back using occlusive Covertest diagent. Whatmann no. 3 filter paper disks measuring 1 cm² were placed in Petri dishes to absorb the diluted, liquid test substance. Using tweezers the disks were pressed against the side of the dishes to squeeze out any excess liquid and inserted into the aluminum chambers.

**Execution of the test**

The skin of the subject's back is cleaned with a 70% solution of alcohol and the samples are applied in two rows, one on either side of the spine. The exact location of the strips will depend on the number of substances to be tested as well
as the presence of nevi or pre-existing areas of discoloration. The samples are left in place for 48 hrs and the subject is instructed to keep the area dry. At the end of this period the subject returns and the strips are removed. Residual test substances are cleaned away and the contact point is circled with dermographic ink so that it can be read later.

**Reading the results**

Skin reactions are observed 15 min and 24 hr after the strips have been removed. The severity of the reaction is scored according to the following scale and any lesions or signs of toxic reactions are carefully examined.

### Evaluation Scale

<table>
<thead>
<tr>
<th>Erythema</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Barely visible</td>
<td>1</td>
</tr>
<tr>
<td>Visible</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
</tr>
<tr>
<td>Intense (possibly with slight crusting)</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Edema</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Barely visible</td>
<td>1</td>
</tr>
<tr>
<td>Visible</td>
<td>2</td>
</tr>
<tr>
<td>Moderate (borders raised circa 1 mm)</td>
<td>3</td>
</tr>
<tr>
<td>Intense (edema outside the test area)</td>
<td>4</td>
</tr>
</tbody>
</table>

**Scoring the results**

At each reading (15 min, 24 hr) each application site is scored according to the above scale for erythema and for edema, and the sum of the two scores (erythema and edema) is calculated. The average of the final scores for all subjects was calculated for each product. The Final Results Table contains the average group scores at each reading for each product tested.

**Classification of the products**

Each product was classified as a non-irritant, a mild irritant, a moderate irritant or a strong irritant according to a modification of the scale originally proposed by Draize. This modified scale, which considers as non-irritants only those products with scores of less than 0.5, allows the investigator to distinguish more easily between mild, non-specific reactions and those that are due to the test substance itself.

<table>
<thead>
<tr>
<th>Modified Draize Classification</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Index</td>
<td>Classification</td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>Non-irritant</td>
</tr>
<tr>
<td>0.5-2.0</td>
<td>Mild irritant</td>
</tr>
<tr>
<td>2.0-5.0</td>
<td>Moderate irritant</td>
</tr>
<tr>
<td>5.0-8.0</td>
<td>Strong irritant</td>
</tr>
</tbody>
</table>

**Selection of volunteers**

The study was conducted on 40 healthy volunteers, male and female, between the ages of 18 and 65. Subjects were excluded from the study if they had: 1) dermatitis or positive histories for allergic tendencies, and / or 2) participated in a similar study within three months prior to the beginning of our study. The purpose of the study and the possible risks involved were explained to all subjects prior to obtaining their consent.

**Results**

Irritation skin test, conducted through occlusive patch-test methods, is helpful to point out moderate or strong irritants. This test is suitable to point out weak irritants, especially if used products potentially irritant but in low concentration as those normally employed. In other words Draize's test needs, to give positive results, higher concentrations than those normally used in the products.

In our study, evaluating some soaps, we used a 2% concentration, probably the same normally present during the use of a soap-bar. Our results demonstrated that any soaps were able to induce, in that concentration and through employed method, important skin irritation (table I).
Soap Chamber Test

This test allowed us to evaluate the irritant potential of repeated applications under stress of a 7% solution of each test substance. Each product was tested in this manner on 30 volunteers for seven days.

Selection of volunteers

Volunteers of both sexes between the ages of 20 and 65 were selected for these tests. Prior to testing, potential candidates were subjected to screening with the modified Draize technique to determine their tolerance of sodium lauryl sulfate. Those with positive results were excluded from the soap chamber test. This preliminary screening facilitated interpretation of the results of the latter test.

Preparation of samples

Aqueous 7% solutions of each product were used for testing. At this concentration, the solutions were excessively dense to be applied easily and had to be heated slightly to liquefy them.

Method of application

Substances were applied to the subject’s skin using During chambers. The During chamber is an aluminum chamber 12 mm in diameter containing two layers of cotton which absorb a considerable quantity of the test substance. The chamber is applied with an occlusive dressing in order to demonstrate the irritant potential of the test substance under conditions of stress. (1,37,38,53)

Test execution:

1. The skin of the volar surfaces of both forearms was cleaned with a 70% alcohol solution. Because of the limited space available for testing, only eight samples were tested at a time: four on each forearm.

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKIN IRRITATION TEST: RESULTS</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>IIM 15 (1)</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>01</td>
</tr>
<tr>
<td>02</td>
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<tr>
<td>03</td>
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<tr>
<td>04</td>
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<td>05</td>
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<tr>
<td>06</td>
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<tr>
<td>07</td>
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<td>08</td>
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<td>09</td>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
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<tr>
<td>12</td>
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<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
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<tr>
<td>15</td>
</tr>
<tr>
<td>16</td>
</tr>
</tbody>
</table>
The contact portion of the testing lasted for five days. On the first day of the test, the chambers were filled and applied to the skin where they remained for 24 hrs. The subject was instructed to refrain from wetting the test area. After 24 hrs the chamber was removed and refilled with the same quantity of the test substance. The chamber was reapplied to the same site and left in place for six hours. This process was repeated on days 3, 4 and 5. Contact during these last four days was limited to six hours each. The test areas were left unprotected during the hours between daily applications. The last application was removed on the fifth day of testing and the results were read 36 hrs later, i.e. on the seventh test day.

The initial application is left in place four times longer than the subsequent four. This serves to increase the permeability of the skin barrier. The 18-hour periods between each subsequent application are aimed at reducing any acute inflammation that might occur and to allow the development of visible drying, wrinkling or desquamation.

**Reading of the test results**

The parameters of possible irritative reactions are:
- Erythema
- Desquamation
- Fissure formation

Each is evaluated according to the following scales:

<table>
<thead>
<tr>
<th>Erythema</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Slight (mottled or diffuse)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate and uniform</td>
<td>2</td>
</tr>
<tr>
<td>Intense</td>
<td>3</td>
</tr>
<tr>
<td>Intense with edema</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Desquamation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Fine</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fissure formation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Fine</td>
<td>1</td>
</tr>
<tr>
<td>Superficial (single or multiple)</td>
<td>2</td>
</tr>
<tr>
<td>Deep with hemorrhage or exudate</td>
<td>3</td>
</tr>
</tbody>
</table>

**Calculation of results**

Each product was tested on 30 subjects. For organizational reasons, these subjects were divided into three groups of ten each. As with the Draize test, the scores for each of the three parameters were added together to obtain the subject score for each substance. These individual scores were then averaged for each group of ten subjects. These mean indices are presented for each product in Table 2.

The average of these three group indices was then calculated for each product (mean irritant index).

**Product Classification**

Based on their mean irritant indices, the sixteen products tested were classified into four categories:
- non-irritants
- mild irritants
- moderate irritants
- strong irritants

The results of this classification are shown in Table 3.

**Discussion**

This classification system has been used in other studies like ours and obviously reflects the skin responses to the various products being examined. In fact, the soaps classified as moderately irritating cause moderately severe alterations of the skin and the sum of the scores assigned to these changes ranges from 3.0 to 4.5.

Below this level, in the score range of 1.0 to
**Table II**

SOAP CHAMBER TEST RESULTS
Mean values for erythema, desquamation and fissure formation

<table>
<thead>
<tr>
<th>Product</th>
<th>Group 1*</th>
<th>Group 2*</th>
<th>Group 3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0.5</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>02</td>
<td>3.8</td>
<td>3.9</td>
<td>4.1</td>
</tr>
<tr>
<td>03</td>
<td>3.6</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>04</td>
<td>4.5</td>
<td>4.7</td>
<td>4.6</td>
</tr>
<tr>
<td>05</td>
<td>4.6</td>
<td>4.7</td>
<td>4.8</td>
</tr>
<tr>
<td>06</td>
<td>6.3</td>
<td>6.1</td>
<td>6.0</td>
</tr>
<tr>
<td>07</td>
<td>6.6</td>
<td>6.4</td>
<td>6.3</td>
</tr>
<tr>
<td>08</td>
<td>6.6</td>
<td>6.5</td>
<td>6.4</td>
</tr>
<tr>
<td>09</td>
<td>3.9</td>
<td>3.8</td>
<td>3.4</td>
</tr>
<tr>
<td>10</td>
<td>4.1</td>
<td>4.1</td>
<td>4.3</td>
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<tr>
<td>11</td>
<td>2.1</td>
<td>3.1</td>
<td>3.2</td>
</tr>
<tr>
<td>12</td>
<td>2.7</td>
<td>2.5</td>
<td>2.9</td>
</tr>
<tr>
<td>13</td>
<td>2.6</td>
<td>2.3</td>
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<td>2.4</td>
<td>3.1</td>
<td>2.8</td>
</tr>
<tr>
<td>15</td>
<td>2.6</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>16</td>
<td>2.5</td>
<td>2.2</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* each group contained 10 subjects.

**Table III**

PRODUCT CLASSIFICATION BASED ON MEAN IRRITANT INDEX
Mean indices based on results of soap chamber test in 30 subjects.

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean index</th>
<th>Classification *</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0.5</td>
<td>non-irritant *</td>
</tr>
<tr>
<td>16</td>
<td>2.3</td>
<td>moderate irritant *</td>
</tr>
<tr>
<td>15</td>
<td>2.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>13</td>
<td>2.5</td>
<td>&quot;</td>
</tr>
<tr>
<td>12</td>
<td>2.7</td>
<td>&quot;</td>
</tr>
<tr>
<td>14</td>
<td>2.8</td>
<td>&quot;</td>
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<tr>
<td>11</td>
<td>3.0</td>
<td>&quot;</td>
</tr>
<tr>
<td>03</td>
<td>3.6</td>
<td>&quot;</td>
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<tr>
<td>09</td>
<td>3.7</td>
<td>&quot;</td>
</tr>
<tr>
<td>02</td>
<td>3.9</td>
<td>&quot;</td>
</tr>
<tr>
<td>10</td>
<td>4.2</td>
<td>&quot;</td>
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<tr>
<td>04</td>
<td>4.6</td>
<td>&quot;</td>
</tr>
<tr>
<td>05</td>
<td>4.7</td>
<td>&quot;</td>
</tr>
<tr>
<td>06</td>
<td>6.1</td>
<td>strong irritant *</td>
</tr>
<tr>
<td>07</td>
<td>6.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>08</td>
<td>6.5</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

<0.5 = non irritant  
0.5-2.0 = mild irritant  
2.0-5.0 = moderate irritant  
5.0-8.0 = strong irritant
3.0, products are classified as weak irritants. Scores less than 1.0 indicate that the product is a non-irritant. (1,37-40)

It is almost impossible not to provoke some form of cutaneous lesion with the method used here. For this reason, the classification of “non-irritant” is given to those products with mean irritant indices of less than 1.0. According to this classification, only product no. 01 met the criteria for a non-irritant.

**Flex Wash Test**

The Flex wash test, first introduced by Kligman et al., is one of the most useful and reliable tests available for evaluating the irritant capacity of a substance. The test was designed to determine, within a relatively brief period of time, whether a product can provoke cutaneous irritation. (1,47,48,49)

**Materials and methods**

Because of its sensitivity, the skin of the antecubital fossa is used for this test. The volunteer washes this area three times a day with a predetermined quantity of the product at a standardized concentration. The cleansing should be performed by delicately massaging the area with the fingers for approximately thirty seconds. The area is then thoroughly rinsed and patted dry.

The above routine is repeated for five days and the area is examined daily for signs of irritation. The test is determined at the first sign of significant irritation.

Eighty subjects, divided into eight groups of ten each, were subjected to the flex wash test in the present study. The subjects of each group tested two products simultaneously, one on each arm.

**Results**

Since each group was composed of ten subjects, the results are expressed as percentages of subjects who had to interrupt the test, because of signs of irritation, before the five days were up.

Product no. 01 was associated with the lowest percentage of interruptions and therefore seems to be the least irritating even with excessively frequent use. The rank of the other fifteen products in terms of increasing irritant potential can be seen in Figure 1.

Since product no. 01 emerged from all of the previously described tests as the least irritating, we used it as a reference for comparison in all subsequent tests.

**Fig. 1 - FLEX WASH TEST (% dropped out)**

**Half-Face test**

The most common problem encountered when we attempt to study the effects of two or more cleansing products on the skin is that the objective reaction, non to mention the subjective evaluation of the effects, varies from subject to subject. The only way to eliminate this problem would be to test all of the products on the same subject using the same skin surface area for each test! Given the impracticality of such an approach, the value of the half-face test becomes obvious: it allows comparison of at least two products at the same time in one patient utilizing the same skin region.
Materials and methods

For this test, 150 volunteers between 18 and 55 years of age were selected. The study group included both males and females. None had active lesions in the facial area and all denied history of allergic contact dermatitis.

The subjects were divided into 15 groups of ten. Each member of a given group was given two soaps, one of which was always product no. 01. In this way, each of the 15 products being compared with product no. 01 was tested in ten subjects.

The subject was instructed to wash one half of his/her face with product no. 01 and the other half with the comparison product. Cleansing was to be carried out twice a day for five consecutive days.

Before washing the face, the subject was instructed to wash his/her hands with a standard soap that contained no fragrances in order to eliminate possible contaminants from the hands which might interfere with our interpretation of the results.

The test was carried out as follows:

1. The hands were washed with one of the study products for 15 seconds.
2. One half of the face was gently washed with one of the study products or with product no. 01.
3. The face was rinsed and gently patted dry without rubbing.

The same procedure was then repeated for the other half of the face (using the second product). At the beginning of the study each subject was given a form to fill out and return at the end of the study period. This form contained questions regarding the effects of cleansing with the two products.

The parameters to be evaluated with the questionnaire were:
- dryness
- moisture
- tightness
- softness
- roughness
- smoothness

The subject was asked to respond to each question with “yes”, “no” or, in some cases, “I don’t know”.

The questionnaire is shown in the following table.

<table>
<thead>
<tr>
<th>Half-Face test questionnaire</th>
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</thead>
<tbody>
<tr>
<td><strong>Subject no.</strong></td>
</tr>
<tr>
<td><strong>Questions</strong></td>
</tr>
<tr>
<td>Does the right/left side of your face feel dry/moist/tight/soft/rough/smooth after washing?</td>
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<tr>
<td>1st washing:</td>
</tr>
<tr>
<td>Which side feels drier/more moist/tighter/softer/rougher/smoothier after washing?</td>
</tr>
<tr>
<td><strong>Answers:</strong> Yes/No/Don’t know</td>
</tr>
<tr>
<td><strong>Surname and name/Tel./Address/Test Center</strong></td>
</tr>
</tbody>
</table>
Results

Based on both the responses to the questionnaire and the objective evaluation of the above-mentioned parameters, product no. 01 seemed to be superior to any of the other fifteen. For each parameter considered, a graph was drawn that shows, together with the data in Table 4, the responses of the volunteers. The graphs show the percentage of positive responses to the questions on each parameter, i.e. affirming that use of the given product was associated with feelings of skin dryness, softness, etc. Since the parameters shown in Figure 2 (tightness), 3 (dryness) and 4 (roughness) are negative ones, the products in these graphs that received fewer affirmative responses are those that were judged more positively, while products with high percentages of affirmative responses in Figures 5 (moisturizing effect), 6 (softness) and 7 (smoothness) are the ones that made positive impressions on the volunteers.

Table IV
HALF FACE TEST: RESULTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>01</th>
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<th>03</th>
<th>04</th>
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<tbody>
<tr>
<td>Dryness</td>
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<td>Hydration</td>
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<td>Tenderness</td>
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<td>Roughness</td>
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</table>

Fig. 2 -

Dermatological evaluation of cosmetic products for skin detergency
For example, if we consider the parameter of skin tightness, product no. 04 emerges as the most unacceptable since 92% of the volunteers found that washing with this product left their skin feeling tight.

Product no. 01, in contrast, was considered the most acceptable from this point of view: only 11% of the volunteers indicated that skin tightness was associated with its use.

**Measurement of sebum levels**

The purpose of skin cleansing is that of removal of “dirt”. However, the dirty portion of the skin is the hydrolipid barrier which contains not only environmental impurities but also epidermal tissue debris. The ideal skin cleanser must, then, remove at least part of the hydrolipid barrier but it should not deprive the skin completely of its protective covering. (2-5,42,43)

The skin of the face is one of the most delicate
areas of the body in which to test a product. Its anatomical structure is delicate, and certain parts of it are rich in sebaceous glands. Facial skin is also one of the cutaneous areas most exposed to environmental aggression. This almost constant exposure, coupled with the sensitivity of facial skin to psychological and nervous factors, makes the skin of the face unique. (42-44,46)

For these reasons, the behavior of a cleansing product in this particular region is especially indicative and can serve as an index of its safety in other less delicate skin areas. In addition, testing on facial skin offers an excellent indication of the delipidizing effect of a cleansing product, which, as we have already seen, is one of the most important parameters to be considered in evaluating the irritant potential of these substances. We therefore decided to analyze the sebum levels of the faces of our volunteers to determine more precisely the delipidization potential of our test products.

**Materials and methods**

One hundred fifty subjects were divided into 15 groups of ten each. These groups were the same ones that were used in the half-face test and were later subjected to analysis of stratum corneum water content as well.

Baseline sebum levels were determined for each subject using the Schwarzhaupt SM 410 Sebometer.

Using a photometric method, this instrument measures the quantity of cutaneous oils deposited on a semitransparent tape which is pressed against the test area and held in place for 30 seconds. Prior to testing, the instrument must be zeroed. The spool of tape is advanced until an unused portion is covering the application head. The tape is inserted in a photoelectric cell chamber. Its presence causes closure of an electrical circuit which switches on a light. The beam is amplified with a mirror and transferred to an amperometer.

The tape becomes more and more transparent as more sebum is deposited on it and, consequently, more light can pass through it to reach the amperometer. The quantity of light that passes through the tape is reflected on a digital display. Higher values indicate that more light is passing through the tape which has become more transparent due to a larger amount of sebum absorbed.

All of the subjects used product no. 01 on one half of their faces. Each group of ten subjects, (sex and age distribution were similar for all groups), used one of the other products on the other half of the face. On the day before test washing began, the baseline sebum levels were determined for each side of the subject’s face. Baseline values were based on five readings taken in adjacent areas of the facial skin. Measurements were made at the same hour of the day for all subjects and not less than eight hours after routine facial cleansing in the morning. The exact same procedure was repeated after the subject had completed the required five days of the half-face test. These final values were then compared to baseline figures.

**Results**

Figure 8 shows the average baseline and final sebometric indices for each of the sixteen products tested. These values are averages of the values for the ten subjects testing each product. In Appendix no. 1, the average values, baseline and final, for each of the 150 subjects are shown.
Certain soaps, such as product no. 13, product no. 15 and product no. 16 were associated with values very similar to those of product no. 01, which caused very little change in the facial skin sebum levels. Once again, product no. 01 emerged as the superior product, although the differences between this product and the ones mentioned above were actually quite limited.

All of the other soaps tested, with the exception of products no. 06 and 10, significantly diminished the sebum levels in the subjects who used them. Products no. 06 and 10, in contrast, caused an increase in sebum levels with respect to baseline values which can be considered to be a rebound effect (Figure 9).

Using these results, we can also make direct comparison between product no. 01 and each of the other 15 as far as their effects on each subject or each group are concerned.

Since, the aforementioned exhaustive analysis provided data only on product 01 compared to one other soap, it was decided to compare the difference in mean levels of sebum before and after five days of washing in the 15 groups of subjects who used various soaps on half their face with those of 150 subjects who used product 01 on half their face.

Some of these data are shown in Figure 9.

Measurement of stratum corneum water content

As pointed out earlier, one requisite for an acceptable skin cleanser is that it not alter the cutaneous barrier and therefore safeguard the integrity of the stratum corneum. Measurement of the level of hydration of this skin layer offers an indirect index of the aggressivity of a tensioactive substance since the skin becomes proportionally drier as alteration of the stratum corneum increases. We thus performed before and after corneometric examinations in the subjects who used the products being tested. (51,52)

Materials and methods

One hundred fifty subjects were used in this part of the study. Each washed one half of his/her face with product no. 01 and the other half with one of the other test products. Each of the latter products was tested in ten subjects, i.e. the same groups created for the half-face test and subjected to sebometric analysis. As with the latter approach, baseline values were determined before test washing began. These values were based on three consecutive readings taken from adjacent areas of the facial skin. Readings were made with a CM 420 corneometer made by Schwarzhaup. This instrument uses a cutaneous probe to measure the capacitance induced by various quantities of water contained in the most superficial layers of the epidermis. After completion of the half-face test, final values were determined for both sides of each subject’s face.

Results

The mean values, baseline and final, emerging from the corneometric analysis are shown in Figure 10. These figures indicate that only product no. 01 caused a significant increase in cutaneous hydration. All of the other products were
associated with modest increases or very slight decreases in cutaneous moisture levels. The significant increase associated with product no. 01 was seen in almost all of the 150 subjects who used it. Again these figure allow us to determine how each of the fifteen products compares with product no. 01 and to rank each product according to its effect on stratum corneum hydration. Appendix no. 2 contains the corneometric values for each of the fifteen test groups (expressed as group means); in the case of product no. 01, the values are the means of all 150 subjects who used this product.

In Figure 11, the sixteen products have been ranked in descending order according to the levels of cutaneous hydration reflected in the appendix figures.

Measurement of Cutaneous pH

The pH of the epidermal surface is determined by the biochemical and physical properties of the hydro lipid film that covers it. Skin pH varies with the area of the body being considered and is usually higher in those regions that contain apocrine sweat glands, such as the axillae or genital area, or where there is a buildup of epidermal cellular debris and sudoration products.

The average pH of skin is 5.5 although values between 4.5 and 6.0 are considered normal. (42,45,46) The physiological pH contributes to skin homeostasis, inhibits the growth of pathogenic organisms and provides chemical defense of the skin depends primarily on the buffer system provided by the eccrine sweat glands. The secretions of these glands are able to restore the pH to physiological values within a relatively brief period of time.

Other substances present in the epidermis also contribute to this buffer system: amino acids, fatty acids, uric acid, lactic acid, SO₄⁻ and Cl⁻ ions. These substances serve primarily to neutralize any excessive alkalinity. Cations, such as Na⁺, K⁺ and NH⁺, and other amino acids exert a buffering effect on excessive acidity. (4,42,46)

Cleansing is generally considered to be the most important factor involved in alterations of cutaneous pH. These alterations can facilitate the development of skin irritation which can then open the way to other pathological processes. It is thus important to evaluate the effect of any skin cleansing product on the pH of the skin. (42,47)

Materials and methods

Cutaneous pH was measured in 80 volunteers between the ages of 18 and 37. All subjects pre-
sented normal pH values in the regions tested prior to our measurements. Ten subjects were used for each test product.

The test period lasted for five days. Each day the subject used the product assigned to his/her group to wash the volar surface of one forearm in the morning and in the afternoon (not less than 8 hrs after the first washing). The other forearm was cleansed in the same way using product no. 01. The subjects used 1 ml of a 2% solution of the product and were instructed to use 30 seconds of manual massage of the area for each washing.

In the afternoon, 30 min. and 1 hr. after the second daily washing, the pH of the test areas were measured with a pH 900 PC skin pH-meter. Each value is a result of ten automatic measurements performed at two-second intervals.

**Results**

The baseline pH values in the study population varied from 4.0 to 5.5. In addition to the differences that emerge between soaps, synthetic detergents and products no 01, Figure 12 shows that the latter product produced less modification of the baseline pH than any of the other fifteen products. The group assigned product no. 01 had an average baseline pH of 5.2. At the end of the five days of washing, this average had risen to 3.35.

This represents an extremely modest increase to levels that are still within normal limits.

The other products under study provoked much more significant modifications in the pH, as can be seen from Figure 13. Use of traditional alkaline soaps led to a full point increase in skin pH from a baseline mean of 5.5 to a final value of 6.5 which is clearly no longer within the normal range.

Figure 13 also shows the differences that emerged between product 01 and the other 15 tested.

One of the most important harmful effects of cleansing is the damage it causes to the stratum corneum. Adhesion between the keratin plaques disappears and they are shed. This situation leads to an increase in TEWL. In order to determine the degree of damage to the stratum corneum provoked by each of these products, we performed two more tests: the Scotch tape test and cutaneous imprints.

**The scotch tape test**

This simple technique involves the application of an adhesive strip to the skin of the patient. When the strip is removed, a certain number of keratinocytes will come with it. The amount removed will be proportional to the degree of damage suffered by the stratum corneum.

In order to improve our evaluation of this para-
measure, the cells that were collected on the adhesive strip were examined under the electron microscope. The strip was attached to a cylindrical support (stub) 2.5 cm in diameter. Even though application pressure has little effect on the number of keratinocytes that will be detached, we used a spring-activated device to standardize the pressure. The scotch tape test was used on all 150 volunteers that participated in the half-face test. Cells were collected before and after the five-day cycles of test washing. The specimens were metallicized directly using a Balzer instrument with a gold electrode. They were then examined with a Cambridge 180 Stereoscan electron microscope.

Results

The numbers of cells that were detached from the stratum corneum before and after the washing cycle were compared for each subject. It was not possible to make comparison between groups, since each individual presents his/her own desquamation pattern. Our findings show that certain soaps were associated with greater desquamation after washing. Others produced little change in the desquamation. Product no. 01 was found to reduce more significantly than the others the number of stratum corneum cells that were shed.

Skin imprints

This approach involves the use of an inert substance capable of reproducing a mirror image of the cutaneous surface. The material (Xanthopren - Bayer) is pressed against a skin surface where it fills all depressed areas (sulci, wrinkles, etc.). These areas appear as ridges in the imprint. If the imprint is used as a mold and filled with another plastic material, the result will be a faithful reproduction of the skin surface. This method allows us to study the surface of a cutaneous area in vivo and to evaluate the changes induced by a cosmetic treatment or, in our case, a skin detergent. We made imprints of the lateral periocular and zygomatic areas of the subjects before and after the half-face test. The reproductions were metallicized with gold and examined with the Cambridge 180 Stereoscan electron microscope.

Results

The morphometric analysis of the cutaneous imprints allowed us to evaluate the damage to the stratum corneum induced by each of the products we tested. Most of the products provoked modifications of the superficial corneocytes which appeared poorly linked to one another, irregular and in some cases desquamated. The products associated with these effects were nos. 04, 07, 09, 02, 10 and 12. More modest changes were found in the subjects who had used products no. 13, 16, 11 and 14. The differences were quantitative rather than qualitative and it would be impossible to rank the products according to these findings. Limited changes or even improvement in the superficial morphology of the skin were observed with the use of both product no. 15 and product no. 01 (Figures 14, 15, 16, 17). The findings are illustrated in the photographs.
Conclusions

Although the results of our studies allow us to define one product as superior to the other fifteen, this is not, in our opinion, the most important finding of our study. We would rather emphasize the utility of these approaches for the identification of products that are potentially irritating or cosmetically unacceptable before they are placed on the market.

We found considerable differences among these products, but none of them was so irritating or cosmetically ineffective as to be judged in a completely negative manner. Overall, we found that several of the products we tested, some of them traditional soaps, others synthetic detergents, are well-tolerated and provide effective cosmetic improvement. In particular, products 01, 12, 13, 15 and 16 were all found to be weak irritants in our tests. Three of these products were synthetic, the other three were traditional soaps.

These findings were consistent with those that emerged from almost all of the tests we performed.

Product 01 consistently found to be the least aggressive and to produce the most cosmetically acceptable results. Between the two there were only very slight differences. Product no. 1 is a synthetic detergent.

This apparent similarity leads us to a general consideration. Cleansing of the skin is a complex and important problem. Choosing a product solely on the basis of whether it is a soap or a synthetic detergent is unwise. What matters more is that the formula upon which the product is based is a valid one, that the ingredients as well as the product itself are carefully controlled and that valid forms of quantitative testing are available to the manufacturer to help him to identify the product best suited to the consumers' needs as projected by the cosmetic industry. The cosmetological technician can carry out such tests and on the basis of the findings that emerge help identify the desired product.
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