

Petrolatum is not comedogenic in rabbits or humans: A critical reappraisal of the rabbit ear assay and the concept of “acne cosmetica”

ALBERT M. KLIGMAN, *Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.*

Accepted March 8, 1996.

Synopsis

The objective was to find out whether petrolatum, previously reported to be comedogenic in the rabbit ear model, would worsen acne. Vaseline petrolatum jelly (Vaseline[®], Chesebrough-Ponds) and Vaseline petrolatum jelly cream (Creamy Vaseline[®], Chesebrough-Ponds) were applied to separate groups of ten acne patients each twice daily for eight weeks.

No evidence of comedogenic potential was found by the follicular biopsy technique. Papulo-pustules significantly decreased in both groups.

Greasiness cannot be equated with comedogenicity. The admonition for acne patients to seek “oil-free” skin care products has no scientific merit with regard to comedogenicity. Cosmetics are an unlikely cause of the relatively high prevalence of post-adolescent female acne.

INTRODUCTION

In a 1972 paper entitled “Acne cosmetica,” Kligman and Mills reported that as many as 50% of commercial cosmetics were comedogenic in the rabbit ear model (1). Since then, this assay has been extensively used by producers of drugs and cosmetics to assess their potential for inducing acneform eruptions (2). Recently, manufacturers have added the human back test to appraise acnegenicity. Reputable manufacturers of cosmetics routinely screen their products for comedogenicity in order to satisfy the consumer that their product is “non-comedogenic,” a useful claim in a highly competitive market.

However, neither the rabbit nor the human assay is entirely satisfactory. Serious controversies have sprung up regarding the reliability and relevance of the rabbit ear model. A voluminous literature reveals remarkably divergent results among laboratories and investigators. Substances deemed to be strongly comedogenic by some are declared by others to be innocuous. Reviewers have not failed to notice the high frequency of strikingly contradictory results. Long ago Frank questioned whether the rabbit ear model had any relevance for humans (3). The most extreme example of the controversies generated by the rabbit ear assay is the publications of Fulton, a prominent acneologist

(4). He has classified a large number of substances according to their comedogenic potential. He states categorically that manufacturers should automatically exclude ingredients that are comedogenic at any concentration. This caveat has brought to the fore a vexing problem for manufacturers, namely, the substantial number of desirable and seemingly safe substances that have been "blacklisted" by Fulton. Nelson and Rumsfield, too, have published lists of "unacceptable ingredients" (5). They go so far as to recommend products that do not contain "unacceptable ingredients." Draelos, too, has sharply commented on the complexities and uncertainties of evaluating cosmetic components by the rabbit ear model (6). Especially notable in Fulton's multitudinous list of comedogenic substances are D & C red dyes and a large variety of fatty substances that contribute to the aesthetic and functional properties of cosmetics. These have not been demonstrated to be acnegenic in humans. Because of these conflicts, I updated the rabbit ear assay in a 1990 publication, adding specifications that, hopefully, would substantially reduce inter-laboratory disagreements (7).

REASSESSMENT OF THE COMEDOGENICITY OF OILS

This wordy introduction brings us to the issue that is central to the focus of this paper.

Using the original model, Kligman and Mills stated that petrolatums and mineral oils from different sources were uniformly comedogenic (1). With the updated model, it turns out that these were "false positives" (see below). This is far more than an academic controversy since it strongly impacts on the credibility of safety claims for skin care products.

Seborrhoea, the excessive production of sebum, is a prerequisite for the development of acne (8). Persons with seborrhoea are also greatly discomforted and try to remove excess oil by frequent washing or by oil-absorbing papers.

It is understandable that acne patients intuitively wish to avoid oily and greasy cosmetics. Indeed, it has become the universal mantra for cosmetic manufacturers to claim that their products are "oil-free." Dermatologists, too, routinely proscribe oil-containing facial products for acne sufferers. Patients are advised to read the labels and avoid medicaments and cosmetics that contain "oils." The assumption is that oily substances are intrinsically comedogenic. A justification for this belief stemmed from an earlier publication that described pomade acne in Afro-American men (9). Adult black men use a variety of greasy products daily to groom curly, kinky hair. Dense crops of open and closed comedones sometimes develop on the glabrous skin adjacent to the scalp.

The case would seem to have been made for a general warning against products that contain greases and oils. As a matter of fact, the shelves are now loaded with a wide variety of products that claim that they are "oil-free." "Oils," however, comprise a great variety of chemically unrelated materials. The implication is that viscosity (greasiness) alone determines comedogenicity.

Morris and Kwan have also become vexed by contradictory reports and question the usefulness of the rabbit ear test for formulating non-comedogenic cosmetics (10).

The present study was undertaken to seek a resolution of these disputes. Petrolatum was selected as the centerpiece of this investigation for two reasons: (1) Petrolatum is the

archetypical hydrophobic grease, and (2) it is widely used in a great variety of topical products for varied purposes. It behooves us to have accurate information about its potential for producing adverse effects.

METHODS

SUBJECTS AND TREATMENTS

Paid volunteers were recruited from a single district in South Philadelphia. Each participant had persistent, moderate acne of at least two years duration. The criteria for entry was at least ten papulo-pustules and 15 comedones. Both mild and severe cases of acne were excluded.

There were two groups of ten each. In each group there were six males and four females, with an average age of about 18 (range 14 to 22). A variety of OTC products had been used by most subjects with varying efficacy. Anti-acne medications were stopped one month before starting the study.

One group received a product consisting of a highly refined petrolatum, twice daily, to the entire face for eight weeks. The second group received a cream product that is an aqueous emulsion containing 30% petrolatum.

The identity of the products was masked to the subjects by putting the contents into new unmarked jars. The volunteers were told that the treatments, though greasy, might be beneficial in soothing acne. We indicated that the use of oily formulations in acne was a new approach and that this was a preliminary study to evaluate efficacy. The subjects were paid a modest fee for their participation.

We showed each subject how to apply a pea-sized amount to both sides of the forehead, followed by spreading with the fingers over the entire face. The face was washed with a mild soap prior to each application. The study was carried out in the winter months, when the greasiness of the products would be less discomforting.

OBSERVATIONS

Lesion counts of the entire face were made at baseline and after eight weeks. A dermatologist experienced in the method counted all comedones, open and closed, recording these together as a single category. Likewise, papules were not separated from pustules, and were simply categorized as papulo-pustules.

Microcomedones were estimated by the cyanoacrylate follicular biopsy technique at 0 and 8 weeks, following the quantitative image analysis methodology of Pagnoni and Kligman (11,12). Briefly, a drop of cyanoacrylate (Krazy Glue) was applied to the mid-forehead and spread out under a plastic slide. After hardening of the polymer in about two minutes, the slide was gently lifted off, carrying with it a thin sheet of the outer horny layer with attached vellus hairs. Those hairs that were encased by horny casts as observed under the stereomicroscope were considered to be microcomedones. The density of microcomedones was expressed as the number per square centimeter.

Global estimates of improvement were made by the subjects and by the dermatologist

at 0, 4, and 8 weeks, using the following grading system: worse, no change, slight improvement, moderate improvement.

STATISTICS

Changes in lesion counts from baseline were assessed by Student's t-test.

RESULTS

Changes from baseline are shown in Figure 1 for microcomedones, visible comedones, and papulo-pustules. A slight but insignificant decrease in visible comedones was noted in both groups. Likewise, the slight decrease in the density of microcomedones was not statistically significant.

By contrast, in both groups papulo-pustules decreased significantly ($p = <0.05$). Global self-assessments yielded the following data:

PETROLEUM JELLY:

- Three recorded no change.
- Six recorded slight improvement.
- One recorded moderate improvement.

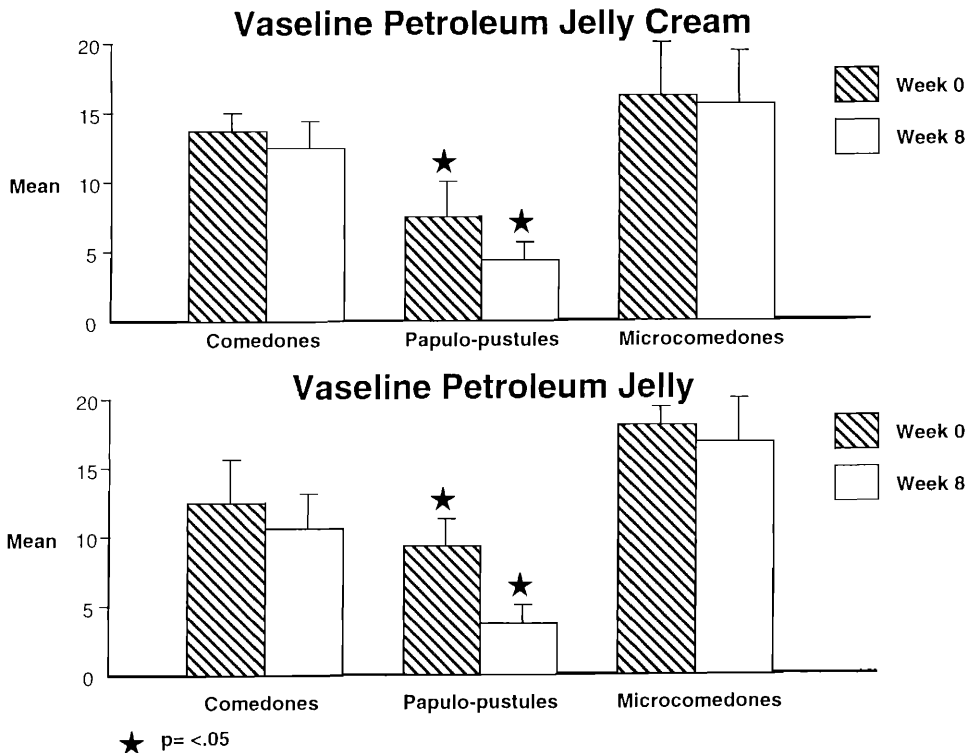


Figure 1. Effect of petrolatums on acne lesion counts after twice-daily applications for eight weeks. Comedones were not affected. Both products significantly reduced papulo-pustules.

PETROLEUM JELLY CREAM:

- Two recorded no change.
- Six recorded mild improvement.
- Two recorded moderate improvement.

The dermatologists' assessments at eight weeks roughly corresponded to the patients' estimates, except that the grades were somewhat lower. No case of worsening was observed.

DISCUSSION

The salient finding in this study is that Vaseline petroleum jelly was not comedogenic when applied twice daily to adolescents with active acne. The emulsion containing 30% petrolatum behaved similarly.

It is well known that acne patients will develop comedones fairly rapidly when potent comedogens, such as crude coal tar, are applied (8). We have found that even moderately comedogenic substances such as esters of fatty acids (for example, undiluted isopropyl myristate) will induce microcomedones on the forehead of patients with active acne when applied twice daily for eight weeks (unpublished observations). The discussion that follows is an attempt to bring some perspective into what has been an area of frustrating contentiousness.

In the 1972 "acne cosmetica" study, the test formulations were inserted inside the ear canal, based on the fact that the follicles there are larger and more numerous. What was not appreciated at the time was that in this protected site loose horny material is commonly retained in the follicular lumens, mimicking comedones. We failed to distinguish between the dense compact horn that comprises a microcomedo, a pathologic impaction, and the normal accumulation of loose horny squames. A more serious error was our failure not to biopsy the opposite untreated ear. This is a necessary control in order to provide a reference for judging the amount of horny material in the treated ear. Without a control biopsy, many materials will be falsely labeled as comedogenic.

Our current guidelines are as follows: The test substances are applied to the underside of the pinna in the concave area just external to the ear canal (7). An inch-long ellipse is excised for histologic evaluation, followed by semi-serial sectioning. This allows many more follicles to be visualized. It should be noted that the follicular population is heterogeneous. As in humans, individual follicles vary considerably in size and responsiveness. The final grading is a global estimate of all the follicles within the section. This is, of course, a subjective estimate. Quantification can be obtained by preparing whole, heat-separated epidermal mounts (13). The diameters of all the follicles can be scanned, enabling calculation of the mean diameter. The daily exposures have been extended from two to three weeks. Using this new procedure, including proper controls, petrolatum was reevaluated for comedogenicity. None of five samples from different producers, including yellow and white petrolatums, were found to be comedogenic.

It is reassuring for the validity of the method that "red veterinarian petrolatum," used as a sunscreen in World War II was found to be strongly comedogenic. This is an

extremely crude grade of petrolatum. It is also reassuring that certain lanolin derivatives such as acetylated lanolin were also comedogenic. These examples validate the specificity of the revised assay.

Attention is called to the reliability of the cyanoacrylate follicular biopsy, assisted by image analysis, for identifying comedogenic substances in humans. When applied twice daily for eight weeks to the foreheads of oily, acne-prone persons, comedogenic materials such as acetylated lanolin and isopropyl myristate increased both the density and size of horny casts (unpublished observations). Thus, there is encouraging evidence that the results of rabbit and human tests are congruent. There is too little awareness that the risk of inducing comedones is concentration-dependent. Substances that are strongly comedogenic when tested neat or in high concentrations become non-comedogenic after sufficient dilution. Neat isopropyl myristate and neat acetylated lanolin are definitely comedogenic. However, the concentrations in facial products are generally well below 15%. At this level, neither agent was found to be comedogenic in either the rabbit or human model (unpublished observations). One cannot determine from a reading of the ingredients whether a given product will be acnegenic or not. What matters solely is the behavior of the product itself.

In the current study both petrolatum and the 30% petrolatum emulsion product significantly reduced the number of papulo-pustules. This is a fairly typical result for non-medicated vehicles. It is of surpassing interest that in double-blind, vehicle-controlled, anti-acne studies, the beneficial effect of the vehicle often approaches that of the active agent! Mills and Kligman reviewed worldwide reports on the efficacy of dozens of anti-acne medicaments (14). There was not a single instance in which the test substance was found to be without benefit. Acne nearly always improves under medical care, regardless of the agent. This is the reason why so many marginally effective agents can be sold in large quantities.

Soreness and tenderness is a frequent complaint of patients who have many inflammatory lesions. Most of the patients in this study volunteered that soreness was moderated by both products. For over a century petrolatum has been extensively used to treat a variety of skin ailments such as burns, abrasions, rashes, and dry skin. Petrolatum also has protective actions against soap and solvent damage and against irritating drugs like tretinoin (unpublished observations). These uses probably reflect its ability to enhance the barrier function of the stratum corneum and perhaps to promote healing. It has been shown that petrolatum becomes incorporated into the intercellular lipid domains between corneocytes, thereby becoming part of the structure of the horny layer (15).

In the original, "acne cosmetica" report, half of the cosmetics were found to be comedogenic. This was proffered as the explanation for the relatively high frequency of acne in post-adolescent women. Several studies have shown that as many as one-third of adult women suffer from "breakouts" especially premenstrually (16,17). "Breakouts" are typical acne lesions, mostly incited by the rupture of microcomedones (18).

It is now highly questionable whether cosmetics are the chief cause of post-adolescent female acne. Reputable manufacturers of a great variety of facial skin care products, moisturizers, sunscreens, foundations, lotions, and toners routinely screen their products for comedogenicity. Women have been taught to look carefully at the label to make sure that the product is "non-comedogenic." Despite these efforts, the prevalence of post-adolescent female acne has, in my experience, increased rather than decreased (19).

Professional adult women seem especially vulnerable to episodic "breakouts," often lasting for decades into menopause. By contrast, acne is uncommon in post-adolescent males. I have ventured the speculation that post-adolescent female acne is due to release of androgenic corticosteroids, induced by work-associated stress, superimposed on household duties and marital commitments. The work of Lucky *et al.* has made it clear that the onset of acne in prepubertal girls is closely correlated with the serum level of adrenal androgens, notably dehydroepiandrosterone (20). Stress is a well known stimulus for the synthesis of adrenal androgens. It must also be said that genuine acne cosmetica has not altogether disappeared. There are literally hundreds of producers of cosmetic products throughout the world, and many do not adequately test their wares. I have randomly collected from the pocketbooks of my patients a surprising number of exotic skin care products by unregistered foreign manufacturers. Some of these were comedogenic in the rabbit assay.

Petroleum jelly is an exceedingly complex mixture of hundreds of saturated hydrocarbons. It is produced by the fractional distillation of petroleum. During the process, petrolatum remains in the bottom portion as a semisolid, bluish-colored material. It is then refined exhaustively to remove color, aromatic hydrocarbons, odoriferous materials, and other "impurities." Petrolatum is known generically as soft white paraffin. Specifications for acceptability are given in the U.S. Pharmacopea. Vaseline® is the trade-name of petrolatum made by Chesebrough-Ponds. Plewig *et al.* in their report on pomade acne in Afro-American males, specifically incriminated Vaseline® among other greasy grooming products (9).

Herein lies an interesting story that is worth recounting. It turns out that Vaseline has become a generic term for almost any hydrophobic grease. Recently, we bought "Vaseline" at six local pharmacies whose customers are mainly Afro-Americans. In only three stores was the product bona fide Vaseline®. The other three were Vaseline-like greases of unknown composition made by unfamiliar manufacturers. We found these three to be comedogenic in the revised rabbit model. Accordingly, in reports that incriminate Vaseline as comedogenic, one must check the source.

This study is not intended as a recommendation of petrolatum for the treatment of inflammatory acne vulgaris. However, it does show the benefits of moisturization for inflammatory acne.

Dermatologists routinely inveigh against oily cosmetics for acne sufferers. Patients are urged to seek oil-free cosmetics. This advice has not been supported by scientific studies. Comedogenicity has nothing whatever to do with oiliness; the latter is a physical attribute and not a chemical entity. Some of the most potent comedogenic substances, dioxin for example, are non-oily. On the other hand, many classical oils, notably vegetable oils, are usually non-comedogenic.

Chemical structure determines comedogenicity. As yet, acnegenicity cannot be predicted by structure. The mechanism underlying comedogenicity is unknown. Why corneocytes stick to each other to form horny impactions is still a mystery.

REFERENCES

- (1) A. M. Kligman and O. H. Mills, Acne cosmetica, *Arch. Dermatol.*, **106**, 843 (1972).
- (2) A. Zatulone and N. A. Konnerth, Comedogenicity testing of cosmetics, *Cutis*, **39**, 591 (1987).

- (3) S. B. Frank, Is the rabbit ear model prophetic of acnegenicity, *J. Am. Acad. Dermatol.*, **6**, 373 (1982).
- (4) J. A. Fulton, Comedogenicity and irritancy of commonly used ingredients in skin care products, *J. Soc. Cosmet. Chem.*, **40**, 321 (1989).
- (5) F. P. Nelson and J. R. Rumsfield, Cosmetics, contents and function, *Int. J. Dermatol.*, **27**, 665 (1988).
- (6) Z. D. Draelos, Assessing the complexity of cosmetic ingredients, *Cosmet. Dermatol.*, **7**, 17 (1994).
- (7) A. M. Kligman, Improved procedure for assessing acnegenicity (comedogenicity) in the rabbit ear model, *J. Toxicol. Cutan. Ocul. Toxicol.*, **4**, 395 (1990).
- (8) G. Plewig and A. M. Kligman, *Acne and Rosacea*, 2nd ed. (Springer-Verlag, New York, 1993).
- (9) G. Plewig, J. E. Fulton, and A. M. Kligman, Pomade acne, *Arch. Dermatol.*, **101**, 580 (1970).
- (10) W. E. Morris and S. C. Kwan, Use of the rabbit ear model in evaluating the comedogenic potential of cosmetic ingredients, *J. Soc. Cosmet. Chem.*, **34**, 215 (1983).
- (11) O. H. Mills and A. M. Kligman, The follicular biopsy, *Dermatologia*, **167**, 57 (1983).
- (12) A. Pagnoni, A. M. Kligman, and S. el Gammal, Determination of density of follicles on various regions of the face by cyanoacrylate biopsy, *Br. J. Dermatol.*, **131**, 862 (1994).
- (13) J. A. Mezick, G. Thorne, M. C. Bhatia, L. M. Shea, and R. J. Capetola, "The Rabbit Ear Micro-comedo Prevention Assay," in *Models in Dermatology*, Vol. 3, H. I. Maibach and N. J. Lowe, Eds. (S. Karger, Basel, 1987), p. 68.
- (14) O. H. Mills and A. M. Kligman, Drugs that are ineffective in the treatment of acne vulgaris, *Br. J. Dermatol.*, **128**, 371 (1983).
- (15) R. Ghadially, L. Halkier-Sorensen, and P. M. Elias, Effects of petrolatum on stratum corneum structure and function, *J. Am. Acad. Dermatol.*, **26**, 387-396 (1992).
- (16) B. A. Newman and F. F. Feldman, Adult premenstrual acne, *Arch. Dermatol. Syph.*, **69**, 350 (1954).
- (17) E. Epstein, Incidence of facial acne in adults, *Dermatol. Digest*, **7**, 49 (1968).
- (18) A. M. Kligman, Post-adolescent acne in women, *Cutis*, **48**, 75 (1991).
- (19) A. M. Kligman, A critical look at acne cosmetics, *J. Cutan. Aging Cosmet. Dermatol.*, **2**, 109 (1989).
- (20) A. W. Lucky, F. M. Biro, G. A. Huster, A. D. Leach, J. A. Morrison, and J. Ratterman, Acne vulgaris in premenarchy girls, *Arch. Dermatol.*, **130**, 308 (1994).