Vol. 27 No. 3 March 1976

Journal of the Society of Cosmetic Chemists

Contents

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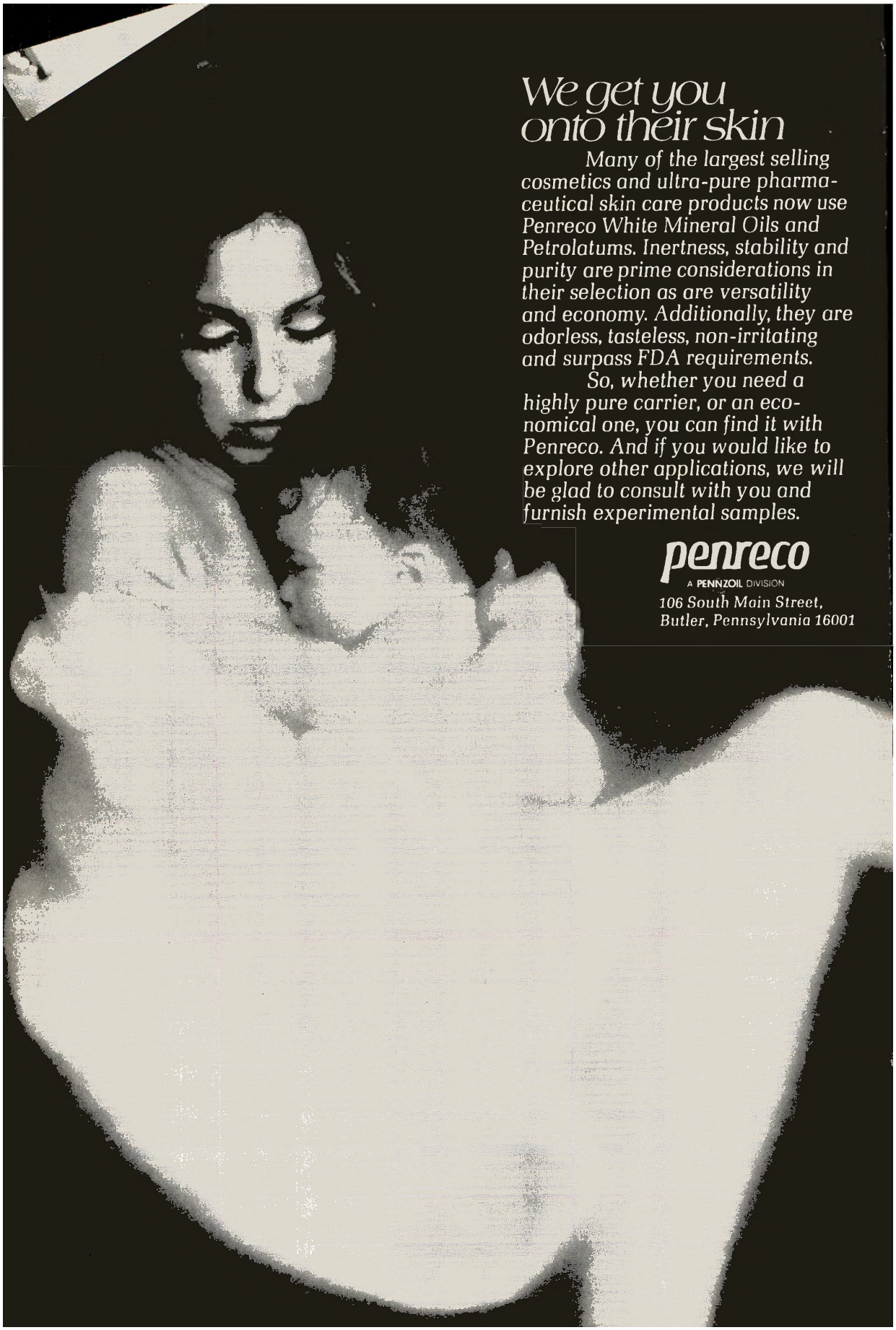

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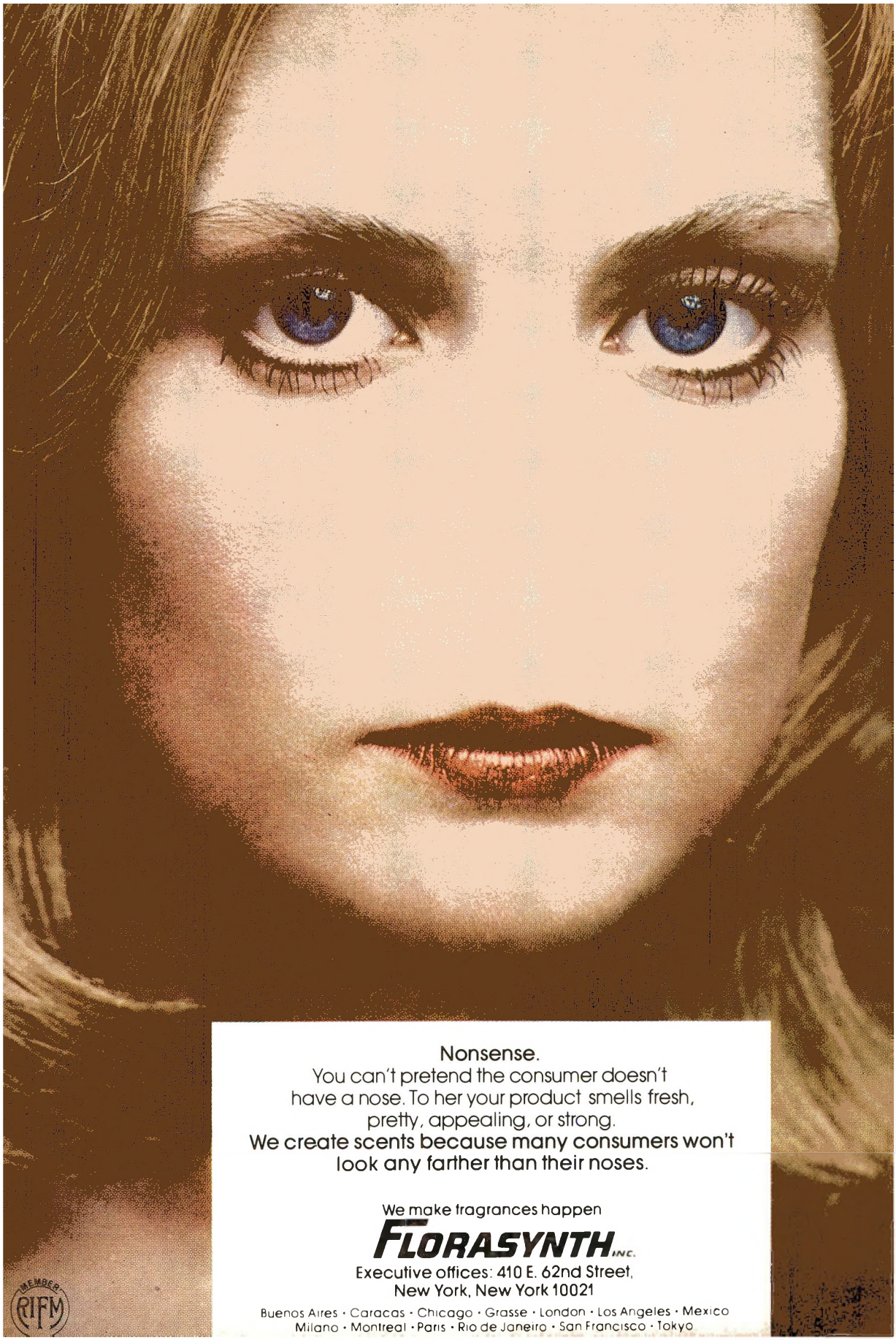

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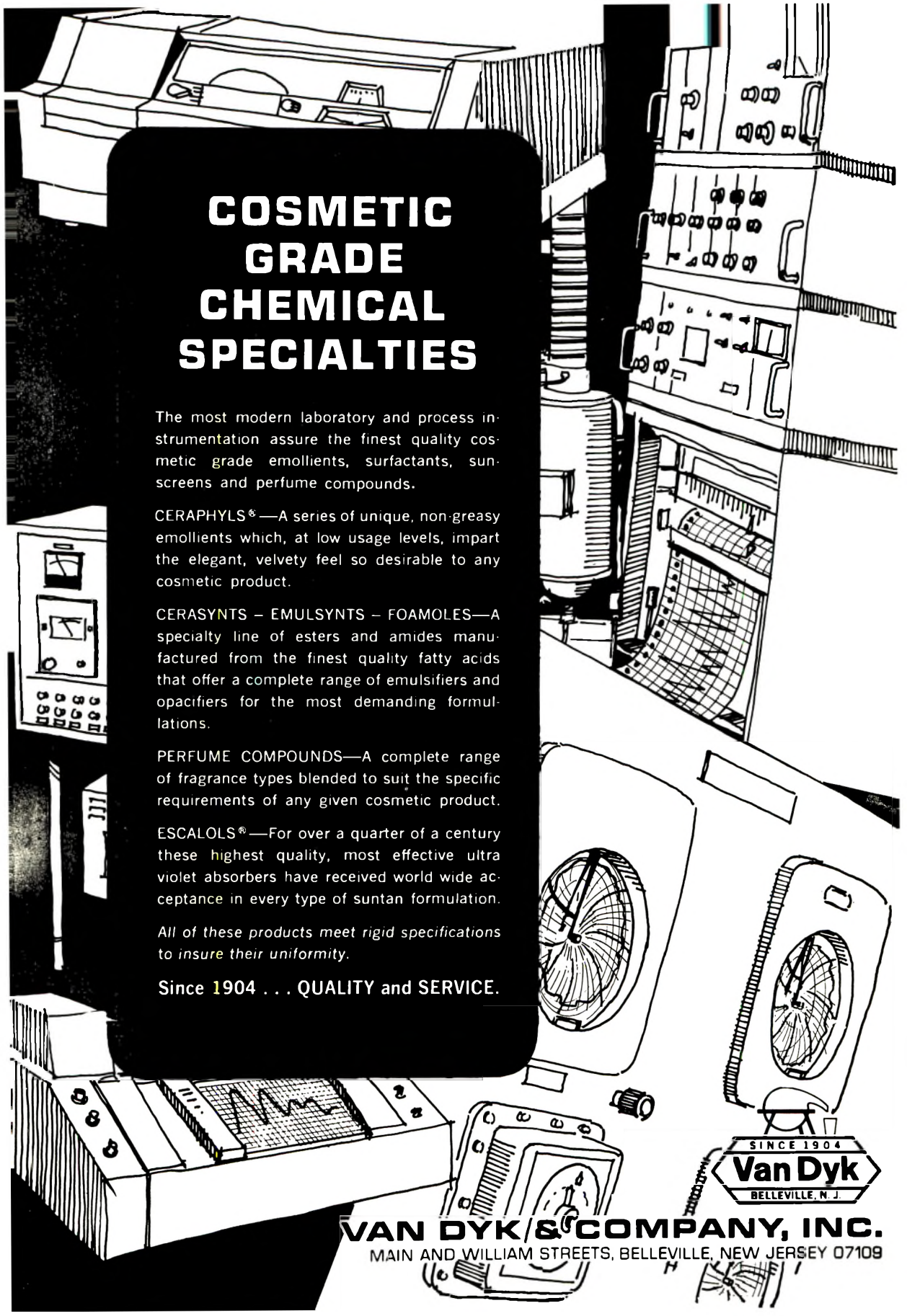

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SYNOPSES FOR CARD INDEXES

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

The nature of dandruff: Albert M. Kligman, Kenneth J. McGinley, and James J. Leyden. *Journal of the Society of Cosmetic Chemists* **27,** 111 (March 1976)

Synopsis—Dandruff is a fine example of the inverse square rule in experimental medicine: interest in a problem is inversely proportional to the square of its prevalence. Rare disorders evoke great interest; great minds and monies are applied to their study. Conversely, familiar things are scorned and neglected. Dandruff also illustrates an adverse effect of therapeutic advances: curiosity collapses when a means of control is developed. The power to subdue a disorder weakens interest in its inner qualities.

Two paths of investigation, which have long occupied our attention, namely, cutaneous microbiology and the horny layer, finally led us to the scalp. We have been serious students of dandruff for almost a decade.

It is our intent in this paper to tell what we have learned and to present an overview of dandruff in the light of our personal experience. We shall be less concerned here with raw data than with ideas of what dandruff is and how it behaves.

New insect repellent derivatives of N-disubstituted β-alanine: Manfred Kliev and Friedrich Kunlow. *Journal of the Society of Cosmetic Chemists* **27,** 141 (March 1976)

Synopsis—A number of derivatives of N-disubstituted β-alanine were synthesized. Among them, several N-alkyl esters of 3-(N-n-alkyl-N-acyl) aminopropionic acid and of 3-(N-n-alkyl-N-carbalkoxy) aminopropionic acid effectively repelled aedes aegypti mosquitos from human skin to an extent equal to that of N,N-d ethyltoluamide. An olfactometer, which permitted simple, efficient, and rapid evaluation, was developed for preliminary screening of repellency. The correlation between repellency of the synthesized compounds and their volatility and their molecular structure was studied. One compound, ethyl 3-(N-n-butyl-N-acetyl) aminopropionate, is particularly noteworthy; it exhibits high mosquito repellency, possesses extremely low toxicity toward warm blooded animals, and is ·vell tolerated by human skin.

J. Soc. Cosmet. Chem., **27**, 111-139 (March 1976)

The Nature of Dandruff

ALBERT M. KLIGMAN, M. D., Ph.D., KENNETH J. McGINLEY, and JAMES J. LEYDEN, M.D.*

Presented, December 1974, SCC Annual Meeting, New York, N.Y.

Synopsis: DANDRUFF is a fine example of the inverse square rule in experimental medicine: interest in a problem is inversely proportional to the square of its prevalence. Rare disorders evoke great interest; great minds and monies are applied to their study. Conversely, familiar things are scorned and neglected. Dandruff also illustrates an adverse effect of therapeutic advances: curiosity collapses when a means of control is developed. The power to subdue a disorder weakens interest in its inner qualities.

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It is our intent in this paper to tell what we have learned and to present an overview of dandruff in the light of our personal experience. We shall be less concerned here with raw data than with ideas of what dandruff is and how it behaves.

I. DEFINITION

Dandruff is excessive clinically noninflammatory scaling of the scalp. In the great majority of cases, diagnosis can be made almost instantly by simply inspecting and scratching the surface. Lesions elsewhere on the body call attention to disorders which also happen to involve the scalp, notably seborrheic dermatitis and psoriasis. Difficulties arise when the latter are chiefly confined to the scalp for inflammatory changes are masked in this location. Scalp skin is thick and redness and exudation may be hidden by layers of scale.

Scaling itself is a very nonspecific sign. Great experience is needed to recognize the special morphologic qualities of different dermatoses, *viz.*, the silvery scale of psoriasis or the greasy scale of seborrheic dermatitis. We always inquire about past and present skin disease.

Wrong diagnoses, while not common, are frequent enough to cause some mischief. It is disquieting how often we have mistaken another process for dandruff, most often seborrheic dermatitis in older subjects. Diagnostic er-

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rors have serious consequences when one is appraising efficacy in a small panel of "dandruff" subjects.

Quite recently, another disease, atopic dermatitis, has impressed us by its ability to mimic dandruff. We became aware of this source of error when puzzling over why "dandruff" patients failed to respond to highly effective antidandruff preparations. It turned out that these subjects had atopic dermatitis, often with a background of infantile eczema in association with a family and personal history of respiratory allergies. Since atopics are notoriously unable to tolerate anionic soaps and surfactants, failure to respond to antidandruff shampoos should immediately arouse the suspicion that the subject does not have dandruff. When we find that all but 1 of 10 subjects improve, we immediately review the case. Our belief is that dandruff is never completely resistant to such potent drugs as selenium sulfide (SeS) and zinc pyridinethione (ZPT).

II. Prevalence

For the most part, statements concerning the proportion of persons who have dandruff are merely "guesstimates." Systematic epidemiologic surveys are expensive and laborious; dandruff is, after all, not a health-threatening disorder. To be meaningful, a large unselected rigorously defined population would have to be examined. The frequency with which physicians encounter dandruff patients in their clinics or practices is no measure of prevalence. Neither is the size of the market for antidandruff products.

It is easy enough to describe dandruff as *excessive* scaling. Tension arises over the qualifying adjective: How much is excessive? Lacking objective measurements, we are driven to subjective estimates. The amount of scaling that will be regarded as bothersome will largely depend on social status, the rule being that concern increases directly with height up the ladder. What the ghetto resident would not notice might be a severe embarrassment to a bank president. A few flakes would mar the dignity of a funeral director while a blizzard of scales would likely be ignored by construction workers. The rich are more reactive than the poor to factors influencing self-image, the beautiful more than the plain, the sexy more than the neutral, etc.

We appraise dandruff by recourse to the ancient and honorable institution of the professional expert, best exemplified by the wine tasters. The powers of these artists exceed that of gas chromatography when it comes to identifying the type, year, and province where the wine was produced. There are virtually no disagreements among the gentleman of this admirable craft.

With dandruff, too, an individual with inborn sensitivity and meticulous habits can develop a high degree of reliability. Expert status is not conferred upon the novitiate until he has assessed many hundreds of scalps, preferably thousands. Competency can be objectively validated. The candidate grades

50 scalps, the face and body being concealed. The subjects are then scrambled and graded again. Concordance should be achieved in at least 45 of the 50 pairs.

More than a keen eye and conscientiousness are required. Strict rules must be followed if serious error is to be avoided. Scalp cleanliness is a compulsive concern of increasing numbers of people. Daily washing will banish all signs of scaling. Moreover, many persons regularly use antidandruff shampoos. The suppressive effects of the more potent ones last for a month or more. Hence, we make certain that active agents have been avoided for at least that period. Then, too, grading must be done at a fixed interval after last bland shampoo; we use 4 days, since this is the approximate "restoration time" in subjects with appreciable dandruff (see below).

We use a 0 to 10 grading scale (1). This is, of course, quite arbitrary and requires some explanation, since we virtually never assign grades greater than 7 or less than 1. The justification for this seemingly stretched out scale is to allow for the entire range of possibilities. For example, if dandruff subjects wear bathing caps and are prevented from washing, the grades will ascent to 9 or 10 within two weeks. Conversely, potent agents may be so effective that the grade falls to 0.

The descriptive equivalents of the various grades are as follows: Grade IV equals mild dandruff, Grade V equals moderate dandruff, and Grade VI equals severe dandruff. Grade III is slight scaling, not enough to warrant corrective efforts. Grade I and II are shady areas where scaling is miniscular and very difficult to judge. Here, we would emphasize that all scalps show some scaling even though this may be very slight.

Using this scoring system, we surveyed a prison population of 1,033 unselected young adult males, mainly in the 25 to 35 age group. About 18 per cent fell into grade IV (mild dandruff), while another 13 per cent were grade V (moderate dandruff). Grades VI and VII accounted for less than 5 per cent. Another 18 per cent were classified as Grade I. Grades II and III were the commonest, about 23 per cent each.

Bourne and Jacobs surveyed what might be regarded as a comparable group (7220 soldiers) and judged that only 2.5 per cent had "gross" dandruff (2). If the latter description conforms to our grade VI, there then exists good agreement that severe dandruff is uncommon. In our survey, less than one in 5 young adults had moderate dandruff (Grade V). Clearly, it is not easy to recruit good panels for antidandruff assays unless one is willing to settle for mild Grade IV dandruff. Cohen looked at 500 young women, and he thought that about 15 per cent had moderate dandruff, a figure close to ours (3). Van Abbe and Dean also emphasized that good dandruff subjects are hard to find (4). The subjective element in self-assessment is brought to light when we put out a call for dandruff subjects to participate in therapeautic studies. Few respondents have Grade V dandruff. Most are Grade III.

Scaling is over-rated in our population.

Women seem to have as much dandruff as men. Prevalence seems to be no different in blacks and whites. However, nothing is known about the frequency of dandruff among the various races of mankind nor of the influence of geography and climate. Similarly, we are quite ignorant about genetic influences. Heredity can certainly not be ruled out.

Although we have not conducted a formal survey of how dandruff varies throughout man's lifespan, we have formed some rather firm impressions. Young infants commonly experience a conspicious episode of scalp scaling. Large flakes are shed over a period of weeks followed by subsidence. "Cradle cap," as this condition is called, has been even less studied than dandruff. Nothing is known about it. We see no reason to call it dandruff or imply any relationship whatever. Our speculation is that it represents a wave of exfoliation of the thickened horny layer which protected the fetus in its watery intrauterine existence. Cradle cap disappears without "active" treatment.

Dandruff is decidely rare in children. Our maxim is that excessive scaling is a prepuberal child is atopic dermatitis until proved otherwise. Seborrheic dermatitis, on the other hand, can be very severe in infants. It is fortunately uncommon. It resembles the adult disease, but is not necessarily the same.

Dandruff begins to emerge at puberty and may be thought of as another cutaneous event that marks the onset of sexual maturation. The pubertal display includes expansion of the sebaceous glands (oiliness), appearance of public and axillary hair, apocrine sweating (axillary odor), deepened pigmentation (melanization), thickened skin, and other signs. All these changes reflect increased cutaneous activity. Presumably, this same enhancement applies to the physiologic function of desquamation; the production and shedding of cornified cells is accelerated. Starting quietly at puberty, dandruff intensifies gradually over the next few years, probably peaking in the late teens and early twenties.

It is a common belief that bad acne and severe dandruff go hand in hand, but no one has performed a comparative study that would validate such a view. The correlation, in any case, is not a strict one. The most one can say is that acne and dandruff are both quite common in late adolescence. An association between the two might be purely coincidental. We think that dandruff levels off in young adults, gradually declining toward middle age. It can be categorically stated that dandruff falls off sharply as old age is approached. We have had intensive experience in an institution for the aged and know with certainty that after 60, Grade V subjects are about one-third as common as in young adults. After 75, dandruff is very rare, while after 85, it practically vanishes altogether. This decremental age trend contrasts sharply with seborrheic dermatitis which increases after middle age and indeed becomes quite

common in the elderly, especially among males. Many of these are misdiagnosed as dandruff, a mistake we made for many years (see below). Fortunately, seborrheic dermatitis responds to the same agents that are effective in dandruff.

III. Seasonal Variations

One widely held belief, which has been sustained by formal epidemiologic investigation, is that dandruff declines in the summer months. The decrease actually begins in the late spring and bottoms out in the summer (1). The seasonal rhythm is easily perceived by comparing very low and high grades. In late spring, for example, Grade I accounts for about 25 per cent in a young, unselected population, while the comparative figure for winter is 10 per cent. Conversely, Grade VIs are more common in early winter than at any other time. These seasonal changes are not artifacts of altered visibility, which might occur from say increased sweating in summer time. Objective measurements of horny cell production also bear out the validity of the seasonal changes. Sales of antidandruff products are another indicator; these fall off in summer.

While biologists are keenly aware of circadian (daily) rhythms, too little attention has been paid to seasonal ones. Molting patterns are well-known in many vertebrates. In man, Orentreich has obtained evidence that physiologic shedding of telogen (club) hairs increases in the fall, causing unnecessary concern in women (5). We venture to say that these annual rhythms are innate and are not responses to exogenous events such as temperature changes.

In any event, the summer downturn in dandruff should be taken into account in therapeutic assays. The most rigorous time for testing is in the fall and winter. Weaker agents will tend to be over-rated when the trial begins in springtime.

IV. Relationship to Oiliness (Seborrhea)

Earlier writers created a distinction between an oily and a dry type of dandruff, respectively called pityriasis olesa and pityricis in the pedantic jargon of the day. It takes only a moments reflection to discern the absurdity of such designations. Scalps, of course, are either oily or not oily, regardless of the presence or absence of dandruff, whether their owners are blondes or brunettes, saints or scoundrels, etc. One is reminded of a charming saying among the Pennsylvania Dutch: "When the cock crows on the dung heap, the weather will either change or it will stay the same." The real question is whether there is a statistical association between oiliness and dandruff.

We undertook to settle the matter. The production of sebum was measured by having each subject dip his entire scalp for 2 min in a liter of ethyl ether 24 hours after defatting the scalp by a similar immersion. After filtering and

evaporation, the residual oil was weighed, giving total scalp sebum production for a 24-hour period. This was repeated after a rest of a few days and the values averaged. 18 subjects with Grade V dandruff were compared to 16 with Grades of II and III. The mean sebum production was 378.3 mg in the latter and 419.4 mg in dandruff subjects. The standard deviations were rather large but similar in both groups. The slightly higher sebum production in dandruff was not statistically significant ($p => 0.05$).

The effect of artificially decreasing sebum production was investigated in 6 young adult females with Grade V dandruff, who also complained of oiliness. Each took 0.1 mg of ethinyl estradiol from the fifth to the twenty-fifth day of each menstrual cycle for three cycles. This caused an average decrease in sebum production of 44 per cent (range 31 to 52). The subjects used a bland shampoo twice weekly during this time. Menstrual irregularities occurred, but were not considered pertinent to the issue. The clinical grades remained stable as did objective measurements of horny cell production (the corneocyte count). The subjects easily perceived that the scalps were no longer oily, but they agreed with our estimate that scaling remained at pretreatment level.

Another three females with marked oiliness and dandruff applied a 0.5 per cent solution of ethinyl estradiol in equal parts of ethanol and propylene glycol to their scalps once daily for 30 days. Corneocyte counts were not done. Oil production was so severely curtailed that two complained of excessive dryness. The dandruff seemed to have actually worsened in these two. We interpret this to mean that oil tends to conceal scales in the same way that any grease obliterates the look and feel of dry scaly skin after its application. Light is scattered more readily when the spaces between the scales are no longer filled with fat. Defatting the scalp with either one may greatly accentuate the appearance of scaliness, even in nondandruff subjects. Thoroughly wetting the scalp with water also transiently decreases the visibility of scales.

In any case, scaling and oiliness are independent of each other. Dandruff is not affected when female hormones are given to decrease the volume of the sebaceous glands. Estrogens, it may be noted, apparently do not affect the proliferative activity of the epidermis.

V. Assessing the Degree of Dandruff: Measurement of Epidermal Kinetics

Cells are continually being shed from the surface. The source of new cells is the germinative layer, the bottom most row of cells directly over the dermis. These cells comprise the reproductive compartment and undergo random mitotic divisions. Some cells in the row immediately above the basal layer also have the capacity to divide. The daughter cells of cell division are forced

out of the basal zone and in their upward passage undergo a complex series of transformations designated by the general term, 'differentiation' and by the dermatologic term, 'keratinization.' As each cell becomes larger, tonofilaments (bundles of the fibrous protein keratin) increase in quantity and thickness, new organelles appear, the cell membranes thicken, and the cells become increasingly stuck to one another. All these and many other changes, which take place in the differentiating compartment, have a specific biologic goal, the production of horny cells. The latter comprise the fabric of the stratum corneum (SC).

Horny cells or corneocytes are dead rigid cells, literally bags of fibrous protein encased in membranes so tough that strong alkalis dissolve out the cellular contents leaving empty sacs. The horny cells are held together by a strong intercellular glue, forming thereby a coherent horny layer The function of the latter is to act as a "barrier" to prevent the passage of substances into or out of the skin.

The SC seals off the body from the environment. Near the surface, the SC begins to crack. This is the desquamating zone, where the cells become loosened in preparation for their being shed. This outer loose porous zone is only three to four cell layers thick.

It is important to understand that horny cells do not come off individually, but in variably sized aggregates, comprising tens and even hundreds of cells. These clumps are, for the most part, invisible, being less than 200 μm (0.2 mm) in diameter. The individual horny cells are about 40 μm in diameter. Aggregates larger than about 0.2 mm are visible to the naked eye as flakes or squames. Depending on the thickness, flakes as large as 2 mm may contain thousands of horny cells. Very large flakes may contain hundreds of thousands of cells. Squames are, of course, the hallmark of dandruff; they become more numerous and larger with increasing severity. We shall have to be concerned with their origin.

One of the first questions which arises is whether dandruff subjects produce a greater quantity of horny cells. Or are corneocytes merely being shed in visible flakes? Some measurement of epidermal proliferative activity is required. Specialists concerned with epidermopoesis have developed various techniques for estimating the rate at which the epidermis renews itself. One can determine the average time for a cell to move from the basal layer to the surface (the transit time). Indeed, it is possible to calculate separately renewal times for the dead horny layer and the viable epidermis. None of these measurements have been made for the scalp. Still, we have generated some data which enables a meaningful comparison of epidermal kinetics in persons with and without dandruff. In the studies summarized below, the subjects were always young adult males.

The mitotic index, the percentage of basal cells in mitosis, affords an estimate of how rapidly the cells in the germinative compartment are reproduc-

Table I
Mitoses Per Thousand Basal Cells

Subjects	Nondandruff Grades II and III		Subjects	Dandruff Grades V and VI	
14	Mean	14.6	18	Mean	30.6
	SD[a]	±4.9		SD[a]	±7.9

[a]Standard deviation.

Table II
Labeling Index
(Number of Labeled Cells per 100 Basal Cells)

Subjects	Nondandruff Grades II and III		Subjects	Dandruff Grades V and VI	
16	Mean	11.0	19	Mean	17.5
	SD	±2.4		SD	±4.4

ing themselves. Because it is difficult to identify cells in early and late mitosis, this determination is rather imprecise. Since only a small percentage of basal cells are dividing at a given time, one must scan thousands of cells to keep the experimental error within bounds.

Comparisons were made between 14 nondandruff subjects (Grade II and III) and 18 dandruff subjects (14 Grade V and 4 Grade VI). The results are shown in Table I. Even though the values were distributed over a wide range, the mitotic index was about twice as great in the dandruff group ($p = <0.01$). This result indicates that proliferative activity is increased in dandruff. This was the first evidence we secured that more cells were being shed from dandruff scalps. It is worth pointing out here that cell turnover on the head region is normally much swifter than on the glabrous skin (6).

Another means of estimating cell turnover is to "tag" cells in the reproductive compartment with tritiated thymidine. Those cells which are in the DNA-systhesis phase of the cell cycle will incorporate the radioactive nucleotide into their nuclei. After suitable histologic processing, one can determine the percentage of radio-labeled basal cells (the labeling index). The technique entails a 0.1-ml intradermal injection of 5 to 10 microcuries of tritiated thymidine followed by excision biopsy 45 min later.

The number of labeled cells per hundred basal cells was determined in 16 subjects without dandruff (Grades II and III) and 19 subjects with Grades V and VI. The results are shown in Table II.

Again, the range was great within each group. There can be little doubt, however, that the proportion of DNA-synthesizing cells was about twice as large in dandruff, indicating much faster cell renewal.

The rate at which labeled cells move through the epidermis in their journey to the surface (transit time) is another indicator of proliferative activity. To follow cell movement, one delays excising the injected area for a variable number of days. One can then see how far up the tagged cells have migrated

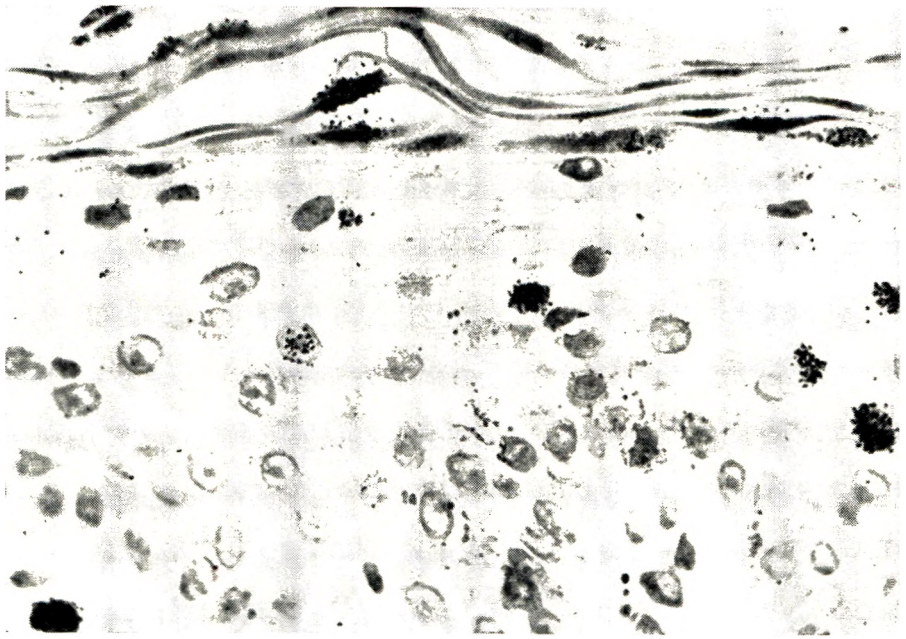

Figure 1. Dandruff, 5 days after injection of tritiated thymidine (630 x); cells with labeled nuclei have already reached top of epidermis

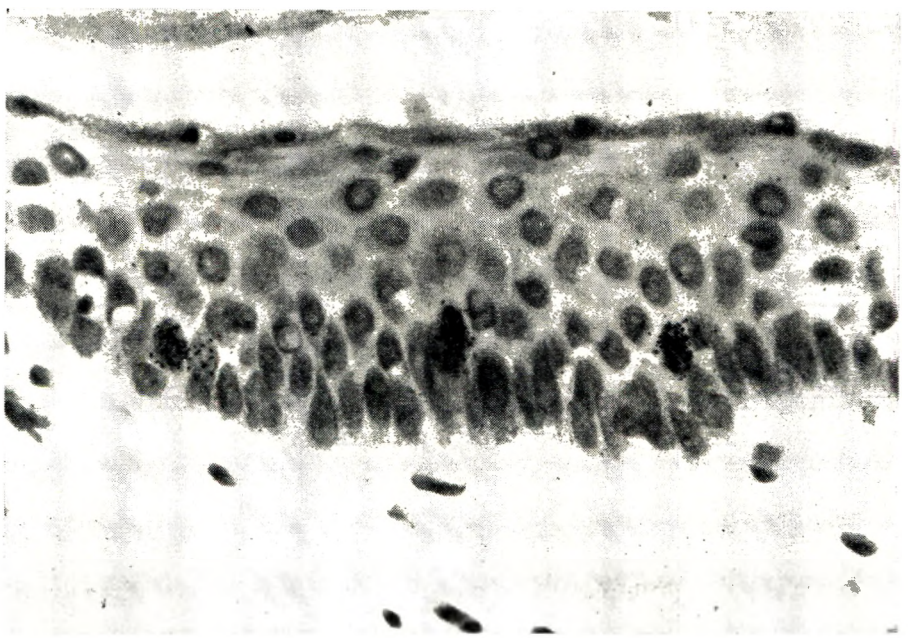

Figure 2. Nondandruff, 5 days after injection of tritiated thymidine (630 x); labeled cells are fewer than in dandruff and have migrated only to about mid-epidermis

until the label is lost at the base of the SC due to dissolution of the nucleus. The method is crude, because labeled cells leave the basal layer at different times, and so certain ones may be near the top, while others have scarcely begun to move.

Biopsies were taken from 8 dandruff subjects and 7 without dandruff 4 to 6 days after injection of tritiated thymidine (Figs. 1 and 2). In every instance, labeled cells migrated more rapidly through the epidermis in dandruff. In the dandruff subjects, after five days, many labeled cells had reached the granular layer just beneath the SC. After seven days, there were far fewer labeled cells, indicating that many had moved into the horny layer. The viable epidermis in dandruff, therefore, turns over in about a week or so.

VI. The Corneocyte Count

The foregoing studies indicate a faster generation of epidermal dandruff. This means, of course, that more horny cells are being shed. A useful way of demonstrating this is the corneocyte count, a procedure which we originally devised to quantify the cutaneous microflora (7).

The sampling technique removes corneocytes in the desquamating loose outer zone. This is accomplished by placing a 3.8 sq cm glass cup on the clipped scalp. One ml of a buffered nonionic detergent is placed in the cup, and the surface rubbed vigorously with a teflon rod for 1 min. This is repeated once more. The samples are pooled and then mechanically agitated to disperse the cells. A few drops of crystal violet are added, and the cells are counted in a Fuchs-Rosenthal hemocytometer.

The corneocyte count is always performed 4 days after a bland shampoo and is expressed as the quantity of horny cells per square centimeter per 4 days. On the scalp, horny cell aggregates tend to be retained, shielded by the hairs. The count then is an indirect measurement of cell turnover. In the same individual, the counts are remarkably repeatable time after time, whether or not the subject has dandruff.

From what has been said, one would expect a positive correlation between clinical grades and corneocyte counts. When the regression line for 296 pairs of observations was plotted, the grades and counts did indeed parallel each other, but the correlation was weak, just achieving statistical significance (r equals 0.415) (1). This seemed a bit odd, but the reason soon came to light. A few individuals with Grades IV and V (mild and moderate dandruff) had unexpectedly low counts, while conversely, some Grade II and III scalps had counts which were more characteristic of the dandruff group. These unusual individuals accounted for the uncomfortably large standard deviations around the mean. As a rule, however, Grade V subjects had counts exceeding 800,000 cells per sq cm while values of less than 500,000 per sq cm were typical of nondandruff subjects (Fig. 3). One cannot decide on the basis of

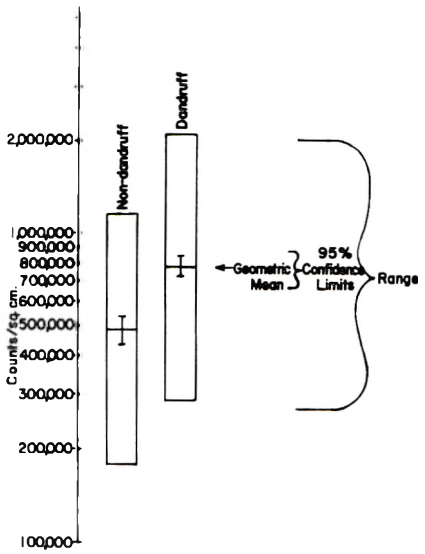

Figure 3. Quantitative determination of number of desquamating cells (corneocyte count) in 112 nondandruff and 126 dandruff subjects

the count alone whether a particular individual has dandruff. The individual variations are too great.

On the other hand, the experienced observer has no uncertainty deciding whether a person has dandruff clinically. The eye, of course, sees flakes or squames, not the small aggregates of horny cells, which the count "sees." The count reflects the quantity of horny cells, while the eye perceives only large aggregates. So, it sometimes happens that a person, whose count is low, might seem to have dandruff because his squames are conspicuous, even though not very numerous. On the other hand, some persons actually have an increased production of horny cells, but do not appear to have dandruff because the cells are shed in small aggregates. We may cite here a particularly convincing demonstration of the latter situation (high count, low grade). When certain chemical irritants, notably anionic detergents, were repeatedly applied to normal scalps, the counts rose sharply, often reaching levels twice that of severe dandruff. Except for an initial brief flurry of scaling, these artificially stimulated scalps did not show clinical signs of dandruff. An increase in the corneocyte count is a reliable sign of scalp irritation, especially valuable since inflammatory changes may be subclinical.

With this intelligence, one can imagine a perverse way to treat dandruff, namely, by chemically damaging the scalp. The method is impractical, but the principle is sound. When we applied 10 per cent sodium lauryl sulfate solutions daily to dandruff scalps, scaling increased markedly for the first week

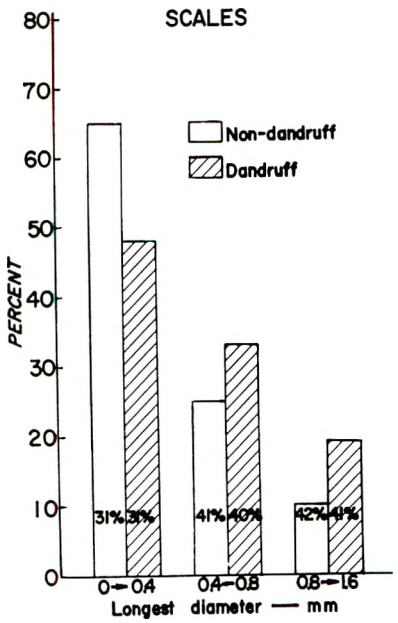

Figure 4. Distribution of squames by size. Dandruff flakes tend to be larger than non-dandruff, but overlap is great. Numbers within columns designate percentage of nucleated cells. Larger squames tend to contain more nucleated cells

or so accompanied by signs and symptoms of irritation. By another 2 to 3 weeks, however, few flakes were to be seen, though the counts generally exceeded 2,000,000 per sq cm. The individuals no longer had dandruff!

Dandruff, it should be clear by now, is the product of two processes: increased production of horny cells and increased production of the squames. These generally go together. We thought it worth while to compare the size of the squames in subjects with and without dandruff using a fine comb to collect scales. More flakes, of course, were obtained from dandruff scalps. The scales were separated into 3 size classes by their longest diameters, 0.2 to 0.4 mm, 0.4 to 0.8 mm, and 0.8 to 1.6 mm. The differences were not as great as we though they could be (Fig. 4). Flakes from dandruff scalps tended to be larger, but the overlap was great. About 20 per cent of dandruff flakes fell into the largest size class compared to about 10 per cent for Grades I and II. The smallest scales, made up about 25 per cent of the total in nondandruff subjects and less than 50 per cent in dandruff subjects.

Clearly, flakes are produced by all scalps, even those with very low grades of dandruff. Surprisingly, the latter may be just as large as in dandruff. As we shall see, they are similar in all other respects.

VII. The Course of Dandruff

We had the opportunity to follow institutionalized dandruff subjects including young adults and the elderly for a year or more. The scalps were assessed both by clinical grades and by corneocyte counts.

Apart from the already mentioned seasonal variations, we have come to the conclusion that dandruff is a rather stable process. Counts and grades vary little over many months. The extraordinary week to week oscillations in the severity of dandruff reported by Van Abbe and Dean are outside our ken (4). We regard scaling to be about as steady a process as sebum excretion or hair growth rates, although more easily modified by external factors.

As we see it, once an individual develops dandruff he will have to live with it till old age slows down epidermal proliferative activity. Dandruff does not come and go, folklore notwithstanding. The saying, "getting ones dander up" applies not to dandruff, but to seborrheic dermatitis, an unrelated condition.

It should be pointed out that the corneocyte counts are likewise quite stable. It would appear that the rate of production of horny cells and scales is a rather fixed characteristic as typical of an individual as his complexion or body odor.

VIII. Is Dandruff a Disease?

A disease exists when there are structural or behavioral changes, which are qualitatively different from the normal, a distinction that is not always easy to make. The finding of tissue pathology, gross or microscopic is decisive.

As we now conceive the process, dandruff is not a disease in the visual sense. No feature can be found which is absent in "normals." The differences are purely quantitative. People with dandruff make more horny cells and more scales. Desquamation is a physiologic process; individuals are distributed along a continuum depending on rates of shedding. There can be no sharp dividing line between dandruff and nondandruff subjects. As with all physiologic processes, the population can be fitted into the normal bell shaped curve. Using corneocyte counts, we have shown that the rates of horny cell production are log normally distributed, that is, a bell shaped curve is obtained when frequency is plotted against the log of the corneocyte count. Accordingly, the averages are expressed as geometric means rather than arithmetically. For statistical analysis, the values must first be transformed into logs.

People with dandruff are located to one side of the bell shaped curve. The proper terminology is not dandruff versus "normal," but dandruff versus nondandruff. Where the line is drawn is, of course, quite arbitrary.

Since dandruff is only an intensified state of desquamation and scaling, it

is altogether inept to speak of "cures." Dandruff can be suppressed but how can a physiologic process be cured (except by death!).

Virtually, nothing is known of the mechanisms, which regulate the rate of epidermal proliferation. Current theories focus on chalones (inhibitors of cell multiplication) and cyclic AMP. Turnover is increased when the concentration of the latter is low. No measurements of these regulators have been made on the scalp.

We have cursorily looked into the question of whether or not dandruff subjects have increased epidermal turnover in other body areas, the trunk for example. Dandruff subjects give no clinical evidence of increased shedding such as fine scaling, dryness, or roughness. Bearded subjects did not have dandruff in the beard area. The labeling index was determined on the back and thighs of 4 subjects with severe dandruff. The proportion of DNA synthesizing cells was within the normal range. It is probable that increased desquamation is limited to the scalp.

Finally, it seems unlikely that an animal model of dandruff can be experimentally created. Definitions are all important here. The appearance of dandruff is easily mimicked. Scaling is a nonspecific response to injury. Chemical and physical trauma will provoke increased cell turnover and desquamation of horny cells in flakes, a dandruff-like condition. Histologically, however, one sees the stigma of tissue injury: inflammatory cells and thickening of the epidermis. In Troller's guinea pig model, the scaling, which followed inoculation of *Pityrosporum* ovale onto skin, anointed with artificial sebum was, in our view, no more than an irritant response to substances liberated from the fatty mixture (8). Rubbing 10 per cent lauric acid in mineral oil daily over the flank of a guinea pig will also induce scaling. Histologic examination immediately shatters the idea that the process is equivalent to dandruff. The tell-tale signs of skin injury are vividly displayed in the microscope.

On the other hand, we know of no reason why dandruff should not occur spontaneously in furry animals. Many pet owners think that dogs have dandruff!

IX. Is Dandruff a Mild Form of Seborrheic Dermatitis?

The statement that dandruff is grade one-half seborrheic dermatitis is trenchant but untrue. These conditions have little in common save scaling. Seborrheic dermatitis is an inflammatory process with a recognizable histologic pattern. As a rule, seborrheic dermatitis will generally show its typical greasy scales on the face and so can easily be recognized. Moreover, unlike dandruff, it is a fluctuating process often being aggravated by emotional stress.

There is a widespread, but nonetheless false belief, that dandruff is patchy, involving some portions of the scalp more than others. This idea has resulted

in the practice of dividing the scalp into a number of segments, with each one receiving a separate grade. One scheme calls for separate evaluations of 25 areas, a 20 to 30 min process (4)! Some delineate 9 areas while the "short" method utilizes 4.

We compared the corneocyte counts on opposite sides of the scalp in 140 individuals with and without dandruff. The correlation between the two sides was exceptionally high ($r = 0.843$). A phenomenally straight line was produced when the right and left sides were plotted against each other. There was no evidence of asymmetry. Also, by clinical grading, dandruff seems to us to be a uniformly diffused (not patchy) process. Seborrheic dermatitis, on the other hand, presents itself in the form of circumscribed lesions with indistinct borders, just as it does on the skin. It, not dandruff, is patchy.

Microorganisms recognize the difference between dandruff and seborrheic dermatitis. While small numbers of S. *aureus* occassionally can be recovered from dandruff, this potentially virulent organism can be isolated in large numbers from 20 per cent of the subjects with seborrheic dermatitis (9). S. *aureus* may comprise as much as one-third of the total flora.

Finally, dandruff wanes with age, while seborrheic dermatitis increases.

X. Histopathology

No aspect of our study has taken more twists and turns than the views we have held at different times concerning the microscopic changes in dandruff. It will be instructive to review the origins of this confusion.

We were satisfied after studying about 50 dandruff specimens that we had a good grasp of the pathologic changes (10). We had examined many normal scalps in the past, and thought we knew the terrain, but our attention then was on the hair follicle, not the epidermis. Naturally, it is quite easy not to see when one is not looking. For a while, we thought that there was in dandruff an increased perivascular infiltrate of mononuclear cells in the upper dermis, a kind of smouldering low grade chronic inflammatory reaction. The idea appealed to us, since at that time, we believed that dandruff was merely mild seborrheic dermatitis. Experts in cutaneous histopathology, who looked at these specimens, agreed with our interpretation, but, of course, they too had no real experience with the anatomy of normal scalps. It is not until one has studied scores of so-called normal scalps that one begins to appreciate the extraordinary individual variations. Many of these show round cells infiltrates to a similar degree. We came to realize that the cuff of adventitial cells, which normally surround small vessels, was greater over the head. The microvasculature of the scalp is very richly developed, and mononuclear cells seem to emigrate into the tissues more readily, a sort of slow extravascular circulation. We made the same mistake in studying acne of the face, where, again, normally there are more mononuclear cells patrolling the tissue, creating a specious appearance of low-grade inflammation.

On the scalp, the undersurface of the normal epidermis is very strongly sculptured. The dermal papillae deeply dent the epidermis so that in cross-section the dermo-epidermal contour is highly undulating with long rete pegs (Fig. 5). Sometimes the appearance is psoriasiform. Initially, we thought that the epidermis in dandruff was thicker (acanthotic). This, too, turned out to be a variable feature and not at all characteristic of dandruff. An important factor in this regard is age. Deeply projecting papillae with long rete pegs are very characteristic of youth. After middle age, the dermo-epidermal contour progressively flattens and the thickness of the epidermis decreases. In old age, the dermo-epidermal junction is flat rather than wavy, and the epidermis may become very thin. These changes, along with decreased cell turnover, explain the disappearance of dandruff in the aged.

It is appropriate, here, to provide objective data on the extent to which age influences the rate of production of horny cells. Using corneocyte counts, we compared elderly subjects with and without dandruff to young adults. By clinical criteria, dandruff was equally severe in both groups (Grade V). It turned out, however, that the counts were appreciably lower for the elderly (Table III). Within each age group, the dandruff subjects had higher counts, the difference being especially prominent in the aged. This is another illustration of lack of a strict correlation between counts and grades. Apparently, the elderly can produce just as many scales even though cell turnover is decreased.

Since scaling is the central feature of dandruff, one might think this would be a dominant finding in histologic sections. Unfortunately, fixation and processing literally ruins the horny layer. Judgments of thickness and quality of the SC are very hazardous. Quite often, it is simply knocked off in sectioning —leaving only fragments.

Originally, we thought that there was a diagnostic histologic feature in dandruff. We said, "the hallmark of dandruff is scattered foci of parakeratosis" (10) (parakeratotic horny cells retain their nuclei). Small mounds of parakeratotic horny cells are to be sureco mmon in dandruff, but identical findings are present in nondandruff. Again, the difference is merely quantitative. To find them in nondandruff may require examination of many sections. They become increasingly sparse as the grades diminish from III to I.

The reader has no doubt begun to sense that the parakeratotic foci are, in fact, the visible flakes or squames, which are universal in all scalps. Like the corneocyte count, their quantity is proportional to the clinical grade. Parakeratosis invariably signals increased prolferative activity and is usually a consequence of underlying inflammatory change.

Parakeratosis is typical of such inflammatory dermatoses such as seborrheic dermatitis and psoriasis in which cell turnover is sharply accelerated. In our previous work, we were mystified as to how these parakeratotic mounds formed in dandruff. They often seemed to be sitting over a normal epidermis

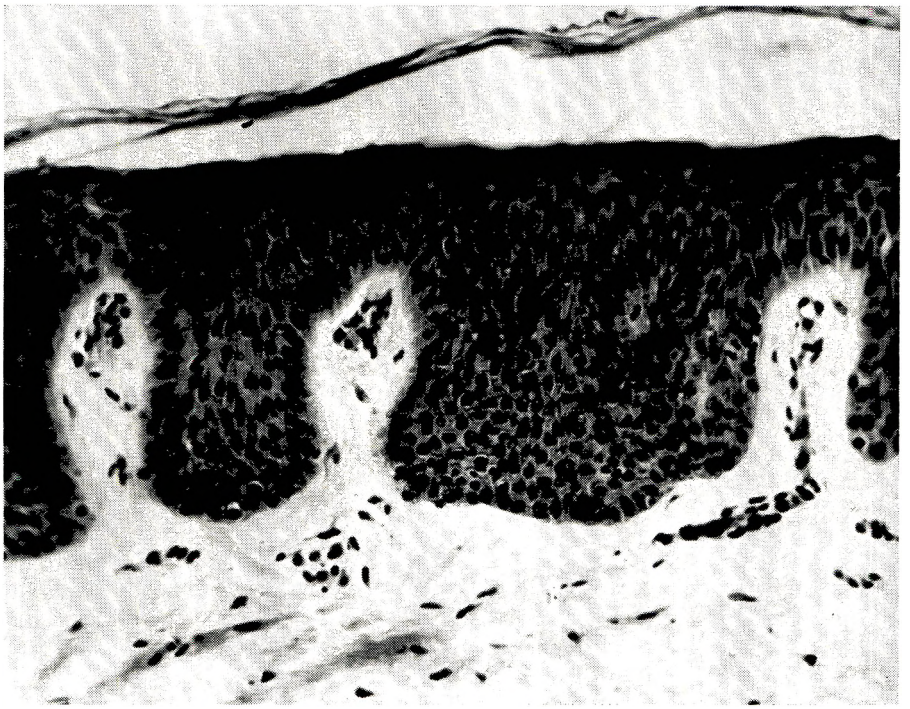

Figure 5. Normal scalp of young adult (H&E, 265 x). Papillae project deeply into the epidermis. Rete ridges are long. Capillaries within papillae drain into subepidermal plexus of venules (partly shown on papilla on right). Thickness of epidermis and waviness of the dermo-epidermal line vary greatly from specimen to specimen

Table III
Influence of Age on the Corneocyte Count

Nondandruff				Dandruff			
Subjects		Corneocytes (/cm²)		Subjects		Corneocytes (/cm²)	
Number	81	GM[a]	520 800	Number	91	GM	873 000
Mean age	28	SD	0.22	Mean age	29	SD	0.22
(Range 21 to	46)			(Range 21 to	48)		
Number	45	GM	367 100	Number	21	GM	636 800
Mean age	63	SD	0.20	Mean age	62	SD	0.17
(Range 51 to	79)			(Range 53 to	72)		

[a]Geometric Mean.

and dermis. This was a faulty observation, which we now hasten to rectify. By studying serial sections of dandruff specimens, one can discern the sequence of events. The parakeratotic scale is the second stage of a transient episode of

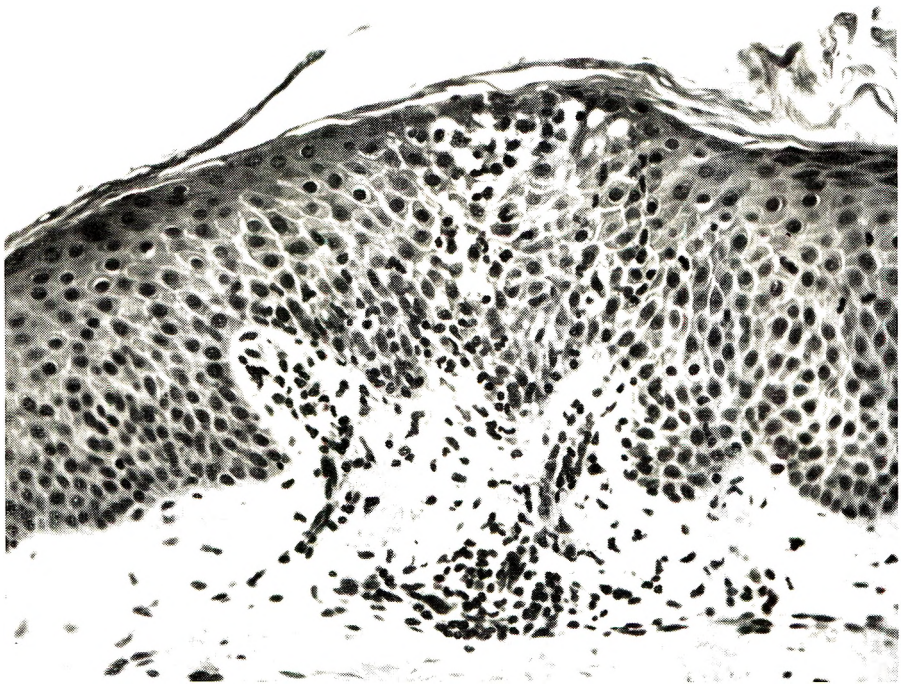

Figure 6. Spongiotic microvesicle (H&E, 260 x). Capillary has "squirted" serum and inflammatory cells into epidermis. Swollen and separated epidermal cells are beginning to degenerate. Infiltrate of mononuclear cells surrounds venule below

focal inflammation. The first change takes place in the papilla, usually involving two or three adjacent ones. The capillary dilates and a mixture of neutrophiles and mononuclear emigrate into the papillary space (Fig. 6). The venule draining this small focus of inflammation shows an increased perivascular infiltrate of round cells. The inflammatory cells leaving the capillary migrate into the epidermis, inducing intercellular edema. The spaces between the cells become wider, an appearance termed spongiosis. Sometimes the cells become completely detached and undergo necrosis forming a small microvesicle (Fig. 6). The granular layer quickly disappears in the affected zone and is replaced by parakeratotic cells. Initially these are infiltrated with polymorphonuclear leukocytes (Fig. 7). Soon after, the inflammation abates and inflammatory cells no longer wander into the epidermis. The epidermis recovers and starts to form a normal horny layer. At this stage, the parakeratotic segment forms a thin cap over the regenerating horny layer before disappearpearing altogether (Fig. 8).

This "pulse" or burst of inflammatory activity is nor peculiar to dandruff. It was beautifully described by Civatte in his classic studies of psoriasis (11).

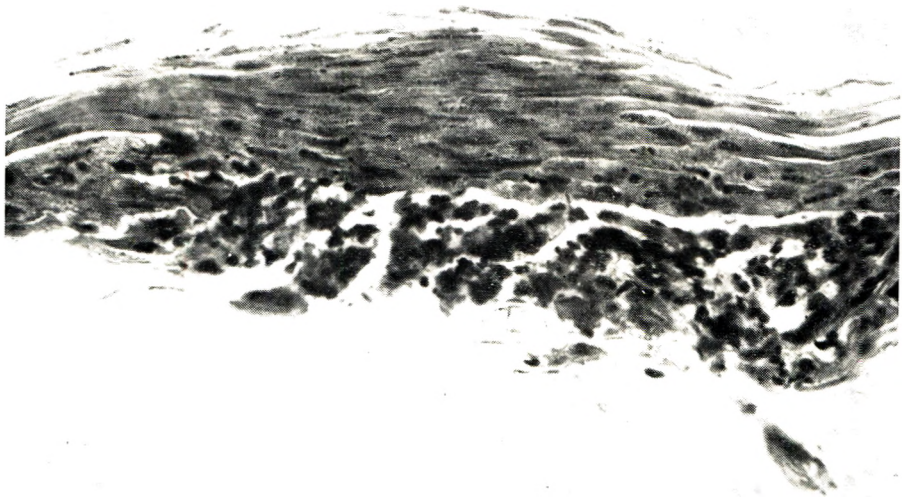

Figure 7. A parakeratotic scale (H&E, 620 x). Squame has many nucleated cells. Large number of neutrophils has gathered below. Squames contain variable amount of dried up exudate, a crust

Following the eloquent style of the French, he epitomized the sequence by the term "squirting capillaries." He envisioned that the capillaries squirted a load of serum and inflammatory cells into the epidermis, thereby creating microvesicles full of neutrophils (Munro's abscesses). Squirting capallaries are also found in seborrheic dermatitis (12). Segments of parakeratosis are typical of both dermatoses, but are only one aspect of a much more complex abnormality. In dandruff, they are superimposed on a normal background, little brushfires on an otherwise serene landscape.

The size of an individual scale depends on the number of papillae involved. One squirting capillary produces a small scale, three adjacent ones a fairly good sized scale. It is rare for more than 3 contiguous papillae to be affected. The distribution of these inflammatory microfoci is haphazard, though they seem to occur with higher frequency next to hair follicles.

We previously remarked that scales from dandruff and nondandruff could not be told apart. When individual small scales were mechanically shaken so as to disperse the cells, we found that about one-third of the horny cells were nucleated. The percentage of nucleocytes was somewhat higher in larger scales, about 40 per cent. This concurs with the histologic impression of more intense inflammatory activity beneath the larger scales.

By collecting scales and sectioning them en masse, one gains a different impression of how extensively these are permeated by neutrophils. Newly

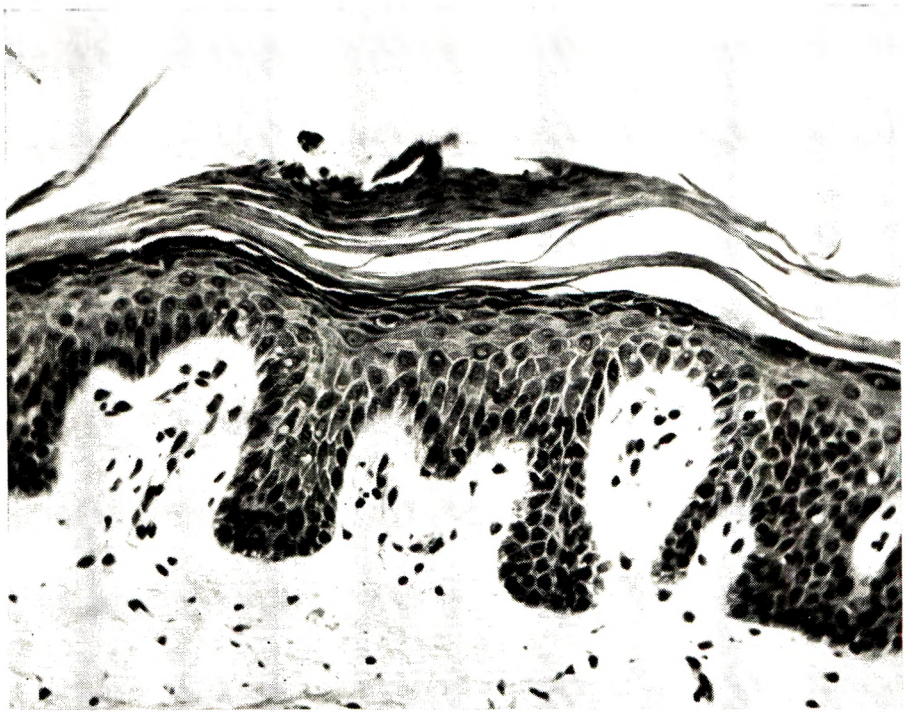

Figure 8. Late stage of squame formation (H&E, 265 x). There is a small mound of parakeratotic cells. Underlying epidermis is normal and capillaries are quiescent

formed scales frequently show a solid mass of polys on their surface. The point here is that dandruff scales are heavily impregnated with inflammatory cells and serum and are actually "crusts." It is of more than passing interest that the biochemical changes in dandruff scales have a resemblance to psoriasis, as one might expect, since the dynamics are much the same.

Subjects with severe dandruff often complain vociferously of scalp itching. Pruritus certainly occurs in nondandruff, also, but is less frequent and less distressing. Itching is, of course, highly variable and perceived differently by individuals. Tentatively, we consider itching to be set off by the squirting capillaries. Inflammatory microfoci in the papillae could readily activate fine sensory nerve fibers.

We call particular attention to an artifact, which bewildered us for quite a while. This consisted of small foci of necrotic cells in the upper epidermis. These dead zones stained poorly, were shallow, and had a concave outline. We puzzled over them at length till we perceived that the horny layer overlying them was ruptured. It finally occurred to us that the horny layer had

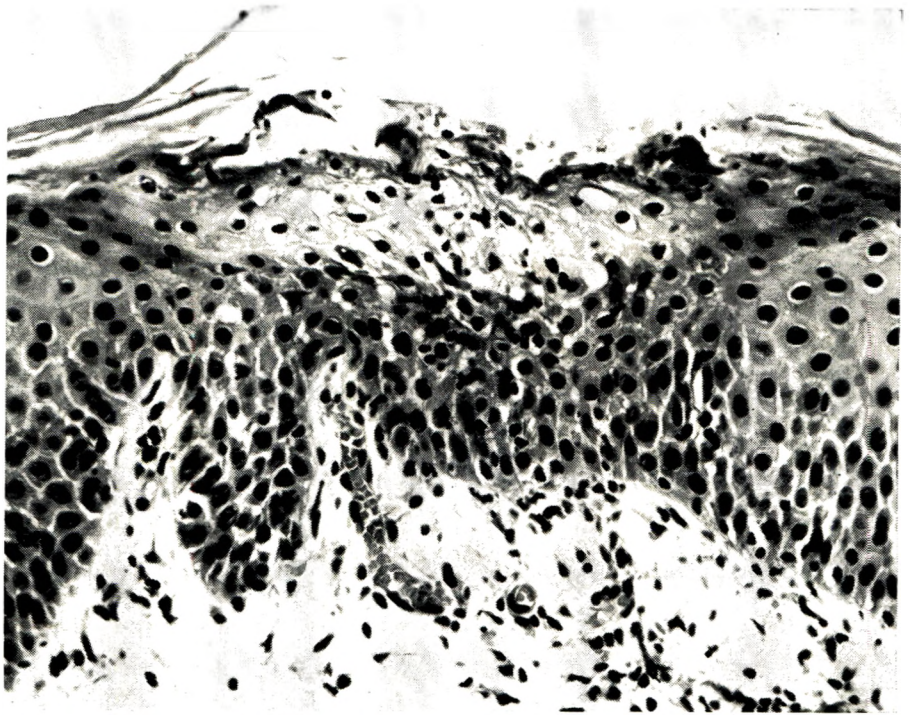

Figure 9. A saucer shaped zone of necrosis underlying ruptured horny layer is hallmark of scratch. Fingernail injury has provoked tiny focus of inflammation (H&E, 630 x)

actually been torn away by the fingernail (Fig. 9). These necrotic foci are caused by scratching, the natural response to itching!

Finally, we must re-examine the data regarding cell kinetics in dandruff. We had no appreciation of squirting capillaries when we scanned slides for thymidine labeled nuclei when we were determining labeling indices. Re-examination of dandruff slides showed that clusters of labeled cells occurred just beneath the parakeratotic mounds. Labeled cells were not only unusually abundant in these foci but were distributed in the second and third row above the basal layer, just as in psoriasis. Inflammation, no matter how produced, invariably stimulates cell turnover.

Clearly, cell proliferation is not uniform in scalp epidermis. There are "hot" spots of reproductive activity. The values we have presented for the labeling index in dandruff are speciously high, for we counted all labeled cells. We now see that the "hot spots" should have been disregarded to obtain a truer appreciation of the proliferative activity in the normal epidermis. The inflammatory foci are, fortunately, far enough apart so as not to vitiate the con-

clusion that cell turnover is increased in dandruff. Once more, we can appreciate that two processes coexist in dandruff; a general increase in mitotic activity and scattered foci of rapidly replicating cells secondary to focal inflammatory change. The first produces more horny cells everywhere, the second produces horny cells in the form of visible scales.

XI. The SC in Dandruff

Any description of the horny layer must take into account the "contaminating" effect of the parakeratotic scales. Their very prominence captivates the eye and leads to faulty descriptions. Ackerman and Klingman, for example, were impressed with the disorderly organization of the horny layer in dandruff, remarking that the horny laminae were folded and wavy, with numerous cracks, often in such disarray as to form whorls (10). They were clearly describing large scales, not the intervening horny layer. In surface replicas, the squames looked like big irregular boulders scattered on a flat landscape. In the electron microscope, the scales somewhat resemble psoriatic horny layer (numerous intracellular lipid droplets and decreased attachment plaques). Clearly, the squames must be studied separately, being a distinctive tissue.

We have made some preliminary observations on the orthokeratotic, apparently normal horny layer in dandruff. Corneocytes were studied after the sample was freed of scales by coarse filtration. Individual horny cells were normal in size, shape, and staining properties. Fractures of the cell membranes were rare. If there are structural abnormalities these must be very subtle. In psoriasis and seborrheic dermatitis, there are easily recognized changes in the sizes and shapes of the horny cells.

We found that the glistening layer was reached with fewer scotch tape strippings on dandruff scalps (10 to 15 strippings versus 26 to 48 in nondandruff) (10). This suggests that the SC in dandruff has fewer cell layers. To secure more quantitative data, we obtained intact discs of horny layers from dandruff and nondandruff scalps by the cantharidin-blister technique. After sectioning and swelling the cells in alkali, the number of cell layers was counted. The results are shown in Table IV. The number of cell layers was found to decrease with increasing scalp grade. Grade II and III scalps had 16 to 20 cell layers, decreasing sharply to 10 in moderate dandruff (Grade V) and to 7 in Grade VII. In large scales, by contrast, there were as many as 25 to 50 cell layers. Apparently, the greater the production of cells, the more readily these tend to separate and be shed, leaving fewer cells to comprise the "barrier." This implies that the scalps of dandruff subjects are more permeable. Assuming this to be true, antidandruff medicaments would penetrate more rapidly and reach the germinative layer in higher concentrations. This

Table IV
Number of Cell Layers in Horny Layer

	Grade	Subjects	Cell Layers	
			Mean	SD
Nondandruff	II	5	20	±2.1
	III	5	16	±1.1
Dandruff	V	8	10	±1.7
	VII	3	7	±0.8

may explain why substances as insoluble as ZPT are so effective in dandruff, even when applied intermittently as a shampoo. Presumably, the horny layer will thicken as dandruff improves, thus lessening permeability.

XII. Effect of Shampooing

Perhaps, the first question to be answered is "What happens when scalp washing is prohibited?" Will every one develop dandruff? We had Grade III subjects wear perforated bathing caps for 21 consecutive days. None developed dandruff, though more scales were dispersed in the hair. We were rather surprised that the grades remained about the same. On the other hand, when Grade V dandruff subjects wore caps for the same time, the grades increased to IX and X (1). Scaling became tremendous accompanied by an offensive odor, no doubt from a great increase in the resident microflora. The corneocyte counts tended to level off after the sixth day. Obviously, the small largely invisible horny cell aggregates are lost more easily that the scales, hence the differing patterns for grades and counts. The nondandruff subject, therefore, is in no danger of developing excessive scaling by being indifferent to scalp hygiene, while the nonwashing dandruff subject will leave a trail of scales beyond him.

When dandruff is allowed to worsen by not washing, one gets an opportunity to observe the extent to which the follicles contribute to scaling. The upper portion of the follicle (the infundibulum) also produces horny cells, and these could add materially to the total load. In fact, in severe dandruff, one can see collarettes of scales forming in the mouths of the follicles. These are quite loose and do not form sheaths around the hairs. It would seem that the infundibular epithelium can make an important contribution to the quantity of corneocytes and squames.

Incidently, the horny material produced by the internal root sheath of the follicle falls apart completely and is therefore invisible. It makes no contribution to scaling.

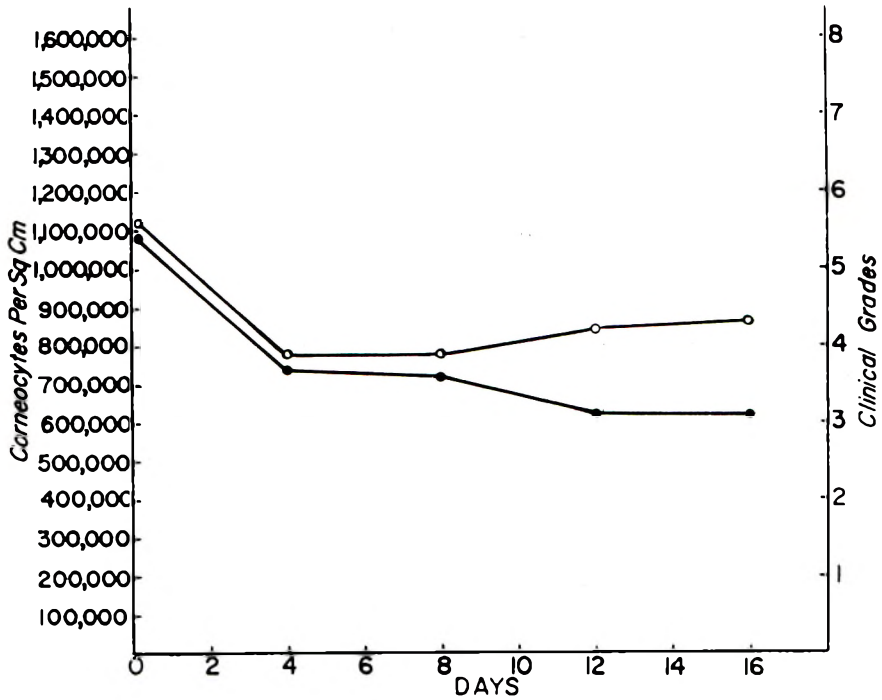

Figure 10. Effect of bland shampooing every other day (dandruff subjects). Counts and grades decreased after two shampoos. Corneocyte count was not affected by further shampooing. Grades continued to decline so that subjects no longer had dandruff by 10th day: (O) Low neocyte counts; (●) clinical grades

Suppose the dandruff subject washes every day or every other day; then, dandruff simply disappears; the scales are removed before they can grow to maturity. We took the trouble to make detailed observations on 10 subjects with Grades V and VI dandruff whose scalps were washed with an especially mild nonmedicated shampoo* every other day for 8 times. The results are shown in Fig. 10. Each shampoo consisted of 2 one-min washings with a rinse between. After 2 shampoos, the corneocyte count fell from 1.1 million cells/sq cm to 800,000/sq cm—not decreasing further. The new equilibrium was still within the dandruff range. Clearly, the horny cells were being produced at the same rate as before. The initial mean grade of 5.5 was reduced to mild dandruff (Grade 3.8) by two shampoos. After six, the mean grade was III; the subjects no longer had clinical dandruff. Repeated shampooing, as one might

*Johnson's Baby Shampoo, Johnson & Johnson, New Brunswick, New Jersey.

Table V

Effect of a Single Nonmedicated Shampoo on Ten Dandruff Subjects

Days	0	1	2	3	4	8
Grades[a]	5.29	**2.00**	**2.57**	**4.00**	5.14	5.57
Corneocyte	1024000	**279600**	**532400**	**762600**	1025000	**1355000**
Pityrosporum	1473000	**343400**	**542400**	**791600**	1486000	**2109000**
Aerobes	413200	**61750**	179200	388600	5081000	799700
C. acnes	40810	28040	5958	10200	18440	8634

[a]Except for grades, the figures are geometric means/cm.^{2} The values in boldface type are significantly different from day zero ($p = < 0.05$).

expect, had a profound effect on the squames. It is the flakes which account for the visibility of dandruff. It would be interesting to find out whether repeated shampooing has any effect on the inflammatory microfoci. Many scalp conscious young adults feel obliged to shampoo every other day or more often, whether or not they have dandruff. The elimination of inflammatory microfoci, and perhaps itching, could provide a "scientific" rationale for what seems to be compulsive scalp cleansing.

We determined on 10 dandruff scalps the effect of a single washing with a nonmedicated shampoo, following the changes daily for the next 8 days. This was a complete analysis including changes in the microflora. The results are shown in Table V.

Within 24 hours, there was a drastic fall in the grades from 5.3 to 2.00. It was not until the fourth day that the grades returned to the pretreatment level. The corneocyte counts followed a similar pattern, a sharp decline by 24 hours, returning gradually to the original level by the fourth day. This confirmed our previous finding that the restoration time in dandruff is about four days. The loss from shampooing is made up during this time. Shampooing dandruff subjects every four days, therefore, should not have an appreciable effect on grades or counts. This expectation was, in fact, empirically fulfilled in a study of 15 dandruff subjects.

It is of passing interest to note that the follicle-inhabiting *C. acnes* was not affected. The aerobic micro-organisms, which live on the surface, were restored by day 2; while the slower growing yeasts took 4 days to reach their original density.

We found that shampooing removed about 40 per cent of the corneocytes that could be obtained by the detergent-scrub technique. However, the corneocyte count 24 hours later was considerably lower than the count immediately after shampooing, falling from 770,000/sq cm to 288,000/sq cm. We theorized that shampooing removes oil and horny debris, which serves as a trap for desquamating cells. Hence, loss of corneocytes is facilitated for a

time until the oil-horn matrix is restored. The effects of shampooing are thus more complex than one ordinarily supposes.

XIII. The Scalp Microflora

Ever since the discovery of the germ theory of disease, microorganisms have figured prominantly as etiologic factors in various scalp disorders. Over 100 years ago, Malassez asserted that yeasts of the genus *Pityrosporum*, caused dandruff. The French master, Sabouraud, believed likewise. To this day, many authorities consider that yeasts are influential in producing dandruff, though the term "infection" is not so likely to be used as in days of old. Some recent workers consider that bacteria, as well as yeasts, collaborate to provoke excessive scaling, citing as proof the enhanced benefits of suppressing both members of the resident microflora in comparison to yeasts alone.

Consulting the literature will confound the novitiate who wishes to know what organisms customarily live on the scalp. The most recent comprehensive study of the scalp microflora yielded an astonishing variety of "resident" organisms on dandruff scalps including 30 kinds of yeasts, 143 molds, 44 bacteria, and 8 actinomycetes (13). It is not a surprise that advocates of an etiologic role for *Pityrosporum* find yeast more often in dandruff scalps.

A limitation of all past microbiologic studies is that none has been quantitative. It is only be determining the quantities of each organism that one can distinguish between those which live on the scalp from those which are mere contaminants from the environment. The hair is an efficient trap for particles and is expected that a few colonies of many different air-borne organisms will be recovered.

Conditions on the scalp are quite favorable for the growth of micro-organisms. There are numerous sweat glands to supply moisture, many sebaceous glands secreting a variety of metabolizable lipids, and of course, the brisk production of corneocytes. The latter contain as much as 20 per cent of water soluble substances furnishing a steady stream of nutrients. In consequence, the scalp teems with organisms, the population being made up of orders of magnitude more dense than on the trunk and extremities.

The time is overdue to decide whether microorganisms do or do not play a role in dandruff. The importance of *Pityrosporum* is so deeply entrenched in theories of causation that the search for new antidandruff agents often utilizes an *in vitro* screen against *Pityrosporum ovale*. ZPT apparently flowed into the channels of commerce via this route.

We compared the microflora of subjects with and without dandruff and came to the following conclusions. The resident microclora of the scalp is really quite simple whether or not dandruff is present(9). Three groups of organisms are always found 1. an anaerobe, *C. acnes;* 2. yeasts of the genus *Pityrosporum*; and 3. aerobic cocci. These are the same organisms which com-

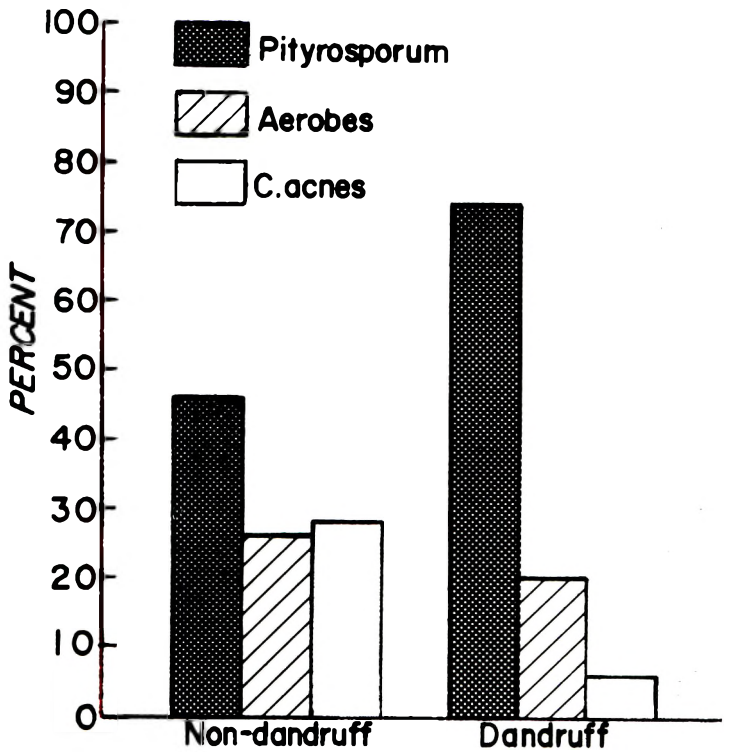

Figure 11. Proportions of organisms comprising the scalp microflora. In dandruff, yeasts make up three quarters of resident organisms. Aerobes remain same while *C. acnes* decreases

prise the dominant members of the microflora in most body regions. It should be emphasized that these three groups are ubiquitous; the prevalence is 100 per cent. Technical errors account for rare failures to isolate all three from all scalp. The scalp, is nonetheless, a distinctive territory, for aerobic diptheroids, common elsewhere, are sparse or missing.

It is only in the proportions of these three groups that dandruff scalps differ (Fig. 11). Whereas *Pityrosporum* made up about 45 per cent of the total population in nondandruff (about half a million organisms per square centimeter) it accounted for 75 per cent of the total in dandruff.

The dominant yeast on the scalp is *P. ovale*. *P. orbiculare* is frequently found but in considerably lower amounts. It should be noted that the total quantity of microorganisms is only slightly higher in dandruff, 1.2 million/sq cm as compared with 1.0/sq cm. The finding of almost twice as many *Pityrosporum* in dandruff could lend support to the belief that yeasts are important. An alternative hypothesis is that increased production of horny cells provide more surface and nutrients for growth.

Curiously, the cocci were not increased in dandruff. Of surpassing interest was the substantial decrease of *C. acnes* in dandruff. In explaining this, it is useful to recall that *C. acnes* is a strict anaerobe. A slight increase in O_2 tension will antagonize its growth. How could this occur in dandruff? Our supposition is that the inflammatory microfoci are just numerous enough to lessen the degree of anaerobiasis in the follicles where *C. acnes* lives.

We undertook to evaluate the rule of micro-organisms by eliminating them one at a time with appropriate antimicrobial agents and finally suppressing the entire microflora with a combination of agents (14). The effect on dandruff was appraised by clinical grades and corneocyte counts.

Suppression of *C. acnes* by oral tetracycline had no effect, as was expected. Inhibition of the areobes by topical neomycin had no influence whatever on grade or counts. Virtual elimination of *P. ovale* by topical amphotericin B for 4 weeks had no discernible effect on dandruff. This was sufficiently important to justify a longer study on the assumption that reduction in scaling might have a long lag time. This possibility was ruled out when it was found that 9 weeks of amphotericin B brought no changes in the grades or corneocyte counts.

We followed the *Pityrosporum* trail even further hoping to put the matter to rest, once and for all. This time, dandruff and *P. ovale* were both suppressed by twice weekly shampooings for 3 weeks with 2.5 per cent SeS shampoo. Then, half the subjects used only a bland shampoo twice weekly, while the other half received topical amphotericin to prevent the regrowth of *P. ovale*. In both groups, grades and counts returned to the pretreatment level at the same rate, in 4 to 6 weeks.

Finally, all three medicaments were given simultaneously resulting in concomitant suppression of aerobes, yeasts, and anaerobes. Dandruff remained unaffected.

These data are strongly apothetical to the belief that micro-organisms play a role in dandruff. In our view, the reign of *P. ovale* is at an end. Increase of *P. ovale* in dandruff is a consequence of increased scaling not its cause.

On the other hand, there is no room for ambiguity concerning the inflammatory microfoci. These do represent a pathologic process. The tissue in these areas is structurally and functionally abnormal. Disease exists at these sites. We have no insights as to their causation.

XIV. Mode of Action of Antidandruff Agents

On numerous occasions, we have measured the effectiveness of ZPT and SeS shampoos on dandruff. These invariably exert a suppressive effect, the latter being more potent. Their mode of action seems to be cytostatic. They decrease epidermal proliferation by inhibiting the multiplication of germinative cells. We found that SeS decreased the corneocyte count to about the same extent in nondandruff subjects (15). There was a corresponding decline

in the labeling index. We found that dandruff could be promptly controlled by other potent cytostatic agents, ordinarily restricted for use in serious diseases such as leukemia. The drugs included methotrexate and topical nitrogen mustard. Likewise, topical corticosteroids are highly effective in dandruff, again chiefly because they reduce the rate of multiplication of germinative cells.

This does not complete the story. Our preliminary findings with crude coal tar raise up some interesting questions. Clinical improvements was noted with tar shampoos, but paradoxically, the corneocytes remained the same or did not fall till much later. Do tars eliminate the inflammatory microfoci and thus abolish visible scaling without changing epidermal proliferation? Do they perhaps influence the cohesiveness of horny cells so that they cannot aggregate into noticeable squames? These questions are now occupying our attention.

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Neue Insektenabwehrmittel -

am Stickstoff disubstituierte ß-Alaninderivate

MANFRED KLIER[1]) und FRIEDRICH KUHLOW[2])

Synopsis — New insect Repellents Derivatives of N-disubstituted β-Alanine. — A number of derivatives of N-disubstituted β-alanine were synthesized. Among them several N-ALKYL ESTERS of 3-(N-n-ALKYL-N-ACYL) AMINOPROPIONIC ACID and of 3-(N-n-ALKYL-N-CARBALKOXY)AMINOPROPIONIC acid effectively repelled AEDES AEGYPTI MOSQUITOES from HUMAN SKIN to an extent equal to that of N,N-DIETHYLTOLUAMIDE. An OLFACTOMETER, which permitted simple, efficient, and rapid evaluation, was developed for preliminary screening of repellency. The correlation between repellency of the synthesized compounds and their volatility and their molecular structure was studied. One compound, ETHYL 3-(N-n-BUTYL-N-ACETYL)AMINOPROPIONATE, is particularly noteworthy; it exhibits high mosquito repellency, possesses extremely low toxicity towards warm blooded animals, and is well tolerated by human skin.

Wirkstoffe zur Abwehr stechender, blutsaugender oder sonst lästiger Insekten, sog. Repellents, haben eine bedeutende kosmetische, gesundheitliche und hygienische Funktion zu erfüllen. Bekannte und bereits seit längerer Zeit im Gebrauch befindliche Repellents sind z. B. Dimethylphthalat, 2-Äthylhexadiol-(1,3) und m-Toluylsäure-N, N-diäthylamid. Ein Nachteil der bekannten Repellents ist deren Weichmacherwirkung gegenüber Kunststoffen, ferner ihre oft ungenügende Haut- und Schleimhautverträglichkeit.

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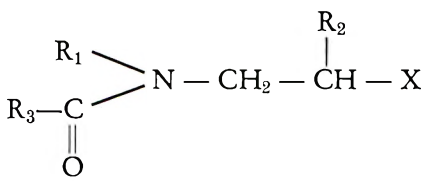
Aufgabe der vorliegenden Arbeit war es, neue Mittel mit starker und lang anhaltender Repellentwirkung, guter Hautverträglichkeit, geringer Toxizität und vernachlässigbarer Weichmacherwirkung zu finden. Es wurde gefunden, daß bestimmte, am Stickstoff disubstituierte β-Alaninderivate eine ausgezeichnete Repellentswirkung gegenüber der Gelbfiebermücke, *Aedes aegypti L.*, aufweisen.

Unsere Aufmerksamkeit wurde auf Alanin und dessen Abkömmlinge durch zwei bereits vor längerer Zeit erschienene Arbeiten gelenkt (2) (3). In diesen wurde α-Alanin als Stechmücken anlockende Substanz angegeben. Die leichtere präparative Zugänglichkeit der β-Alaninderivate war Anlaß, uns zunächst dieser Substanzklasse zuzuwenden.

Die untersuchten, am N disubstituierten β-Alaninderivate werden durch die allgemeine Formel I beschrieben:



I

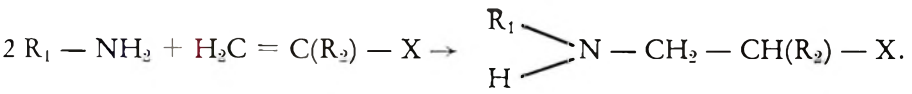
in der R_1 für einen Alkyl-(C_1—C_6), gegebenenfalls substituierten Aryl- oder Cycloalkylrest steht, R_2 H oder eine Methylgruppe und R_3 eine Alkyl-, Aryl- oder Alkoxygruppe mit höchstens 8 C-Atomen bedeuten, sowie X für -CN oder -$COOR_4$ steht, wobei R_4 einen Alkylrest mit bis zu 6 C-Atomen darstellt. Einige der durch die Formel I charakterisierten Substanzen sind in der Literatur bereits bekannt (4).

MATERIAL UND METHODEN

Chemische Synthesen

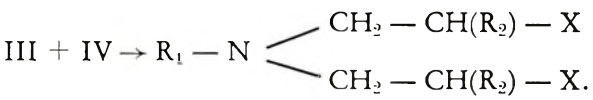
Die am N disubstituierten β-Alaninderivate werden in zwei Reaktionsschritten hergestellt: 1. durch Addition von primären Aminen II, R_1NH_2, an die reaktive C=C-Doppelbindung von Acrylsäurederivaten III, $H_2C{=}C(R_2)$—X. R_1-, R_2 und X haben die in I angegebene Bedeutung. Die Additionsreaktion wird ohne Zusatz von Verdünnungsmitteln bei 5 bis 30° C und Normaldruck ausgeführt. Mit bei Raumtemperatur sehr flüchtigen Ami-

nen ($R_1 = CH_3$, C_2H_5) wird in einem Druckgefäß gearbeitet. Die Reaktionspartner setzen sich bei Wahl eines Molverhältnisses von 2:1 zwischen II und III zu Addukten (Zwischenprodukten) IV um:



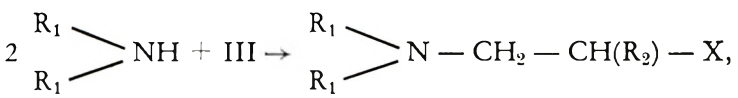
II III IV

Bei ungenügendem Aminüberschuß entstehen neben IV unerwünschte [2:1]-Addukte der Struktur V als Nebenprodukte:



V

Die Isolierung und Aufarbeitung der Zwischenprodukte IV erfolgt nach Verjagen des Amin-Überschusses durch Destillation im Vakuum. Unter den Verbindungen IV befinden sich mehrere mit ausgeprägter Repellentwirkung gegenüber *A. aegypti L.* Wenn man die obige Additionsreaktion mit sekundären Aminen IIa statt mit primären ausführt:



IIa IVa

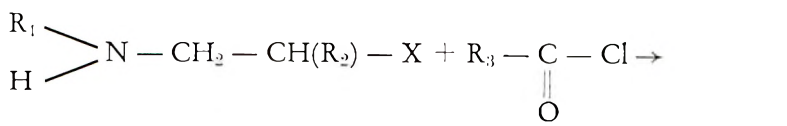
erhält man die Addukte IVa, wovon mehrere gleichfalls eine deutliche Repellentwirkung aufweisen. In *Tabelle 1* sind einige Addukte der Typen IV und IVa aufgeführt, die Repellentaktivität aufweisen.

Tabelle 1

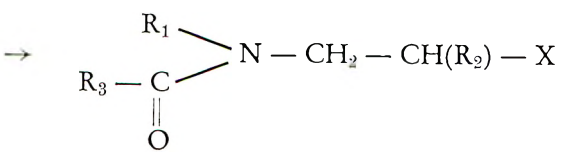
Nr.		Siedepunkt °C/Torr	Formeltyp
1	3-N-Äthyl-aminopropionsäure-2-äthyl-hexylester	97—100/0,6	IV
2	3-N-n-Propyl-aminopropionsäure-äthylester	60—62/0,2	IV
3	3-Pyrrolidino-propionsäure-n-butylester	90/0,8	IVa
4	3-Piperidino-propionsäure-n-butylester	88/0,3	IVa
5	3-(N, N-Dimethyl-)aminopropionsäure-n-hexylester	65—68/0,1	IVa
6	3-(N, N-Diäthyl-)aminopropionsäure-n-butylester	61/0,2	IVa

Die Verbindungen IV und IVa werden in einer Ausbeute von 65—80 % erhalten; sie sind bei Raumtemperatur wasserhelle Öle, die mit alkalischer Reaktion in Wasser mäßig gut löslich sind. Ihre Beständigkeit in wäßrigem Milieu ist begrenzt; daher sind sie zur Anwendung auf der menschlichen Haut ungeeignet und wurden nicht eingehender untersucht.

Die Darstellung der Endprodukte nach Formel I aus den Zwischenprodukten IV erfolgt durch ihre Umsetzung der mit Carbonsäurechloriden VI bzw. mit Chlorameisensäureestern VII:



IV VI bzw. VII



I

Diese Umsetzung wird vorzugsweise in Gegenwart von Triäthylamin oder Pyridin bei 0—30° C in Benzol als Verdünnungsmittel ausgeführt. Die Endprodukte I werden durch Vakuumdestillation isoliert und gereinigt. In der *Tabelle 2* sind einige der nach dem beschriebenen Verfahren hergestellten Verbindungen der Formel I zusammengestellt.

Tabelle 2

N, N-disubstituierte β-Alaninderivate nach Formel I [3] [4]

Nr.	Substituenten				Ausbeute %	Kp. °C/Torr
	R_1	R_2	R_3	X		
1	$n-C_4H_9$	H	CH_3	$COOC_2H_5$	89	108—110/0,2
2	C_2H_5	H	$n-C_3H_7$	$COOCH_3$	78	122—125/3
3	C_2H_5	H	CH_3	$COOC_2H_5$	84	105—106/0,6
4	$n-C_3H_7$	H	CH_3	$COOC_2H_5$	81	101—102/0,3
5	CH_3	H	CH_3	$COO-n-C_4H_9$	82	112—116/0,15
6	$n-C_3H_7$	H	C_2H_5	$COOCH_3$	79	135—138/3
7	$n-C_4H_9$	H	CH_3	$COO-n-C_4H_9$	82	136—138/0,3
8	C_2H_5	CH_3	CH_3	$COOC_2H_5$	73	118—120/3
9	C_2H_5	CH_3	C_2H_5	$COOC_2H_5$	75	123—126/2
10	CH_3	H	$C_4H_9CH(C_2H_5)$	$COOCH_3$	68	106—108/0,05
11	C_2H_5	H	CH_3O	$COOC_2H_5$	87	95— 98/3
12	C_2H_5	H	C_2H_5O	$COOC_2H_5$	89	118—120/5
13	$n-C_4H_9$	H	$n-C_3H_7$	CN	77	125—130/0,2
14	$i-C_4H_9$	H	CH_3	$COOC_2H_5$	77	110—112/0,05
15	$sek.-C_4H_9$	H	CH_3	$COOC_2H_5$	90	118/0,05
16	H	H	$n-C_3H_7$	$COOC_2H_5$	35	114/0,1
17	H	H	C_2H_5	$COO-n-C_4H_9$	42	115/0,3
18	$C_6H_5-CH_2$	H	CH_3	$COOC_2H_5$	84	142—146/0,3
19	$C_6H_5-CH_2$	H	CH_3	CN	86	121—124/0,01
20	CH_3	H	C_6H_5	$COOCH_3$	80	134—137/0,3
21		H	CH_3	$COOC_2H_5$	77	134—136/0,4

[3] Nr. 16 und 17 sind Mono-Substitutionsprodukte, aber strukturell den Substanzen der Formel I zuzuordnen.

[4] Elementaranalyse und IR-Spektren entsprechen der geforderten Struktur; die Reinheit der Präparate beträgt nach gas-chromatographischer Bestimmung $\geqslant$ 95 %.

Die in *Tabelle 2* genannten Substanzen sind wasserhelle, praktisch geruchlose Öle von geringer Wasser- und Mineralöllöslichkeit. Sie lösen sich gut in Äthanol und höheren Alkoholen, z. B. in 2-n-Octyl-dodecanol sowie in höheren Estern, z. B. in Isopropylmyristat.

Substanz Nr. 1 in *Tabelle 2*, 3-(N-n-Butyl-N-acetyl-)aminopropionsäureäthylester wurde, stellvertretend für die übrigen dort genannten Verbindungen, näher untersucht: Sie ist beständig gegen Licht, Luft und Feuchtigkeit. Nach 1stündigem Kochen einer 20 %ø wäßrigen Suspension sind keine Zersetzungsprodukte nachweisbar. Korrodierende Wirkung auf Metalle (Eisen-, Stahlblech) konnte nicht beobachtet werden.

Untersuchung der insektenabweisenden Wirkung

Olfaktometertests

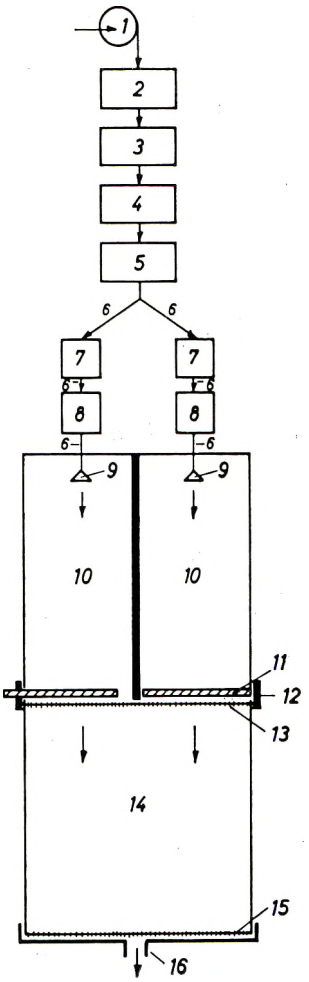
Die zahlreichen synthetisierten Verbindungen der Formeltypen I bzw. IV, IVa wurden zunächst einer orientierenden Vorprüfung ihrer insektenabweisenden Wirkung unterworfen. Als geeignete Screening-Methode erwies sich die Prüfung in einem Olfaktometer, wobei eine möglichst einfach zu bedienende und schnell arbeitende Versuchsanordnung gewählt wurde. Als Testinsekten dienten *A. aegypti*-Weibchen einer Subkolonie des Hamburger Tropeninstituts.

Im Olfaktometer wird die sogenannte intrinsic repellency (5) ermittelt, also die dem Testpräparat eigentümliche Repellentaktivität im Gegensatz zu der Dauer dieser Wirkung, die ein solches Präparat auf einem bestimmten Substrat unter festgelegten Bedingungen entfaltet.

Das benutzte Olfaktometer ist eine Modifikation der Versuchsanordnung von Howell und Goodhue (6). In einem geeigneten Testkäfig wurden jeweils 60 hungrige *A. aegypti*-Weibchen zwei gleichartig konditionierten Luftströmen ausgesetzt, deren einer mit dem Dampf des zu untersuchenden Testpräparates beladen war. Ein gut wirksames Repellent führte zur spontanen Fluchtreaktion der Testtiere in den wirkstofffreien Kontrolluftstrom.

Versuchsanordnung

Das Strömungssystem des Olfaktometers ist in der *Abbildung* skizziert: 1 = Frischluftquelle (Preßluft ca. 5 atü); 2 = Reduzierventil; 3 = Aktivkohlefilter; 4 = Siliconölheizbad, auf 33° C thermostatiert; 5 = Luftbefeuchter, mit destilliertem Wasser gefülltes, auf 30° C thermostatiertes Glasgefäß mit eingetauchter Glasfritte, 6 = mit Schaumstoff wärmeisolierte Silicongummischläuche; 7 = Durchflußmesser und -regler; 8 = Hygrometer und Thermometer; 9 = Luftstromeinlässe durch mit Glasfritten (äußerer Durchmesser 9,5 cm) versehene Glastrichter; 10 = Vorkammern 30×30×15 cm; 11 = Wechsel-Stahlrahmen 30×15 cm, mit Baumwollgaze bespannt (Maschenweite ca. 0,6 mm; sie dienen der Prüfsubstanzdosierung für den Teststrom; 12 = Dichtungsrahmen, Abdichtmaterial Silicongummi; 13 = Wechselrahmen, mit Diolen®-Gaze bespannt, Frontabschluß des Testkäfigs; 14 = Testkäfig 30×30×30 cm; 15 = Rückseitenverschluß des Testkäfigs, mit 5-cm-Bohrung zum Einführen der Testtiere versehen, mit Watte-Pfropfen verschließbar, sonst ausgeführt wie der Frontseitenverschluß 13; 16 = Abzugshaube 32×32 cm mit zentraler 5-cm-Bohrung mit Anschluß an die Entlüftungsleitung. Die Teile 10, 12, 13, 14, 15 und 16 bestehen aus Acrylglas von 4 mm Wandstärke.



Vorversuche

In den Vorversuchen wurden die der vorliegenden Apparatur am besten angepaßten Versuchsbedingungen ermittelt:

1. Luftströmung: Kontroll- und Testluftstrom wurden in gleicher Weise konditioniert. Die relative Feuchte wurde auf 65—70 % eingestellt, die Strömungsgeschwindigkeit auf 4—6 cm/sec begrenzt (im Testkäfig mit NH_4Cl-Nebel gemessen). Um etwaige Turbulenzen in der Luftströmung und Totvolu-

mina im Testkäfig möglichst gering zu halten, wurde die Einheit aus Vorkammern und Testkäfig aus der Horizontallage in die Vertikale gedreht (Abzugshaube nach oben gerichtet).

2. Die Lufttemperatur im Testkäfig wurde auf 28—29° C eingestellt.

3. Die Wirkstoffapplikation und -dosierung erfolgte durch Tränken (Imprägnieren) eines der beiden gazebespannten Rahmen 11 mit 10 % äthanolischer Lösung der Testsubstanz; nach Verdunsten des Lösemittels verbleiben ca. 1,1—1,2 g Substanz auf der Gazefläche von 15×30 cm (450 cm^2), d. h. ca. 3 mg/cm^2. Die gleichmäßig über die gesamte Frontfläche der Testkammer eingebrachte Testsubstanz unmittelbar vor der Stirnfläche des Testkäfigs trägt zu einer homogenen Verteilung des Wirkstoffs über den gesamten Querschnitt des mit Testsubstanz beladenen Luftstromes bei.

Versuchsausführung

60 *A. aegypti*-Weibchen, 4 bis 10 Tage alt, die 24 h vor Testbeginn nur Wasser als Nahrung erhalten hatten, wurden 2 h vor Beginn des Versuchs in den Testkäfig gebracht. Nach Erreichen der geforderten Luftströmungswerte befanden sich durchschnittlich 70 % der Testtiere auf der gesamten Stirnfläche des Testkäfigs (13, s. *Abbildung)* verteilt. Nach Dosierung der Testsubstanz über den Wechselrahmen 11 wurde das Verhalten der Tiere während 2 min beobachtet.

In Versuchen mit bekannten, stark wirksamen Repellents wie m-Toluylsäure-N, N-diäthylamid (DEET) und Dimethylphthalat (DMP) als Modellsubstanzen (unter gleichen Versuchsbedingungen wie oben angegeben) wurde gefunden, daß die Testkäfighälfte, die vom Teststrom überstrichen wird, spätestens 15 sec nach der Repellent-Dosierung von allen Stechmücken verlassen bzw. gemieden wird; demnach wurden von den hier untersuchten Verbindungen des Formeltyps I nur solche als starke Repellents klassifiziert, bei denen das gleiche Ergebnis wie mit DEET und DMP erzielt werden konnte.

Nach 2 min wurde der mit Testsubstanz versehene Rahmen 11 gegen einen nicht imprägnierten ausgetauscht. Der Verschlußrahmen 13 wurde ebenfalls durch einen unkontaminierten ersetzt[5]). Vor Beginn des nächsten Versuchs wurde die Apparatur während 10 min mit reiner Luft gespült. Dann wurde die nächste Substanz in den zuvor als Kontrollstrom dienenden Teilstrom dosiert. Eine bestimmte Mückenpopulation (60 Tiere, s. oben) wurde zu je 4 auf-

[5] Der Rahmen ist nach Reinigung in 15%igem wäßrigem Äthanol wieder verwendbar.

einanderfolgenden Versuchen verwendet. Anschließend wurde deren Reaktion gegenüber dem Repellent DEET kontrolliert. Bei stärkerer Beanspruchung der Testtiere nimmt deren Aktivität merklich ab.

Prüfung der Repellent-Langzeitwirkung an Aedes aegypti im Laboratorium (Armtest)

Der Unterarm einer Versuchsperson wurde mit je 2 ml 10% äthanolischer Lösung der Testsubstanzen gleichmäßig eingerieben. Die Hand wurde durch einen Gummihandschuh geschützt. Der mit Wirkstoff behandelte Unterarm wurde in einem mit ca. 500 hungrigen Mückenweibchen (Alter 4—14 Tage) gefüllten Testkäfig eingeführt. Die Beobachtungsdauer betrug je 3 min. Der Test wurde 5 min nach Wirkstoffapplikation ausgeführt und nach 1, 3 und 5 h wiederholt.

Ergebnisse

Die Insektenabwehrwirkung

Die Substanzen Nr. 1—13 in der *Tabelle 2* zeigen bei der Prüfung im Olfaktometer eine starke intrinsic repellency, d. h., ihre Wirkung ist unter den gegebenen Versuchsbedingungen von der der bekannten Repellents DEET und DMP nicht unterscheidbar. Die Substanzen Nr. 14 und 15 zeigen eine deutliche, aber geringere Wirkung als die erstgenannten Verbindungen. Eine quantitative Bestimmung der Wirksamkeit dieser Stoffe, etwa durch Angabe des Repellent-Index (6) bezogen auf den Index von DEET = 1,00, wurde nicht vorgenommen, da nur die am stärksten wirksamen Verbindungen (Nr. 1—13, *Tabelle 2*) einer weitergehenden Prüfung ihrer Abwehrwirkung unterzogen werden sollten. Die Verbindungen Nr. 16—21 zeigten im Olfaktometer keine Abwehrwirkung. Die Substanzen Nr. 1—13 wurden dem Langzeittest unterworfen. Dabei wurde zum Vergleich das Repellent DEET in gleicher Weise geprüft.

Die Ergebnisse sind aus der *Tabelle 3* ersichtlich. Sie beruhen auf den bei 4 freiwilligen Testpersonen bei 4maliger Wiederholung erhaltenen Einzelwerten.

Tabelle 3

Prüfung der Langzeitwirkung (xxx = voller Mückenschutz, kein Niedersetzen, xx = partieller Mückenschutz, häufiges Niedersetzen, jedoch keine Stiche)

Nr. gemäß *Tabelle 2*	Repellentwirkstoff	Wirkung nach Stunden		
		1	3	5
1	3-(N-n-Butyl-N-acetyl)-aminopropionsäure-äthylester	xxx	xxx	xxx
2	3-(N-Äthyl-N-n-butyryl)-aminopropionsäure-methylester	xxx	xxx	xxx
3	3-(N-Äthyl-N-acetyl)-aminopropionsäure-äthylester	xxx	xxx	xx
5	3-(N-Methyl-N-acetyl)-aminopropionsäure-n-butylester	xxx	xxx	xxx
11	3-(N-Äthyl-N-carbomethoxy)-aminopropionsäure-äthylester	xxx	xxx	xxx
12	3-(N-Äthyl-N-carbäthoxy)-aminopropionsäure-äthylester	xxx	xxx	xxx

Von den in der *Tabelle 2* genannten Verbindungen zeigen demnach nur die Substanzen Nr. 1, 2, 5, 11, 12 und annähernd auch Nr. 3 eine starke, unter den Versuchsbedingungen dem DEET gleiche Langzeitwirkung gegenüber *A. aegypti*. Die anderen Substanzen nach *Tabelle 2* haben eine geringere Wirkung.

Toxizität, Hautverträglichkeit

Orientierende Vorversuche ergaben für die Substanz Nr. 1, *Tabellen 2* und *3* besonders niedrige Toxizitätsdaten; daher wurde diese Verbindung einer ausführlicheren toxikologischen Prüfung unterworfen[6]).

1. Akute Toxizität an Ratten, peroral, LD_{50} = 14,0 ml/kg, niedrigste toxische Dosis, peroral, ~ 10 ml/kg Körpergewicht p. o.

2. Lokale Verträglichkeit auf der Rückenhaut (intakt und skarifiziert) des Kaninchens (einmalige Anwendung, Einwirkungsdauer 24 h); 10 % in 50 vol.-%igem wäßrigem Äthanol: Substanz wurde reaktionslos vertragen.

3. Akute Toxizität bei lokaler Applikation (unverdünnt) auf 1/10 der Körperoberfläche von Ratten LD_{50} > 10,0 ml/kg Körpergewicht; niedrigste toxische Dosis > 6,35 ml/kg Körpergewicht.

[6] F. Leuschner, Laboratorium für Pharmakologie und Toxikologie, 2104 Hamburg 92, Gutachten (1973—1974).

4. Verträglichkeitsprüfung an Kaninchen bei 4-wöchiger peroraler Applikation der unverdünnten Substanz: die Dosis von 0,5 ml/kg Körpergewicht/Tag wurde reaktionslos vertragen, niedrigste toxische Dosis zwischen 0,5 und 1,5 ml/kg Körpergewicht/Tag.

Einwirkung auf Kunststoffgegenstände (Anlöse- bzw. Anquellvermögen)

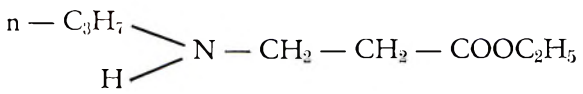
Ein Kugelschreiber aus Kunststoff und ein 10×10 cm großes Stück einer Kunststoffregenhaut aus Weich-Polyvinylchlorid wurden 6 h in eine 30 vol-%ige Äthanollösung von 3-(N-Äthyl-N-acetyl)-amino-propionsäureäthylester (Nr. 3, *Tabelle 2*) gelegt. Als Vergleichssubstanzen dienten, in gleicher Verdünnung, DEET und DMP. Substanz Nr. 3 der *Tabelle 2* zeigte keine Einwirkung auf die Kunststoffgegenstände; durch DEET und DMP wurden die Kunststoffe angegriffen: ihre Oberflächen waren rauh, klebrig und gequollen.

DISKUSSION

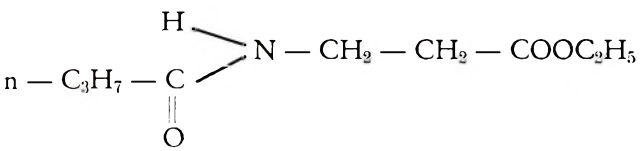
Die Substanzklasse des Formeltyps I umfaßt eine große Anzahl von Verbindungen mit Repellentwirkung gegenüber *A. aegypti*. Die wirksamsten Stoffe sind in den *Tabellen 2* (Nr. 1—13) und *3* zusammengefaßt. Aus *Tabelle 2* ist zu entnehmen, daß der Substanztyp I erhebliche Variationen der Substituenten R_1, R_3 und X (und in geringem Umfang R_2) erträgt, ohne die Repellenteigenschaften zu verlieren. Die Reste R_1 und R_3 (mit R_3 = Alkyl) sind besonders variationsfähig, wobei mit Alkylketten C_1—C_4 die normal unverzweigten Reste ein Optimum der Repellentaktivität erbringen. Dies belegt ein Vergleich der Substanzen 1 und 14 bzw. 15 der *Tabelle 2*, die sich nur durch den Substituenten R_1 unterscheiden. Die unterschiedliche Wirksamkeit dieser 3 Substanzen muß auf deren strukturelle Isomerie zurückgeführt werden, da der Unterschied in ihrer Flüchtigkeit zu vernachlässigen ist. Die Substanz Nr. 10 enthält, unter den starken Repellents als einzige einen längeren Substituenten R_3 (2-Äthyl-hexyl). Im übrigen führen die Substituenten R_1 und R_3 mit höherer Anzahl C-Atome, speziell die cycloaliphatischen oder aromatischen, zu einer starken Abnahme der Insektenabwehrwirkung bzw. zu völlig unwirksamen Verbindungen (vgl. die Substanzen Nr. 18 bis 21 in *Tabelle 2*). Diese sind weniger flüchtig als die übrigen aus *Tabelle 2* ersichtlichen Verbindungen und bestätigen die Beobachtungen von Johnson *et al.* (7) und von Gualtieri *et al.* (8), wonach Stoffe mit einem Siedepunkt > 300 bis 350° C bei Normaldruck im allgemeinen keine ausreichende Repellentwirkung mehr aufweisen.

Es besteht eine interessante Struktur-Repellent-Wirkungsbeziehung bei am Stickstoff m o n o substituierten Verbindungen der Formel I und IV.

In der Verbindung Nr. 2 der *Tabelle 1* und in der Verbindung Nr. 16 der *Tabelle 2:*



Nr. 2, *Tabelle 1;* $Kp_{0,2}$ 60—62° C



Nr. 16, *Tabelle 2;* $Kp_{0,1}$ 114° C

sind R_1 = n-Propyl, R_3 = H und R_1 = H, R_3 = n-Propyl; R_2 = H und X = $COOC_2H_5$ sind gleich. Die Substanz Nr. 16, *Tabelle 2*, hat zwar einen höheren Siedepunkt als Nr. 2, *Tabelle 1;* dieser liegt aber noch innerhalb des Siedebereichs wirksamer Verbindungen *(Tabelle 2).* Da es uns nicht gelungen ist, bei anderen, analog Nr. 16, *Tabelle 2* strukturierten Verbindungen (z. B. Nr. 17, *Tabelle 2)* eine Repellentwirkung nachzuweisen, liegt die Vermutung nahe, daß die Alkylaminofunktion eine notwendige Bedingung für die Repellentwirkung des Substanztyps I darstellt, die allerdings durch die N-Acylfunktion (R_3-CO-N-) verstärkt wird, vgl. die allgemein stärkere Wirkung des Substanztyps I gegenüber Typ IV bzw. IVa.

Der Einfluß des Substituenten R_2 (= H oder CH_3) auf den Repellenteigenschaften der Verbindungen der Formel I ist weniger stark ausgeprägt; allerdings sind unter den stärksten Repellents in *Tabelle 3* keine Substanzen mit R_2 = CH_3 vertreten. Ähnlich ist der Einfluß des Substituenten X. In *Tabelle 2* findet sich nur eine stark insektenabwehrende Substanz mit der Nitrilgruppe (X = CN, Nr. 13), in *Tabelle 3* tritt diese nicht auf. Hat X die Bedeutung $COOR_4$, liegt das Optimum der Wirksamkeit bei Verbindungen mit R_4 = Methyl bis n-Butyl.

ZUSAMMENFASSUNG

Eine Reihe von N-disubstituierten β-Alaninderivaten wurde synthetisiert. Von diesen zeigten einige 3-(N-n-alkyl-N-acyl)-aminopropionsäure-n-alkylester und 3-(N-n-Alkyl-N-carbalkoxy)-aminopropionsäure-n-alkylester auf menschlicher Haut gegen Aedes aegypti L., eine sehr hohe, dem m-Toluylsäure-N, N-diäthylamid vergleichbare Abwehrwirkung. Zur orientierenden Bewertung der Repellentwirkung wurde ein schnell arbeitendes und einfach zu handhabendes Olfaktometer entwickelt. Die Struktur-Wirkungsbeziehung der hergestellten Substanzen und die Abhängigkeit ihrer Abwehrwirkung von der Flüchtigkeit wurden untersucht. Die Verbindung 3-(N-n-Butyl-N-acetyl)-aminopropionsäureäthylester wird auf Grund ihrer besonders hohen Insektenabwehrfähigkeit in Verbindung mit ihrer äußerst geringen Warmblütertoxizität und hohen Hautverträglichkeit besonders hervorgehoben.

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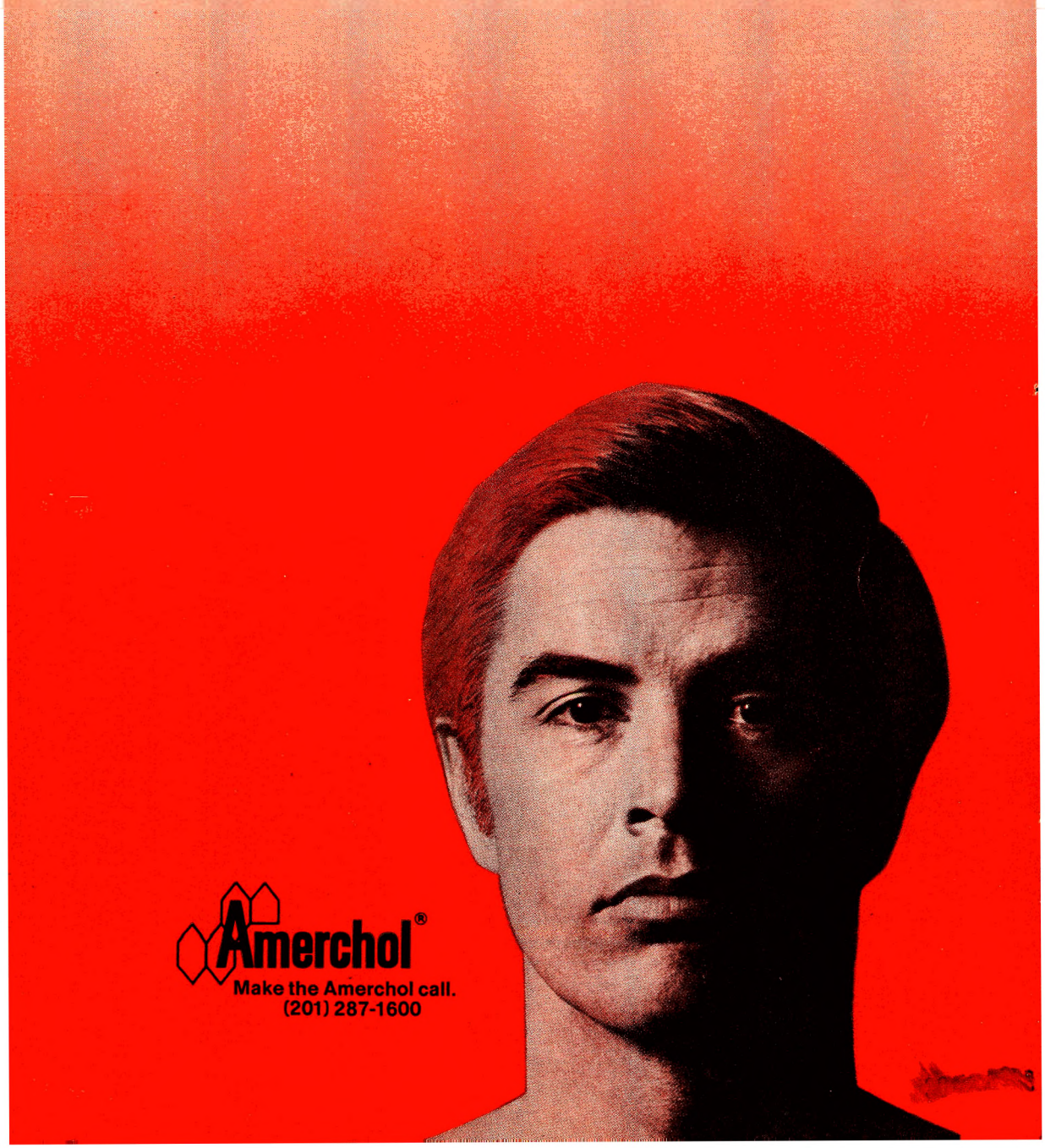

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