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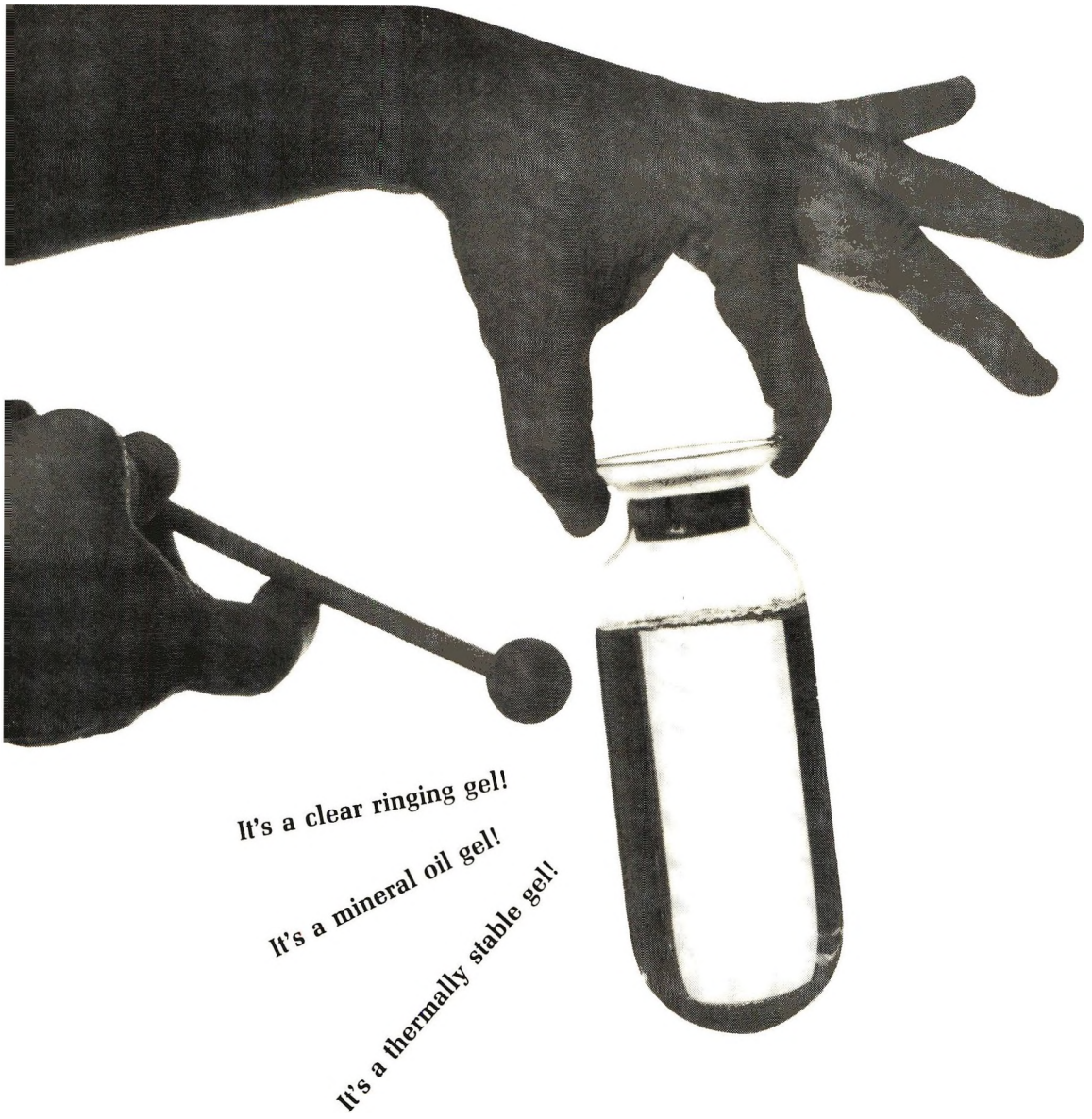

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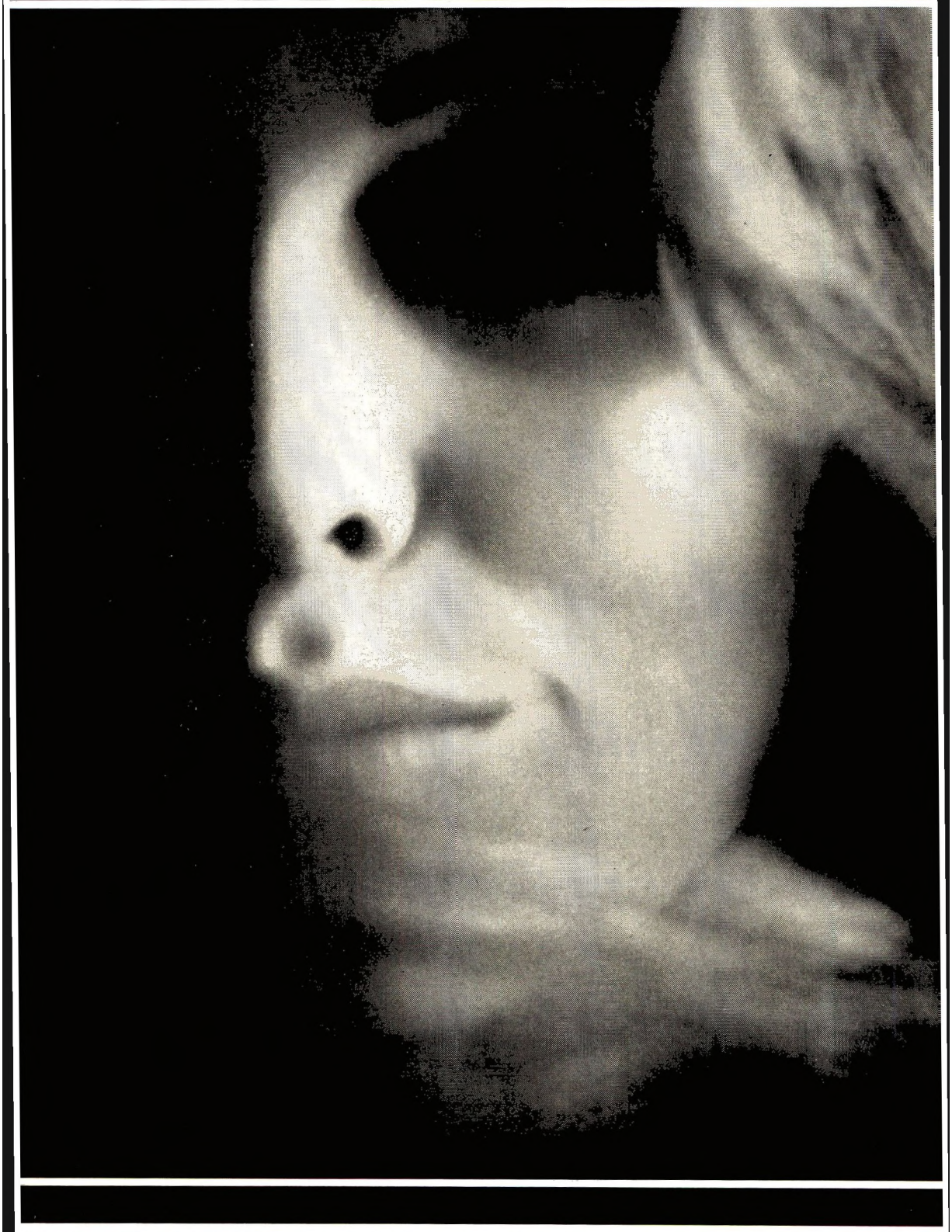

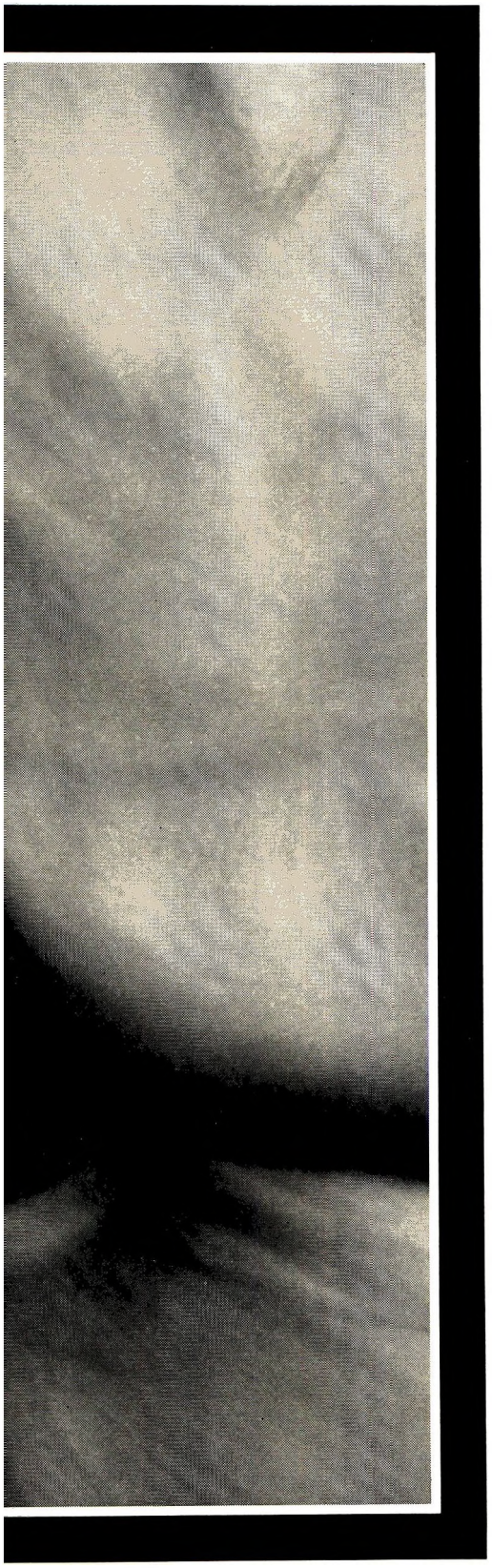

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 3 × 5 in. index cards for reference, without mutilating the pages of the Journal.

Measuring the meaning of fragrance: J. Stephan Jellinek. *Journal of the Society of Cosmetic Chemists* **18,** 755 (Dec. 9, 1967)

Synopsis—Three different techniques for measuring the meaning of fragrances are reviewed. Profiling, Semantic Differential, and Multidimensional Scaling are discussed with special emphasis on the last. The application of these techniques by perfumers and in consumer testing is described.

Hand degerming evaluation utilizing a split-use method: James Brown, Jr., Ronald J. Eriksson, Frank Yackovich, and David Taber. *Journal of the Society of Cosmetic Chemists* **18,** 769 (Dec. 9, 1967)

Synopsis—Utilizing a modified split-use method, two bacteriostatic soaps were evaluated for their utility in degerming the hands. One soap contained 0.75% (by weight) of hexachlorophene and 0.75% of 3,4,4′-trichlorocarbanilide; the other consisted of equal parts by weight of 3,5-di- and 3,4′,5-tribromosalicylanilides, 4,4′-dichloro-3-(trifluoromethyl)carbanilide, and 3,4,4′-trichlorocarbanilide for a total concentration of 2%. A significant reduction in bacterial counts was achieved by both soaps as compared to a nonmedicated soap. It is emphasized that rigorous handling of data requires that the confidence interval be identified when per cent reduction is used as a basis for describing degerming efficiency.

The color of red hair: Peter Flesch, Elizabeth J. Esoda, and Sidney A. Katz. *Journal of the Society of Cosmetic Chemists* **18,** 777 (Dec. 9, 1967)

Synopsis—The iron pigments extracted with boiling acids from human red hair and chicken feathers are closely related and are possibly identical. Evidence is presented that these unique substances are well-defined chemical entities and not artifacts of keratin hydrolysis. The iron pigment is probably the major pigment of human red hair. Its limited extractability from red hair is due to its destruction during extraction and not to the small amount present in the hair. In all of its forms the iron pigment has been proved to be a metallo-protein. It can be broken down to a compound with a relatively small molecular size which retains all the essential properties of the originally extracted pigment. Synthesis of an iron-protein in melanocytes raises many questions which cannot be answered at present.

Use of electronic data processing in anti-dandruff clinical research: Martin Greif and Harry J. Prokop. *Journal of the Society of Cosmetic Chemists* 18, 785 (Dec. 9, 1967)

Synopsis—Fifteen different compositions were studied in a pilot clinical program in terms of their ability to control the symptoms of dandruff. The clinical protocol is discussed briefly. Electronic data processing of the double-blind monadic clinical study is described in some detail. For comparison purposes, the data were analyzed both by EDP and manual means. The relative economics are discussed.

Pseudomonads in cosmetics: Saul Tenenbaum. *Journal of the Society of Cosmetic Chemists* 18, 797 (Dec. 9, 1967)

Synopsis—Pseudomonads are bacterial organisms found in soil, water, air, food, in and on the body. They break emulsions, and produce foul odors and slime while decomposing cosmetics and pharmaceuticals. The organisms have the capacity to develop resistance to agents inimical to other microorganisms.

Preparations are placed on preservation study to determine the ability of a product to withstand consumer use and abuse. Some materials can inactivate the preservatives used to protect the product. The only effective way of knowing whether a product is protected is to inoculate the formulation with organisms and examine for viability.

The ability of pseudomonads to adapt to and proliferate in preparations is such that maintenance of the inoculum is insufficient for adequate preservation status. The only properly preserved preparation is one that is essentially self-sterilizing. A self-sterilizing preparation can be achieved, in most cosmetic products, without an increase in costs or loss of marketability.

A statistical approach to the evaluation of cutaneous responses to irritants: W. M. Wooding and D. L. Opdyke. *Journal of the Society of Cosmetic Chemists* 18, 809 (Dec. 9, 1967)

Synopsis—This study was done to test the application of certain common statistical experimental designs to the field of human patch testing where they do not appear to have been used previously, and to investigate certain variables affecting irritation test results. Sodium lauryl sulfate, a typical irritant of general interest, was used. Two experiments are described in which several factors thought to affect irritation results were tested; these included irritant concentration, certain time factors, and types of patch used. Irritation was basically scored on a five-point scale. Results showed that experimental and subject-to-subject variation could be greatly reduced by adequate experimental design, that several factors of interest were influential in the system, and that the error of measurement (estimation by a judge scoring the patch sites) was much smaller than had been expected. One of the significant effects of considerable interest was the finding that the degree of observable irritation was a function of the interval between removal of a patch and the time the site was scored.

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St. Louis Chapter of SCC Is Organized

Henry Maso (4th from left), president of the Society of Cosmetic Chemists, presents the charter for the newly formed St. Louis Chapter of the S.C.C. to Warren Hintz (3rd from left), Chairman of the St. Louis Chapter. Also pictured are (left to right) Hubert Merrell, treasurer; Jesse Starkman, president elect of the S.C.C.; Warren Hintz; Henry Maso; Raymond Auer, Chairman elect; and Chris Christensen, secretary.

MARTIN M. RIEGER HONORED BY SCC

Dr. M. M. Rieger honored. Dr. M. M. Rieger (left) was honored by the Society of Cosmetic Chemists for his five years of distinguished service as Editor of the JOURNAL OF THE SOCIETY OF COSMETIC CHEMISTS. Mr. Henry Maso, President of the Society of Cosmetic Chemists is shown presenting the plaque.

I.F.F. AWARD 1966

The 1966 I.F.F. Award of $1,000 was presented to Dr. Christopher M. Papa (left) by Henry Maso, President of the Society of Cosmetic Chemists. Dr. Papa's paper, "The Action of Antiperspirants," was judged to be the best paper published in the JOURNAL OF THE SOCIETY OF COSMETIC CHEMISTS during 1966.

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Measuring the Meaning of Fragrance

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Presented September 20–21, 1966, Seminar, New York City

Synopsis—Three different techniques for measuring the meaning of fragrances are reviewed. Profiling, Semantic Differential, and Multidimensional Scaling are discussed with special emphasis on the last. The application of these techniques by perfumers and in consumer testing is described.

Introduction

The title of this paper may be a little mystifying; we do not usually think of fragrance as having a meaning, nor of meaning as being something measurable. The phrase "the measurement of meaning," was borrowed from Osgood, who described the uses of a new linguistic technique, the semantic differential, in a book by this title (1). We shall have more to say about the semantic differential and its use in measuring meaning later on.

But what about the meaning of fragrance? It is quite obvious to anyone who has worked with fragrance that it would be highly inappropriate to use a Tabu-type fragrance in a dishwashing detergent or an insecticide spray, just as it would be a hopeless undertaking to launch, under a sophisticated name and with a romantic advertising story, a perfume that smells like lemons and lavender. That quality of the Tabu fragrance which clashes with the concept of a detergent or an insecticide is what may be called its meaning. It is the message which

* General Foods Corporation, 250 North St., White Plains, N. Y. 10602.

the fragrance conveys to the consumer, the connotations and associations which it evokes.

In this sense, the meaning of a fragrance is its most important quality; it is its reason for being. The function of a fragrance is always to convey a message about the woman (or man) who wears it or about the product in which it is used. If it succeeds in conveying the right message forcefully, the fragrance will be successful (2). If we accept this to be true, it becomes obvious that a perfumer, no matter whether his job is to create fragrances or to select them, must have a good understanding of their meaning and of the message they convey. To be more exact, since the product with which he is involved will have to be sold to a specific public, he must understand the meaning which his fragrances hold for that public. Understanding the meaning of a fragrance is very closely related to that most valuable gift a perfumer can have: the gift to predict what will sell.

To get this understanding, the perfumer traditionally has had to rely on three sources of information: introspection (what message does this fragrance convey to me?), observation of market performance (what kinds of fragrances are successful in what products?), and an understanding of certain chemical relationships (phenolic odorants occur in smoke and hence connote danger; citrus oils smell refreshing since they are associated with sour fruits; fatty aldehydes and indol are related to components of bodily excretions and hence have erogenous meanings (2). All of these approaches are indirect, and all have their pitfalls. Introspection fails if the perfumer is not completely in tune with his public; market performance depends on many things other than the fragrance; and the effect of chemical relationships on meaning has been largely in the realm of speculation and could not be proven. The direct approach—to go to a consumer or to a group of consumers and ask: What does this fragrance mean to you?—has not been considered feasible in the past.

There are several reasons for this. Every perfumer has had bad experiences with the layman's ability to identify odors or to react to them in any consistent way. He has repeatedly run into normal, intelligent people who fail to recognize the odors of the most familiar flowers or even of their own perfume, or who on Monday definitely prefer fragrance A over B only to reverse their judgment, with equal conviction, on Tuesday. After a few of these encounters, a perfumer tends to get discouraged about using laymen's judgments for guidance (3). Also, if you give people a fragrance and ask them to describe it, you usually

don't get much that is useful. Most respondents will tell you that it is "very nice" or "fragrant" or maybe that "I don't like it much"; and if you are lucky, you get some more specific descriptions, such as "smells like something you eat," "like my grandmother's garden" or "like a drugstore." If, as is the case, every respondent who says anything at all says something different, how can you combine and interpret the comments?

There are, then, real difficulties. Still, if fragrance is, in essence, a message, it is highly important to search for techniques which will reliably measure the meaning of this message to the consumer. And it is a milestone in perfumery that such techniques have recently been developed and are beginning to get used.

Odor Profiles and the Semantic Differential

In 1960, Paukner (4) obtained from respondents unschooled in perfumery descriptions of the odorants, citral, *p*-methyl quinoline, eugenol, geraniol, menthone, and hexenyl formate, which were very interesting since they partly confirmed but also partly conflicted with the meanings which these materials hold for professional perfumers. More important, these descriptions could be said to have statistical reliability. There are several noteworthy features about Paukner's approach. For one thing, he did not use just a handful of people in his test; he used 287 respondents. In tests of this type, there is real value in large numbers. People disagree about such questions as "To what extent is the odor of citral stimulating?", but if you take a sufficiently large group, the opinions of those who find the odor extremely stimulating will be counterbalanced by a group of people who hardly find it stimulating at all. Averaging all the votes, you arrive at a value which is valid in the sense that a very similar value can be obtained by posing the same question about citral to a different group of respondents.

Poffenberger, in 1932, conducted an experiment which, although it does not deal with odor, nicely illustrates this point (5). He presented his test subjects with ten different shapes and asked them to rank these in order of decreasing area (Fig. 1). Poffenberger then scored each individual's performance by calculating the rank correlation coefficient between the actual order and the order guessed by the subject. Lining up the shapes in perfectly correct order would result in a coefficient of $+1.00$, doing it all wrong (reverse order) would give a coefficient of -1.00, and guessing at random would usually give values between $+0.40$ and -0.40. Judging the area of these complex shapes is not easy. When

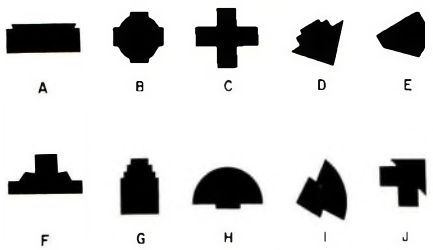

Figure 1. Shapes of different areas (after Poffenberger)

seven people were asked to rank the shapes their scores ranged from −0.03 to +0.67, with an average score of +0.36. The interesting point is that when the judgments of these seven people were combined by addition, a ranking was obtained which scored +0.79, which is not only very much better than the average score of the respondents but is even distinctly better than the score of the best judge. When a ranking was obtained by combining the judgment of 20 individuals it scored +0.92, which is remarkably close to perfect. There was nothing unique about this experiment and about the results obtained. It has been repeated many times and you can repeat it at home, if you wish. You always find that the judgment of the group will be closer to the truth than that of the individual judges. The reason is that the mistakes of different judges tend to go in different directions. Among our seven judges there may have been three who underestimated the size of shape A, two who overestimated it, and two who judged it correctly. When all the answers were combined, the negative and the positive errors largely canceled one another, so that the group answer was close to the truth. In experiments where you can't establish objectively how correct an answer is, you will find that the average judgments of larger groups are generally in better agreement with one another than the opinions of individuals. This is the reason why Paukner worked with 287 respondents.

Another important feature of his experiment was that he did not allow his respondents to choose freely the words with which to describe the odors. If 287 people start associating freely with drugstores, grandmother's gardens, things they eat, etc., it becomes impossible to combine their votes. He used a questionnaire in which the respondents were given words such as *delicate, bitter, cold;* the instructions were to indicate on a clearly defined scale (0 = not at all appropriate, 4 = completely appropriate) the extent to which each word fits each odor.* With such

* In similar experiments, many investigators prefer to use word pairs (*delicate–rough, cold–warm*) rather than single words; each procedure has certain advantages.

a highly structured questionnaire it becomes easy to arrive at a group judgment simply by adding individual votes.

The technique of providing the respondent with words rather than letting him choose his own carries with it the danger of losing information; a respondent will not be able to express the meaning an odor has for him if you don't provide him with the words with which to express it. It is, therefore, important to use a well-chosen list of words and to make it long enough to include all aspects of odors that might be relevant. Paukner worked with a list of 66 adjectives. Actually, an extensive list may make the respondent more articulate than a free "open end" questionnaire by reminding him of ways to describe an odor which are meaningful to him but which he would not have thought of had he been left to his own devices; however, we have to admit that there is a danger of "leading the witness" in this procedure. There is yet another advantage inherent in long lists: if they contain words that are either similar or nearly opposite in meaning, the results can be checked for consistency. An odor for which the word *strong* is rated as highly appropriate should probably have low ratings for *delicate* and *mild*. If it doesn't, it may be worth checking whether the respondents have understood the instructions and whether no mistakes were made in the tabulating or processing of the data. Often, where a single piece of information is not meaningful or statistically significant, a pattern of responses such as may be obtained in a longer questionnaire can convey valuable information. Naturally, there are practical limits to the length of a list of words: the longer it is, the more time-consuming and costly the interview and subsequent data processing and interpretation become. After having used his questionnaire in several large-scale tests the experimenter learns which adjectives are the most important and relevant for his particular kind of problems and which ones he can omit without losing much information. Using his lengthy questionnaire, asking 287 respondents to describe six odors and calculating the averages of their responses, what did Paukner achieve? He obtained profiles of odorants in terms of adjectives which give the perfumer, for the first time, a reliable, direct indication how these odorants strike his public, what kind of meaning they convey to nonperfumers (Fig. 2). This is certainly important, but it is only a first step. A very natural next step is to apply the same technique of questioning to complete perfume compounds rather than single ingredients. Thus Paukner (6) took a simple lavender composition and proceeded to add increasing amounts of an ambermuskcivet complex to it. According to the perfumer (2) this

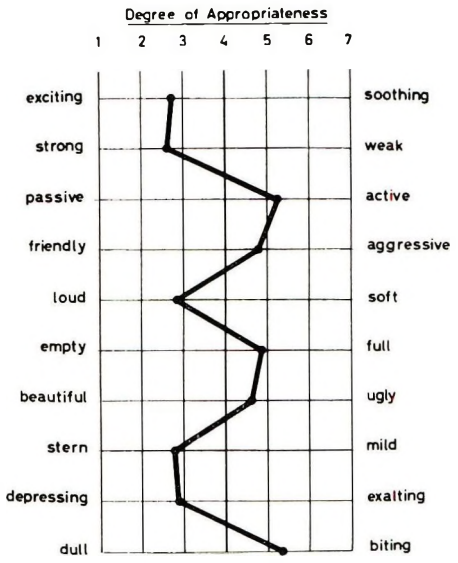

Figure 2. Partial profile of amber (after Paukner)

should make the composition increasingly sexy; but does the public agree? Paukner tested it and found it to be so. In these tests, he used a mathematical extension of the profile generation technique: He applied a factor analysis to his scores and thus obtained a semantic differential (1).

We are here getting into territory that may seem obscure to the nonpsychologist and the nonstatistician; the underlying ideas, however, are fairly simple. If we take a number of odors, each of which is described in terms of the appropriateness of a series of adjectives, we can, by carefully looking at the data, discover certain patterns. Odors which are described as fresh, warm, or cheerful, are likely also to have high ratings on the adjectives *pleasing* and *harmonious* and to have low ratings on *poor* or *artificial*. This is because the words *fresh*, *warm*, and *cheerful* have overtones of goodness and pleasantness which are also inherent in *pleasing* and *harmonious* but not in *poor* and *artificial*. This underlying notion of pleasantness, which will tend to make the ratings on adjectives such as *fresh*, *warm*, *cheerful*, and *harmonious* move together as we pass from one fragrance to another, is, in a mathematical sense, a factor which can account for a certain proportion of the difference between different odors. In a psychological sense, it is one of the basic dimensions in terms of which odors are perceived. Certainly, pleasantness is usually a very important factor, but it is by no means the

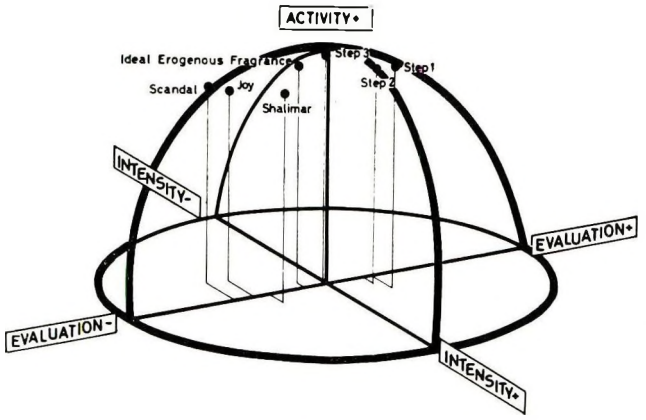

Figure 3. Odor description space (after Paukner)

only one. Two fragrances may be about equally pleasant, but one has a high rating on *fresh*, *stimulating*, and *spicy* while the other may have low ratings on these adjectives but be very *calming*, *soft*, and *mild*. The underlying dimension in which these two fragrances differ might be described as *activity*. The important thing about these dimensions is that they are not speculative contructs of the investigator's imagination but actual patterns and regularities underlying the respondent's reactions to the fragrances, isolated by rigorous mathematical techniques and labeled only afterwards by the investigator. It is not naive or presumptuous to say that these are true, meaningful dimensions of people's responses to odors.

It is common to extract five or six factors (or uncover five or six basic dimensions) in a semantic differential on odors. One of the basic features of factor analysis is that the factors are always extracted in order of decreasing importance: the first factor explains most of the differences between the responses, the second factor a smaller portion, etc. Thus it is usually legitimate to disregard the factors beyond the third or fourth one. There is a tendency among workers in the field to use three factors for this makes possible a visual representation in three dimensions. The three factors are represented by mutually orthogonal axes and the things judged by points. An odor which is rated high in pleasantness will be represented by a point close to the positive end of the "pleasantness axis"; an unpleasant odor will lie close to the other end. Odors represented by points which lie close together are perceived by the respondents to be similar in the message they convey, although they may be, as fragrances, distinctly different (Fig. 3).

Thus, the semantic differential technique provides a very elegant way of uncovering, describing, and graphically representing the meaning of different fragrances to the consumer. It makes it possible for the perfumer to assess whether a certain change he has made in a fragrance actually moves it in the desired direction, as far as the public is concerned. It also makes possible a new and truly creative type of imitation of successful perfumes. If a soap manufacturer brings out a highly successful toilet soap with a novel type of fragrance, we can use this fragrance as a model, not as we have always done by trying to make one that smells just like it, but by finding out just how the consumer describes this fragrance and then developing one which may smell quite different to the perfumer but which is described by the consumer as being similar to the model in terms of the basic dimensions important to him! Better yet, we will try to make a fragrance which the consumer sees as being similar to the model in all relevant respects but which he likes just a little better. This new fragrance, although different, will be equally appropriate to toilet soap and should at least be equally successful* if one of the basic hypotheses of linguistics is true, namely, that "an individual will behave toward a new object or event in a manner that is similar to the way he behaves toward objects and events that he encodes in the same way" (7). To be good at this new type of imitation, the perfumer has to have a thorough understanding of what it is about a fragrance that will make the consumer describe it in a certain way. It certainly is not easy to acquire this kind of understanding; but running exactly the kind of test which Paukner and several others have been conducting during the past few years can be of great help.

The semantic differential opens another intriguing possibility. It is quite feasible to have respondents describe, in terms of the adjectives of the questionnaire, not only different fragrances but also such concepts as "the ideal erogenous fragrance" or "a very masculine after-shave lotion." After running through the factor analysis, these concepts can then be represented by points in the semantic space, along with the points representing actual fragrances. By determining in what way the actual fragrances differ from the concepts and then making the adjustments necessary to bring them close (this may sound simple, but it requires all the art and understanding of a master perfumer) new fragrances can be created. If the semantic differential truly and fully de-

* Naturally, if this new fragrance is incorporated in a new toilet soap, this soap will be as successful as its model only if everything else about it, soap quality, name, price, packaging, etc., is also right.

scribed the respondents' feelings, if the respondents were representative of the population for whom the new fragrance is intended, and if it is true that people act similarly toward things they describe similarly, the new fragrance thus developed should be highly successful.

Let us now take another close look at the semantic differential. What are the basic dimensions it uncovers? Are they always the same? In principle, the dimensions emerging will depend both on the odors or other stimuli tested and on the adjectives used in the questionnaire. If the range of adjectives is sufficiently broad and diverse so that the respondent can clearly express through them any and all feelings he has about odors, then the adjectives should not place any limitation on the factors emerging. But what odors (or other things) are tested does make a difference. Paukner found that his first three dimensions were characterized, respectively, by the adjectives *beautiful/harmonious*, *spicy/alert/strong*, and *fresh/hard/aggressive*. He labeled the first dimension *evaluation*, the second *activity* and the third *intensity*.* Randebrock, in a similar study, found that his first dimension was characterized by the contrasting word pairs *elated-depressed*, and *uplifting-depressing;* the second one by the word pairs, *ferocious-gentle* and *stern-mild;* and the third dimension by *bracing-insipid* and *full-empty* (8). These dimensions are similar to Paukner's, but they are not identical. In some of our own studies on foods and flavors we found *wholesome–risky* and *everyday–party* emerging as important dimensions.

Odor Classification and Multidimensional Scaling

Recently there has been a resurgence of interest in the old question: Are there any basic odor types in terms of which all odors can be described (9)? A great deal is known about the four basic tastes, about the primary colors, and about the physical foundation of acoustics, but odor has always defied meaningful, objective, verifiable classification. Couldn't the new techniques of uncovering the basic dimensions of fragrance lead us to a recognition of the "primary odors," if such exist? Most of the investigators who have worked in this area have not used the semantic differential to construct their "odor space" but have used a technique which, starting from different premises, leads to a similar representation of odors as points in a space. This is the technique of multidimensional scaling (10). In this technique the respondents are

* These three dimensions, with the third one usually labeled *potency*, are also the main ones which Osgood obtained when examining all kinds of concepts and things other than odors, using the semantic differential.

presented not with single odorants but with pairs. They are asked to indicate simply how similar to one another or how different they find the members of a pair to be. If there are a total of three odors to be studied, this would make three pairs and three "distances" between odors (A–B, B–C, and A–C). If there are 25 odors, there are 300 distances to be determined. There are various ways of getting estimates of magnitude of difference from respondents. Whatever method is used, judging these differences or distances is a difficult task, not unlike the lining up of different shapes in order of area which was discussed before. Again, we have to take a fairly large number of judges and calculate average judgments if we want to get reproducible results.

By the time we complete the interviews and calculations on, say, 25 odorants, we will have 300 figures representing the pair-wise distances between 25 points (the odorants). Two points and a given distance between them can be represented on a (one-dimensional) straight line; three points, with three pair-wise distances between them, can be accurately represented by a triangle in a (two-dimensional) plane; four points, with six interpoint distances, will be represented by a pyramid in a three-dimensional space. To represent 25 points accurately, when the 300 pair-wise distances between them are given, takes a 24-dimensional space. In multidimensional analysis we actually start out with such a space, but through mathematical techniques, too complex to describe here but similar in principle to the techniques a cartographer uses in depicting a three-dimensional reality on a two-dimensional map, we simplify it down to a space with maybe four or five dimensions. In this process we inevitably introduce some distortion but we make our information much easier to understand and to handle. By the time we get down to three dimensions, we may have introduced some fairly severe distortions but we have something we can look at.

Such a three-dimensional representation is, in many ways, similar to the "odor-space" we discussed before, the end-result of the semantic differential. There is one important difference: while the meaning of the dimensions of the space obtained in the semantic differential can be understood by reference to the adjectives or word pairs that are closely related to these dimensions, the space model obtained by multidimensional scaling has dimensions which are unlabeled and which may have no meaning. In a way this limits its usefulness; but in another way, it is the direct result of a fundamental advantage which the multidimensional scaling technique has over the semantic differential. In the semantic differential, the respondent has to give his answers in terms

of the adjectives the experimenter has provided. The experimenter will make every effort to select adjectives which will cover all aspects and all dimensions of the odor which may be relevant to the respondent, but he can never be sure that he has succeeded in this. Also, the adjectives or word-pairs used in the questionnaire may introduce distortions by influencing the respondent, e.g., by suggesting that certain things are relevant which are actually of no importance to him. In the multidimensional scaling technique, the respondent is given no words. He judges differences and similarities between odors along dimensions which he may not even consciously formulate but which are, *ipso facto*, the most relevant to him. It is for this reason that those who have set out to explore olfaction without preconceived ideas, in search of primary odor qualities, have preferred to use multidimensional scaling.*

Woskow (11) used odorants and described them in a nine-dimensional space of which the first three dimensions were the most important. Although the dimensions are not labeled, their meaning can be interpreted by looking at what odorants lie close to the ends of the space along each of the dimensions. Woskow's first and mathematically most important dimension (it accounted for a large portion of the differences between the odorants) was clearly an evaluative one. It had vanillin, safrol, methyl salicylate, and benzaldehyde at one end and butyric acid, pyridine, and scatol at the other. The second dimension has camphor, guaiacol, and menthol at one end and aliphatic alcohols (C_2–C_9) and vanillin at the other. Woskow suggests the label *cooling, woodsy* for this axis; but *prickling-medicinal vs. soothing-unctuous* might be preferable. The third and following dimensions are difficult to interpret. In fact, in interpreting a multidimensional model, a good case can be made for not assigning any meaning to dimensions but only to interpoint distances.

Schutz, also trying to uncover primary odors, measured the quality of 30 odorants using both a semantic differential technique and multidimensional scaling (12). From the multidimensional scaling experiment he derived nine main factors which he labeled: fragrant, etherish, sweet, burnt, rancid, oily, metallic, spicy, and sulfurous or goaty.

On the basis of this work, Schutz proposed a nonverbal method of describing odors: choose some standard odors which are distinctly different from one another; for each odor to be described, have respondents

* Even using this technique the experimenter cannot help influencing the outcome of his experiment: he is the one who selects the odorants to be tested, and he has to interpret the results.

judge the pair-wise distances (degrees of difference) between this odor and each of the standard odors; use a group of respondents and average their judgments to get reproducible values. The set of average distance judgments of the new odor with respect to the standard odor describes, and in a sense, defines the new odor. Schutz used nine odorants, one for each of his factors, as standards, but other standards may also be used with this method which Schutz called the "matching standards method." Amoore (13) has applied the matching standards method to test his own theory about primary odors.

Yoshida (14) also examined a divergent group of odorants by the multidimensional scaling technique in an attempt to ascertain whether earlier proposed schemes for odor classification had any objective basis. He also investigated a group of modern luxury perfumes and a group of spices and herbs by the same technique. In each case, he extracted four or five factors, of which the evaluative one was the most important one.

So far, then, attempts to find primary odors as the underlying dimensions in a multidimensional space model of odor have only been moderately successful. They have, however, shown that multidimensional scaling can be used to construct space models of odors. We can then use these models for different purposes, i.e., to measure how consumers at large react to given fragrances, in what way and in what direction a change in a fragrance affects the consumers' perception of it, or to get guidance in the development of new fragrances for a specific purpose.* True, the lack of words in the model to guide us can become a handicap, but there are ways of overcoming this difficulty. A research group at General Foods is currently using a modified multidimensional scaling technique in flavor work with very promising results.

Product Testing

The new techniques for measuring the relations between, and the meaning of things and concepts in general, and of fragrances in particular, can be of real value not only to the perfumer, but also to those in the perfumery and cosmetic industry who are concerned with the consumer testing of new or improved products. As Randebrock (8) pointed out, the use of a questionnaire containing many different scales (29 in his case), rather than only questions about degree of liking or preference has the advantage of yielding stable, reproducible results with panels of as few as 30 respondents; whereas the traditional tests require several

* A novel technique of new product development which makes extensive use of multidimensional scaling is described by Barnett (15).

hundred respondents to give stable findings. That is a practical advantage. A more significant, fundamental difference lies in the fact that the traditional tests, by centering only on the questions, "How much do you like this sample?" or "Which do you prefer?", have, in effect, assumed that the evaluative dimension, the question of pleasingness, is the only relevant one, the only one which determines consumer behavior toward the product. We know that this is not true. One cannot help wondering how far beer, cigarettes or coffee would have gone in the marketplace if at their first exposure to the consuming public they would have been accompanied by a questionnaire asking, "How much do you like this new product?" Since aspects other than pleasantness do influence consumer acceptance of a product, new techniques to measure these other aspects can and will lead to important advances in acceptance prediction.

Conclusion

The techniques for measuring the meaning of fragrance discussed in this paper, namely, profiling, the semantic differential, and multidimensional scaling, are new and we still have to learn a lot about how to use them. We have to learn, among other things, how to choose those adjectives for our questionnaires which will give the most useful information; how best to select the samples or concepts to be included in a test (for what we learn about one sample depends on what other samples are tested alongside with it); and how to interpret the space models obtained in multidimensional scaling. We shall learn these things, and in so doing we shall be forging powerful tools for the perfumer and for the marketing expert.

To the perfumer, these techniques will provide a more direct and clear means of communication with the consumer than he has had up to now. By setting up, through the kinds of tests we have discussed, a feedback system and a continuing dialogue with his public, the perfumer will learn more and more precisely what it is about a fragrance that makes the public perceive it as sexy, as refreshing, as dull, or as masculine. If the marketing group has determined that the new baby powder that is being developed should be perceived by the public as being more cool, gentle, and wholesome than those currently available, the perfumer can use his dialogue with the public to guide him towards a fragrance which is cool, gentle, wholesome, and baby-like. Also, the dialogue makes possible a creative rather than slavish imitation of competitors' successful products. To the marketing expert, these testing methods will provide

better, more subtle, and more reliable means of predicting consumer acceptance of new products and new fragrances. The tests will be the more valuable the more different the new product is from existing, familiar ones.

Finally, and maybe most importantly: if both the laboratories and marketing groups start using these tests and adopt the relationship to the consumer which the tests imply, a better understanding and closer cooperation between the two groups will be the inevitable outcome. No one can deny that this would be a good thing.

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Hand Degerming Evaluation Utilizing A Split-Use Method

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Synopsis—Utilizing a modified split-use method, two bacteriostatic soaps were evaluated for their utility in degerming the hands. One soap contained 0.75% (by weight) of hexachlorophene and 0.75% of 3,4,4′-trichlorocarbanilide; the other consisted of equal parts by weight of 3,5-di- and 3,4′,5-tribromosalicylanilides, 4,4′-dichloro-3-(trifluoromethyl)carbanilide, and 3,4,4′-trichlorocarbanilide for a total concentration of 2%. A significant reduction in bacterial counts was achieved by both soaps as compared to a nonmedicated soap. It is emphasized that rigorous handling of data requires that the confidence interval be identified when per cent reduction is used as a basis for describing degerming efficiency.

Introduction

To investigators in the field of soap germicides, many techniques are available for evaluating skin degerming after use of an antibacterial soap. Among these are clinical observations, tape strippings, swab methods, contact plate methods, and plate counts obtained from handwashings. Four of these methods involve counting bacterial colonies, while clinical trials involve observations of the progress of bacterial diseases of the skin.

The moderation or the prevention of skin diseases is one of the attributes of an effective bacteriostatic soap. However, for the initial evaluation of a new bacteriostatic system in soap, the length of time needed for a meaningful clinical study makes this tool of questionable value.

* Armour Grocery Products Company, 3115 S. Benson St., Chicago, Ill. 60609.

Tape stripping as used by Updegraff (1) involves applying an adhesive tape to the skin surface then removing the tape with an adhering layer of skin containing colonies of bacteria. This method allows one to determine the distribution of bacterial colonies in relation to area and depth of the epidermal layers.

Skin swabbing (2) is performed with cotton or calcium alginate swabs. The swabs are placed in water or hexametaphosphate solution, the suspension is shaken thoroughly to distribute the organisms, and an aliquot is plated bacteriologically. Variations in bacterial counts can occur because of nonreproducibility in the pressure used in applying the swabs to the skin and because the number of bacteria removed from the swabs may vary with the efficiency of the shaking.

A more reproducible swabbing technique is utilized by Pachtman, *et al.* (3). An aliquot of a 0.1% aqueous solution of Triton X-100®* is pipetted into a glass cylinder 23 mm in diameter pressed firmly against the skin. The skin is abraded gently with a glass rod or Teflon policeman for two minutes, then the solution is removed, diluted, and plated bacteriologically.

Contact plates are made by adding liquefied nutrient agar to a Rodac plate† or small container, allowing the agar to harden, then pressing the plate with uniform pressure to the skin area to be tested. Plates are incubated at 37 °C for 48 hours, whereupon colonies are counted. Ulrich (2) stated that the method is reproducible for the first five plates taken sequentially from the same area of skin. Only bacteria on the surface of the skin are enumerated by this method.

This paper deals primarily with handwashing as a technique for evaluating skin degerming.

Price (4) developed a procedure whereby bacteria are removed from the hands and forearms by scrubbing with a surgical scrub brush in a series of basins containing a standard volume of water. Bacteria are removed at a decreasing rate as determined by counting the bacteria in each basin, plotting a curve which can be used to calculate the total bacterial flora on the area tested. Counts are taken on ten to fourteen basins.

If an antibacterial soap is used, the reduction in bacterial count as compared to the number obtained when a nonmedicated soap is used gives a measure of the effectiveness of the antibacterial product.

* Iso-octyl phenoxy polyethoxy ethanol. Registered trade mark of Rohm & Haas Co., Philadelphia, Pa.

† Distributed by Baltimore Biological Laboratories, Inc., Baltimore, Md. 21218.

Cade (5), also utilizing a multiple basin technique, determined that degerming could be evaluated after one to two weeks of test soap application. By counting the bacteria in the first, fourth, and fifth basins of a series, a curve was developed from the data obtained. Since bacterial removal beyond the fifth basin was constant when an effective bacteriostatic soap was employed, it was considered unnecessary to go beyond five basins (5). Per cent reduction was calculated by comparing counts obtained with a bacteriostatic soap to those obtained with a control (nonmedicated) soap.

The multiple-basin procedure was further modified by Roman, *et al.* (6) who used a baseline control of 1,580,000 bacteria per fifth basin for all participants on the panel and evaluated the degerming efficiency of a bacteriostatic soap in five days. This number was obtained from nonmedicated soap washings. Similarly, Kooistra, *et al.* (7) used a control reference figure of 1,300,000 bacteria for the fifth basin, a value derived from more than 500 individual fifth basin handwashings. The assumption behind this method is that participants in any experiment have essentially the same number of bacteria on their hands at the outset. However, this hypothesis is not confirmed by the second basin data for control soaps presented in their paper.

In the four handwashing tests mentioned the underlying assumption is that the bacterial counts from the hands of the individual or the group would have remained constant during the entire test period if a nonmedicated soap had been used exclusively.

Unlike the handwashing techniques just described, the procedure of Quinn, *et al.* (8) allows each participant to serve as his own control during the five-day evaluation period. This is accomplished by requiring the panelist to wash one of his hands, generally the left, with nonmedicated soap, reserving the other hand for the bacteriostatic soap. When either hand is being washed, the other is covered with a neoprene glove. Results are obtained by comparing counts from the hand using nonmedicated soap with those from the one on which bacteriostatic soap is applied.

Prior to the evaluation period, nonmedicated soap is used on both hands for seven days. Neoprene gloves are used by panelists when performing such chores as dishwashing, scrubbing, shampooing, etc.

The Quinn procedure (8) has been used by the authors to evaluate skin degerming. Results obtained with two antibacterial soaps by means of this split-use method are described here.

Experimental

The procedure provides for two types of washing sessions: application and sampling. Application washing sessions are those in which both the medicated and nonmedicated soaps are applied to the hands. These sessions make certain that panelists are exposed to the test soaps several times each day. Sampling washing sessions are identical to application sessions except that nonmedicated soap is applied to both hands and the basin water from each hand is plated and cultured for bacteria.

The daily application is as follows: 1500 ml of tap water are added to each of two basins. Each subject covers his right hand with a sterile neoprene glove and wets both the gloved and ungloved hands. Twenty milliliters of a 10% solution of nonmedicated soap are poured into the cupped hands which the subject then washes up to the wrists. After 20 seconds of washing, additional water is taken up and the washing continued for another 75 seconds. Hands are then rinsed in the wash basin for 20 seconds and the water is allowed to drain into the basin for 20 seconds. The procedure is repeated, but this time the left hand is covered with a sterile glove and a 10% solution of bacteriostatic soap is used.

During the sampling periods, both hands are washed individually with nonmedicated soap solution only.

The panelist is instructed to follow the same washing procedure at home, which includes the use of neoprene gloves with medicated and nonmedicated soap bars. Soap solutions are used in the laboratory because quantitative dispensing and lathering are made easier.

For colony counting, aliquots of wash water are added to Tryptic Soy Agar* containing 0.07% lecithin and 0.5% Tween-80®,† both of which are used as bacteriostat neutralizers. Plates are run in duplicate and incubated for 48 hours at 37 °C before counting.

Basin sampling sessions for bacterial counts are conducted on Monday, Wednesday, and Friday noons. The schedule of washing and sampling is shown in Table I.

For the experiments reported here, a nonmedicated soap‡ was used as the control. Two medicated soaps were evaluated, the first (Soap A)

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* Difco Laboratories Incorporated, Detroit, Mich.

† Polyoxyethylene (20) sorbitan mono-oleate. Registered trade mark of Atlas Chemical Industries, Wilmington, Del. 19899.

‡ Ivory®, Procter & Gamble, Cincinnati, Ohio.

Table I
Split-Use Washing and Bacterial Sampling Schedule

Day	Time	Left hand	Right hand
Monday	am	Ivory	Ivory
	Noon	Ivory (count)	Ivory (count)
	pm	Ivory	Test soap
Tuesday	am	Ivory	Test soap
	Noon	Ivory	Test soap
	pm	Ivory	Test soap
Wednesday	am	Ivory	Test soap
	Noon[a]	Ivory (count)	Ivory (count); test soap
	pm	Ivory	Test soap
Thursday	am	Ivory	Test soap
	Noon	Ivory	Test soap
	pm	Ivory	Test soap
Friday	am	Ivory	Test soap
	Noon	Ivory (count)	Ivory (count)

[a] After count wash on Wednesday, right hand washed with test soap.

containing 0.75% hexachlorophene and 0.75% 3,4,4′-trichlorocarbanilide, a synergistic bacteriostatic system. The second bacteriostatic soap (Soap B) contains equal parts by weight of 3,5-di and 3,4′,5-tribromosalicylanilides, 4,4′-dichloro-3-(trifluoromethyl)carbanilide, and 3,4,4′-trichlorocarbanilide for a total of 2%.

Results and Discussion

A total of twenty-four men and women of various occupations were divided into four panels for the experiment. Two methods of analyzing the data were used to determine degerming efficiency of the two medicated soap bars: per cent reduction and an analysis of variance. Before analyzing the data by an analysis of variance, logarithms of the bacterial counts were taken.

In Table II are presented the results of the analysis. A statistically significant difference was found between the degerming properties of the two bacteriostatic soaps as compared to the nonmedicated soap. For this test to show a statistical difference between medicated and nonmedicated soaps, the probability that a difference does not exist should be less than or equal to 0.05.

The analysis also showed that there was no significant variation among panelists for all four tests. This indicates that in these panels

Table II

Summary of Analysis of Variance of Quinn Test (Fifth Day Data)
(Bacteriostatic soaps A & B vs. non-medicated soap[a])

Soap A			Soap B		
Test no.	Variation between soap	Variation among panelists	Test no.	Variation between soap	Variation among panelists
1	$P < 0.01$	N.S.[b]	3	$P < 0.01$	N.S.
2	$0.025 < P < 0.05$	N.S.	4	$0.05 < P < 0.1$	N.S.
Pooled	$P < 0.01$	N.S.	Pooled	$P < 0.01$	N.S.

[a] Nonmedicated soap: Ivory®, Procter & Gamble, Cincinnati, Ohio.
Soap A: Active ingredients: 0.75% 3,4,4′-trichlorocarbanilide, 0.75% hexachlorophene.
Soap B: Active ingredients: 0.67% 3,5-di- and 3,4′,5-tribromosalicylanilides, 0.67% 4,4′-dichloro-3-(trifluoromethyl)carbanilide, 0.67% 3,4,4′-trichlorocarbanilide.

[b] N.S.: Not significant.

Table III

Range of Degerming Efficiencies in Per Cent Reductions (Fifth Day Data)

Soap A		Soap B	
Test no.	95% confidence limits	Test no.	95% confidence limits
1	88.5–98.5	3	84.5–99.4
2	83.0–91.5	4	86.5–97.5
Pooled	87.4–96.2	Pooled	87.7–98.9

Soap A: Active ingredients: 0.75% 3,4,4′-trichlorocarbanilide, 0.75% hexachlorophene.
Soap B: Active ingredients: 0.67% 3,5-di- and 3,4′,5-tribromosalicylanilides, 0.67% 4,4′-dichloro-3-(trifluoromethyl)carbanilide, 0.67% 3,4,4′-trichlorocarbanilide.

the pairing, or using an individual as his own control, was not absolutely necessary. Contrary to what might be expected, the number of transient bacteria present on the hands of the subjects did not significantly influence the variation among panelists. Resident as well as transient bacteria were removed because of the extended period of handwashing.

To obtain a meaningful comparison of degerming ability between two test soaps using per cent reduction as the method of analysis, a confidence range or interval should be used in place of a single average value to avoid making false conclusions based on apparent differences between average values. This minimizes the possibility of making the assumption that a real difference exists between two soaps when the observed differences may be due merely to random biological variations.

Results of the tests are summarized in Table III using per cent reduction as the method of analysis. Only the 95% confidence limits are shown so as to emphasize the importance of using a range in the interpretation of per cent reduction calculations.

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The Color of Red Hair*

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Synopsis—The iron pigments extracted with boiling acids from human red hair and chicken feathers are closely related and are possibly identical. Evidence is presented that these unique substances are well-defined chemical entities and not artifacts of keratin hydrolysis. The iron pigment is probably the major pigment of human red hair. Its limited extractability from red hair is due to its destruction during extraction and not to the small amount present in the hair. In all of its forms the iron pigment has been proved to be a metallo-protein. It can be broken down to a compound with a relatively small molecular size which retains all the essential properties of the originally extracted pigment. Synthesis of an iron-protein in melanocytes raises many questions which cannot be answered at present.

Introduction

Why is red hair red? What is the chemical difference between the pigments of blonde and black hair? These natural variations in hair color usually extend to all epidermal pigment cells. For this reason, while ultraviolet light turns the skin of brunettes dark brown, it imparts a copper color to blondes, and burns red individuals. Yet we know very little about the chemistry of these basic types of human pigmentation.

Recent chemical studies have led to the conclusion that it will be a most difficult, possibly insurmountable, task to unravel the structure of the black and brown melanins, also called eumelanins, because of the variable course that polymerization may take during pigment formation (1). At least the precursors of eumelanins and the nature of the building stones aggregating to form the black pigment are known. In the case of

* Investigation supported by Public Health Service Research Grant No. AM 10046 from the Division of Research Grants, National Institutes of Health.

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the yellow-red pigments (pheomelanins)* not even the chemistry of the mother substance has been clarified. Tryptophane has been suggested as a candidate, but there is no evidence that it has any such role except in lower species (2). Studies to test whether it gives rise to pheomelanins in man have led to negative results (3).

There is however in bright red human hair a characteristic entity, the "possible pink constituent" of Sorby who discovered it almost 90 years ago. His classical description (4, 5) lists most of the unique properties of this pigment: its pink color when extracted from bright red human hair with hot "20 times diluted sulfuric acid;" the color change and subsequent precipitation upon addition of alkali to the solution; and its broad absorption band in the visible region of the spectrum. Sorby correctly suspected that the pink substance may be a degradation product and not the original form in which the pigment exists in the hair.

In 1943 unaware of this early account, Rothman and the senior author isolated this substance again by boiling red hair with 0.1*N* HCl for 1–2 hours. The pink solution turned reversibly yellow above pH 2, precipitated at the neutral point, dissolved at an alkaline pH, had an absorption band in acid solution with a maximum at about 535 mμ, and contained iron in the trivalent form. Very little information was obtained about its chemical composition. Because of its iron content, the material was named trichosiderin (6).

Trichosiderin met with a less than unprejudiced acceptance by the scientific world. An attempt was made to relegate it to the status of an artifact of keratin hydrolysis (7). No mention was made of the fact that the pink colored products in keratin hydrolysates turn yellow at pH 7 and can be obtained by boiling horny products (or other proteins with aromatic amino acids) for 4–6 hours with 40–60 times stronger acids than are required for the extraction of trichosiderin. Such procedures destroy the red hair pigment. Nevertheless this allegation found its way into the literature (2, 8) casting doubts on the existence of trichosiderin as a valid chemical entity.

The belief that trichosiderin was merely an incidental by-product of pigmentation in red hair was reinforced by the observation that after an apparently exhaustive extraction of trichosiderin, the color of red hair was not diminished noticeably. Also, the presence of iron in an epidermal pigment seemed incompatible with the copper enzyme-controlled synthesis of the eumelanins.

* The distinction between eumelanins and pheomelanins is based on color differences, solubility in acids and alkalies and nature of precursors (2).

During the past 20 years, other investigators have extracted similar pigments from red chicken feathers (9, 10). These results suggested that red iron pigments were also present. More recently, Barnicot (11), extracted from human red hair a yellow solution with weak alkali at room temperature. Upon boiling in weak acids for five minutes, this solution turned red and assumed the characteristic absorption spectrum of trichosiderin. Barnicot's finding has provided further proof that trichosiderin is not an artifact of keratin hydrolysis.

The progress of the past 20 years in the isolation and purification of tissue components persuaded us to resume the study of this unique substance. It was hoped that the pigment of red chicken feathers would prove sufficiently similar to trichosiderin to serve as an experimental model, thus overcoming the difficulty of obtaining sufficient quantities of red hair.

Recent Research on Red Hair Pigment

The first aim of the work, the isolation of various forms of iron pigments from chicken feathers has been achieved (12). All these forms have absorption bands at about 280 mμ. This paper describes the overall direction and some general aspects of these studies.

In all major respects the pigment of red chicken feathers behaved as its human counterpart. It had the same indicator properties in its "siderin" form, precipitated at pH 7, had the same absorption spectrum, contained iron, and could be degraded to derivatives analogous to those of trichosiderin. This finding is of considerable biologic importance. It suggests not only that the iron pigment is phylogenetically very old, but also sheds new light on the relative importance of trichosiderin.

Considerably larger amounts of pigment could be extracted from feathers than from hair. Boiling for ten minutes with 0.1N HCl yields 6–10 times more pigment than two hours of boiling of hair. This finding was not surprising, because on a macroscopic and molecular level feathers are looser structures than hair and would release their pigment more easily. However, feathers of Rhode Island Red chickens are not noticeably redder than bright red hair. It appears that a large proportion of trichosiderin may be retained by the compact hair shafts. Consequently, during the prolonged extraction the pigment may be attacked by the extracting hot acid and made insoluble in the hair shaft.

This hypothesis was tested by prolonging the extraction of hair and feathers with boiling 0.1N HCl for several hours. Although the solutions obtained in the later stages are pale yellow or virtually colorless,

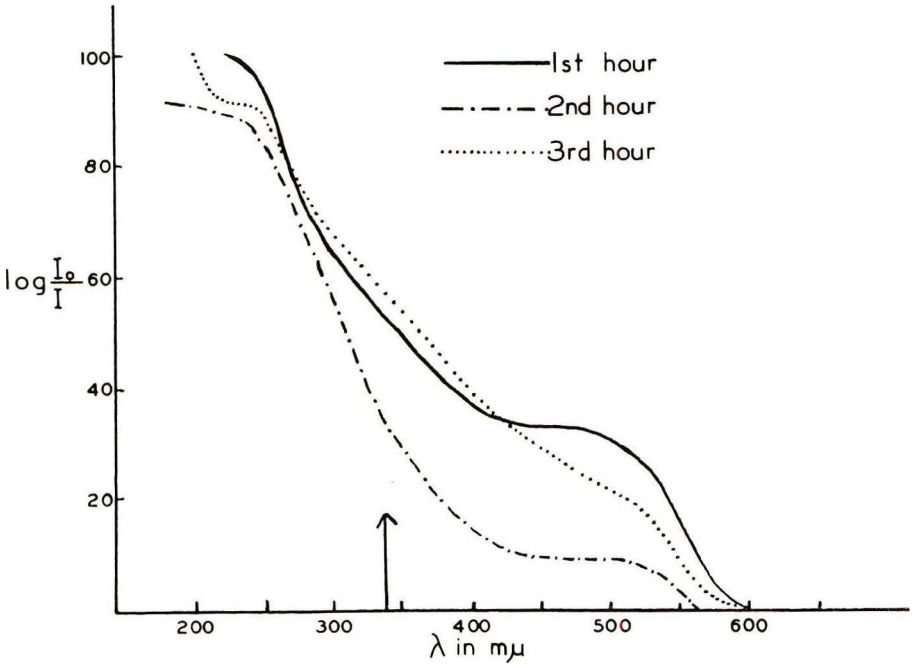

Fig. 1. Absorption spectra of trichosiderins in 0.1 *N* HCl with increasing length of extraction from hair. At arrow the concentration of the solutions was decreased to obtain readings at shorter wavelength

upon neutralization small amounts of pigment may be precipitated from them. These late precipitates are increasingly brown; their absorption bands at 535 mμ flatten and eventually disappear (Fig. 1), and their iron content steadily decreases (Table I). The organic portion of the pigment has no characteristic absorption bands and is complexed in acid solution through its combination with iron. Therefore it is likely that when the iron content drops below a certain level (about 0.1%), the pigment becomes insoluble and cannot be extracted anymore.

Indirect evidence for this view comes from Dutcher and Rothman's iron determinations in hair of different colors (13). Black and blonde hairs were found to contain, on the average, 2.71 and 2.43 mg Fe/100 g, respectively; red hair, on the average, 9.78 mg/100 g *i.e.*, about 7 mg more per 100 g hair than the other varieties. Assuming an iron content of about 0.5% in the original pigment *in situ*, 7 mg iron corresponds to 1400 mg pigment. Actually, red hair yields 40–60 mg trichosiderin per 100 g of hair; therefore only 3–5% of the total amount may be extracted. As the iron content of the pigment in the hair itself is unknown,

Table I

Iron Content of Hair and Feather Pigments after Varying Lengths of Extraction Time

Length of extraction with boiling 0.1*N* HCl (hr)	Hair pigment (Fe %)	Feather pigment (Fe %)
1	0.28	0.51
2	0.24	0.17
3	0.15	0.15

this calculation may be faulty.* Yet the basic observation is correct that the pigment is destroyed gradually during extraction. The authors believe that *the iron pigment is a major, possibly the major, pigment of bright red human hair.*

In attempts to elucidate the chemical nature of trichosiderin, the research concentrated on the chromophore group which contains iron complexed with an organic moiety. The presence of iron in the molecule is responsible for its acid solubility, absorption band in the visible, indicator property, and neutral iso-electric point. The iron-free or iron-poor parts of the pigment have few characteristic features; they are insoluble in acids, dissolve with a yellow-brown color in alkalies, and have no absorption band in the visible.

Trichosiderin proved to be a metallo-protein; preliminary analyses indicate that it is composed solely of iron, amino acids, and maybe some amines.† The protein nature is firmly established by a CHN ratio of 47.2–49.2, 6.8–7.3, 15.4–16.3 in all forms of the pigment, a positive Lowry reaction (14), typical infrared spectra, and amino acid analyses.

Before carrying out extensive amino acid analyses, efforts were directed at obtaining a chromophore of the smallest possible molecular weight which still retains all the specific properties of trichosiderin. The method is essentially one of repeated acid extraction. The chromophore is soluble in acids and highly resistant to them at room temperature. Acid extraction of the chromophore with successive removal of the iron-poor parts is carried out at different levels: first with boiling 0.1*N* HCl during the extraction of trichosiderin or feather siderin; then with 5*N* HCl or 10% KSCN which splits off large amounts of brown, iron-poor material. The resulting chromophore becomes dialyzable in 0.1*N* HCl.

* Dutcher and Rothman's data are based on an iron content of 10%, an unrealistic figure. At best, such a high iron content could be found in purified preparations only (5, 15); in the present work it was impossible to obtain such samples.

† Improved analytical methods may account for the discrepancies between earlier reported data (5, 15) and those reported herein.

With each successive step more and more of the brown, nondialyzable component is removed and the chromophore becomes increasingly purple with sharpened absorption bands and a threefold increase in iron content. The dialyzable chromophore passes through a Sephadex G-50 column as a single sharp band, and its ultraviolet and visible bands are in the same fractions. Present experiments suggest that further diminution of its molecular size may be achieved; the size already obtained is smaller than that of any heretofore described human epidermal pigment.

Amino acid analyses may reveal much about the nature of the chromophore. By comparing hair pigments with feather pigments and by analyzing the iron-free and iron-rich portions, it should be possible to single out those amino acids which combine with iron to form the chromophore group. Preliminary amino acid analyses already have revealed regularities in some of the split products.

Biological Implications

The synthetic pathway of this pigment raises many questions. If the iron pigment is elaborated by pigment cells, then the synthesis of an iron-protein by nucleic acids must follow a radically different course than the copper-catalyzed polymerization of eumelanin in the melanosomes. This view must be reconciled with everyday observations and clinical experience which strongly suggest that these two pathways must coexist (2). Here only a few pertinent observations will be mentioned. There is a whole gamut of red shades, ranging from "carrot-red" or "orange" through Titian-red to red-brown (16). While these varieties could be explained with differences in the chromophore content of the iron pigment, it is conceivable that a varying admixture of eumelanin pigment also is present. The frequently observed gradual darkening of red (and blonde) individuals with advancing age favors the view that the two pigmentary processes coexist. The strongest proof for this theory is the occasional irreversible conversion of red hair to brown after the administration of chloroquine (2).

Is it then the same pigment cell which produces a metallo-protein and a melanin? The answer is not known. Although red pigment cells in man and fowl appear to differ from the melanocytes synthesizing black melanins (17, 18), the data are too fragmentary to permit a definite conclusion. It is also possible that macroscopic red color occasionally may be produced without iron. From time to time the authors have obtained specimens of bright red hair which did not yield any iron pigment. A case in point also may be the reddening of the hair of infants suffering

from kwashiorkor, a severe nutritional deficiency (19). That redness does not necessarily presuppose an iron pigment is proved by the Irish setter whose red hair contains no trichosiderin.

Although two pigmentary pathways must be postulated, there is no reason why these pathways must follow a common initial course, as suggested by Fitzpatrick *et al.* (2). All the available facts may be explained by assuming two coexisting but independent pathways for red and black pigments.

Another unsolved problem is presented by the "missing links" between chicken and man. Trichosiderin may occur in other species as well, but as yet none has been found.

Red-haired individuals may be biologically inferior. The hair covering the entire body of our ancestors regardless of its color, offered adequate protection against ultraviolet light. As hair became a rudimentary structure, the epidermis became exposed to sun against which the iron pigment apparently does not offer adequate protection. The present scarcity of red-haired people may be due to their suppression by the more viable dark-colored races.

Discovery of a previously unknown substance, such as the iron pigment of red hair, always raises more questions than can be answered. Most of these problems are accessible to experimental study. It is hoped that their solution will be attempted before another 25 years will elapse.

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Use of Electronic Data Processing in Anti-Dandruff Clinical Research

MARTIN GREIF, B.S.,* and HARRY J. PROKOP†

Presented November 30, 1966, New York City

Synopsis—Fifteen different compositions were studied in a pilot clinical program in terms of their ability to control the symptoms of dandruff. The clinical protocol is discussed briefly. Electronic data processing of the double-blind monadic clinical study is described in some detail. For comparison purposes, the data were analyzed both by EDP and manual means. The relative economics are discussed.

Introduction

The etiology of dandruff and its treatment have been discussed in scores of technical communications during the past century. In an early scientific discussion of dandruff, Malassez (1) was the first to indict *Pityrosporum ovale* as the cause of dandruff. Over the years from 1874 to the present day, numerous authors have taken sides for or against this hypothesis. Each has presented evidence to support his contentions. The literature is replete with references (2–8) covering both sides of this controversy.

A systematic review of the literature discloses that numerous additional theories on the cause of dandruff have been offered, and countless treatment procedures were reported as being effective. Thus there are theories relating to various microbial causes (9–14). Wallace (15) considers dandruff to be a manifestation of seborrhea or psoriasis bearing no

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causal relationship to the microorganisms found on the scalp. He recommends the keratolytic approach. Van Abbe (16) suggests that an allergic mechanism plays a significant role in dandruff causation. Some investigators (17, 18) maintain that the condition is a disordered normal desquamating process often associated with excessive oil secretion on the scalp. Harry (3) cites several authors who attempted to show a relationship between digestive disorders and dandruff.

Considering the many hypothetical causes of dandruff symptomatology, it is no surprise that treatment approaches include antimicrobial as well as keratolytic therapy; steroid; antihistaminic; antimitotic; special dietary; and recently, enzymatic (19, 20) approaches. It is widely agreed that the first consideration in effective treatment for dandruff requires thorough cleansing of the scalp to remove both loose and adherent scale. The second phase generally involves the use of a suitable agent to inhibit further production of scale. It is in the latter phase where most differences of opinion arise. Some investigators employ various biochemical means to inhibit scaling. Others prefer to incorporate the therapeutic agent in a shampoo, while still others apply it as a post-shampoo rinse. Another school of thought recommends a pre-shampoo treatment.

This investigation was designed on the theory that topical post-shampoo rinse therapy is most easily controlled. A standard pre-treatment shampoo was used. The rinses were simple aqueous solutions or suspensions of different active ingredients supplemented with a conditioner. They were designed to place the therapeutic agents in intimate contact with the hair and scalp. Approximately 750 subjects entered and 717 completed the study.

Early in the planning stages it became apparent that more than one clinical trial with basically the same design would eventually be required. Masses of data would be generated, and these would undoubtedly be cumbersome and costly to process manually. A review of the protocol suggested that with properly designed record forms the study could be programmed for electronic data processing. Once the programming costs were amortized, subsequent data processing costs and time requirements would be relatively small. If the program were flexible enough, it might be adaptable to other studies with other products. This paper will briefly discuss the clinical protocol and then consider the data processing in some detail. For comparison purposes the data were analyzed by both EDP and manual means. The relative economics will also be discussed.

Procedure

At the start of the study the subjects were divided into fifteen balanced groups with all groups using the same shampoo but each a different test rinse. At the initial visit the consulting dermatologist examined each subject and noted the severity of both loose and adherent dandruff. Using a scale of zero to four plus, only those subjects with three plus or four plus in both loose and adherent dandruff were admitted to the study. Additional information, such as scalp conditions, dermatoses, and presence of itching, was noted on the record forms. Once a subject was qualified to participate in the study, a dandruff history was prepared for that subject. The details of the actual clinical trial, which lasted eight weeks, are not germane to this discussion. However, the subjects were examined by a dermatologist five days after treatment during weeks Nos. 1, 2, 4, 6, and 8, and his observations were recorded. Upon completion of the trial, each participant was required to complete a questionnaire wherein he or she provided subjective responses to various questions. The completed forms were checked and then forwarded for key punching and data processing.

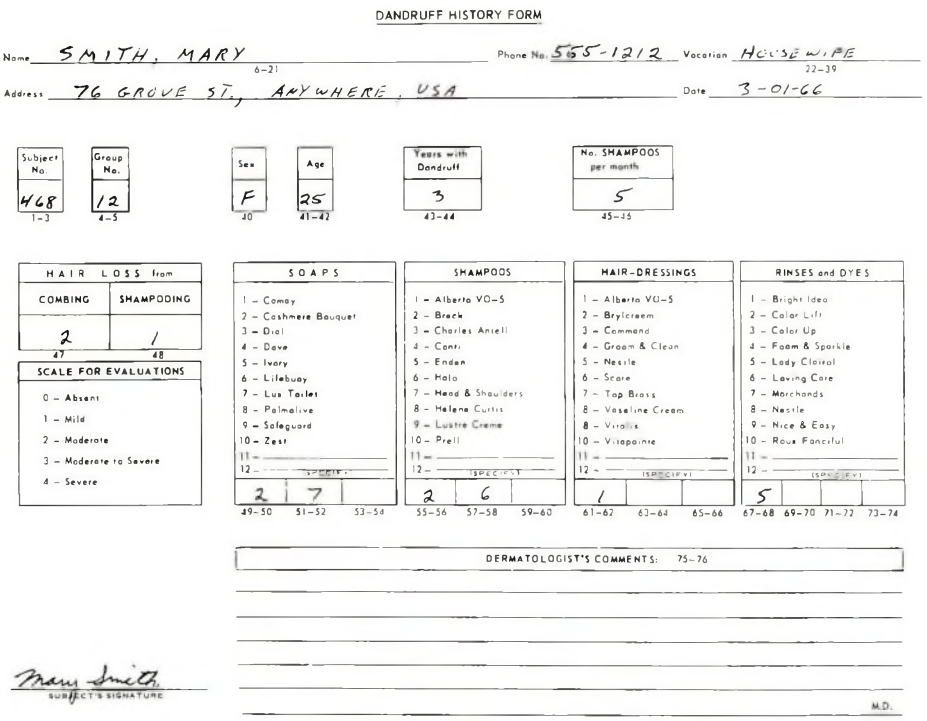
Electronic Data Processing

Transmittal Forms

Three custom transmittal forms were designed to record the pertinent information for each subject in the study (Figs. 1, 2, and 3). The information from each transmittal sheet was key punched into an 80-column data card. For each subject there were three cards of punched data. The subjects were identified by name and number. Transmittals were designed to allow flexibility in the subjects' entries and still provide meaningful data for computer processing. Encoded on the transmittals were numbers associated with answer boxes or comment entries which ranged from 1 to 80. These are the card columns in which the data were punched. Figure 4 shows a typical punched-in data card.

Dandruff History Form

This form contained the subject's identification including name, number, address, phone number, vocation, sex, age, etc., and a brief history relating to scalp and hair. The history included information such as: years with dandruff, number of shampoos per month, the subject's own appraisal of his extent of hair loss from (a) combing and (b) shampooing, and the names of products used on the hair.


DANDRUFF HISTORY FORM

Name SMITH, MARY (6–21) Phone No. 555-1212 Vocation HOUSE WIFE (22–39)

Address 76 GROVE ST., ANYWHERE, USA Date 3-01-66

Subject No. (1–3)	Group No. (4–5)	Sex (40)	Age (41–42)	Years with Dandruff (43–44)	No. SHAMPOOS per month (45–46)
468	12	F	25	3	5

HAIR LOSS from COMBING (47)	HAIR LOSS from SHAMPOOING (48)
2	1

SCALE FOR EVALUATIONS
0 – Absent
1 – Mild
2 – Moderate
3 – Moderate to Severe
4 – Severe

SOAPS	SHAMPOOS	HAIR-DRESSINGS	RINSES and DYES
1 – Comay	1 – Alberto VO–5	1 – Alberto VO–5	1 – Bright Idea
2 – Cashmere Bouquet	2 – Breck	2 – Brylcreem	2 – Color Lift
3 – Dial	3 – Charles Antell	3 – Command	3 – Color Up
4 – Dove	4 – Conti	4 – Groom & Clean	4 – Foam & Sparkle
5 – Ivory	5 – Enden	5 – Nestle	5 – Lady Clairol
6 – Lifebuoy	6 – Halo	6 – Score	6 – Loving Care
7 – Lux Toilet	7 – Head & Shoulders	7 – Top Brass	7 – Marchands
8 – Palmolive	8 – Helene Curtis	8 – Vaseline Cream	8 – Nestle
9 – Safeguard	9 – Lustre Creme	9 – Vitalis	9 – Nice & Easy
10 – Zest	10 – Prell	10 – Vitapointe	10 – Roux Fanciful
11 = (SPECIFY)	11 = (SPECIFY)	11 = (SPECIFY)	11 = (SPECIFY)
12 = (SPECIFY)	12 = (SPECIFY)	12 = (SPECIFY)	12 = (SPECIFY)
2 7 (49–50, 51–52, 53–54)	2 6 (55–56, 57–58, 59–60)	1 (61–62, 63–64, 65–66)	5 (67–68, 69–70, 71–72, 73–74)

DERMATOLOGIST'S COMMENTS: (75–76)

Mary Smith
SUBJECT'S SIGNATURE

M.D.

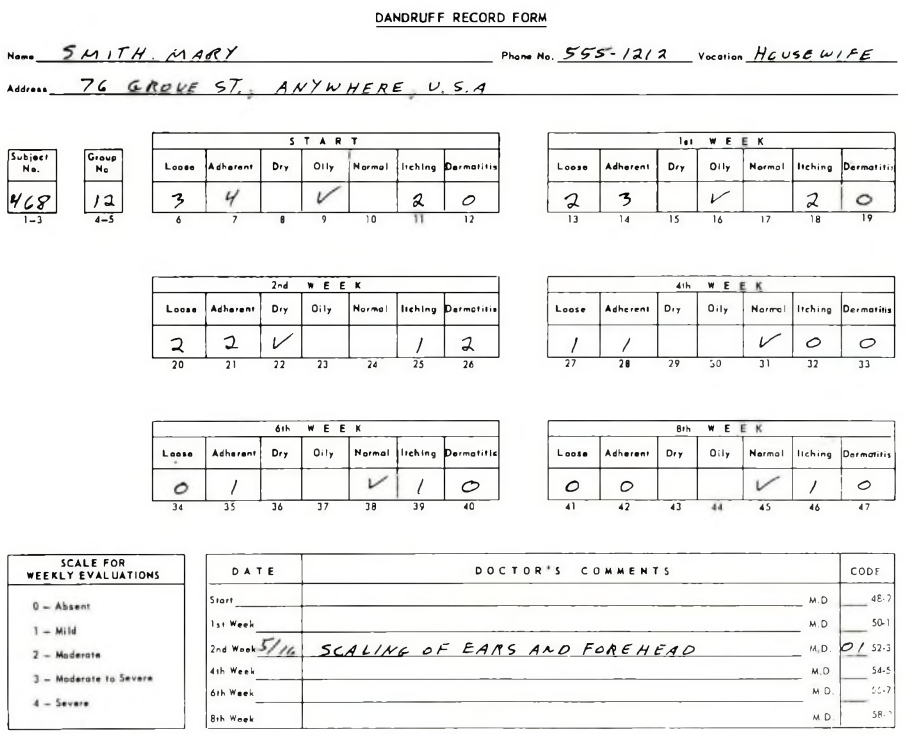
Figure 1. Patient history transmittal form

Dandruff Record Form

In addition to the subject's identification, this form contained the dermatologist's evaluation of the subject's hair and scalp condition at each observation interval. Included in the evaluation were the extent of dandruff scaling (both loose and adherent); the condition of the scalp whether dry, oily, or normal; and the degree of itching. The dermatologist was also afforded a place to note the presence of supraorbital dermatoses, which are often associated with dandruff. If such dermatitis was reported, it was described under doctor's comments. Six observations were made by the dermatologist during the eight-week study.

Dandruff Questionnaire

The Questionnaire posed key questions about the subject's personal response to the test product and a comparison to previously used products. The subject was asked to note the extent of hair loss (if any) during the last week of the study.



DANDRUFF RECORD FORM

Name SMITH, MARY Phone No. 555-1212 Vocation HOUSEWIFE

Address 76 GROVE ST., ANYWHERE, U.S.A

Subject No.	Group No
468	12
1–3	4–5

START	Loose	Adherent	Dry	Oily	Normal	Itching	Dermatitis
	3	4		✓		2	0
	6	7	8	9	10	11	12

1st WEEK	Loose	Adherent	Dry	Oily	Normal	Itching	Dermatitis
	2	3		✓		2	0
	13	14	15	16	17	18	19

2nd WEEK	Loose	Adherent	Dry	Oily	Normal	Itching	Dermatitis
	2	2	✓			1	2
	20	21	22	23	24	25	26

4th WEEK	Loose	Adherent	Dry	Oily	Normal	Itching	Dermatitis
	1	1			✓	0	0
	27	28	29	30	31	32	33

6th WEEK	Loose	Adherent	Dry	Oily	Normal	Itching	Dermatitis
	0	1			✓	1	0
	34	35	36	37	38	39	40

8th WEEK	Loose	Adherent	Dry	Oily	Normal	Itching	Dermatitis
	0	0			✓	1	0
	41	42	43	44	45	46	47

SCALE FOR WEEKLY EVALUATIONS

0 – Absent
1 – Mild
2 – Moderate
3 – Moderate to Severe
4 – Severe

DATE	DOCTOR'S COMMENTS	CODE
Start	M.D.	48-7
1st Week	M.D.	50-1
2nd Week 5/16	SCALING OF EARS AND FOREHEAD M.D. 01	52-3
4th Week	M.D.	54-5
6th Week	M.D.	56-7
8th Week	M.D.	58-9

Figure 2. Patient record transmittal form

Coding System

A coding method was established for tabulating entries on the transmittals in order to simplify key punching and to eliminate the need for a large volume of alphabetic information in core storage. It also simplified computer program logic and reduced machine processing time.

Each test product was assigned a two-digit number called group number. The subjects were assigned three-digit numbers called subject number. A scale for evaluations to record severity of each condition under study was included and is shown in Figs. 1, 2, and 3. Entries in spaces provided for doctor's comments, previously used hair and scalp products, and the subject's recorded likes and dislikes about the test product were all assigned two-digit numbers for coding. Through the use of this coding system, the printed reports were far more comprehensible in that they were not cluttered with verbose descriptions.

DANDRUFF QUESTIONNAIRE

Date 4-20-66

Name SMITH, MARY Subject No. 468 (1-3)

Address 76 GROVE ST., ANYWHERE, U.S.A. Group No. 12 (4-5)

Phone No. 555 1212 Vocation HOUSEWIFE Sex ☐ Male ☑ Female Age 25

How would you rate the test product which you used the past 2 months? (Check only one).

One of the best I've tried	☑	1
Good, but not one of the best	☐	2
Fair	☐	3
Poor	☐	4

(6)

During the month before you started this test, what one product did you use most for treating dandruff or washing your hair:

ENDEN (7-18)

How would you rate the test product you used the past 2 months with the one listed immediately above. (Check proper boxes)

	Test Product MORE EFFECTIVE	Test Product AS EFFECTIVE	Test Product LESS EFFECTIVE	
Comparison of DANDRUFF results				
A. Reduction in amount of dandruff	☑	☐	☐	19
B. Length of time dandruff was reduced	☐	☑	☐	20
Hair MORE MANAGEABLE	☐	☐	☑	21
Hair FEELS CLEANER	☑ (1)	☐ (2)	☐ (3)	22

Please write down everything you can think of that you LIKED about this test product:

	CODE	
REDUCED DANDRUFF	02	23-24
SCALP FEELS GOOD	06	25-26
		27-28
		29-30

Please write down everything you can think of that you DISLIKED about this test product:

	CODE	
CAUSED ITCHING	33	31-32
HARD TO COMB	38	33-34
		35-36
		37-38

Using the "Scale For Evaluations" enter the number in boxes 39 and 40 which best describes your experience with hair loss during the last week of the study.

HAIR LOSS from	
COMBING	SHAMPOOING
1	0
39	40

SCALE FOR EVALUATIONS

0 – Absent
1 – Mild
2 – Moderate
3 – Moderate to Severe
4 – Severe

mary Smith
SUBJECT'S SIGNATURE

Figure 3. Patient questionnaire transmittal form

Transmittal Processing

After the study was completed, transmittals were edited for completeness and accuracy. The doctor's comments on the record forms and the subject's recorded likes and dislikes on the questionnaires were coded at this time using master code lists. Next, the pertinent information was key punched into 80-column data cards. The cards were verified to insure accuracy. Because there are only 80 columns per card, a separate card was required for each transmittal form.

Unit Record Machine Processing

The key punched cards were sorted to group number and subject number sequence and separated into three card types—history, record, and questionnaire—on a high-speed card sorter. Then each deck was listed on a tabulating machine for the purpose of visually editing the key punch data. After necessary corrections were made, the history deck was matched against the record and questionnaire decks on group and subject numbers to insure that all three cards for each patient had been key punched for processing. The computer was programmed to note inconsistencies or logic errors in the raw data. If a card for a patient was missing in any of the three decks or if an identifying number was incorrectly key punched, this would constitute selected unmatched cards. Reasons for unmatched cards, other than the two mentioned above, could be as follows:

(a) A subject voluntarily dropped out of the study.
(b) A subject failed to follow the prescribed protocol instructions and was dropped.
(c) A transmittal form was inadvertently not key punched.

All unmatched cards ejected in the sort and match process were checked at this time, and necessary corrections were made before processing could continue (Fig. 5).

The next phase in unit record processing was to segment the history cards into desired age groups and years with dandruff groups for correlation reports. This was accomplished by sorting the history cards into the age or years with dandruff sequence and separating the cards into the desired groups. Then each group was matched against the record cards on test product and subject number in order to separate the record deck into the same groups. The segmented record cards were then coded using the gang punching method on a reproducer. Thus, the assigned codes were used as control factors in computer processing rather than the

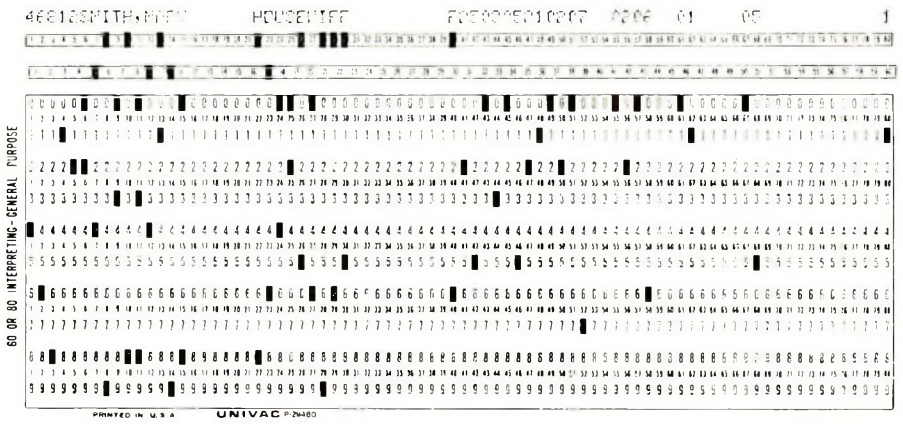


Figure 4. Patient history data card

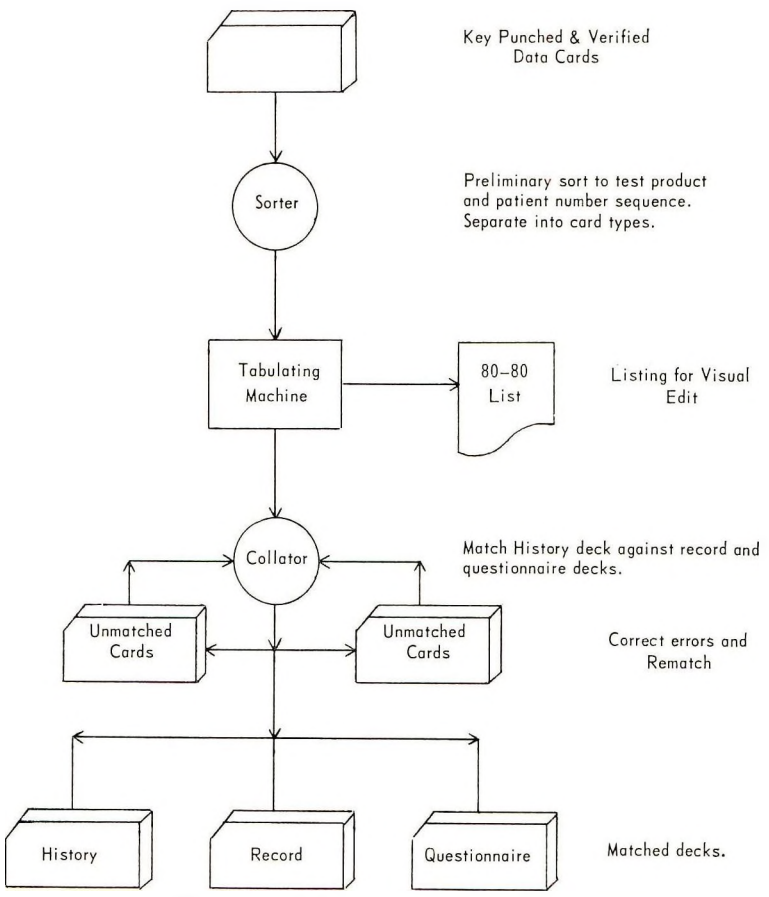


Figure 5. Edit phase of unit record card processing

actual age or years with dandruff for a patient. A typical example follows:

Age groups (years)	*Code*	*Years/dandruff*	*Code*
29 and under	1	5 and under	1
30 to 39	2	6 to 10	2
40 and over	3	11 to 15	3
		16 and over	4

The last phase of card processing was to sort the cards to a report sequence for computer processing.

Computer Processing

The computer programs were written to accept magnetic tape as input since the processing rate with tape is much faster than that with cards. Furthermore, use of tape minimized the handling of bulky cards. The card images were captured on tape *via* a card-to-tape utility program. Five input tapes were produced. Each pass required resorting the cards to another report sequence. The final sequence of all reports was by test product group. Under the control of the program(s), cumulative totals, counts, and averages were printed for each observation by test product, and an improvement factor was calculated. In addition to printing the subject's data in detail, summary tables were also produced. The computer was programmed to signal inconsistencies in this phase as in all other phases.

Generated Reports

Nineteen reports were printed on the computer in the analysis of each of 15 antidandruff products. Three reports (A, B, C) showed those subjects whose condition of scalp at start was either dry, oily, or normal and the trend each followed to the end of the clinical study; three reports (H, I, J) showed the correlation of condition of scalp at start to the degree of dandruff with the trends to conclusion; six reports (D, E, F, G, K, Q) showed the trends from start to eighth week under the parameters "degree of dandruff," "degree of itching," "dermatitis," and "overall improvement"; two reports (O, P) showed the trends in reduction of dandruff *vs.* (a) age groups and (b) years with dandruff from test product sequence. The last five reports (L, M, N, R, S) showed tallies of responses to the questionnaire from the test product sequence (Fig. 6).

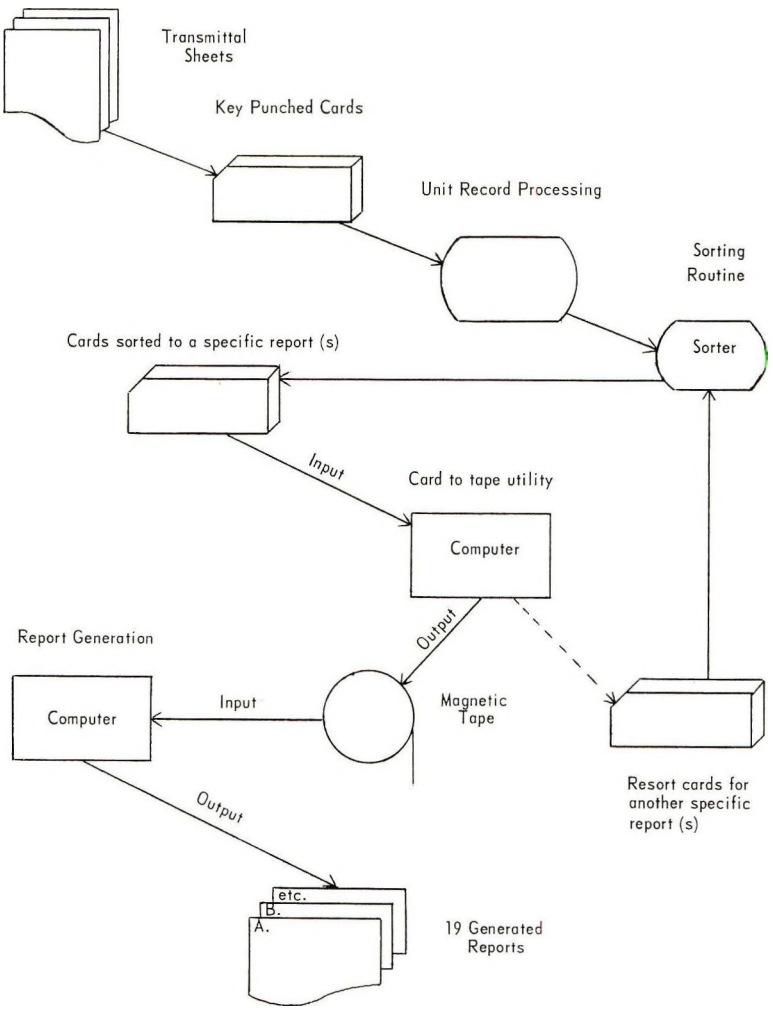


Figure 6. Computer system flowchart of the dandruff pilot clinical program

Listed below are the generated reports by identification letter and title.

Item	*Title*
A	Condition of scalp at start—dry
B	Condition of scalp at start—oily
C	Condition of scalp at start—normal
H	Correlation of condition of scalp at start to quantity of loose dandruff
I	Correlation of condition of scalp at start to quantity of adherent dandruff
J	Correlation of condition of scalp at start to quantity of loose and adherent combined
D	Quantity of loose dandruff
E	Quantity of adherent dandruff
F	Quantity of loose and adherent dandruff combined
G	Degree of itching
K	Overall improvement rating
Q	Degree of dermatitis
O	Reduction of dandruff *vs* age groups
P	Reduction of dandruff *vs* years with dandruff
L	Hair loss during combing and shampooing (Start *vs* eighth week)
M	Test product rating
N	Comparison of dandruff results
R	Recorded likes about test product
S	Recorded dislikes about test product

Comparison: Time and Cost

The estimates described below are based on a dandruff clinical study involving 717 subjects. It should be noted that the cost of manually compiling and analyzing data remains constant for each clinical study of the same size and nature, regardless of the number of studies conducted. The net cost of each electronic compilation would be the actual running cost plus a *pro-rata* share of the initial programming cost. This share of programming cost diminishes as the number of studies increases assuming no changes in programming are required. The time requirements and dollar costs per study (assuming five studies are to be run) are shown below:

	Manual method	*Electronic method*
Cost per study	$2500	$800
Time requirement per study	6 weeks	1.8 weeks

Conclusion

What has been described here is properly referred to as a "pilot program." It was instituted to determine feasibility, costs, and time requirements. The computer program did not include tests of statistical significance or plotting of curves. Such a program can be modified to

include all the statistical treatments previously done manually. It has been shown that computer processing can significantly reduce the amount of time required to complete analysis of results in a large clinical trial, eliminate human mechanical errors, and at the same time save a considerable amount of money. When a program is used in more than one trial, the savings in both time and dollars become even more dramatic.

(Received May 22, 1967)

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Pseudomonads in Cosmetics

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Presented before the New York Chapter, October 3, 1966

Synopsis—Pseudomonads are bacterial organisms found in soil, water, air, and food, in and on the body. They break emulsions and produce foul odors and slime while decomposing cosmetics and pharmaceuticals. The organisms have the capacity to develop resistance to agents inimical to other microorganisms. Preparations are placed on preservation study to determine the ability of a product to withstand consumer use and abuse. Some materials can inactivate the preservatives used to protect the product. The only effective way of knowing whether a product is protected is to inoculate the formulation with organisms and examine for viability. The ability of pseudomonads to adapt to and proliferate in preparations is such that maintenance of the inoculum is insufficient for adequate preservation status. The only properly preserved preparation is one that is essentially self-sterilizing. A self-sterilizing preparation can be achieved, in most cosmetic products, without an increase in costs or loss of marketability.

Introduction

Pseudomonads are bacterial organisms frequently responsible for deterioration of food, petroleum products, pharmaceuticals, and cosmetics. They participate in the degradation of polystyrene, dibutyl phthalate, polyvinyl chloride, formaldehyde resins, cutting oils, jet fuel, kerosene, and interfere in the manufacture of paper and plastics (1). Military and related civilian research into contaminated jet fuel have demonstrated that pseudomonads are an important member of the contaminating flora. The degree of contamination has, on occasion, been severe enough to degrade the fuel and cause operating problems.

Pseudomonads are heterotrophic, asporogenous, polarly flagellated organisms with or without slime, with or without pyocyanine, with or without green-yellowish fluorescence, and with or without a yellow-brown colony. Only in a few cases can the genus be easily recognized by

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one of its striking properties such as pigment formation. From an industrial point of view *pseudomonads can be considered, in a general sense, as motile, gram-negative rods giving a positive oxidase test.* Psychrophilic pseudomonads are ubiquitous and can be isolated from soil, fresh and salt water, food, skin, and feces.

Reports of pseudomonads in pharmaceuticals and cosmetic products, with the exception of ophthalmic preparations, have been scarce; and one would believe that pseudomonal contamination of pharmaceuticals and, in particular, cosmetics are infrequent. The most frequent contaminant of ophthalmic preparations and, as such, responsible for serious eye injuries and even loss of vision has been *Pseudomonas aeruginosa* (2, 3). Accordingly, nonsterile preparations are now regarded as adulterated and misbranded within the U. S. Food, Drug, and Cosmetic Act. Further, multiple dose ophthalmic products must contain agents that will inhibit the growth of microorganisms (4).

During the past decade a number of preservation failures in cosmetic products have been reported. Bryce and Smart (5), for example, found that contaminated hair products, of all varieties, invariably contained gram-negative organisms some of which were pseudomonads. This period coincides with the period of changeover from anionic to nonionic emulsifiers (6). These nonionic surfactants, derived from fatty acids, are responsible for the superiority of current cosmetic and pharmaceutical preparations as stable, smooth, appealing, and effective formulations compared to those of 10 years ago. But nonionics have changed many preparations which could not support microorganisms into veritable culture media for growth, and the contamination organisms found most frequently have been pseudomonas.

Although there have been relatively few published reports dealing directly with pseudomonas contamination, the many publications concerning problems in preservation which have appeared within this same period, frequently mention this genus. Within the 1957–1960 period, for example, a dozen different pseudomonads were isolated from commercial and experimental formulations and this experience has occurred in several other laboratories. Thus, pseudomoniasis has been and is a continuing problem.

Susceptible Preparations and Their Contaminants

Pseudomonas growth has been found in or reported as responsible for the degradation of shampoos, facial lotions, sun preparations, baby products, ophthalmic solutions, make-up products, cleansing creams, emol-

lient creams, cleansing pads, cosmetic eye preparations, "wrinkle remover" solutions, cleansing sponges, protein solutions, pharmaceutical and cosmetic gels, hydrocarbon cleansing oils, and so forth. These include simple solutions, O/W and W/O emulsions, triphase systems, gels, and hydrocarbon oils.

The preparations from which pseudomonads are usually recovered are O/W emulsions at a pH of 7.5–8.5, which contain a significant amount of nonionics. This does not mean that formulations containing anionic or cationic emulsifiers are not subject to attack, but the presence of nonionics has been found to be most conducive to pseudomonas contamination. This menstruum is so favorable, that pseudomonas, because of its resistance to biostatic agents, is the usual contaminant and one that grows in the product as a pure culture. At times staphylococci, aerogenes, yeasts, and molds have been isolated but never mixed with pseudomonas. Experience has indicated that a poorly preserved product is naturally contaminated only by a specific organism, e.g., a cream susceptible to pseudomonas is not susceptible to staphylococci or yeast, although a fulminating pseudomonas infection may pave the way for subsequent mold growth. The consequences to the product of heavy growth of pseudomonas are likely to be the development of a foul odor, formation of a deposit or turbidity, a change in flow and break-down of the emulsions by enzymatic activity, decolorization and/or the development of a brown color in the presence of hydrocarbon oils.

A bacterial population such as is encountered in a O/W emulsion is continuously changing through adaptation or mutation. It is possible, therefore, for organisms to emerge which may develop resistance to a particular combination of inhibitors and the emulsion will ultimately spoil. The spoilage may occur after the peak of the microbial population has been reached and the microbial count is declining. Occasionally materials are seen containing as many as 5 million organisms/ml without obvious signs of deterioration. Few would believe that such material is fit for sale.

A perusal of the publications on pseudomonas growth in cosmetic and other industrial products reveal the capabilities of this genus. They break down hydrocarbons (7) including petrolatum (8), remain viable for months in aircraft fuel (9), utilize alkanes such as hexane, and aromatics like benzene (10). They form inducible enzymes to benzoic and anthranilic acid (11), produce lipases and oxidize fatty acids (12, 13), maltose, lactose, cellobiose, and melibiose (14). Then their enzymes liquify gelatin, attack casein, perform amylolysis (15), and are most active at

25°C and at a pH of 7–8. They are micro-aerophilic and few organisms have their capacity for growth at 0°C (16).

The susceptibility of some formulations to pseudomonas contamination may be specific. A pseudomonad isolated from one preparation may not readily grow in any other. Pseudomonads that have been isolated from a make-up product, for example, will only grow within the specific product. Pseudomonads isolated from a cream could only grow within that cream. It has been necessary on occasion to prepare product-containing culture media to achieve profuse growth and after adaptation to laboratory media, the pseudomonad organism must frequently be inoculated into the original cream to maintain the resistance of the organism. Because of these properties, it is a practice of the author's laboratories to name isolated pseudomonads according to the product of origin. For example, there is a culture named *Pseudomonas aquasolarus*, a variety of aeruginosa, whose name indicates the origin of the culture.

Pseudomoniasis, An Industrial Disease and Its Control

The growth of pseudomonads in a product becomes an infection and the termination of which is the destruction of the product. The mechanisms of industrial control are similar to those used by a health department in a communicable disease program.

1. Preservatives increase the resistance of products to infection.
2. Manufacturing at 180°F reduces or eliminates the microbial content of the ingredients.
3. Sanitary compounding and filling minimizes contamination of the preparation.
4. Housekeeping and sanitation is enhanced by company issued sanitary regulations and periodic inspection by sanitarians.
5. Education of manufacturing personnel can help achieve a high level of sensible, clean production methods. Successful results can most readily be obtained when plant personnel understand the reasons for sanitation of equipment and for clean manufacture.
6. An investigative microbiology program tests the adequacy of product preservation, controls the microbiological quality of products at the time of manufacture, conducts shelf studies, and examines partially used products secured from consumers.

In sum, the mechanisms which insure the microbiological quality of manufactured products are:

1. Microbiological control
2. Sanitary manufacturing
3. Sanitary inspection
4. Personnel education

Preservation

Regardless of sanitary manufacturing methods, a poorly preserved preparation provides many opportunities for microbial infection. As described earlier an O/W emulsion at a pH of 7–8 containing nonionics provides a particularly suitable menstruum for pseudomonas growth. Preservatives used to restrain growth are the well known phenolics, parabens, bisphenols, and organo-metals. Organic acids can be effective at pH <7 while quaternaries tend to be incompatible with a nonionic slightly alkaline system. It is necessary to bear in mind that few cosmetically acceptable bacteriocides are active against pseudomonads specifically, even in the absence of surfactants. Anionics in general appear to inhibit selectively the metabolism of gram-positive organisms, and those which are bacteriocides tend to have a narrower spectrum than cationics. The bacterial action of anionics is influenced to a greater extent by changes in pH.

Some nonionics actually *inactivate* the bacteriostatic action of bisphenols and Tween® 80,* for example, is a more effective antidote than blood serum for nullifying the antibacterial properties of these compounds (17). However, Tween 80 in low concentrations, such as 0.02%, render pseudomonads actually more susceptible to antimicrobial agents. This enhancing effect at low levels is noteworthy since at higher levels the surfactant was found by Kohn *et al.* (18) to be an antagonist to the very same bacteriostatic agents. Studies have shown that *Pseudomonas aeruginosa* is capable of growth in solutions and dispersions of nonionic surfactants of the Tween type and can split the ester linkages of these agents (19). Practically all the nonionics of ethylene oxide or propylene oxide condensates of fatty acids and alcohols inactivate many preservatives in current use. It was found, in a study of 36 nonionics and 26 preservatives, that the nonionic surfactants reduced the efficiency of all preservatives when the ratio of surfactant to preservative exceeded certain critical values (20).

Of course, one is now cognizant that with time, inactivation of many biostats occurs and what appears as a well-preserved preparation today can be spoiled by an inoculum 2–3 months after manufacture (21). Emulsions of the water-in-oil type are relatively resistant to natural pseudomonas attack since the continuous oil phase acts as a barrier to penetration of the organism into the water phase, and impedes the spread of growth through the system. However, the features which pre-

* Tween 80 is a registered trademark of Atlas Powder Company, Wilmington, Del.

vent invasion also retard the antimicrobial activity of the biostats, so that a W/O emulsion which is more difficult to infect also appears to be more difficult to preserve. Other factors affecting the activity of preservatives in W/O systems are those controlling the availability of the preservatives in the aqueous phase, in particular, the O/W partition coefficient of the preservative, the phase-volume ratio, and the temperature. Antimicrobial compounds with high oil-water partition coefficients concentrate in the oil often with insufficient quantities being distributed in the aqueous phase to inhibit microbial growth. The partition coefficient of most biostatic agents will be higher for vegetable oils than for mineral oils, making vegetable oil creams more difficult to preserve than those prepared from mineral oil. Knowing the concentration of a biostat, the dissociation constant, the concentration of nonionics and the partition coefficient, the amount of the agent dissolved in the aqueous and micellar phases at a given pH can be calculated. Evans (22) found, for example, that a 0.5% concentration of *p*-hydroxy benzoate in an aqueous 6.0% Tween solution gave a concentration equivalent to 0.1% of the benzoate in the water phase.

Noble and Savin (23) reported a hospital pseudomonas outbreak which illustrates these concepts. A well preserved steroid cream was diluted with lipids. The diluted cream containing 0.1% chlorocresol was found contaminated with *P. aeruginosa*. Although 0.02% chlorocresol in aqueous solution was sufficient to prevent growth of organism, the chlorocresol migrated into the oil constituents of the cream leaving the aqueous portion with insufficient biostat. Examination of the clinical records suggested that the cream had caused minor infections over a period of several months. Similarly diluted creams from two of eight other hospitals were also found contaminated with pseudomonads.

In preserving a product one frequently overlooks the nutritive quality of the preparation. Cosmetic and pharmaceutical formulations often range from water solutions to high protein soups. The growth potential of the product itself is a prime agent influencing the ease or difficulty of preservation, for a nutrient-free preparation is easier to preserve than one containing nutriles. The "magic ingredients" now current in many formulations can on occasion be nutrients, stimulating the growth of organisms and obstructing the action of preservatives.

Existing screening tests in agar or broth which determine a compound's inherent antimicrobial activity are helpful only in eliminating completely inactive materials. They will not predict, with any reliability, performance in the actual cosmetic or pharmaceutical emulsion.

The preservation of formulations depend on many factors and *the only reliable way to determine the resistance of a product to organism growth is to inoculate and test the product itself*.

In the author's laboratories a minimum incubation period of 13 weeks is utilized to determine the possible loss of preservation potency with time. Re-inoculation of the samples during incubation often demonstrates declining effectiveness of the preservation system. On one occasion during a prolonged product inoculation test the preservative repressed the inoculum for 17 weeks, but shortly thereafter vigorous growth and deterioration of the preparation ensued. Adaptation of pseudomonads can take place quickly, or very slowly over several weeks, and it is the latter which one has to watch (6). This may account for the sporadic spoilage reported in the field when control samples on shelves are in perfect condition (19).

Preservation is interpreted by some as preventing the growth of micro-organisms. Does that definition fully protect the consumer? When pseudomonads introduced into a product survive, but do not multiply, can one really say, from what is known of the organism, that the preparation is adequately preserved? Kohn *et al.* (24), consider a preservative too slow-acting to be used in ophthalmic solutions if it could not sterilize a given pseudomonas suspension within one hour. Preparations are placed on preservation study to determine the ability of the product to withstand consumer use and abuse. The numbers of pseudomonads one finds in a product may have been introduced as increments during use or developed as a result of growth. Regardless of the origin, the presence of a large number of pseudomonads over a significant period of time predicates a quality, health, and legal hazard. A small number of organisms surviving within a preparation may in time adapt to and proliferate in the product. It therefore follows that the only adequately preserved preparation is one that is essentially self-sterilizing. The sterilizing time however need not be measured in hours but in days or even weeks.

The test organisms which the author employs have been taken from working formulations which were at one time contaminated, so that the screen contains strains adapted to and encountered by the laboratory's preparations. Screening methods should not be fixed since new strains of pseudomonads continually are isolated from domestic and foreign sources.

It must be accepted that unforeseen and exceptional organisms or conditions may turn up during routine manufacture to inactivate a

preservation system. It is the author's view that the preservation ability of a product should be well in excess of the minimum necessary to inhibit the anticipated flora, and ideally one aims at a product that is self-sterilizing. In the author's experience, a self-sterilizing preparation can be achieved in most cosmetic and pharmaceutical products, without any significant increase in cost or loss of marketability.

Sanitation

Preservation should not be expected to compensate for insanitary or careless manufacturing or packaging. Aseptic production, with the exception of eye preparations, is neither economical nor necessary. The product will therefore be microbiologically challenged, the magnitude and quality of which will vary with the ingredients, water supply, cleanliness, and season. This challenge may be most severe for it is here that continuing adaptation of the pseudomonads to the formulations takes place. The number of organisms from these sources has a bearing on their subsequent ability to proliferate within the preparation. Very small inocula have little opportunity to multiply. Large inocula increase the assault on the preservation system and increase the probability that adaptive resistant pseudomonads will be introduced into the product.

Ingredients themselves can be a potent source of contamination. In time the troublemakers are found, and microbial specifications are then added to future purchase contracts. Anionic detergents have frequently been a particular problem. Containers are rarely a problem but closures are. A liner may support growth or, by absorbing moisture from spatterings of the product on the surface, alter the potency of the preservatives. Pseudomonads with borderline resistance can develop there, and once enhanced, infect and multiply within the preparation itself.

Water supply is a particularly important source of *Pseudomonas*. The difficulty of eradicating this organism from water is underscored by the work of Belium and Koshi (25), who found that the resistance of *Pseudomonas* to chlorine ranged from 45–150 ppm. Water supply systems should be monitored microbiologically, from the main to the manufacturing tank inlet. Attention should be paid to the state of pipe work, valves, hoses, loops, and other sites of water stagnation. The water supply often contains a small number of organisms but without adequate control, water can issue from a tap or hose with thousands of pseudomonads and other gram-negatives/ml. A demineralizer may be a frequent source of this development. It provides a good medium for multiplica-

tion and during warm weather can discharge counts of 100,000 or more gram-negatives. The best water control system the author has devised has been the installation of a hot water storage tank and manufacturing processes have been revised to use hot water wherever possible.

The manufacturer must take steps to ensure that no build-up of resistant organisms occurs in the production and distributing systems. This can be effected by factory hygiene using hot water, detergent and formalin treatment of filling machines, tanks, pumps, filters, mills, pipes, etc., immediately after use. Microbiological tests on equipment should be made to insure the effectiveness of the cleansing operation. The presence of thousands or hundreds of organisms/swab or ml calls for immediate action. Tens may be unavoidable although zero counts can be obtained from areas whose cleanliness is deemed crucial.

Ayliffe *et al.* (26) described an outbreak of meningitis in 14 neurosurgery patients. The infectious agent was *Pseudomonas aeruginosa*. An examination of the environment revealed the epidemic strain in a shaving brush used for preparation of the scalp and on the floor of a room occupied by an infected patient. Twenty other strains were isolated from hand creams, sinks, floors, soap trays, sink cloths, and the cap of a bottle of antiseptic.

Good general cleanliness is important in controlling pseudomonas contamination. And by general cleanliness is meant that of shelves, bins, tables, beneath tables, the under side of shelves, etc. At these points spillage of products mixed with dust, dirt and moisture provide a menstruum for the development of resistant pseudomonad strains.

Two incidents illustrate the diligence necessary in meeting a pseudomonas problem within a plant.

1. A strain of pseudomonas had adapted to a pharmaceutical steriod cream. The microbial content was monitored through the mixing kettle, filter, pump, homogenizer, aging tank, pipelines, and filling machines. Each element in the process was scrupulously cleaned and sterilized, and yet the very next batch had to be rejected. The number of organisms in the product was so large that it could only have been introduced by a massive inoculation. The only element in the chain that had not been dismantled had been an in-place pipe delivering the emulsion from tank to filler. The line, however, had been cleaned and formalin-sterilized and zero plate counts were obtained from the effluent to prove it. It was found, on breaking into the pipe, that the water had cleaned the bottom oval of the pipe, while the top oval, because of the low pitch, contained a layer of cream. Recovery of viable organisms could not be

made immediately after the sterilizing treatment, but within a short time the infection reestablished itself within the top oval, and a water wash thereafter contained thousands of pseudomonads/ml. Scouring the pipe eliminated the problem and the next batch which was contaminated again had to be rejected. Microbial examination of the entire process failed to reveal any source of inoculation. A gimlet-eyed chemist solved the problem. He found an economy-minded, filling-line supervisor had utilized, as a lubricant for the cap of the vial, rejected material which had escaped destruction. This material, which should have been disposed of weeks earlier, contained hundreds of thousands of organisms/ml. Thereafter, there were no problems.

2. The laboratory reported a significant microorganism content in an experimental make-up product. The preparation in time killed the pseudomonad which had an identifiable characteristic. The source proved elusive. Two bottles containing a suspension of an ingredient used in the preparation were found tucked away in a rarely used part of the stockroom. The bottles were coated with a mixture of spillage and dust. The contents were sterile but a swab of the spillage yielded thousands of the particular pseudomonad/square centimeter. It is probable that with time these organisms, if not eliminated, would have completely adapted to the preparation.

Conclusion

This paper represents the experience of a microbiology laboratory in dealing with industrial pseudomoniasis over more than a decade. During this period, respect for pseudomonads as adaptive organisms has continued to grow. The lesson to be learned is that eternal vigilance is the price of quality and, occasionally, even of safety.

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A Statistical Approach to the Evaluation of Cutaneous Responses to Irritants

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Synopsis—This study was done to test the application of certain common statistical experimental designs to the field of human patch testing where they do not appear to have been used previously, and to investigate certain variables affecting irritation test results. Sodium lauryl sulfate, a typical irritant of general interest, was used. Two experiments are described in which several factors thought to affect irritation results were tested; these included irritant concentration, certain time factors, and types of patch used. Irritation was basically scored on a five-point scale. Results showed that experimental and subject-to-subject variation could be greatly reduced by adequate experimental design, that several factors of interest were influential in the system, and that the error of measurement (estimation by a judge scoring the patch sites) was much smaller than had been expected. One of the significant effects of considerable interest was the finding that the degree of observable irritation was a function of the interval between removal of a patch and the time the site was scored.

Introduction

It is well known that the use of patch testing for the detection of primary irritation or sensitization of human skin to various substances, as usually practiced, is subject to severe limitations of numbers (1). For example, it may readily be shown by elementary probability calculations that the use of the usual patch testing techniques, even were several thousand subjects to be used, might easily fail to predict a serious proportion of reactors in the population.

This problem has not been solved by the techniques to be described in this paper, but it is hoped that it may have been ameliorated. The

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authors have felt for some time that utilization of modern methods of experimental design and data analysis can be of great value in skin research as well as in practical testing. Surprisingly, very little has been done with these techniques in this area, despite the relatively heavy use of statistics in related biological fields.*

Many irritants have been patch tested and many investigators have suggested test methods. There are now almost as many methods as there have been investigators. Pioneers have included Schwartz (2), Shelanski (3), Draize (4), Voss (5), and Rostenberg (6), all of whom have made important contributions. However, there remains a need for a standard, easily managed, and statistically valid procedure for the assay of irritation potential, one which will give uniform and reproducible results within a short time.

The purposes of this investigation were:

1. To study the advantages of using certain well-known experimental designs and subsequent statistical analyses which do not appear to have been previously used in skin testing.

2. To describe certain minor modifications of current human patch testing techniques which the authors feel may improve experimental reliability and quantitation.

3. To investigate certain variables which appear to operate in a particular irritant system, principally as an example to demonstrate the experimental techniques and statistical analysis.

Preliminary Experiments

Irritant

To accomplish the above objectives, particularly the first (*viz.*, to demonstrate the efficiency of formal experimental design and analysis), it was believed advisable to use a specific single irritant. Sodium lauryl sulfate† was chosen because of its common use in many household products and toiletries, because it is believed to have no important sensitization effects, and because it is well-known to dermatological investigators.

Scoring

Again, in order to use a familiar system, it was felt desirable to have a numerical scale of measurement similar to those used in the past.

* Recently, another approach to quantifying skin testing has been described, and will be published soon (16).

† Duponol C®—E. I. du Pont de Nemours and Co., Inc., Wilmington, Del. 19898.

It was believed, however, that the degree of irritation could be judged visually much more precisely than has previously been supposed, and the scale shown in Fig. 1 was therefore adopted.

In using this scale, the observer was required to estimate the degree of irritation on the treated site compared to untreated surrounding

Figure 1. Irritation scoring scale

skin. During the reading of any site, other treated sites were covered with tissue to eliminate direct comparisons. *The estimates were always made to the nearest 0.25 unit on the scale of Fig. 1.* (It was shown later that this degree of precision was reasonable.)

In planning preliminary work in order to identify possible real variables affecting irritation with sodium lauryl sulfate, the following factors were considered:

Size of patch
Type of covering (occlusion)
Nature of patch
Quantity of test solution
Concentration of irritant
Patch contact time
Observation time (elapsed time after removal of patch)
Tightness of patch
Nature of subjects' skin
Activity of subjects

It was intended that many of the potential variables be held constant so that the exposition of the statistical procedure would not be confused because of undue complexity. It is to be understood that in any real system under investigation it may be practical and frequently necessary to test more sources of variation than the number included in this case. The experimental designs then used would simply be extensions or modifications of those shown here.* In the present case, some preliminary work was done in order to try to select a small number of important factors from the above list. This work is treated briefly below.

Development of Type of Patch

Some initial work was done with oval-shaped open patches, such as are used often in routine patch testing. These were applied over ½ in.

* There are many modifications of factorial designs, as well as certain nonfactorial arrangements available which do not require extremely large experiments when many variables are to be evaluated.

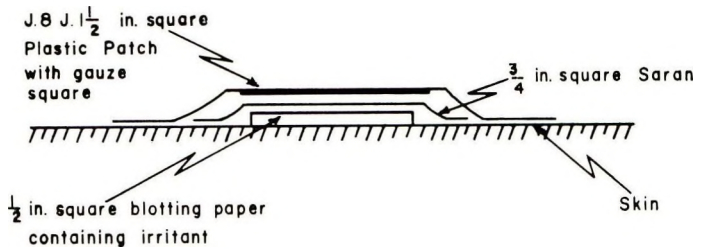

Figure 2. "Regular" patch

pieces of blotting paper to which a constant quantity of irritant solution (4 drops) was applied. The skin was swabbed thoroughly with acetone just prior to patching. Several different areas of the body were used. These patches were found unsatisfactory for reasons which included the following:

1. In many cases the solution evaporated or soaked into the gauze backing after a few hours.
2. There was much apparent variation in pressure exerted on the blotting paper due to the shape of the patch and a tendency for the adhesive to creep.

Following this, ½ in. sq. plastic Band-Aids®* were tested. These were much more satisfactory for adhesion and pressure variation, but loss of solution by evaporation or absorption into the patch was still a problem. Finally, the blotting paper was occluded by the application of a ¾ in. sq. of Saran® † film under the Band-Aid, as shown in Fig. 2. This arrangement was adopted as one of the final two types of patch used, and appeared to solve the problem of loss of solution; blotters remained moist even after 24 hours. Although this degree of occlusion is not usually employed in patch testing, the results of this and subsequent work have indicated that this arrangement, under the conditions of these experiments, may be generally superior to the customary type.

There was strong evidence that the reproducibility of reactions to the new "regular" patch was superior to that of those previously used. Hypothesizing that this may have resulted from maintenance of more constant and intimate skin contact, it was decided to try a further modification designed to have a slightly increased pressure against the skin, and, it was hoped, a much more uniform degree of contact. This

* Band-Aids®—Johnson and Johnson & Co., New Brunswick, N. J. 08903.
† Saran®—The Dow Chemical Co., Midland, Mich. 84641.

"pressure" patch consisted of the regular patch plus two gauze squares, then a taped-on plastic poker chip (Fig. 3). Preliminary experiments indicated that substantially different results might be expected from this arrangement. Since the pressure difference was believed slight, we attributed this to the presumed greater uniformity of contact. The authors are not necessarily recommending this arrangement for general use, of course, and are aware that the use of any substantial degree of pressure is frequently believed undesirable. However, it is felt that this experience, especially when viewed in the light of the two formal experiments to be described below, demonstrates the importance of maintaining a constant contact.

Selection of Variables

Preliminary work indicated that the degree of irritation was probably also influenced by the period of time following the removal of the patch. It was therefore planned to use this factor as an additional variable. Further, contact time and irritant concentration were relevant factors, since it was well known that these variables would affect the system; however, nothing was known to the authors of their interaction with each other and with the other two variables.

To summarize, preliminary experimental evidence suggested use of the following four variables in the first formal experiment:

A. Concentration of irritant
B. Type of patch
C. Patch contact time
D. Observation time (after removal)

In this first factorial experiment, the following potential variables were to be carefully controlled at as constant a level as possible from run to run:

1. Single judge (scorer)*
2. Type of irritant
3. Pre-treatment of site (5-second swabbing with facial tissue wet with acetone)
4. Four drops of solution on each blotter square
5. All patches applied in a single session

* All scoring in the work to be described was done by an experienced pharmacologist, although a small preliminary study indicated no significant difference, and very high correlation (over 95%) between his scores and those of a technician who had been instructed by the pharmacologist and had had less than one day's practice.

6. Solutions of irritant carefully prepared in an analytical laboratory
7. All tests done at same time of year (within a 2-month period)
8. All tests done on subjects' backs.
9. All subjects male
10. All subjects 18 to 45 years old
11. All subjects healthy Caucasians
12. Extremely hirsute subjects excluded
13. All patch characteristics (dimensions, etc) held to close tolerances for each type of patch
14. All subjects laboratory workers
15. All subjects on overnight tests asked to keep patches dry
16. All patches applied by one person
17. All subjects limited to eight patches for comfort

Design of First Experiment

General

The simplest possible design which would incorporate the four selected variables and allow elimination of intersubject response differences was planned. This goal, together with the eight patches per subject restriction, determined the general outline of the design used.

A careful search of the literature did not disclose the previous use of design techniques similar to those contemplated. The authors therefore felt it doubly important to make the initial trials with as simple a factorial design as possible, without sacrificing the major advantages of multi-factor experimental designs as compared to classical (one-factor-at-a-time) procedures.

Objects of the Experiment

The principal purposes of this experiment, which determined the design, were:

1. To minimize experimental error by isolating subject-to-subject differences.
2. To determine the suitability of analysis of variance techniques for handling the data resulting from the experiment.
3. To determine "significance" (i.e., probability of reality) of the variables tested (and their interactions).

Only a small number of subjects were to be used in this initial formal design, because it was important to determine whether any of the selected factors could be tested in a small experiment, and it was desir-

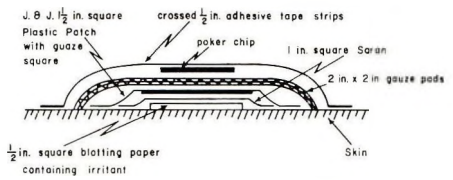

Figure 3. "Pressure" patch

able to make future comparisons with other small groups as a measure of the variability among separate experiments. The preliminary work had already suggested that such variability would be small once the subject-to-subject effect was removed.

Isolation of subject differences called for the use of "blocking" and "confounding," which are widely used in statistical designs in bioassay and in chemical applications research. Essentially, blocking is a technique whereby, in the present case, the influence of subject-to-subject differences on the results is isolated, so that, if successful, it has little or no effect upon the differences of interest represented by such factors as irritant concentration, contact time, etc. Confounding is a technique of "mixing" effects of minor interest with blocks or subjects in order to increase the precision of measurement of more important variables. If it is assumed before an experiment that intersubject differences are substantially greater than intrasubject variation, smaller values of experimental error can be expected when these techniques are used.* The remaining intrasubject error, once this major source is eliminated is that likely to result from relatively small differences in location on the skin, patch application techniques, estimation of scores, etc.

It is not intended that this presentation provide a complete "cookbook" description of the experimental design and statistical analyses used; these may be obtained from several sources listed in the bibliography (7–15). Therefore, the experimental design, final analysis of variance tables, and conclusions are given, but the statistical calculations are omitted

This experiment was to include the above four variables, each to be tested at two levels in a four variable factorial design. The whole

* The sensitivity of the experimental design and analysis in testing the various factors for significance depends: (a) upon the magnitude of the apparent effect due to changing a factor from one level to the next, and (b) upon the magnitude of the experimental error. The former is controllable only by the spacing set between two levels of the factor and upon its inherent ability to cause variation, but the latter depends upon the design characteristics.

design was to be repeated (replicated) three times, with two blocks or subjects in each replicate, confounding the four-factor interaction between each pair of blocks. The levels of the four variables were:

A (*Concentration of Irritant*)
- A_1 = 1% Duponol C
- A_2 = 4% Duponol C

B (*Type of Patch*)
- B_1 = Regular patch (cf. Fig. 2)
- B_2 = "Pressure" patch (cf. Fig. 3)

C (*Patch Contact Time*)
- C_1 = 6 hours
- C_2 = 24 hours

D (*Observation Time*)
- D_1 = 2 hours after removing patch
- D_2 = 18 hours after removing patch

The factorial combination of the above resulted in 16 "runs" to be repeated in each of the three replicates. Each replicate consisted of two "blocks" or subjects, with eight runs applied to each. The quadruple interaction, ABCD, was confounded between each pair of blocks; i.e., its effect, which was assumed a priori to be of no intrinsic interest, was "mixed" with that of the subjects.

In the diagram which follows, standard two-level factorial notation is used. Thus, one of the sixteen runs was $A_1B_1C_1D_1$ (1% Duponol, regular patch, 6 hours' contact time and 2 hours' observation time). Another, for example, was $A_2B_2C_1D_1$ (4% Duponol, pressure patch, 6 hours, 2 hours). These two runs are designated in standard notation as run (1) and run ab, respectively. This notation includes the appropriate lower case letter if the factor referred to is used at the high level, and omits this letter if the factor is used at its low level. Thus, the 16 runs in each replicate were:

1 ($A_1B_1C_1D_1$)	d ($A_1B_1C_1D_2$)
a ($A_2B_1C_1D_1$)	ad ($A_2B_1C_1D_2$)
b ($A_1B_2C_1D_1$)	bd ($A_1B_2C_1D_2$)
ab ($A_2B_2C_1D_1$)	abd ($A_2B_2C_1D_2$)
c ($A_1B_1C_2D_1$)	cd ($A_1B_1C_2D_2$)
ac ($A_2B_1C_2D_1$)	acd ($A_2B_1C_2D_2$)
bc ($A_1B_2C_2D_1$)	bcd ($A_1B_2C_2D_2$)
abc ($A_2B_2C_2D_1$)	abcd ($A_2B_2C_2D_2$)

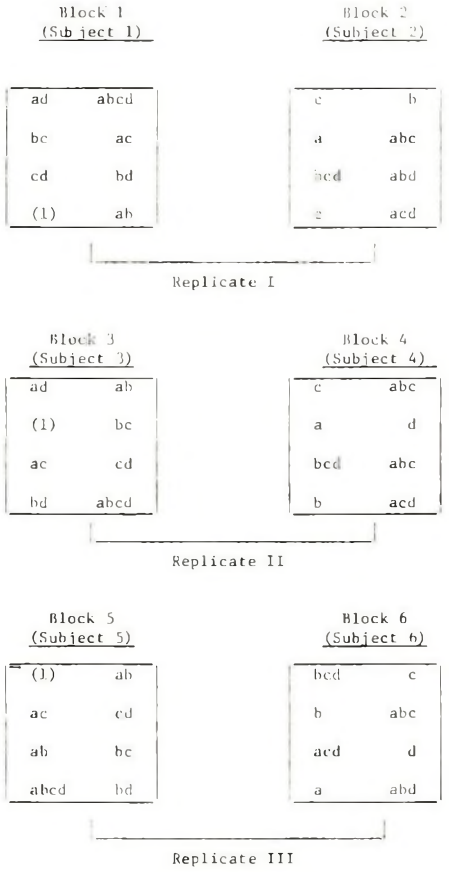

Figure 4. Final experimental design—first experiment

Figure 4 shows the final design. The order of the runs (i.e., the sequence and position used in applying the patches to each subject) was randomized, as was the order of the two blocks in each replicate, by using a table of random numbers. The rectangles of Fig. 4 represent the backs of the subjects; four patches were applied along each side of each back, below the scapulae.

Data and Analysis of First Experiment

The experiment was performed as shown in Fig. 4. The patches were applied in the positions shown. Each area to be patched was swabbed with acetone as described previously, and four drops of the irritant solution were placed on each blotting paper square just before patching. Observations were made as scheduled by Variables C (con-

tact time) and D (observation time). All scoring was done by comparing a patched area with an adjacent untreated and untaped skin area. All patches not being read were draped to reduce subjectivity. The order of reading the sites on a given subject was not controlled.

Table I shows the scores obtained. The scoring system used was that of Fig. 1.

A conventional analysis of variance was carried out on the data of Table I.* Table II summarizes the analysis. Note that the mean square for error was 0.2335. This represents an error standard deviation of about 0.48 scoring units, and accounts for all sources of variation not specifically listed in Table II (scoring errors, position effects, patch tightness, etc.). Therefore, the portion of this error attributable to scoring error must have been less than this value unless all other sources of variation were zero. It thus appeared that the reading of patches to the nearest 0.25 unit was a reasonable procedure.

It should also be noted that the total blocks mean square was substantial, as expected, suggesting that the separation of this effect (i.e., subject-to-subject differences) from the error by the blocking and confounding techniques was important.

In drawing conclusions from this experiment, it was decided not to ignore any effects showing significance at the 85% confidence level or better, since the only consequences of falsely assuming an effect to be real would be its inclusion in further work.

There was a strong contact time effect, as expected, but the only interaction involving this was the triple interaction. Since such interactions are rare, this was viewed with some skepticism, but it was nevertheless investigated further (see second experiment below). The remaining significant effects were all included in the same triple interaction, so that the most informative interpretation of the experiment with respect to these effects was done by examining this alone. To do this, averages of all data for each combination of factors, A, B, and D were computed and plotted. The averages and the plots are shown in Fig. 5.

As shown in Fig. 5, at 2 hours' observation time, increasing concentration of the irritant produced a sharp increase in irritation when the pressure patch was used but a *decrease* when the regular patch was used. After 18 hours, however, although there is a possibility of a slightly

* There has been hesitation in the past toward the use of analysis of variance with scored data; however, it has been shown that the non-normality of such data does not seriously interfere with the validity of conclusions drawn, and such procedures are now widely used.

Table I

Original Scores—First Experiment

Block and Rep.	Designation of Run																
	(1)	a	b	ab	c	ac	bc	abc	d	ad	bd	abd	cd	acd	bcd	abcd	Totals
Rep. I																	
Block 1	1.25	...	...	1.00	...	1.25	1.50	...	...	1.00	1.00	...	1.00	...	...	1.25	9.25
Block 2	...	1.00	1.00	...	1.25	...	...	1.25	1.25	...	...	1.00	...	1.25	1.00	...	9.00
Total	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	18.25
Rep. II																	
Block 3	1.00	...	...	1.25	...	2.50	1.50	...	...	1.25	1.00	...	3.25	...	...	3.50	15.25
Block 4	...	1.50	1.25	...	3.00	...	...	3.00	1.00	...	...	2.50	...	3.25	3.50	...	19.00
Total	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	34.25
Rep. III																	
Block 5	1.00	...	...	2.00	...	1.00	1.50	...	...	1.00	1.00	...	1.50	...	...	2.00	11.00
Block 6	...	1.00	1.00	...	3.00	...	...	2.75	1.00	...	...	1.25	...	3.00	2.00	...	15.00
Total	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	26.00
Totals	3.25	3.50	3.25	4.25	7.25	4.75	4.50	7.00	3.25	3.25	3.00	4.75	5.75	7.50	6.50	6.75	78.50

Table II

Analysis of Variance

Source of Variation	DF	SS	MS	F′	Sig. (P)[a]
Between concentrations (A)	1	0.5208	0.5208	2.23	~0.15
Between types of patch (B)	1	0.0469	0.0469	<1	...
Between contact times (C)	1	9.6302	9.6302	41.24	<0.001
Between observation times (D)	1	0.1875	0.1875	<1	...
Concentration × patch type (AB)	1	0.7500	0.7500	3.21	~0.08
Concentration × contact time (AC)	1	0.0208	0.0208	<1	...
Concentration × observation time (AD)	1	0.1302	0.1302	<1	...
Patch type × contact time (BC)	1	0.1302	0.1302	<1	...
Patch type × observation time (BD)	1	0.0208	0.0208	<1	...
Contact time × observation time (CD)	1	0.1875	0.1875	<1	...
Concentration × pressure × contact time (ABC)	1	0.0208	0.0208	<1	...
Concentration × pressure × observation time (ABD)	1	0.6302	0.6302	2.70	~0.12
Concentration × contact time × observation time (ACD)	1	0.0469	0.0469	<1	...
Pressure × contact time × observation time (BCD)	1	0.0000	0.0000	<1	...
	14	12.3228			
Among replicates	2	8.0026	4.0013	2.16	...
ABCD (within replicates, between blocks)	1	1.1719	1.1719	<1	...
Between block differences	2	3.7109	1.8555	...	...
Total among blocks	5	12.8854	2.5771	...	...
Residual (error)	28	6.5366	0.2335	...	...
Total	47	31.7448	...	...	...

Notes: (1) All main effects and interactions except ABCD tested against residual. (2) ABCD and replicates tested against the "between block differences M.S." (3) Definitions of column head symbols: DF = degrees of freedom, SS = sum of squares of deviations from the mean, MS = mean square, F′ = value obtained in F test of significance, and P = probability of the observed difference having occurred by chance.

[a] This column shows the probability that the given effect *is not real*. If P (probability of Ho or unreality) was over about 0.15, no entry was made in this column and the effect was considered "not significant."

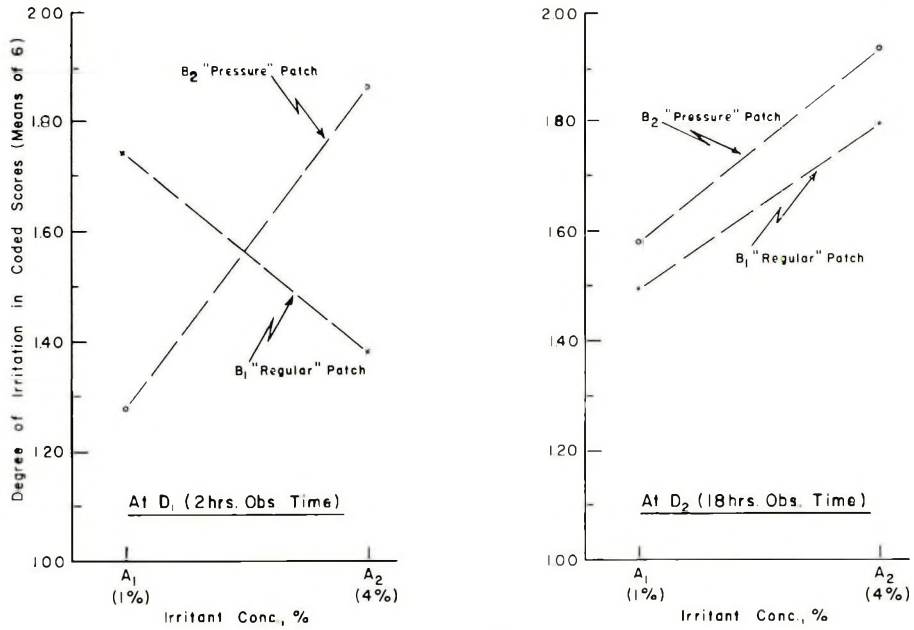

Figure 5. Triple interaction (ABD). Concentration × type of patch × obs. time

greater irritation effect with the pressure patch, the difference, if any, between pressure and regular patches is the same at both concentrations.

Design of Second Experiment

The second design was worked out and run after noting and discussing the findings just given. Several important differences were to be built into this new experiment. From the beginning, it had been felt desirable to run an experiment in which concentration was used at more than two levels, so that the nature of the concentration response (e.g., curved or linear) could be studied. Such a procedure carries additional advantages in that it is often possible to obtain greater sensitivity in the analysis of the data.

Additional levels of concentration were usable only if one or more of the original four variables was eliminated and no new ones added, or if the new experiment was made larger. For practical reasons, the latter was undesirable. Since contact time had been found not to interact with any of the other variables in the first experiment, it was possible to eliminate this factor from the second one, because all necessary information about its main effect response was already available.

This left three variables for the new experiment, with contact time held constant at some fixed level (preferably close to one of those used in the first experiment). It was decided to use 24 hours, because the longer time gave higher irritation values compared to the shorter period, and thus took greater advantage of the range of the scale used. The three remaining variables to be used in this experiment, and the levels decided upon were:

(*A*) *Concentration of Irritant* (*Duponol C*)
- A_1 = 0.5%
- A_2 = 1%
- A_3 = 2%
- A_4 = 4%

(*B*) *Type of Patch*
- B_1 = Regular
- B_2 = Pressure

(*C*) *Observation Time*
- C_1 = 2 hours
- C_2 = 24 hours

The spacing of the levels of concentration (each double the previous one) was adopted because: (a) it was believed that, like many chemical concentration and biological dosage effects, the increase in response might well become progressively smaller as concentration increased (this was also indicated by some of the preliminary work), and (b) this type of spacing made the data amenable to a particularly simple type of statistical analysis for the curvature effects.

The same system of blocking and replication as before was used; i.e., three repetitions of two blocks (two subjects) each. None of the subjects were the same as those used before.

The basic experimental design was that known as a "split plot," in which a main effect instead of an interaction is confounded between ("mixed with") blocks. Thus, it will be noted (Fig. 6) that only one of the two types of patch was used on a given subject in each pair. This procedure results in loss of sensitivity for the detection of the confounded effect (but not complete loss of information, since there was replication). It was decided to use the patch type variable, B, for the splitting, because the previous experiment indicated that this main effect was of little value in interpretation of the other factors, and it had already been established as a strongly significant variable, so that there was little doubt of its reality and little need for further verification.

After randomizing the assignment of each run to each position on each subject's back (i.e., each block), as well as the order of application of the patches, the experimental design was prepared and is shown in Fig. 6.

Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
211 411	422 321	212 311	121 122	211 212	221 421
212 312	421 221	111 312	322 421	412 111	422 321
311 112	222 121	112 411	321 221	311 411	322 122
111 412	322 122	211 412	222 422	312 112	121 222
Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Replicate I		Replicate II		Replicate III	

Key to Identification of Runs:

First digit (A): 1 = 0.5% 2 = 1% 3 = 2% 4 = 4%

Second " (B): 1 = regular patch 2 = pressure patch

Third " (C): 1 = 2 hours 2 = 24 hours

The procedure for this experiment was analogous to that used previously with the exception that the scoring system was changed for convenience. Since it had been established to the authors' satisfaction that readings to the nearest 0.25 score unit could be made, the scores for this experiment were recoded before analysis as shown below:

Original Score	New Score	Original Score	New Score
0.00	1	2.25	10
0.25	2	2.50	11
0.50	3	2.75	12
0.75	4	3.00	13
1.00	5	3.25	14
1.25	6	3.50	15
1.50	7	3.75	16
1.75	8	4.00	17
2.00	9		

Figure 6. Experimental design

Table III

Original Data after Coding

Block and Rep.	A_1 (0.5%)				A_2 (1%)				A_3 (2%)				A_4 (4%)				Totals
	B_1 (reg)		B_2 (pres)		B_1 (reg)		B_2 (pres)		B_1 (reg)		B_2 (pres)		B_1 (reg)		B_2 (pres)		
	C_1, 2 Hrs.	C_2, 24 Hrs.	C_1, 2 Hrs.	C_2, 24 Hrs.	C_1, 2 Hrs.	C_2, 24 Hrs.	C_1, 2 Hrs.	C_2, 24 Hrs.	C_1, 2 Hrs.	C_2, 24 Hrs.	C_1, 2 Hrs.	C_2, 24 Hrs.	C_1, 2 Hrs.	C_2, 24 Hrs.	C_1, 2 Hrs.	C_2, 24 Hrs.	
Rep. I																	
Block 1	8	2	...	...	7	5	...	...	12	7	...	...	10	10	...	...	61
Block 2	...	...	2	6	...	...	1	8	...	...	2	8	...	...	7	5	39
																	100
Rep. II																	
Block 3	3	3	...	...	2	4	...	...	2	7	...	...	3	8	...	...	32
Block 4	...	...	4	6	...	...	3	9	...	...	3	10	...	...	4	9	48
																	80
Rep. III																	
Block 5	2	2	...	...	3	3	...	...	3	2	...	...	2	5	...	...	22
Block 6	...	...	2	1	...	...	2	2	...	...	2	2	...	...	2	2	15
																	37
Totals	13	7	8	13	12	12	6	19	17	16	7	20	15	23	13	16	217
	20		21		24		25		33		27		38		29		

Results of Analysis of Second Experiment

Data

The second experiment was carried out as described, and resulted in the raw data shown in Table III (after coding). All readings were made by a single experienced observer, as before.

Analysis

An "over-all" analysis of variance of the data shown in Table III was done following the usual computational procedures. The sums of squares for the concentration effect and its interaction were then partitioned into segments representing linear, quadratic, and cubic (cubic is equivalent to higher curvature and scatter) portions. This final analysis is shown in Table IV.

Discussion of Results

(1) The partitioning of the concentration effect and its interaction with the other two variables resulted in a very strong test of significance for the linear effect of concentration (probability 1% that effect was not real). None of the double interactions with concentration were significant, however, and although the triple interaction gave a weak significance test as before, the authors were less disposed to accept it, since the previous indication was not strongly reinforced despite the partitioning.

(2) The patch type-observation time interaction was significant, unlike results in the first experiment (if the triple interaction results for that experiment are ignored).

(3) The experimental error mean square was estimated as 4.02, as shown in the table. This is strikingly similar to that of the previous experiment (remembering that a new scoring scale was used here); the estimated standard deviation for error would be $\sqrt{4.02}$, or approximately 2.0 units, corresponding to 0.50 original units, almost identical to the error found in the first experiment. Thus, again, the use of estimates to the nearest $\frac{1}{4}$ of an original scale unit was demonstrated to be practical. Applying the same reasoning as before, this result indicates, as before, that the average error of measurement was less than about 0.5 original scale units, including the error of estimation.

Two of the effects showing considerable significance (linear concentration, A, and the patch type times observation time interaction, BC) are plotted in Figs. 7 and 8. The significant observation time effect

itself is not plotted, since this is better illustrated in the plot of its interaction.

It will be noted that Fig. 7 (which is a plot of means of the original data with a straight line visually fitted to them) bears out the conclusions as to linearity of the concentration effect suggested by the analysis of

Table IV
Analysis of Variance

	Source of Variation	DF	SS	MS	F′	P
Main Effects	*Among concentrations* (A)					
	Linear (A_l)	1	33.00	33.00	8.28	<0.01
	Quadratic (A_q)	1	0.02	0.02	<1	...
	Cubic (A_c)	1	0.20	0.20	<1	...
	Between patch types (confounded with blocks)					
	Between observation times (C)	1	25.52	25.52	6.35	<0.03
Double Interactions	Concentration linear × patch type (A_lB)	1	5.71	5.71	1.42	...
	Concentration quadratic × patch type (A_qB)	1	0.19	0.19	<1	...
	Concentration cubic × patch type (A_cB)	1	0.51	0.51	<1	...
	Concentration linear × observation time (A_lC)	1	5.11	5.11	1.27	...
	Concentration quadratic × observation time (A_qC)	1	4.69	4.69	1.17	...
	Concentration cubic × observation time (A_cC)	1	0.81	0.81	<1	...
	Patch type × observation time (BC)	1	22.69	22.69	5.64	<0.05
Triple Interactions	Concentration linear × patch observation time (A_lBC)	1	9.20	9.20	2.29	5 ~0.1
	Concentration quadratic × patch × observation time (A_qBC)	1	9.18	9.18	2.28	~0.15
	Concentration cubic × patch × observation time (A_cBC)	1	1.63	1.63	...	...
		14	118.46	...	...	...
	Among replicates	2	129.54	64.77	2.83	...
	Between blocks (within replicates)	1	3.52	3.52	<1	...
	Within blocks	2	45.80	22.90	...	...
	Total among blocks	5	178.86	...	...	...
	Residual (error)	28	112.66	4.02	...	...
	Total	47	409.98	...	...	...

Notes: Definitions of column head symbols: DF = degrees of freedom, SS = sum of squares of deviations from the mean, MS = mean square, F′ = value obtained in F test of significance, and P = probability of the observed difference having occurred by chance.

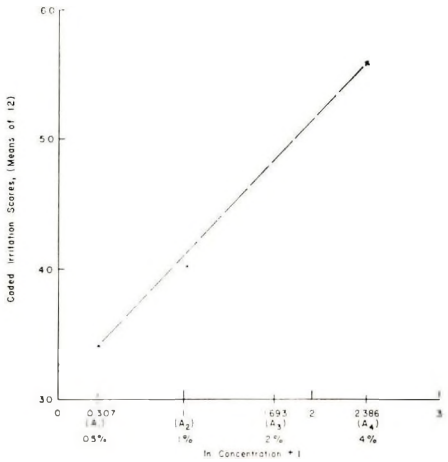

Figure 7. Variable A. Plot of concentration effect. (Log linear portion significant at <0.01 level.) No significant curvature

variance. Because of the significant linear effect and the nonsignificance of higher effects, the visual fitting with a straight line was justified. Note, however, that the "linearity" is based upon logarithmic spacing of the four concentrations, with +1 added to keep all values positive.

Discussion and Conclusions

These experiments demonstrate three points which the authors believe are of great importance in irritation testing, and perhaps for patch testing in general, in addition to the specifics connected with the particular variables used. These are the following:

(1) It appears possible with a properly designed experiment to obtain meaningful estimates of irritation on a scale of 0 to 4, to less than the nearest 0.50 scale unit.

(2) Differences among subjects and among repetitions of experiments have always been regarded as a source of difficulty in irritation testing. This work demonstrates that it is possible, by using very well-known and commonly-used statistical techniques, to isolate completely the first of these sources of variation and to prevent their interference with differences sought among various experimental conditions.

(3) The use of common multi-factor experimental techniques together with appropriate statistical analyses appears practical and useful in studying patch test data.

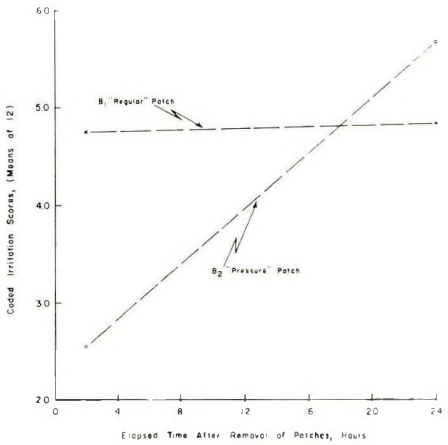

Figure 8. Variable B × C. Plot of observation time × patch = type interaction. (Significant at 0.05 level)

The more specific and less important conclusions to be drawn from this work are connected with the specific variables used. Discounting the first experiment except for the contact time variable, because of the relatively low significance levels obtained, and remembering that the analyses were of the kind known to statisticians as Type I (i.e., more or less limited to the particular levels of the variables and particular populations used), the following conclusions were drawn:

(1) The increase of irritation with increasing concentration of sodium lauryl sulfate (within the range of concentrations tested) appears to be a logarithmic function, with the *rate of increase of irritation decreasing as concentration increases.*

(2) The changes in observable irritation which occur *after* the patches are removed have not been emphasized in the literature. This work suggests that it is important to give the skin adequate time to develop a full irritation manifestation and to take this factor into account, especially in view of its interaction with the type of patch used.

(3) The use of the "pressure" patch, as explained above, was meant not as a device to apply pressure *per se*, but as an attempt to determine whether normal minor variations in pressure under a patch affect the results, so that if so, means for controlling it and thus minimizing variation could be arranged. As this work shows, a relatively small increase in pressure was apparently very important, especially at certain observation times. This suggests that more study and experi-

mentation in the design of patches and the techniques used in applying them is desirable.

(4) It has frequently been felt that lack of uniformity in patch test responses is the result of a "pressure effect." This work has demonstrated that one of the greatest sources of variation is subject-to-subject variation, which is easily isolated by proper experimental designs, which in turn has the effect of sensitizing the experiment so that a given variable may be tested with a much smaller group of subjects than otherwise. Of course the use of small groups presupposes that they are a fair sample of the population to which the results are to be extrapolated.

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Book Reviews

PHARMACEUTICAL CHEMISTRY, VOLUME I: THEORY AND APPLICATION, edited by Leslie G. Chatten, Marcel Dekker, Inc., New York. 1966. 504 pages, indexed. Price $14.50.

The scope of this text can most accurately be described as analytical pharmaceutical chemistry. It is the first volume of a two-volume series which is intended as a text for senior undergraduate and graduate pharmacy students. It deals with theoretical and practical considerations of gravimetric analysis, acid-base titrations and pH, precipitation and complex formation, acidimetry and alkalimetry, nonaqueous titrimetry, complexometric analysis, alkaloidal assay, miscellaneous methods, ion exchange, chromatography, and analysis of fixed oils and volatile oils. Volume II will cover the theory and application of instrumental techniques.

This book is composed of thirteen chapters, each of which has been written by a different contributing author. The joint authorship has an international character with contributors from the United States, Canada, and England. Each of the authors is a teacher of pharmaceutical chemistry or a related discipline of pharmacy.

Considering this organizational approach, the material within the book is surprisingly well presented and integrated. Only a slight tendency toward repetition is evident, and this is often justified for the sake of completeness within each chapter. An ample number of references are listed at the end of each chapter for those interested in further pursuing a particular topic.

The reviewer believes that this book provides a significant advance over some previously available undergraduate texts in analytical pharmaceutical chemistry in that emphasis has been placed on basic theory and applications of specific methods rather than on official assay procedures in the official compendia. The text helps to give insights into the official United States Pharmacopoeia and British Pharmacopoeia assay methods, but only as examples of the basic methods which are described in length.

For individuals who have not been directly involved in analytical chemistry, this book can serve as an excellent review and provides the opportunity to assimilate new methodology which has been added to the field in recent years.

This text is recommended as a general review and reference text for those interested in updating their knowledge of analytical chemistry and analytical pharmaceutical chemistry.—PAUL THAU—CIBA Pharmaceutical Company.

OIL, DETERGENTS AND MAINTENANCE SPECIALTIES. VOLUME 1: MATERIALS AND PROCESSES, edited by Benjamin Levitt. Chemical Publishing Company, Inc., New York. 1967. 280 pages indexed. Price $13.75.

This is the first of two volumes dealing with all aspects of fats and oils including raw materials, manufacturing processes, test methods and applications. This volume deals primarily with materials and manufacturing processes, although many formulations are included. Volume II will deal with additional applications.

After a brief introduction, this book is divided into eight chapters: animal fats and oils; vegetable fats and oils; fatty acids and alcohols; surfactants; production of fats and oils; soap manufacture; synthetic detergents and analysis of oils and detergents.

Since all of the topics covered in these chapters have already been the subjects of full length volumes, the author could include only a small fraction of the information available on each topic. Unfortunately very few references are listed, and many of the classic texts on oils and fatty acids are not mentioned at all.

Yet, there is much useful information in this book, and it should be of value to individuals who are not directly involved with fats, oils and detergents but would like a handy reference work on these subjects.—T. KAUFMAN—Drew Chemical Corp.

MATHEMATICS AND STATISTICS FOR TECHNOLOGISTS, by H. G. Cuming and C. J. Anson. Chemical Publishing Company, Inc., New York, N. Y. 1967. 490 Pages, indexed and illustrated. Price $12.50.

This is a most unusual and valuable book for the industrial scientist, but it is not entirely what its title implies. A more appropriate name, which would describe over 75% of the contents (and, in my opinion, all of the more valuable material) might be, "A Concise Review of College Mathematics for Technologists."

The value of the book lies in the fact that most of the material (351 of the 490 pages) comprises a lucid and succinct presentation of nearly every important topic in college mathematics normally encountered by the undergraduate chemist or engineer, but aimed specifically toward the needs of the adult professional who may wish a relatively painless refresher course. I believe that any such person who has studied the topics covered, even several decades ago, will find the book easy to study as well as an excellent reference work. Despite the brevity of most of the topics, all are treated with clarity and vigor, although with no attempt to develop

complete proofs in every case. The subjects covered range from elementary algebra through analytic geometry, trigonometry, intermediate algebra, differential and integral calculus with applications, and differential equations. Each topic includes a good supply of examples, and each chapter ends with a group of problems for practice, with answers given at the back of the book.

There are a few short chapters on elementary statistics, comprising a total of 114 pages, including an introductory section and short chapters on probability, frequency distributions, control charts, sampling, significance tests, analysis of variance and simple linear regression. In sharp contrast to the above material, these give the impression of having been included as an afterthought. They are brief, incomplete and quite inexact. The adult reader would do better to ignore these chapters and, after covering the mathematical material, to consult some more expert text on applied statistics, such as Mandel's "The Statistical Analysis of Experimental Data" (Interscience, 1954).

A regrettable omission is the lack of a chapter on matrix algebra, a most important topic in modern applied statistics. Also, there is no material on solid analytical geometry. In spite of these omissions and the shortcomings of the statistical section, however, this text is most highly recommended to the audience for which it is intended.—W. M. WOODING—Carter-Wallace, Inc.

RESEARCH IN SURFACE FORCES. VOL. 2. Edited by B. V. Deryagin. Pp. viii × 320. 1966. Consultants Bureau, New York. $27.50.

This is a compilation of the papers presented at the Second Conference on Surface Forces which was sponsored by the Institute of Physical Chemistry of the Academy of Sciences of the USSR. Volume 1 contains the papers presented at the First Conference on Surface Forces which was held in March of 1960 and which celebrated the 25th anniversary of the Laboratory of Surface Phenomena of the Institute of Physical Chemistry.

Academician B. V. Deryagin, in addition to being the editor, is the author of many of the papers. His lead article points out that the emphasis in Volume 2 is on the three dimensional aspects of surface forces.

The volume is divided into five sections of about eight papers each: "Theoretical Problems in Surface Phenomena," "Electrosurface Forces," "Experimental Studies of the Properties of Thin Films," "Surface Phenomena in Dispersed and Porous Systems," and "Surface Phenomena in Adhesion and Friction." Over half of the papers are from Deryagin's laboratory at the Institute of Physical Chemistry: however, approximately ten additional laboratories in the USSR are represented.

A wide variety of problems in both fundamental and applied Surface Chemistry formed the subjects of the papers in this volume. This reviewer

found that there were papers in almost all of his specific current interests. Further, he felt that in most cases important, new information was contained in the papers. In spite of its high price, this must be considered a convenient source of very important literature in the field of Surface Chemistry.—ALFRED H. ELLISON—Gillette Research Institute.

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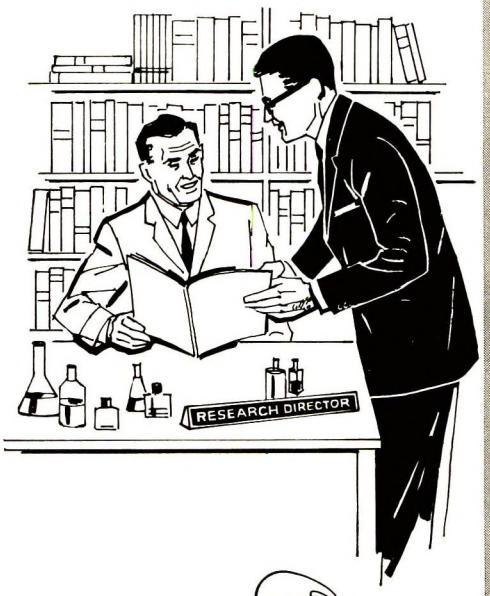

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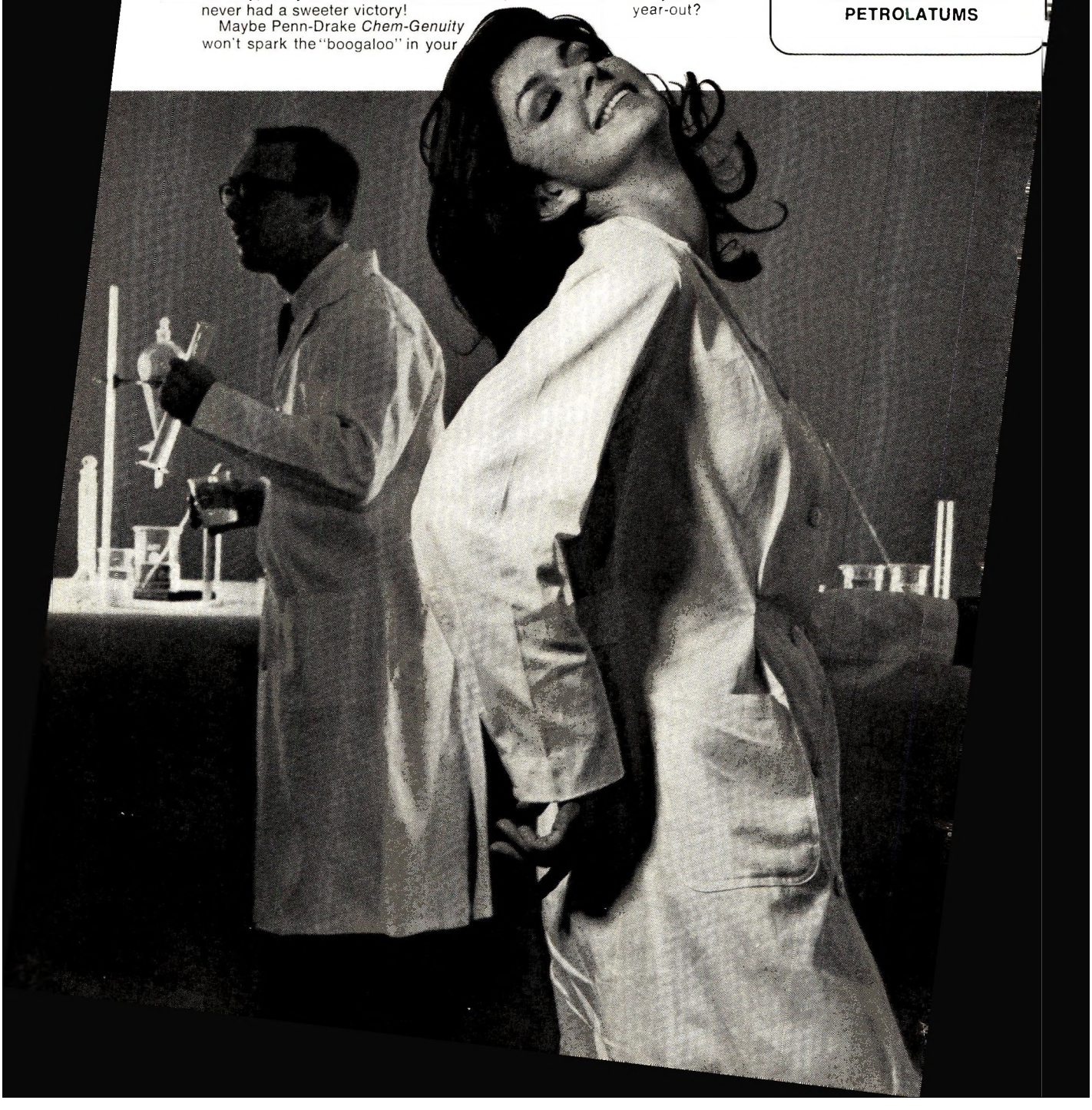

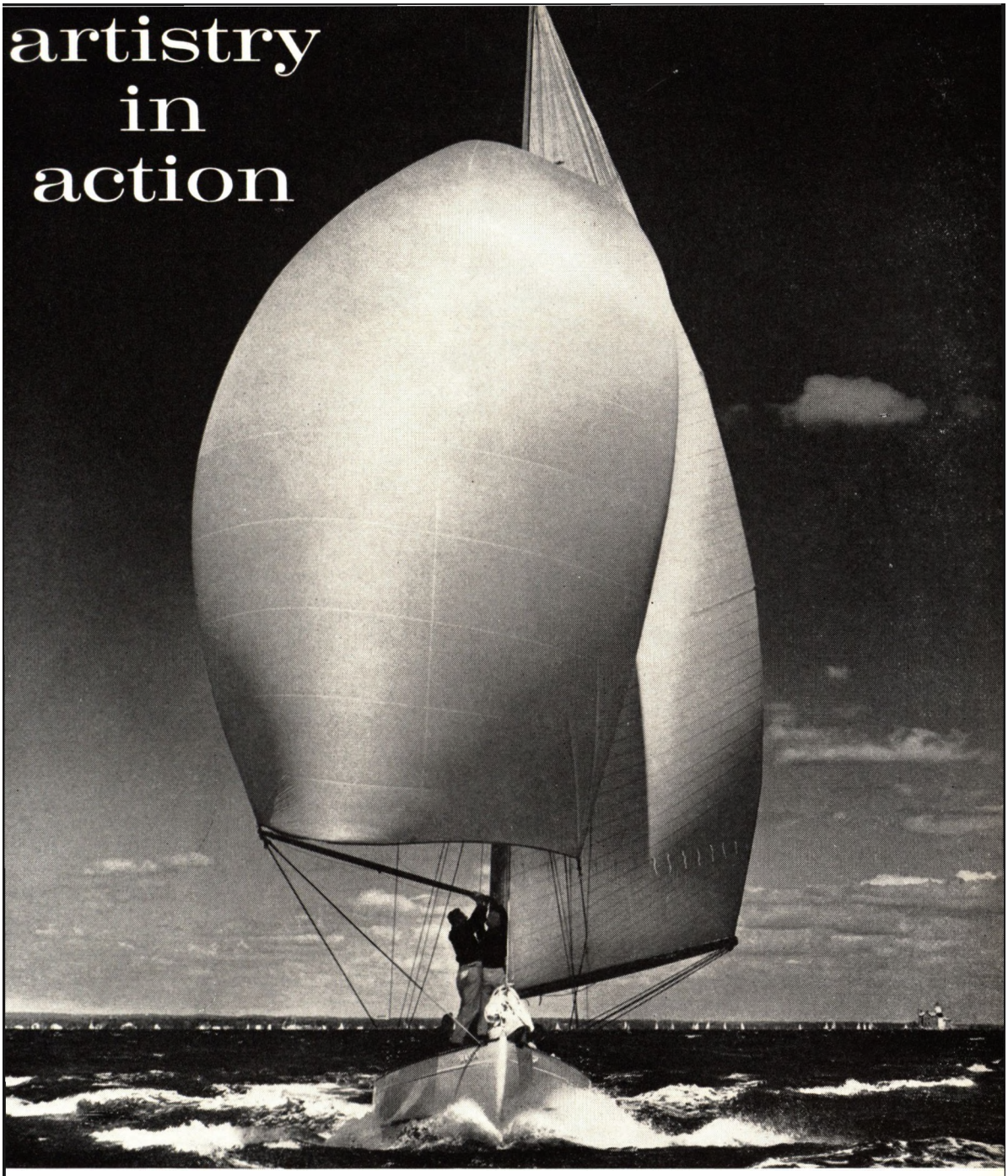

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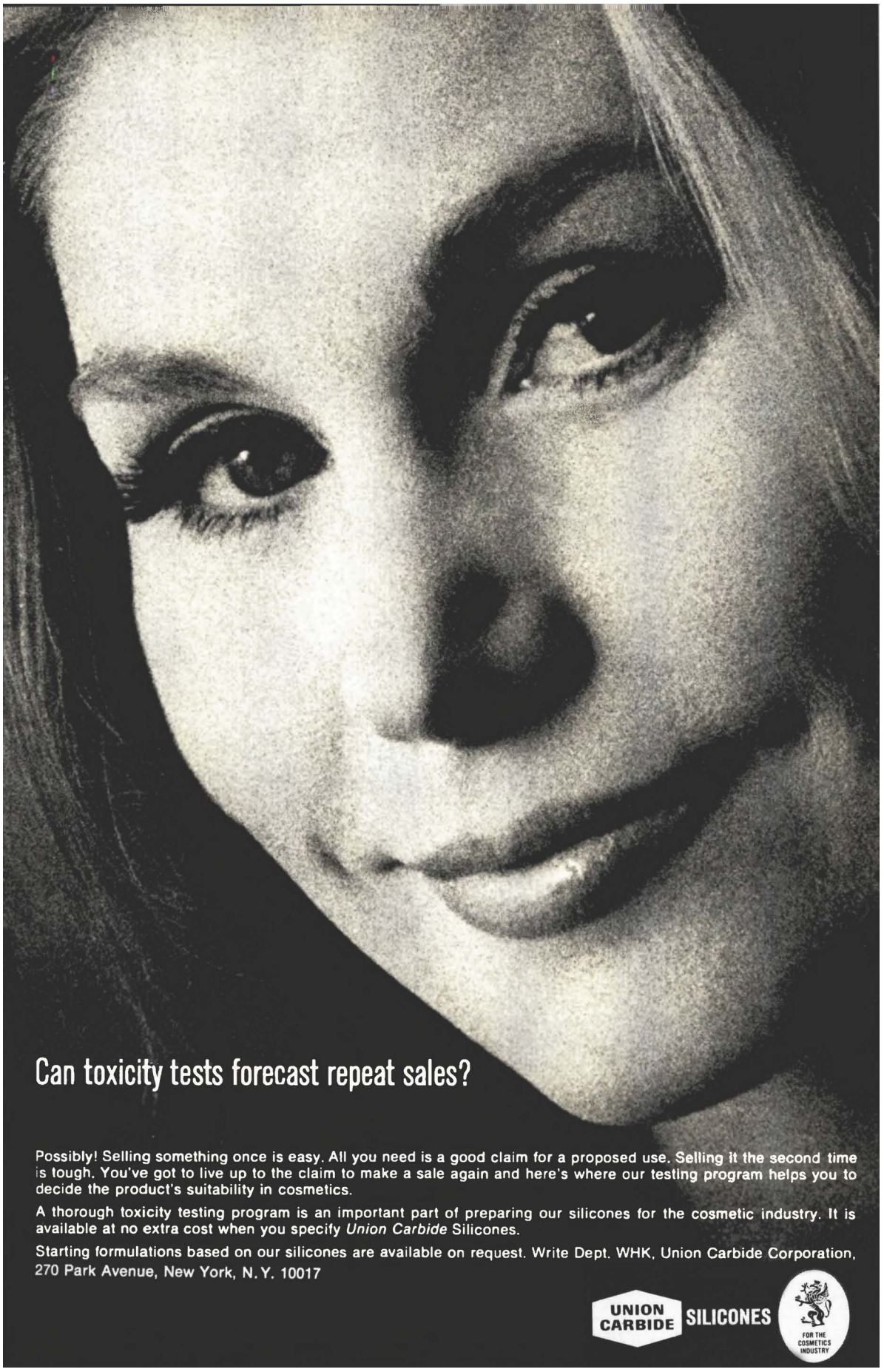

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