CUTANEOUS PHARMACOLOGY AND TOXICOLOGY

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This is the first review of cutaneous pharmacology and toxicology in this series. The choice of topics is highly personal—based on my experience and interests. This has been a period of greatly increased activity in this field; space limitations make this approach mandatory.

IRRITATION

Irritation is defined as nonimmunologically mediated dermatitis resulting from contact with a chemical. Some older texts imply that irritant dermatitis occurs in all subjects on first exposure. Actually, this holds only for the severest of irritants sometimes found in industry and laboratories but not for most drugs and consumer products. Some but not all irritants are also contact sensitizers.

Recent advances in the understanding of irritant dermatitis are conceptual rather than mechanistic or technical. By custom, standard toxicology texts classify chemicals as irritant or nonirritant; perusal of the Merck Index gives numerous examples (1). This nomenclature remains useful for industrial chemicals. For cutaneous drugs and consumer products it is realistic to accept that under appropriately exaggerated circumstances all chemicals—even water—are irritating. Current bioassays utilize this principle; a new chemical or product is compared to a similar agent for which there has been extensive human use. Rather than list a numerical score with somewhat arbitrary limits for acceptability, the new agent may be judged more, less, or equally as irritating as the standard.

Another conceptual advance considers chemicals as having a cumulative irritancy potential rather than an irritancy potential from single exposure. When dermatitis develops after repeated exposures of weeks to years, contact sensitization is generally assumed. While sensitization *may* occur in this situation, cumulative irritancy is even more likely with numerous chemical classes.

The Draize, Woodard & Calvery assay enjoys general acceptance (2). Finkelstein, Laden & Miechowski noted the limitations of the Draize method and started the trend to repetitive applications (3). Kligman & Wooding adapted repetitive irritancy testing (10 applications) to man (4).

Lanman, Elvers & Howard extended this to 21 application days in man, employing an all-or-none reading of dermatitis as their criterion to determine whether irritation has occurred (5). This was modified by Phillips, Steinberg, Maibach & Akers to utilize a five-point grading scale each day (6). This latter study and a sequel by Steinberg et al provide the most complete documentation of the relationship of animal and human irritancy assays (7).

In general the rabbit assay yields good correlation for man with chemicals and concentrations at either extreme—a great deal of irritation or almost no irritation (6). The rabbit can be employed for further definition of the middle ground by repetitive applications. Comparative human and repetitive rabbit data are summarized in the recent studies of Steinberg et al, Marzulli & Maibach (6, 8).

The rabbit is the animal commonly used for these assays; undoubtedly many others would be adequate. The advantages are cost and ease of handling; most important, the rabbit skin develops erythema and induration that is easily quantitated and is similar to that of man. The guinea pig could be utilized for similar reasons (9).

If methods based on these principles were generally used, the frequency and severity of irritant dermatitis would decrease. It is commonly held that most nonindustrial dermatitis is on an immunologic rather than an irritant basis in nature. Our experience in diagnostic patch testing in a university-based contact dermatitis clinic and in predictive testing for irritation and allergy convinces us that the opposite is the case: cumulative irritation is much more frequent than contact sensitization, that is, with most consumer, skin-care, and cosmetic products.

PHOTOTOXICITY

Phototoxicity is a nonimmunologic, light-induced skin response (dermatitis) to a photoactive chemical likened to an exaggerated sunburn. The photoactive chemical may reach the skin by direct application or via the blood stream, following ingestion or parenteral administration. Whether systemically or topically administered, the chemical may require metabolic transformation to become photoactive. It is a form of irritation that requires light.

Biophysical aspects of the phototoxic reaction were reviewed by Harber & Baer (10). Pathak & Fitzpatrick characterize phototoxic substances (11). Epstein has reviewed the general aspects of photosensitization (12).

Until recently there was considerable confusion as to the mechanism of phototoxicity in man and the method of diagnostic proof. Reproduction of the disease was sporadic, suggesting the possibility of an allergic mechanism rather than irritation.

Clarification of the issues rests on two technical and conceptual phenomena: appreciation of percutaneous penetration of the chemical and an appropriate light source. With adequate penetration and sufficient light energy, phototoxicity is

ubiquitous with some phototoxic agents. Penetration can be obtained with intradermal injection, or by removal of much of the stratum corneum with repeated applications of a sticky tape (cellophane tape). The appropriate light source can either be natural sunlight with its erythema rays (below 3100 Å) removed by an appropriate filter (window glass or appropriate plastic film) or artificial light from which the erythema rays have been removed. The high intensity black light is a convenient source (13, 14).

With this information phototoxicity can readily be demonstrated in man in a predictive assay (13). In less permeable skin sites such as the forearm, it is necessary to remove the stratum corneum; in more permeable anatomic areas including the neck and scrotum, higher concentrations of the phototoxic agent may obviate the need for stripping.

Presumably almost all animals could be used as a model for predictive toxicity. With bergamot the hairless mouse and the rabbit appeared somewhat more sensitive than the guinea pig. The pig (swine) was less responsive. The squirrel monkey appeared resistant; the hamster showed changes requiring histologic examination as dermatitis was not noted on gross examination. In practical terms this allows for a more than reasonable selection of test animals for laboratories to choose from. Until additional experience with test animals is obtained, new chemicals likely to be used widely in man might also be examined in human skin.

Similar techniques are suitable for the identification of systemic phototoxic chemicals (15, 16).

What is not clear is whether clinical examples of phototoxicity that have escaped recognition will be found; for example, Hjorth & Moeller identified a peculiar eruption which they termed *bikini dermatitis* (17). They demonstrated the pathophysiology as phototoxicity secondary to a dye in a bathing suit. We suspect that other syndromes may have the same mechanism. A likely syndrome requiring such investigation is the cholasma or melasma appearing with greater frequency in men.

If industrial and government laboratories employed the current predictive methodology, photoxicity would become an antique relegated to ancient textbooks.

DIAGNOSTIC TECHNIQUES IN ALLERGIC CONTACT DERMATITIS

During the last decade dermatologists realized that standardization of the methods of patch testing was inevitable if the results obtained in various clinics are to be comparable. The initial effort of preparing a standardized technique was performed by Magnusson & Hersle (18).

Anatomical Site of Testing

The region used for testing may determine the number of weak reactions to a substance; thus, testing on the anterior thighs yields only 50% of the reactions obtained in the region of maximum reactivity on the upper back. Areas less suitable for testing are the medial surface of the upper arm and the whole surface of the calves.

Adhesive Tape

The concentrations used for patch testing are usually established on the results obtained by occlusive testing. If nonocclusive systems are used, fewer patients will develop irritation, but less of the test materials are absorbed, and false negative reactions may occur.

Some vehicles may impart an added occlusive effect to the tests performed under a nonocclusive tape. Liquids are particularly prone to give false negatives with porous tapes.

The concentrations used for testing are all-important. Usually the concentration required extensively exaggerates the exposure during conditions of use. Thus, a concentration of 5% nickel sulfate may be needed to reproduce a dermatitis caused by the few molecules of nickel released from nickel-plated bra clips.

The concentration selected for patch testing always represents a compromise intended to eliminate false negative reactions but also to avoid false positive reactions and sensitization.

Test Units

Many different test units have been devised by industry and by individual dermatologists. Few have been subjected to systematic comparative study. One study (Magnusson) revealed a striking incidence of false negative reactions with several test units used to the present day. In Magnusson's and Hersle's study AL-TEST (IMECO) was by far superior to the other units evaluated (18). This system ensures adequate contact between test substance and skin, adequate occlusion, and facilitates the reading by leaving an area of skin around the test material free of contact with the adhesive tape. A recent test unit devised by Pirilä is promising, by occupying a smaller area of skin. So far, this test unit has not been compared with those hitherto used.

Epidemiology

The incidence of sensitivity to lanolin, paraben esters, and other substances which are by themselves rarely sensitizing is determined by the number of cases of stasis dermatitis and leg ulcers referred for testing. Thus few cases are seen and tested, while testing is routine in Copenhagen and Munich. Several other sensitivities are particularly frequent in patients with leg eczema, such as reaction to balsam and to local anesthetics.

A study comparing the sensitivities found at six Scandinavian clinics showed that the 20 most frequent allergens were the same in all clinics, although their relative rank could vary. In a later study comparing the sensitivities demonstrated at a number of European clinics with those obtained in Scandinavia, benzocaine was found to be particularly frequent in Germany and Sweden, while sulfonamides were frequent sensitizers in Germany and in Italy where sulfonamides are commonly used for first aid treatment.

Education of the public has failed to reduce the incidence of contact dermatitis. Nickel garter clasps were abandoned, but not because of the risk of sensitization. Primula obconica dermatitis has become rare not because of a shortage of customers

for this plant but because of the reluctance on the part of the florists to stock a plant that carries a well-known risk to the shop employees. If a strong sensitizer is inadvertently marketed by a manufacturer, extensive damage may be avoided by alerting the public through radio or TV. To my knowledge this has only happened once, namely in Norway when a dishwashing agent containing a sensitizing impurity had given rise to a series of cases of dermatitis shortly after it had been released for general sale. Obviously most products of this character have been carefully screened before being put on the market. It is debatable whether a general alert should be given when medium or weak sensitizers are detected on the market. The phrasing of the warning would need to be unnecessarily frightening in order to persuade a housewife to discard a soap powder, toilet soap, etc.

In two epidemics in Denmark caused by an antiseptic toilet soap and an optic whitener in several soap powders it proved possible to withdraw the products within a few months after the cause of dermatitis had been detected.

The reader is referred to a series of collaborative research efforts documenting these points (18–27). These studies provide our first insights into the relative frequency of allergic contact dermatitis to a given agent; these are more satisfactory epidemiologic data than available from single investigator case reports. Much remains to be done before totally satisfactory epidemiologic data based on actual usage are available.

ALLERGIC CONTACT DERMATITIS: PREDICTIVE ASSAYS

Allergic contact dermatitis receives its share of enthusiastic effort in skin research. This serves at least two purposes: (*a*) aiding in the management of human allergic contact dermatitis and (*b*) serving as a convenient animal or human model of delayed hypersensitivity itself. The latter aspect receives more emphasis than the former. This review summarizes but one aspect of this broad area—the ability to predict the proclivity of a chemical to produce this syndrome in man. Although many animals including the mouse can be sensitized, the guinea pig remains the animal of choice for this purpose. The correct performance of the assay is fraught with conceptual and technical difficulty. Fortunately, in appropriately experienced laboratories the assay will correctly identify most moderate-grade human sensitizers (28). Numerous techniques have been reported, but few investigators have validated their model. The original methodologic studies of Landsteiner and Chase led to the officially accepted FDA test (29, 30). Subsequent modifications led to defined methods for applying the allergen topically (31); others have combined topical exposure with intradermal injection with Freund's adjuvant (31a–32). The most complete validation with man exists for the latter. It is likely that no single technique will be applicable to all classes of potential sensitizers; a more reasonable approach will be the identification of those factors requisite for a relevant assay for a given class.

All human sensitization assays are adapted from the guinea pig assay. A voluminous literature extols a dozen or so modifications; a current summary stresses that —as with the guinea pig assay—validation with standard sensitizers is essential (33). The test must be tested.

Most modifications stress either the number of patch test exposures, timing, or the addition of a use-test phase. The critical factor in avoiding false negative responses is the concentration tested (34, 35). By increasing the concentration to some multiple of the planned usage, the Draize test correlates well with the clinical sensitization experience. Kligman has employed this factor and added detergent-related enhancement in a maximization test. He currently has modified the operational steps attempting to avoid several previous pitfalls (36).

CUTANEOUS METABOLISM

Recent studies have shown that skin is indeed a metabolically vigorous tissue. Some aspects of its biochemical activities have been reviewed and discussed by Hsia (37), who pointed out that biochemistry of the skin is a potentially fertile field for new investigation. The ability of the skin to utilize glucose has been well documented (38–40) and the enzymes necessary for glycolysis and for pentose shunt have been demonstrated (41–43). It has been reported that glucose 6-phosphate dehydrogenase of rat skin can be effectively inhibited by the steroid, dehydroepiandrosterone (44). The study indicated that about half of the NADPH necessary for lipid synthesis in the skin might be supplied from the pentose shunt. It is interesting that the uptake of glucose by the skin is enhanced in vitro by insulin (45, 46), and by prostaglandin E_2 (47) although the mechanisms involved are not clear. It has also been shown that the skin is an active site for the synthesis of sterols (48, 49), prostaglandin E_2 (47), and a variety of other lipids (50–52). Of interest is the finding that lipid synthesis in the skin can be inhibited by clofibrate in vitro (53), suggesting that the skin may be a useful model tissue for evaluation of potential hypolipemic drugs.

There have been many studies on steroid metabolism in the skin in recent years. These studies are interesting because many biological activities of the skin and its appendages are regulated by steroid hormones, such as the enlargement of sebaceous glands in response to androgens (54), the relationship between the development of male pattern baldness and androgens (55), the swelling of sexual skin in female chimpanzees caused by estrogens, and detumescence by progesterone (56). In clinical dermatology, preparations containing corticosteroids are among the most frequently prescribed topical medications. It is now well established that the skin not only responds to steroid hormones, but also transforms them into various metabolites. Indeed, cutaneous metabolism of steroids has become an active field of investigation in the past decade. The hormones so far studied include the estrogens (57, 58), the androgens (59–64), the corticoids (65–67), and the progestins (68, 69). In each case, active metabolism has been observed. The subject of steroid metabolism in human skin has been reviewed (70, 71). It is significant that some of the metabolites formed in the skin have greater hormonal potency than the parent steroid; for example, dehydroepiandrosterone, a weakly androgenic steroid secreted by the adrenal cortex, is transformed by skin into the potent androgen, testosterone (61, 71), which is further transformed into 5α-dihydrotestosterone (59, 60). The last-mentioned steroid is currently regarded as the target tissue–active androgen that affects genetic expression in the target cells (72, 73). These findings suggest that skin does not simply receive messages from steroid hormones that are supplied by the

endocrine glands, but that it actively alters the hormone molecules, so that the hormonal activities are modified to suit the need of the tissue. This view is supported by the finding of variations of steroid metabolism during the hair cycle (74, 75); the formation of 5α-dihydrotestosterone from testosterone is most active during the resting phase (telogen), whereas the metabolism of estradiol to form estrone is most active during the growing phase (anagen).

Progress has been made in the understanding of steroid enzymes of the skin. The studies of 17β-hydroxysteroid dehydrogenase (76) and testosterone 5α-reductase (77, 78) revealed specific structural requirements for steroid inhibitors of these enzymes. Using these findings, Voigt & Hsia (79, 80) tested the antiandrogenic effects of 4-androsten-3-one-17β-carboxylic acid and its methyl ester and found that these compounds, when applied topically, prevented the action of testosterone propionate from enlarging the flank organ of female hamsters. In a similar line of research, Mauvis-Jarvis et al (81) showed inhibition of the 5α reduction of testosterone in men treated topically with progesterone. It is hopeful that the enzymic studies may provide a rational basis for the development of therapeutic agents for controlling endocrine abnormalities.

Relevant to drug metabolism in the skin is a recent report by Levin & Conney (82), who demonstrated the enzyme system that hydroxylates benzopyrene in neonatal foreskin. When the skin specimen was cultured in a medium containing the carcinogen, an increase in the hydroxylation activity was induced.

Ziboh & Hsia (83) reported that topical application of prostaglandin E_2 cleared the scaly lesions in the skin of rats fed a diet deficient in essential fatty acids. The importance of essential fatty acids in maintaining healthy skin had been demonstrated long ago (84). It appears possible that the action of these acids may be via the synthesis of prostaglandins which are required for normal keratinization of the epidermis. The therapeutic usefulness of prostaglandins in dermatology is yet to be explored.

IN VIVO PERFUSION TECHNIQUE

The skin perfusion technique was first used by Fox & Hilton to investigate mediators of eccrine sweating in man (85). Greaves and Sondergaard modified the method to make it suitable for prolonged periods of perfusion (86). Two needles (length, 36 mm; internal diameter, 1 mm) are inserted in parallel in the deep dermis 10 mm apart, pointing in opposite directions lengthwise in the flexor surface of the forearm. Both needles have 4 holes 0.635 mm diameter equally spaced along opposite sides of the shaft. Sterile Tyrode solution, warmed to 32–34°C, is infused through one needle and recovered through the other into siliconized glass tubes in an ice bath. Continuous and uniform withdrawal of the perfusate was obtained by applying suction (0.5 atm pressure) with a peristaltic pump. To increase recovery the area of perfusion was confined by applying elastic bands round the forearm proximal and distal to the needles.

The Tyrode is infused at a rate of 2 ml per min and 40–80% of the infused solution is recovered in the perfusate. Plethysmographic studies showed that the volume of the forearm remained constant after the first 15 min of perfusion.

The perfusate issuing from the inflamed skin is then assayed using the cascade superfusion technique of Vane in which the perfusate trickles over a series of different isolated organ preparations mounted inside a heated, humidified cabinet (87). The perfusate is also stored for further pharmacological and biochemical analysis.

Using this technique Greaves and his colleagues have obtained much new information on the nature of pharmacological events in cutaneous inflammation. The special value of this method compared with earlier methods lies in its directness and as well as in its supplying human data on inflammation.

In early experiments Greaves carried out skin perfusions of three types of urticaria: whealing of cutaneous mastocytosis, factitious urticaria, and cutaneous anaphylaxis. In all three urticarial reactions histamine was recovered from involved skin (86, 88) although other, so far unidentified, activity was found in perfusates of urticaris pigmentosa. In these reactions there was a close correlation between histamine release and whealing.

A more unexpected situation was found in allergic contact eczema (89). Patch tests were applied to the flexor surface of the forearm in sensitized subjects. Perfusion of inflammed skin at these sites was carried out 48 hr later. Pharmacological analysis of the perfusates with solvent partition, thin-layer chromatography, and parallel quantitative bioassay revealed that a mixture of prostaglandins E_1, E_2, F_{1a}, and F_{2a} were present in the perfusates. These findings are of great potential significance since prostaglandin E is highly vasoactive (90).

Findings in cutaneous inflammation due to exposure to UVR varied according to the time interval between irradiation and perfusion. During the first 8 hr there was no detectable pharmacological activity. At 8–20 hr most perfusate contained histamine. At 20–48 hr prostaglandin-like acidic lipid material was found in addition to histamine (91). These results illustrate how skin perfusion can be used to obtain a "pharmacological profile" of an inflammatory reaction.

However, there are some limitations in interpretation of results using this method. These have been fully reviewed by Greaves & Søndergaard (92). Recovery experiments indicate that only a small percentage of released or locally synthesized activity is recovered in the perfusate, because of factors that include dilution by the subcutaneous "pool" of Tyrode solution, local enzymic degradation, and diffusion. Furthermore the variation in concentration of an agent in successive perfusate samples does not necessarily reflect changes in rate of formation or release. These changes could, for example, be due at least in part to variation in rates of diffusion through tissues, variation in permeability of lymphatics on blood vessels, or variation in chemical or enzymic degradation. Released pharmacological agents may themselves modify the pharmacological situation in inflammed skin; for instance, prostaglandin E has histamine-liberating properties (90).

Although skin perfusion has undoubtedly produced unique evidence on the pharmacology of inflammation, much work is required to improve the methodology and in particular the quantitativeness of the technique. Achievement of this aim would have the additional advantage of enabling the method to be used in the analysis of mode of action of anti-inflammatory drugs.

RETINOIC ACID

Vitamin A (retinol) was one of the first vitamins to be characterized chemically and physiologically. Still little is known about its metabolism and actual mechanisms of action accounting for the variety of biochemical events controlled by this drug. Many of its supposed attributes have been deduced from studies of vitamin A–deficient animals and humans, but this may lead to false conclusions because of the difficulty in limiting the deficiency to only vitamin A.

Studies indicate that Tretinoin (all *trans* retinoic acid) may be the active form of the vitamin in most of the body tissues since it can replace vitamin A except for the functions of vision and reproduction. The clinical effects of tretinoin have been best studied in the skin where it seems to have the unique potential to induce as well as control epithelial growth.

Metabolic studies have demonstrated that retinoic acid is metabolized rapidly and is present in low levels in tissues and plasma. Only recently have methods become available for the separation and detection of metabolites of vitamin A that are sensitive and relatively free of artifacts. Retinyl palmitate, an ester of retinoic acid, retinal, retinol, retinoic acid, and a polar metabolite appear in various tissues of the rat 12 hr after a dose of 2 μ of 11-^{14}C-retinyl acetate. There is also evidence that retinal is oxidized in intestinal epithelium and skin to retinoic acid. Retinoic acid not only can mimic many of the biologic functions of retinol but also has a sparing action on the vitamin A reservoir in the liver. This conversion of retinol to retinoic acid is apparently irreversible.

The main products of retinoic acid metabolism may be β-glucuronide which is found in the bile (93) and products of decarboxylation found in urine (94). Homogenates of kidney and liver also appear to have the ability to decarboxylate retinoic acid (95).

SUMMARY

The fields of cutaneous pharmacology and toxicology existed as long as man used topical therapy; some medicaments were helpful and others harmful. This review documents recent progress in these fields in terms of the experimental method. Emphasis has been given to conceptual and methodologic progress rather than a list of new molecules. As signs of the advent of the maturity of these fields, a graduate school course has recently been completed, one text has been published (7), and at least two are in preparation. It is likely that the next review of this topic in this series will reflect this considerable progress in terms of relevance to man.

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Literature Cited

1. Merck Index, Merck Co.
2. Draize, J. H., Woodard, G., Calvery, H. P. 1944. *J. Pharmacol. Exp. Ther.* 82:377–90
3. Finkelstein, P., Laden, K., Miechowski, W. 1965. *Toxicol. Appl. Pharmacol.* 7:74–78
4. Kligman, A., Wooding, W. 1967. *J. Invest. Dermatol.* 49:78
5. Lanman, B. M., Elvers, W. B., Howard, C. S. 1968. In *Proc. Joint Conf. Cosmet. Sci.*, Toilet Goods Assoc., Washington DC, pp. 135–45
6. Phillips, L., Steinberg, M., Maibach, H., Akers, W. 1972. *Toxicol. Appl. Pharmacol.* 21:369–82
7. Steinberg, M., McCreesh, A., Akers, W., Maibach, H. 1975. In *Animal Models in Dermatology*, ed. H. Maibach, pp. 1–11. Edinburgh: Livingstone
8. Marzulli, F., Maibach, H. 1975. *Food Cosmet. Toxicol.* 15:533–40
9. Roudabush, R., Terhaar, C., Fassett, D., Dziuba, S. 1965. *Toxicol. Appl. Pharmacol.* 7:559–65
10. Harber, L., Baer, R. 1972. *J. Invest. Dermatol.* 58:324–42
11. Pathak, M., Fitzpatrick, T. 1972. *Radiat. Drug Ther.* 6:1–6
12. Epstein, J. 1972. *Arch. Dermatol.* 106:741–48
13. Marzulli, F., Maibach, H. 1970. *J. Soc. Cosmet. Chem.* 21:685–715
14. Burdick, K. 1966. *Arch. Dermatol.* 93:424–25
15. Maibach, H., Sams, W., Epstein, J. 1967. *Arch. Dermatol.* 95:12–15
16. Kligman, A., Goldstein, F. 1973. *Arch. Dermatol.* 107:548–50
17. Hjorth, N., Moeller, H. 1975. In press
18. Magnusson, B., Hersle, K. 1965. *Acta Derm. Venereol.* 45:123–27
19. Fregert, S. et al 1969. *Trans. St. John's Hosp. Dermatol. Soc.* 55:17
20. Magnusson, B. et al 1969. *Acta Derm. Venereol.* 46:396
21. Wilkinson, D. S. et al 1970. *Acta Derm. Venereol.* 50:287
22. Magnusson, B. et al. 1966. *Acta Derm. Venereol.* 49:396
23. Wilkinson, D. S. et al 1970. *Trans St. John's Hosp. Dermatol. Soc.* 56:19
24. Fregert, S. et al 1968. *Arch. Dermatol.* 98:144
25. Malten, K. et al 1968. *Berufsdermatosen* 16:135
26. North Am. Contact Dermatitis Res. Group 1973. *Arch. Dermatol.* 108: 537–40
27. North Am. Contact Dermatitis Res. Group 1976. *Contact Dermatitis.* In press
28. Bueller, E. 1975. See Ref. 7, pp. 56–66
29. Landsteiner, K., Jacobs, J. 1936. *J. Exp. Med.* 64:625
30. Draize, J. 1959. In *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* Austin: Assoc. Food Drug Officials US
31. Buehler, E. 1965. *Arch. Dermatol.* 91:171

31a. Maguire, H. C. Jr. 1973. *J. Soc. Cosmet. Chem.* 24:151

31b. Magnusson, B., Kligman, A. 1969. *J. Invest. Dermatol.* 52:268

32. Kligman, A. 1966. *J. Invest. Dermatol.* 47:393
33. Marzulli, F., Maibach, H. 1976. *Contact Dermatitis.* 2:In press
34. Marzulli, F., Maibach, H. 1974. *Food Cosmet. Toxicol.* 12:219–27
35. Marzulli, F., Carson, T., Maibach, H. 1968. In *Proc. Joint Conf. Cosmet. Sci.* Sponsored by TGA and Soc. Cosmet. Chem. in cooperation with FDA, Washington DC
36. Kligman, A., Epstein, W. 1975. *Contact Dermatitis* 1:231
37. Hsia, S. L. 1971. *Essays Biochem.* 7:1–38
38. Fusaro, R. M., Johnson, J. A. 1970. *The Dermal Glucose Compartment in the Dermis*, ed. W. Montagna, J. P. Bentley, R. L. Dobson. New York: Meredith
39. Freinkel, R. K. 1960. *J. Invest. Dermatol.* 34:37–42
40. Pomerantz, S. H., Asbornsen, M. T. 1961. *Arch. Biochem. Biophys.* 93: 147–52
41. Yardley, H. J., Godfrey, G. 1963. *Biochem. J.* 86:101–3
42. Halprin, K. M., Ohkawara, M. D. 1966. *J. Invest. Dermatol.* 46:43–50
43. Adachi, K., Uno, H. 1968. *Am. J. Physiol.* 215:1234–39
44. Ziboh, V. A., Dreize, M. A., Hsia, S. L. 1970. *J. Lipid Res.* 11:346–54
45. Kahlenberg, A., Kalant, N. 1966. *Can. J. Biochem.* 44:801–8
46. Ziboh, V. A., Wright, R., Hsia, S. L. 1971. *Arch. Biochem. Biophys.* 146: 93–99
47. Ziboh, V. A., Hsia, S. L. 1971. *Arch. Biochem. Biophys.* 146:100–109
48. Gaylor, J. L. 1963. *J. Biol. Chem.* 238:1643–48
49. Kandutsch, A. A., Russell, A. E. 1960. *J. Biol. Chem.* 235:2256–61

50. Wilkinson, D. I. 1970. *J. Invest. Dermatol.* 54:132–38
51. Hsia, S. L., Fulton, J. E. Jr., Fulghum, D., Buch, M. M. 1970. *Proc. Soc. Exp. Biol. Med.* 135:285–91
52. Vroman, H. E., Nemecek, R. A., Hsia, S. L. 1969. *J. Lipid Res.* 10:507–14
53. Fulton, J. E. Jr., Hsia, S. L. 1972. *J. Lipid Res.* 13:78–85
54. Strauss, J. S., Pochi, P. E. 1969. *Arch. Dermatol.* 100:621–36
55. Ludwig, E. 1968. In *The Role of Sexual Hormones in Pattern Alopecia in Biopathology of Pattern Alopecia,* ed. A. Baccaredda-Boy, G. Moretti, J. R. Frey, pp. 50–60. Basle: Karger
56. Graham, C. E., Collins, D. C., Robinson, H., Preedy, J. R. K. 1972. *Endocrinology* 91:13–24
57. Frost, P., Weinstein, G. D., Hsia, S. L. 1966. *J. Invest. Dermatol.* 46:584–85
58. Weinstein, G. D., Frost, P., Hsia, S. L. 1968. *J. Invest. Dermatol.* 51:4–10
59. Gomez, E. C., Hsia, S. L. 1968. *Biochemistry* 7:24–32
60. Wilson, J. D., Walker, J. D. 1969. *J. Clin. Invest.* 48:371–79
61. Cameron, E. H. D., Baillie, A. H., Grant, J. K., Milne, J. A., Thomson, J. 1966. *J. Endocrinol.* 35:xix–xx
62. Sansone, G., Reisner, R. M. 1971. *J. Invest. Dermatol.* 56:366–72
63. Faredin, I., Fazekas, A. G., Toth, I., Kokai, K., Julesz, M. 1969. *J. Invest. Dermatol.* 52:357–61
64. Takayasu, S., Adachi, K. 1972. *Endocrinology* 90:73–80
65. Hsia, S. L., Hao, Y. L. 1966. *Biochemistry* 5:1469–74
66. Hsia, S. L., Hao, Y. L. 1967. *Steroids* 10:489–500
67. Malkinson, F. D., Lee, M. W., Cutukovic, I. 1959. *J. Invest. Dermatol.* 32:101–7
68. Frost, P., Gomez, E. C., Weinstein, G. D., Lamas, J., Hsia, S. L. 1969. *Biochemistry* 8:948–52
69. Rongone, E. L. 1969. *Proc. Soc. Exp. Biol. Med.* 130:253–56
70. Hsia, S. L. 1971. In *Steroid Metabolism in Human Skin in Modern Trends in Dermatology,* ed. P. Borrie, 4:69–88. London: Butterworth
71. Julesz, M., Faredin, I., Toth, L. 1971. *Steroids in Human Skin.* Budapest: Akad. Kiado
72. Liao, S., Fang, S. 1969. In *Receptor-Proteins for Androgens and the Mode of Action of Androgens on Gene Transcription in Ventral Prostate in Vitamins and Hormones.* ed. R. S. Harris, I. G. Wool, J. A. Loraine, P. L. Munson, pp. 17–90. New York & London: Academic
73. Bruchovsky, N., Wilson, J. D. 1968. *Biol. Chem.* 243:2012–21
74. Rampini, E., Voigt, W., Davis, B. P., Moretti, G., Hsia, S. L. 1971. *Endocrinology* 89:1506–14
75. Rampini, E., Davis, B. P., Moretti, G., Hsia, S. L. 1971. *J. Invest. Dermatol.* 57:75–80
76. Davis, B. P., Rampini, E., Hsia, S. L. 1972. *J. Biol. Chem.* 247:1407–13
77. Voigt, W., Fernandez, E. P., Hsia, S. L. 1970. *J. Biol. Chem.* 245:5594–99
78. Voigt, W., Hsia, S. L. 1973. *J. Biol. Chem.* 248:4280–85
79. Voigt, W., Hsia, S. L. 1973. *Endocrinology* 92:1216–22
80. Hsia, S. L., Voigt, W. 1974. *J. Invest. Dermatol.* 62:224–27
81. Mauvais-Jarvis, P., Kuttenn, F., Bandot, N. 1974. *J. Clin. Endocrinol. Metab.* 38:142–47
82. Levin, W., Conney, A. H., Alvares, A. P., Merkatz, I., Kappas, A. 1972. *Science* 176:419–20
83. Ziboh, V. A., Hsia, S. L. 1972. *J. Lipid Res.* 13:458–67
84. Burr, G. O., Burr, M. M. 1929. *J. Biol. Chem.* 82:345–55
85. Fox, R. H., Hilton, S. M. 1958. *J. Physiol.* 142:219
86. Greaves, M. W., Søndergaard, J. 1970. *Arch. Dermatol.* 101:418
87. Vane, J. R. 1964. *Br. J. Pharmacol.* 23:360
88. Søndergaard, J., Greaves, M. W. 1971a. *Acta Derm. Venereol.* 51:98
89. Greaves, M. W., Søndergaard, J., McDonald-Gibson, W. 1971. *Br. Med. J.* 2:258
90. Søndergaard, J., Greaves, M. W. 1971b. *Br. J. Dermatol.* 84:424
91. Søndergaard, J., Greaves, M. W. 1971c. *J. Pathol.* 101:93
92. Greaves, M. W., Søndergaard, J. 1971. *Acta Derm. Venereol.* 51:50
93. Dunagin, P. E., Zachman, R., Olson, J. 1965. *Science* 148:65
94. Sundaresan, P. R., Sundaresan, G. M. 1973. *Int. J. Vitam. Nutr. Res.* 43:61
95. Kleiner-Bossaler, A., Reluca, H. 1971. *Arch. Biochem. Biophys.* 142:371