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Model System for the Investigation of Dandruff

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Synopsis—A model system has been described for the production of a DANDRUFF-like syndrome on GUINEA PIGS. This system, in addition to SCALP ORGANISMS, requires the presence of a LIPID mixture. The sloughing reaction observed is probably due to the liberation of free FATTY ACIDS and the relative amount of C_{18} MONOENOIC ACID increase at the irritated site. The replacement of the microbial moiety of the test system with a C_{18} monoenoic acid (oleic) also produces a similar type of desquamation and strongly suggests that this or a similar compound may be responsible for the sloughing reaction.

Introduction

The noninflammatory, scaling desquamation of the horny layers of the human scalp, commonly known as dandruff, has been under investigation for a considerable period of time. Despite the plethora of investigational effort which has been devoted to this disease, there remains much disagreement concerning its etiology.

One of the major obstacles to definitive investigations of the etiological aspects of dandruff has been the lack of a suitable and meaningful experimental model. Previous attempts to use humans and/or laboratory animals for this purpose have met with only indifferent success.

Although humans have been used as test animals for numerous studies, they possess certain, inherent disadvantages as suitable subjects, the greatest of which is the almost universal occurrence of dandruff. This ubiquity dictates that the scalp must be "cleared" of naturally occurring dandruff by artificial means before test conditions can be imposed upon it, thus creating a less than desirable experimental model (1).

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Kile and Engman (2) described studies in which they inoculated humans with live and killed *Pityrosporum ovale,* an organism commonly implicated as the causative agent of dandruff (3). The most extensive scaling was produced by rubbing a viable culture of *P. ovale* on the intact scalp skin. Other workers (4, 5) have also inoculated humans and with at least some success; however, as Rocha *et al.* (6) point out, there seems to be considerable doubt concerning the identification of the fungus used in these studies. Emmons (1) states that the trauma incident to intracutaneous injection and scarification, which was a characteristic of many of the preceding studies, often results in some scaling and pigmentation without the inoculation of scalp organisms. Therefore, interpretation of data from many of these studies is difficult. In studies with a confirmed culture of *P. ovale* inoculated on the scalp and back of test subjects, Emmons could not show the presence of lesions on the back and could demonstrate no increase in the severity of scalp lesions. From these data, he concluded that *P. ovale* is a saprophyte of the scalp with no etiological significance in seborrhea. Rocha *et al.* (6) applied *P. ovale* in a lanolin paste to scarified and nonscarified areas of human backs and also injected suspensions of live *P. ovale* cells intradermally into the same skin areas. In all cases, after a short incubation period characterized by erythema and induration, these symptoms disappeared with no further reaction or scaling. Martin-Scott (7) applied *P. ovale* to the skin of human volunteers under a nylon adhesive dressing with similarly negative results.

The use of laboratory animals to simulate dandruff-like conditions has been rather limited. Martin-Scott (7) injected concentrated cell suspensions of *P. ovale* into mice and rabbits with no signs of pathogenicity and no evidence of antibody formation. Durfee and Cousins (8) were somewhat more successful in their attempts to produce dandruff with a *P. ovale* culture applied to the scarified skin of rabbits. These workers found that the induced infections were controlled with a number of antiseptic materials. Leone (9) was unable to produce a pathogenic reaction when *P. ovale* was inoculated into human and guinea pig skin. He concluded that *P. ovale* is a saprophyte of normal skin and that any increase in the pathogenicity of this organism is connected with an increase in the lipid content of the scalp. Spoor (10) also considers lipids to be important in the production of dandruff on animals and concludes that the high level of skin fat or sebum on humans provides an environment for the initiation of dandruff.

If the assumption is made that dandruff is indeed a response to an infectious process, then the need for a suitable, experimental animal system to determine the etiological aspects of this process becomes obvious. It is the object of this report to describe the development of such a system and some experimental parameters which govern it.

Experimental

Animals

The animals used in this work were mature albino guinea pigs of at least 12 months age. An area approximately 7 × 14 cm was clipped on the dorsal surface of the test animals no longer than 24 hours before the application of the test materials. The shaved area was normally divided into 4 or 6 spaces, delineated with a felt marking pen. A record of the history of applications to each animal was maintained which detailed the type and date of applications and the reactions noted. Normally 3 or 4 replicates of each treatment condition were run on different animals.

Test Systems

In most cases, the experimental systems included a lipid mixture designed to mimic natural human sebum. The formula for this mixture is shown in Table I. A 0.2-ml single application of this mixture plus the experimental material was placed in a circular area, 25 mm in diameter, within each delineated space on the shaved back of the guinea pig. The progress of each test was followed daily with a final reading of the degree

Table I
Artificial Sebum Formula

	%, w/w
Oleic acid	15.2
Coconut fatty acids	1.5
Middle cut fatty acids (Hydrofol acids 580)[a]	13.7
Coconut oil	3.0
Lard	27.3
Lanolin	20.3
Cholesterol	1.9
Squalene	4.0
Wheat germ oil	1.0
Petrolatum	8.0
Glycerol	4.1

[a] Ashland Chemical Co., Columbus, Ohio.

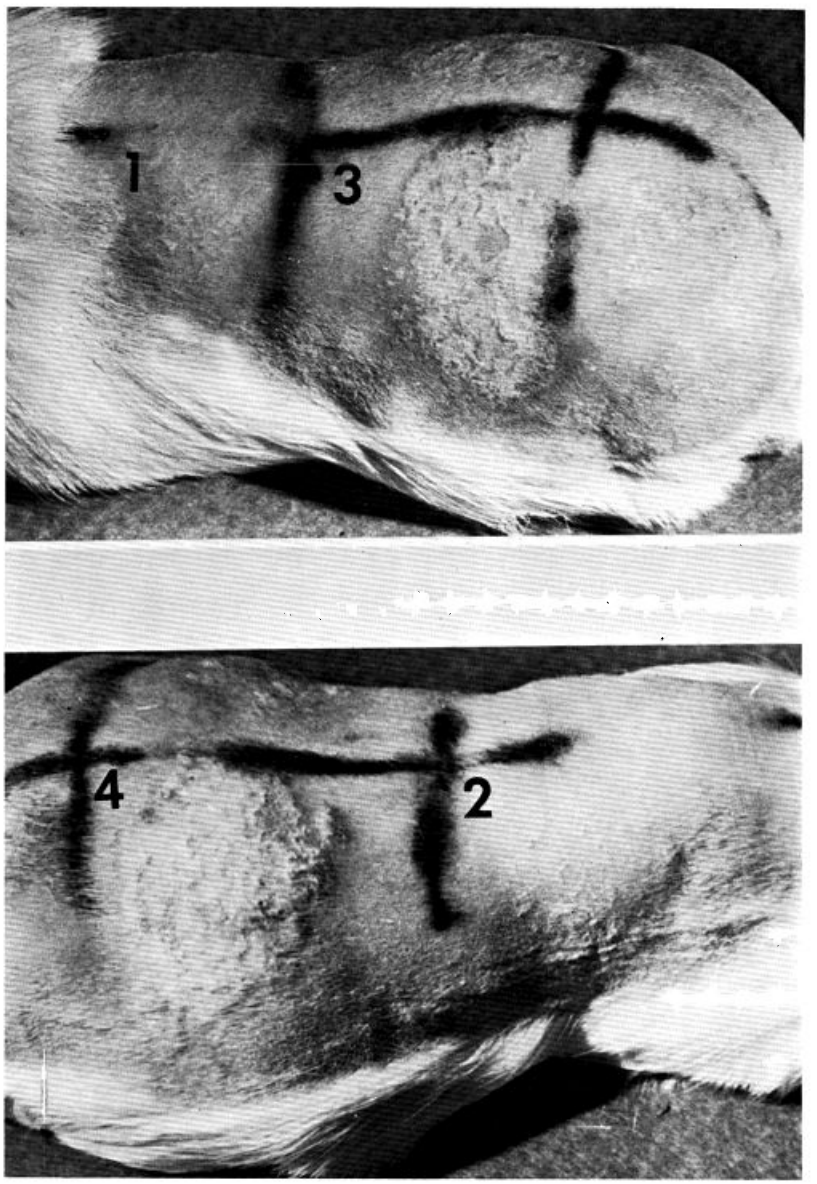

Figure 1. Illustrations of guinea pig sloughing produced by increasing increments of *P. ovale* cells added to artificial sebum. Photographs taken 5 days after initial application of the cell plus lipid mixture. Quadrant numbers refer to subjective severity score in each quadrant

of sloughing being recorded 5 days after application. The sloughing reaction was graded subjectively on a 0–4 scale after the treated areas were reclipped, with the most severe reactions being given the highest score. Photographs showing the severity of sloughing relative to scores are shown in Fig. 1.

Oleic acid (reagent grade) was applied to the test sites at the indicated concentrations in a propylene glycol vehicle. Control sites indicated that the vehicle was not irritating.

Organisms

Representative strains of the major constituents of the scalp microbial population were utilized in this study. These strains were isolated from scalp washings obtained by placing a glass collar (25-mm diameter) firmly in place on the scalp and scrubbing with 5 ml of 0.05*M* phosphate buffer (pH 7.0) for 30 sec. Fresh cell preparations harvested from broth media were routinely used.

The *P. ovale* strain was isolated using the medium described in Table II.

Table II

Formula of Medium for Growth of *P. ovale* and Scalp Diphtheroid

	g/l.
Trypticase soy broth (BBL)	30.0
Tween 40	1.0
Oleic acid	0.1
1*N* Lactic acid	20.0
Streptomycin sulfate[a]	0.005
Penicillin G[a]	0.045
Cycloheximide[a]	0.025
pH 6.0	

[a] Omitted from diphtheroid medium.

For growth and maintenance of pure cultures, the antibiotics in the above formulation were omitted. Incubation was normally for approximately 14 days at 37°C at which time the cells were harvested by centrifugation or filtration.

Diphtheroids were cultured on the above medium minus antibiotics. In every instance, these organisms were grown anaerobically at 37°C and harvested by centrifugation after 5 days of incubation. These cultures have been identified as *Corynebacterium acnes*.

Cocci were classified in accordance with the Baird-Parker (11) system of classification. The organisms conformed to the characteristics of *Staphylococcus* subgroup II. Growth was in Brain Heart Infusion (Difco) medium at 37°C with the cells harvested by centrifugation after 48 hours of incubation.

Unless otherwise specified, organisms were added to artificial sebum at a concentration of 100 mg/g. The microbial concentrate was obtained by centrifuging growth medium at 10,000 rpm in a refrigerated centrifuge for 20 min. The spent medium was decanted and the centrifuged cells were then weighed and added directly and with vigorous stirring to the artificial sebum. This mixture was then applied to the delineated areas on the dorsal surface of the guinea pig.

Fatty Acid Analyses

Extractions of guinea pig skin were carried out by placing a glass collar 25 mm in diameter firmly on the test site. A 5-ml volume of 4:1 diethyl ether:methanol was placed within the collar and the area was scrubbed for 30 sec with a glass rod flattened at the tip. The extract was then withdrawn and filtered and the constituent free fatty acids were converted to the corresponding methyl esters by the methods of Metcalf *et al.* (12) Gas chromatography was carried out with an F&M model 720 dual column gas chromatograph* utilizing a thermal conductivity detector. A 10-foot stainless steel column of 1/4-in. diameter, packed with a 1:9 mixture of diethylene glycol succinate† and 60/80 mesh Gas Chrom P,‡ and operating at a temperature of 185° or 190°C, was used in these studies.

Results

Early experiments had shown that the addition of buffered suspensions of scalp microorganisms directly to guinea pig skin produced no visible irritation or other reaction. In view of the high concentrations of fatty materials on the human scalp and the relatively low concentrations of lipid on guinea pig skin, an attempt was made to approximate more closely the conditions existing on the human scalp by supplementing the guinea pig skin lipids with the artificial sebum formulation. The various strains of scalp organisms were then admixed with the ar-

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‡ Applied Sciences Laboratories, State College, Pa.

Table III
Effect of Cell Suspensions on the Initiation of Guinea Pig Sloughing in the Presence and Absence of Artificial Sebum

	Sloughing Reaction	
	Antibiotic[a] Treated	No Antibiotic
P. ovale + saline	0	0
P. ovale + artificial sebum	3	3
Staphylococcus JTM + saline	0	0
Staphylococcus JTM + artificial sebum	1	0
Diphtheroid + saline	0	0
Diphtheroid + artificial sebum	2	2
Control, artificial sebum	0	0

[a] 0.05% solution of tetracycline.

tificial sebum before application to the guinea pig. The results (Table III) show that suppression of the natural flora of the guinea pig skin does not appear necessary for a positive test reaction and that concentrated *P. ovale* cells produced the strongest reaction of the three groups of microorganisms tested.

The most important conclusion from these data is that the artificial sebum must be present before the microorganisms tested can induce sloughing. The sloughing reaction appeared within 4 to 5 days and reached maximal severity within 6 to 7 days. Under normal circumstances the reaction began to subside within an additional 10 days, with total clearing normally occurring within 2 to 3 weeks from the time of initial application. The scales observed were large, waxy segments of stratum corneum resembling human dandruff to the unaided eye. Microscopically, the guinea pig skin scales bore a remarkable resemblance to human dandruff scales. Thickened, horny, keratin layers were observed in both human dandruff and the sloughed guinea pig skin flakes.

Attempts to accelerate and/or accentuate the sloughing reaction by preabrading the skin or applying the irritant mixture to other areas of the guinea pig were unsuccessful. Koch's postulates have been fulfilled repeatedly using either the *P. ovale* or diphtheroid cell suspensions in artificial sebum. There were no obvious differences in micro- or macroscopic appearance traceable to the type of inoculum used.

The relative ability of representative scalp microorganisms to produce sloughing is shown in Fig. 2. It is apparent that the staphylococcus was unable to produce appreciable sloughing with even very high cell concentrations. The suspension of *P. ovale* was the most effective in this

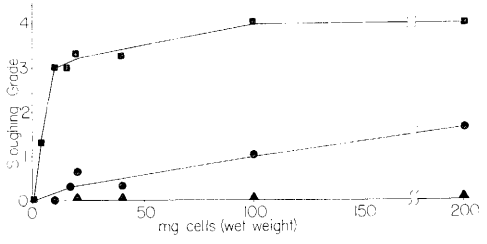

Figure 2. Effect of cell concentration (mg cells per gram of artificial sebum) on sloughing severity

● Diphtheroid
▲ Staphylococcus
■ *P. ovale*

respect, with a maximum sloughing reaction obtained with 100 mg of cells (wet weight) per gram of artificial sebum matrix. Other studies have shown that the addition of combinations of the three microbial types to the lipid matrix did not appear to produce more than an additive effect.

The above data showed that a microbial inoculum was required to produce a change in the artificial sebum which could induce sloughing. A possible source of this material was fatty acids released by microbiological lipolysis of the artificial sebum triglycerides. Gas chromatography data pertaining to extracts of irritated areas treated with organisms plus artificial sebum are shown in Table IV. These data show a

Table IV

Relative Composition of the Free Fatty Acid Component of Extracted Guinea Pig Irritation Sites

Carbon Chain Length	Control (AS[a] only)	*P. ovale* + AS	Diphtheroid A + AS
10	1.41	0.31	0.44
12	2.23	0.98	4.34
14	14.39	7.56	9.52
16	19.52	21.15	24.75
16 (1═)	1.63	2.45	2.29
18	9.92	12.79	10.30
18 (1═)	21.98	38.47	36.06
18 (2═)	3.01	4.32	3.49
20	3.44	1.66	1.73
22	0.83	0.45	0.17
24	8.69	7.18	5.66
Irritation grade	0	3	2

[a] AS = artificial sebum.

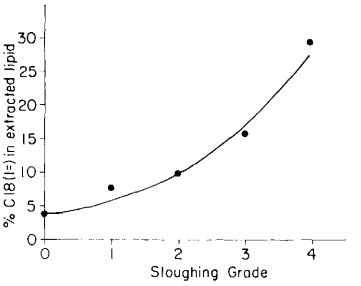

Figure 3. Relative concentration of C_{18} monoenoic acid in ether:methanol extracts of sloughing sites irritated with increasing amounts of *P. ovale* cells

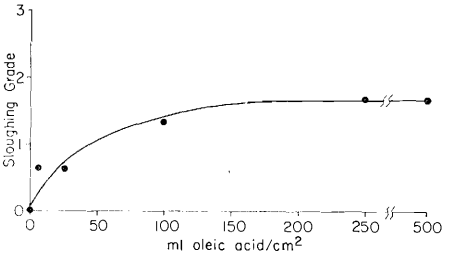

Figure 4. Effect of increasing concentrations of oleic acid on relative sloughing grades

marked increase in the relative content of C_{18} monoenoic acid in extracts from those sites (*P. ovale* and diphtheroid) which produced extensive sloughing. Further data (Fig. 3) indicate that in the case of *P. ovale*–irritated sites, the degree of desquamation correlated with the concentration of C_{18} monoenoic acid in the extracts of the sites. Addition of C_{18} monoenoic acid (oleic) to artificial sebum alone produced a definite irritation and sloughing (Fig. 4) which appeared grossly similar to that induced by microorganisms in artificial sebum. Other C_{18} monoenoic acids such as vaccenic, elaidic, and petroselinic when similarly tested produced comparable sloughing at approximately the same concentration as oleic.

Concentrated extracellular products obtained by dialysis of centrifuged, spent culture medium in a vacuum chamber similarly produced increases (Table V) in relative concentration of C_{18} monoenoic acids and, in the case of the diphtheroid, an increase in C_{18} dienoic acid content.

Table V

Effect of Concentrated Extracellular Products of Scalp Microorganisms on Sloughing and Fatty Acid Content of Artificial Sebum

	Quantity of Extracellular Product Per Gram of Artificial Sebum			
	P. Ovale		Diphtheroid	
	0.1 ml	1.0 ml	0.1 ml	1.0 ml
Av sloughing grade	1.7	1.7	1.3	1.7
% Change in free fatty acids	−0.9	+.4	+1.1	+3.9
% Relative increase in C_{18} (1═)	2.86	8.66	2.52	4.77
% Relative increase in C_{18} (2═)	None	None	0.45	4.39
Tributyrinase activity, μg/min/ml	...	0.9	...	2.5

Discussion

The observed guinea pig sloughing reaction initially develops as patent erythema appearing within 3–4 hours after application of the lipid-microorganism matrix. The erythematous reaction is followed by desquamation of the stratum corneum not unlike the primary irritation produced by sodium lauryl sulfate. Guinea pigs appear to be particularly suitable as test animals because they react to the lipid system with a greater degree of predictability than other common laboratory animals: they are docile and restraints are unnecessary. In cases of extremely severe irritation, open lesions were occasionally observed which were the result of scratching the irritated area by the animal. The lesions normally healed uneventfully and did not impair the usefulness of the particular animal in subsequent testing. Normally, a recovery period of 6 to 7 weeks following initial test applications was allowed before the animal was again subjected to testing.

The most severe sloughing reactions appeared when *P. ovale* was admixed to the artificial sebum. Although this organism did not extensively hydrolyze tributyrin *in vitro*, it did appear to produce a greater relative increase in C_{18} monoenoic acid than the more actively lipolytic but less irritating diphtheroid. The demonstrated ability of *P. ovale* to produce guinea pig sloughing would appear to agree with the recent work of Gosse and Vanderwyk (13) who found that application of the antibiotics nystatin and neomycin to the scalps of 11 human subjects produced a 63.4% average reduction in dandruff. The subsequent deliberate recolonization of these subjects with a nystatin-resistant *P. ovale*

mutant resulted in an average increase of 88.1% in dandruff production, thus indicating the importance of this organism as an etiological agent.

The appearance of appreciable levels of free fatty acids in the artificial sebum was most probably the result of the hydrolytic activities of the microbial component of the system. Although fatty acids were present in the artificial sebum to which microorganisms were not added (Table IV), it appeared that an additional quantity of free fatty acids was required, particularly C_{18} monoenoic acid, to initiate the reaction. This would suggest that some manner of fatty acid threshold must be exceeded to initiate sloughing. These data were further confirmed when cell-free fractions of extracellular medium constituents, containing demonstrable tributyrinase activity, produced an increase in per cent of C_{18} monoenoic acid and total free fatty acid content, and prolonged sloughing of at least moderate severity (Table V). It is thus apparent that extracellular lipases were capable of producing irritation in the absence of the organisms which initially synthesized them.

The implications of these data for human dandruff are obvious. Human sebum has been shown by Kellum (14) to be free of fatty acids as it is secreted by the sebaceous gland, yet appreciable levels are present in surface lipids. The probable source of these fatty acids is sebum triglycerides hydrolyzed by microbial enzymes since the three groups of organisms most commonly found on the scalp, *P. ovale,* diphtheroids, and staphylococci, are competent producers of lipase (15–17). Other data (18) have shown that fatty acids are irritants; however, our attempts to apply specific concentrations of certain fatty acids to the human scalp have been unsuccessful due to the difficulty of maintaining these concentrations on the scalp at all times. However, when attempts (Fig. 4) were made to produce guinea pig sloughing with the artificial sebum component which increases to the greatest degree on guinea pigs, oleic acid, a concentration-dependent sloughing reaction could readily be produced.

In addition, dandruff is a disease appearing usually at puberty when sebaceous secretions on the scalp are increasing rapidly. It can be speculated that the concurrent onset of dandruff and extensive lipid secretion are more than coincidental. It should be remembered, however, that other physiological alterations occurring at this time could possibly induce dandruff-like conditions.

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References

(1) Emmons, C. W., The isolation and pathogenicity of *Pityrosporum ovale, Pub. Health Rep.*, **55,** 1306 (1940).
(2) Kile, R. L., and Engman, R. F., Further studies of the relation of *Pityrosporon ovale* to seborrheic eczema, *Arch. Dermatol. Syphilol.*, **37,** 289 (1938).
(3) Reddish, G. F., The etiology of infectious dandruff, *J. Soc. Cosmet. Chem.*, **3,** 90 (1952).
(4) MacLeod, J. M. H., and Dowling, G. B., An experimental study of the *Pityrosporon* of Malassez: Its morphology, cultivation, and pathogenicity, *Brit. J. Dermatol.*, **40,** 139 (1928).
(5) Moore, M., and Kile, R. L., *Pityrosporum ovalis* as a causative agent of seborrheic dermatitis, *Science,* **81,** 277 (1935).
(6) Rocha, G. L., Silva, C., Lima, A. O., and Goto, M., Experimental studies on *Pityrosporum ovale:* Its pathogenicity and antigenic capacity, *J. Invest. Dermatol.*, **19,** 289 (1952).
(7) Martin-Scott, I., The *Pityrosporum ovale, Brit. J. Dermatol.*, **64,** 257 (1952).
(8) Durfee, T., and Cousins, R. F., Practical tests of dandruff preparations, *Stand. Remedies,* **22,** 3 (1936).
(9) Leone, R., Presence and significance of *Pityrosporon ovalis* in pityriases of the scalp, in figured seborrheic eczema and in various squamous dermatoses, *Minerva Dermatol.*, **27,** 93 (1952).
(10) Spoor, H. J., Dandruff updated, *Amer. Perfum. Cosmet.*, **81,** 81 (1966).
(11) Baird-Parker, A. C., A classification of micrococci and staphylococci based on physiological and biochemical tests, *J. Gen. Microbiol.*, **30,** 409 (1963).
(12) Metcalf, L. D., Schmitz, A. A., and Pelka, V. R., Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis, *Anal. Chem.*, **38,** 514 (1966).
(13) Gosse, R. M., and Vanderwyk, R. W., The relationship of nystatin-resistant strain of *Pityrosporum ovale* to dandruff, *J. Soc. Cosmet. Chem.*, **20,** 603 (1969).
(14) Kellum, R. E., Human sebaceous gland lipids: Analysis by thin-layer chromatography, *Arch. Dermatol.*, **95,** 218 (1967).
(15) Weary, P. E., Comedogenic potential of the lipid extract of *Pityrosporum ovale, Ibid.*, **102,** 84 (1970).
(16) Kellum, R. E., Strangfeld, K., and Ray, L. F., Acne vulgaris. Studies in pathogenesis: Triglyceride hydrolysis by *Corynebacterium acnes in vitro, Ibid.*, **101,** 41 (1970).
(17) Troller, J. A., and Bozeman, M. A., Isolation and characterization of a staphylococcal lipase, *Appl. Microbiol.*, **20,** 480 (1970).
(18) Kellum, R. E., Acne vulgaris. Studies in pathogenesis: Relative irritancy of free fatty acids from C_2 to C_{16}, *Arch. Dermatol.*, **97,** 722 (1968).