(30) White, I. N. H., Boobis, A. R., Davies, D. S. (1984) Biochem. Pharmac. 33, 459–464.
(31) White, I. N. H. (1982) Biochem. Pharmac. 31, 1337–1342.
(32) Correia, M. A., Farrell, G. C., Olson, S., Wong, J. S., Schmid, R., Ortiz de Montellano, P. R., Beilan, H. S., Kunze, K. L., Mico, B. A. (1981) J. Biol. Chem. 256, 5466–5470.
(33) Back, D. J., Breckenridge, A. M., Crawford, F. E., Orme, M. L'E., Rowe, P. H., Smith, E. (1978) J. Pharmacol. Exp. Thera. 207, 555–565.
(34) Back, D. J., Breckenridge, A. M., Challiner, M., Crawford, F. E., Orme, M. L'E., Rowe, P. H., Smith, E. (1978) J. Steroid. Biochem. 9, 527–531.
(35) Kunze, K. L., Mangold, B. L. K., Wheeler, C., Beilan, H. S., Ortiz de Montellano, P. R. (1983) J. Biol. Chem. 258, 4202–4207.
(36) Ortiz de Montellano, P. R., Beilan, H. S., Matthews, J. M. (1982) J. Med. Chem., 25, 1174–1179.
(37) El-Masri, A. M., Smith, J. N., Williams, R. T. (1958) Biochem. J. 68, 199–204.
(38) Wade, A., Symons, A. M., Martin, L., Parke, D. V. (1979) Biochem. J. 184, 509–517.
(39) Sullivan, H. R., Roffey, P., McMahon, R. E. (1979) Drug Metab. Dispos. 7, 76–80.
(40) McMahon, R. E., Turner, J. C., Whitaker, G. W., Sullivan, H. R. (1981) Biochem. Biophys. Res. Commun. 99, 662–667.
(41) Ortiz de Montellano, P. R., Kunze, K. L. (1980) J. Am. Chem. Soc. 102, 7375–7377.
(42) Abdel-Aziz, M., Williams, K. (1969) Steroids, 13, 809–820.
(43) Schmid, S. E., Au, W. Y. W., Hill, D. E., Kadlubar, F. F., Slikker, W. (1983) Drug Metab. Dispos. 11, 531–536.
(44) White, I. N. H. (1982) Chem. Biol. Interact. 39, 231–243.
(45) White, I. N. H., Campbell, J. B., Farmer, P. B., Bailey, E., Nam, N.-H., Thang, D. C. (1984) Biochem. J. 220, 85–94.
(46) Sacher, R. M., Metcalf, R. L., Fukuto, T. R. (1968) J. Agr. Food Chem. 16, 779–786.
(47) Fellig, J., Barnes, J. R., Rachlin, A. I., O'Brien, J. P., Focella, A. (1970) J. Agr. Food. Chem. 18, 78–80.
(48) Marinello, A. J., Gurtoo, H. L., Struck, R. F., Paul, B. (1978) Biochem. Biophys. Res. Comm. 83, 1347–1353.
(49) Helton, E. D., Goldzieher, J. W. (1977) J. Toxicol. Env. Hlth. 3, 231–241.
(50) White, I. N. H., Smith, A. G., Farmer, P. B. (1983) Biochem. J. 212, 599–608.
(51) Ortiz de Montellano, P. R., Kunze, K. L. (1981) Biochemistry 20, 7266–7271.
(52) Lewars, E. G. (1983) Chem. Rev. 83, 519–534.
(53) Oritz de Montellano, P. R., Marigold, B. L. K., Wheeler, C., Kunze, K. L., Reich, N. O. (1983) J. Biol. Chem. 258, 4208–4213.
(54) Liebler, D. C., Guengerich, F. P. (1983) Biochemistry, 22, 4582–4589.
(55) Kappus, H., Bolt, H. M., Remmer, H. (1973) Steroids 22, 203–225.
(56) Maggs, J. L., Grabowski, P. S., Park, B. K. (1983) Biochem. Pharmac. 32, 2793–2800.
(57) Jaggi, W., Lutz, W. K., Schlatter, C. (1978) Chem. Biol. Interact. 23, 13–18.
(58) Committee on the Safety of Medicines (1972) Carcinogenicity Tests of Oral Contraceptives, London, HMSO.
(59) Sherlock, S. (1978) in Primary Liver Tumors (Remmer, H., Bolt, H. M., Bannasch, P., Popper, H. eds), Falk Symposium number 25, pp. 201–216. MTP Press, Lancaster, U.K.
(60) Vessey, M. P., Doll, R. (1969) Br. Med. J. 2, 651–657.
(61) Mann, J. I., Inman, W. H. W. (1975) Br. Med. J. 2, 245–248.

Skin Structure and Metabolism: Relevance to the Design of Cutaneous Therapeutics

Daniel A. W. Bucks[1]

Abstract: The outer layer of the epidermis or stratum corneum is the major barrier to percutaneous absorption. It has been shown that there are numerous enzyme systems beneath the stratum corneum in the viable epidermis capable of metabolizing drugs. A number of prodrug and soft drug topical therapeutic agents have been designed. After these agents penetrate the stratum corneum, they are metabolized by the cutaneous esterase systems to the desired metabolites.

Interest in drug metabolism by cutaneous tissues is increasing as a result of pharmacokinetic, pharmacological, therapeutic, and toxicological considerations. The ability of the skin to metabolize xenobiotics is largely unexplored. Most current knowledge on cutaneous biotransformation is restricted to steroids and polycyclic aromatic hydrocarbons. This lack of knowledge hinders a proper understanding of the fate of topically applied drugs and limits our ability to design rational cutaneous therapies. To fully appreciate the problems involved in studying the metabolism of drugs by skin, it is sensible to first consider the structure and function of skin.

[1] Departments of Pharmacy and Pharmaceutical Chemistry, School of Pharmacy and Department of Dermatology, School of Medicine, University of California, San Francisco, CA 94143

Structure and Function of Skin

Mammalian skin consists of two distinct tissue components, the epidermis and dermis, that are derived from different germinative layers (Fig. 1). The thinner, outer, stratified squamous epithelium, epidermis, is derived from ectoderm. The thicker underlying dermis consists mainly of connective tissue of mesodermal origin. In addition, there are eccrine and apocrine sweat glands, sebaceous glands, and hair follicles in skin. These structures are located primarily in the dermis, although each is derived embryologically from ectoderm.

The epidermis, approximately 0.12 mm thick, contains no blood or lymph vessels. The epidermis consists of three components: the basal layer (stratum basale), the viable layer (stratum lucidum, stratum granulosum, and stratum spinosum), and the horny or barrier layer (stratum corneum). All cells of the epidermis originate from the basal layer by the process of epidermal differentiation or keratinization.

The mechanism controlling epidermal cell proliferation and differentiation is unclear. Recent studies have suggested that cyclic nucleotide (1–3), prostaglandin (4–6) and polyamine (7, 8) metabolism may be critical determinants of normal epidermal proliferation. Epidermal growth factor (EGF) is critical to growth and maturation of the epidermis. *In vitro* studies have

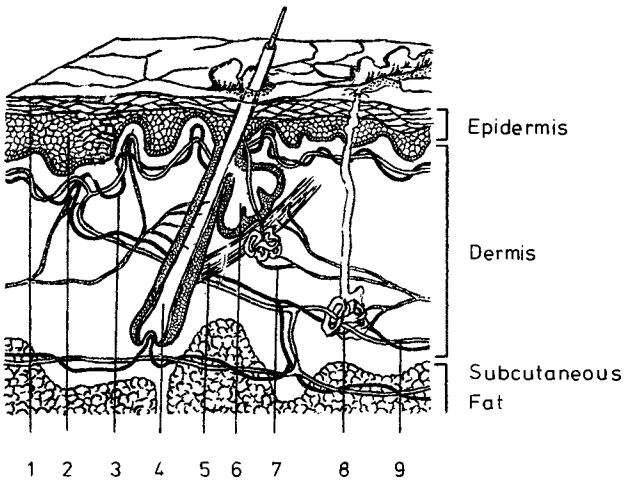


Fig. 1 Diagramatic representation of skin: 1 – horny layer (stratum corneum), 2 – living layer, 3 – capillary, 4 – hair follicle, 5 – smooth muscle, 6 – sebaceous gland, 7 – nerve fiber, 8 – eccrine gland, 9 – lymphatic

demonstrated that addition of EGF to cultured human epidermal cells increases their lifespan (9) and that these effects may involve a cAMP hormone-receptor interaction (10). An extensive review of the physical properties and biological effects of EGF has been written by Carpenter (11).

The differentiating epidermal cells reach the surface after 28 days and will be shed as cornified cells with a low water content. The stratum corneum is 0.01 to 0.02 mm thick. The protein-rich intracellular spaces within the corneocytes of the stratum corneum are hydrophilic, whereas the cell membranes and the extracellular lipid are hydrophobic so that the stratum corneum can be represented as a hydrophilic-lipophilic multilayered structure. This suggests that only substances having both lipophilic and hydrophilic properties will easily penetrate the stratum corneum. Because of the barrier nature of the stratum corneum, topically applied compounds may accumulate, i.e., the stratum corneum may serve as a reservoir from which substances can be subsequently absorbed over long periods of time (12, 13). Thus the dead stratum corneum, by means of its physicochemical properties, forms the critical structure for epidermal barrier function minimizing percutaneous absorption of environmental agents and preventing water loss.

The epidermis rests on and is tightly bound to the dermis. This thicker component of skin consists of cells interconnected by collagen and elastin structural proteins embedded in a ground matrix of glycosoaminoglycans. The dermis is highly vascularized and functions in the regulation of body temperature. The dermis is also a primary site in which cutaneous inflammation occurs in response to skin injury.

The dermis is a site of vigorous metabolic activity. Sebaceous glands located in the dermis synthesize a complex series of lipids. Hair follicles synthesize large amounts of protein and are among the most rapidly replicating of mammalian tissues.

Skin Metabolism

Potential problems in biotransformation studies. Skin has a complex structure; moreover, regional variations in the structure of the skin can pose problems in biotransformation studies. Table I lists the major regional variations.

Differences in percutaneous absorption may have an effect on biotransformation studies in skin. Feldmann and Maibach (15) showed that percutaneous absorption may differ by a factor of 100 depending upon anatomical site. Moreover, great differences in percutaneous absorption have been observed between animal species (16). Penetration and bioavailability of a drug at the site of application are prerequisite to possible metabolism. Therefore, the observed rate of drug biotransformation may differ greatly between different sites and animal species.

Table I. Regional Variations in Skin Structure (14)

Stratum corneum	bacterial flora (greater in moist areas)
	thickness (greater on palms and soles)
Epidermis	thickness
	cell distribution
	size and distribution of appendages
	dermoepidermal junction
Dermis	thickness
	elasticity
	vascularization

Methods employed in skin metabolism studies. Both *in vitro* and *in vivo* techniques have been utilized to study skin metabolism. Early *in vitro* studies employed standard biochemical techniques used in previous hepatic metabolism studies. Either skin fragments or skin homogenates were incubated with a test compound, and metabolites formed by enzymes present in the skin were then characterized. Leung and Ando (17) developed a rotating-disc method in an attempt to account for both skin permeation and cutaneous metabolism. A major improvement was the use of short-term skin organ cultures to study cutaneous biotransformation reactions with viable and structurally intact skin (18, 19). Holland et al. (20) have developed an *in vitro* apparatus for kinetic evaluation of percutenous absorption and metabolism of compounds with viable skin. However, it should be noted that Bundgaard et al. (21) have reported the leaching of hydrolytic enzymes from the skin during *in vitro* percutaneous absorption experiments. There are few reported *in vivo* skin metabolism studies. Morsches and Holzmann (22) reported the cutaneous metabolism of benzyl peroxide *in vivo* when they found only benzoic acid in the serum following topical application. Wester et al. (23) calculated a 20.6% percutaneous first pass effect for nitroglycerine by comparing plasma nitroglycerine and radiolabel AUCs following intravenous and topical nitroglycerine administration; and, by comparison to urine radiolabel excretion, the estimate of nitroglycerine percutaneous first pass effect was 16%.

Biotransformation reactions in the skin. There are a series of functionalization (phase 1) reactions (oxidations, reductions, hydrolysis) and conjugation (phase 2) reactions (glucuronide and sulfate formation, methylation and glutathione conjugation) conducted in the skin (24–26). Table II lists the main chemical groups, with examples, used in skin biotransformation studies. Biotransformation reactions conducted by skin and the respective enzyme systems involved are listed in Table III.

Table II. Compounds Used in Biotransformation Studies

Androgens:	testosterone, DHA, DHT, etc.
Estrogens:	17B-estradiol, estrone
Gestagens:	progesterone
Antiandrogens:	androstenedione, deoxycorticosterone
Polyarenes:	benzo(a)pyrene, dibenz(a,h)anthracene
Corticosteroids:	cortisol, synthetic corticosteroids
Xenobiotics:	aminophenol, styrene glycol, etc.

Table III. Biotransformation Reactions by Skin (14)

Reaction	Enzymes Involved
Phase 1 Oxidation Reactions:	
aliphatic C-atoms	mixed function oxidases
alicyclic C-atoms	mixed function oxidases
aromatic rings	hydroxylases, mixed function oxidases
alcohols	dehydrogenases
deamination	monoamine oxidases
dealkylation	deethylases, demethylases
Phase 1 Reduction Reactions:	
carbonyl groups	ketoreductase
C-double bonds	5(α)reductase
Phase 1 Hydrolysis Reactions:	
ester bonds	esterases
epoxides	expoxide hydrases
Phase 2 Conjugation Reactions:	
glucuronide formation	UDPG-transferases
sulfate formation	sulfo-transferases
methylation	catechol o-methyl transferases
glutathione	glutathion-S-transferases

Enzyme induction and inhibition. The polycyclic aromatic hydrocarbons (PAHs) are hepatic enzyme inducers and cutaneous enzyme inducers. Pohl et al. (27) demonstrated that several enzyme systems could be induced in skin by the topical application of 3-methylcholanthrene (3-MC) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). 3-MC caused a slight increase in cutaneous P-450 levels and a two-fold increase in aromatic hydrocarbon hydroxylase (AHH) activity; both enzyme systems returned to control levels in 72 hours. Percutaneous application of TCDD elicited an increase in cutaneous P-450 activity and AHH activity eight-fold in 24 hours and thirty-fold in 72 hours. The topical application of TCDD doubled the activity of hepatic P-450. 3-MC was unable to induce 7-ethoxycoumarin deethylase activity in skin, whereas TCDD increased the deethylase activity four-fold in 24 hours and seven-fold in 72 hours.

Bowden et al. (28) studied the effects of topically applied mixed function oxidase modifiers on the induction of AHH activity in skin. Although phenobarbital induces hepatic enzyme activity, it had no effect on cutaneous AHH activity, and both AHH activity and induction were inhibited by 7,8-benzoflavin. 5,6-Benzoflavin, 7,12-dimethylbenz[*a*]anthracene and 1,2,5,6-dibenzanthracene induced the activity of cutaneous AHH 350, 600, and 1200 %, respectively. Thus, the activities of inducers and inhibitors may affect the rates of activation and detoxification of drugs in the skin.

Cutaneous Metabolism in The Design of Topical Drug Therapy

There are at least two major problems to be solved in the design of therapeutic agents to be used in topical drug therapy. Many potential therapeutic compounds are not practical, because they do not penetrate the stratum corneum barrier to a significant degree. The second major problem occurs when the therapeutic agent possesses undesirable systemic activity. With compounds that poorly penetrate the skin's outer layer, it might be better to deliver the drug in a more permeable inactive form that, once in the skin, it is metabolized to the active agent, e.g. a prodrug. In the case of the therapeutic agent that has undesirable systemic activity, it might be better to design an agent with limited, site specific, activity, e.g. a soft drug (see below).

Prodrugs. A prodrug may be defined as an inactive agent that is activated by an enzymatic process to the active drug in a predictable and controllable manner.

Cromolyn (cromoglycic acid) has weak anti-inflammatory activity (29). Haider (30) found that topically applied cromolyn was effective in the treatment of atopic eczema. Bodor et al. (31) postulated that cromolyn would have antipruritic activity as well as anti-inflammatory activity based on its mechanism of action and its structure; however, because of its polar character and short biological half-life, it would not effectively penetrate the skin. Enhanced penetration was accomplished by the development of more lipophilic prodrugs of cromolyn (31). The carboxy groups were esterified. The resulting lipoidal prodrugs significantly increased the amount of cromolyn penetrating the skin. The hexanoyloxymethyl ester (Fig. 2) was found to be a promising therapeutic agent.

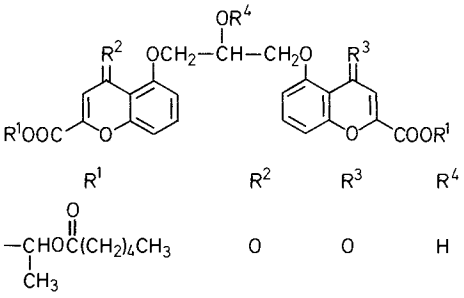
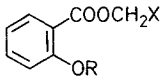


Fig. 2 Structure of the hexanoyloxymethyl ester of cromolyn

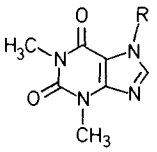
Salicylic acid and acetyl salicylic acid (ASA) are useful agents in topical drug therapy. Salicylic acid is used for its antiseptic, germicidal, keratolytic, and anti-inflammatory properties. Keratolytic agents damage the cornified layer of the skin, which is then sloughed off to whatever depth the agent has acted. Salicylic acid has good anti-inflammatory properties, but ASA was found to have greater anti-inflammatory properties than salicylic acid and in some cases even hydrocortisone (32). Several prodrug forms (Fig. 3) of ASA and salicylic acid have been developed (33). *In vivo* studies of the methylthiomethyl and methylsulfinylmethyl 2-acetoxybenzoate ester prodrugs of ASA found these freely penetrable compounds hydrolysed to ASA by the skin esterases (34). Significant metabolism of all of the salicylic acid derivatives occur in skin. They concluded that further *in vivo* studies should be performed to answer the question as to whether therapeutic levels either as keratolytic, anti-inflammatory, or analgesic agents can be achieved by these prodrugs of salicylic acid and ASA.



R = CH_3CO, X = CH_3S–
R = CH_3CO, X = CH_3SO–
R = CH_3CO, X = CH_3SO_2–
R = H, X = CH_3S–
R = H, X = CH_3SO–
R = H, X = CH_3SO_2–

Fig. 3 Prodrugs of ASA and salicylic acid

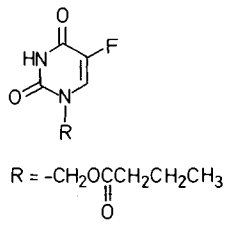
Prodrugs of theophylline are under development for the treatment of inflammatory conditions and psoriasis. Sloan and Bodor (35) designed and synthesized hydroxymethyl and acyloxymethyl prodrugs of theophylline (Fig. 4) that, *in vitro*, exhibit increased lipid and water solubilities. Although the prodrug approach has not yet been optimized, these prodrugs increased the delivery of theophylline into the skin *in vivo*, and once theophylline was delivered, it was an effective antiproliferative agent.



R = CH_2OH
R = $CH_2O_2CC(CH_3)_3$

Fig. 4 Structure of the theophylline prodrugs

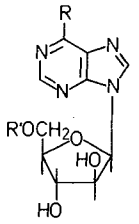
Topical application of 5-fluorouracil has been useful in the treatment of various diseases such as actinic keratoses, epithelial neoplasms, and psoriasis. Although 5-fluorouracil is useful, it does not penetrate the skin well because of its low lipophilicity. Mollgaard et al. (36) designed and synthesized *N*-1-acyloxymethyl prodrugs of 5-fluorouracil and report that 1-butyryloxymethyl-5-fluorouracil (Fig. 5) readily penetrated the skin, and once in the skin it was metabolized to 5-fluorouracil. Enzymatic cleavage of the ester group results in the formation of 1-hydroxymethyl-5-fluorouracil which is decomposed instantaneously into formaldehyde and 5-fluorouracil. The authors suggested that *N*-acyloxymethyl derivatives of 5-fluorouracil may be promising prodrug candidates for the enhanced topical delivery of 5-fluorouracil.



R = $-CH_2OCCH_2CH_2CH_3$ (O)

Fig. 5 Structure of 1-butyryloxymethyl-5-fluorouracil

In the treatment of genital herpes (herpes simplex type 1 and type 2) two antiviral agents have had limited effectiveness: ara-A and acyclovir. Vidarabine (9-B-D-arabinofuranosyladenine, ara-A) has broad spectrum activity against DNA viruses such as herpes simplex; however, when applied topically for the treatment of herpetic skin, it was inactive. This was attributed to poor transdermal penetration (37), because when injected intradermally vidarabine improved the course of the infection (38). Vidarabine-5'-valerate (Fig. 6) is an



R = NH_3, R' = $CH_3(CH_2)_3CO$

Fig. 6 Structure of vidarabine-5'-valerate

example of an ara-A prodrug. Because of the increased lipophilicity, it will penetrate the stratum corneum more readily. Once in the epidermis, the prodrug may be converted by an esterase into the parent drug (39, 40). Another prodrug of ara-A has been designed and synthesized by Shannon et al. (41). The compound is ara-A-2',3'-diacetate (ara-ADA). The increased lipophilicity allows the compound to penetrate the skin, where it is metabolized to the parent drug. *In vivo* tests indicate that ara-ADA is as effective as acyclovir in treating primary infection lesions (Fig. 7).

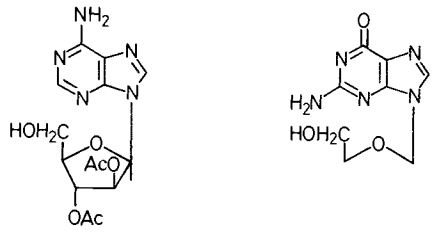


Fig. 7 Structure of ara-A-2',3'-diacetate (ara-ADA) and acyclovir

Sloan et al. (42) have developed and synthesized pivaloyloxymethyl prodrugs of 6-thiopurines. They report the development of two soft-alkylated derivatives (Fig. 8) of 6-mercaptopurine that deliver 5 and 13 times more 6-mercaptopurine than 6-mercaptopurine itself.

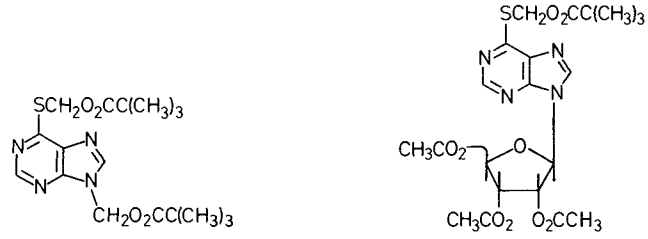


Fig. 8 Prodrugs of 6-mercaptopurine

Soft drugs. The term *soft drug* was coined by Bodor (43). He defines soft drugs as biologically active chemical compounds (drugs) which are characterized by a predictable *in vivo* destruction (metabolism), after they achieve their therapeutic role, to inactive moieties.

One of the first soft drugs designed was fluocortin butyl ester, a new type of topical steroid. Fluocortin butyl ester is an ester of a steroid acid and the primary alcohol butanol. *In vitro* and *in vivo* studies (44) using skin from guinea pigs and humans showed a partial hydrolysis of the ester in the skin. The degree of ester cleavage was more pronounced in stripped than intact skin due to the increase in percutaneous absorption. Saturation of the esterases was not observed. The first metabolic step was ester cleavage to form an inactive product (Fig. 9). The rapid biotransformation of fluocortin butyl ester to systemically inactive metabolites suggests a safe topical corticoid therapy even in highly susceptible patients like infants, pregnant women and the elderly.

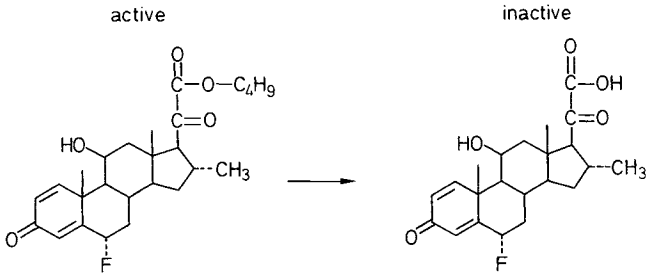


Fig. 9 Biotransformation of fluocortin butyl ester in man

Metabolic disposition studies of benzoyl peroxide indicate that it may be classified as a soft drug. *In vivo* and *in vitro* percutaneous absorption studies of benzoyl peroxide (45) indicate that it penetrates into the skin and is transformed into benzoic acid within the skin layers. Thus, in the treatment of acne vulgaris with benzoyl peroxide, its action is site-specific; however, it is worth noting that benzoyl peroxide is a potent contact sensitizer in experimental studies, and this effect may occur in up to 1 % of acne patients (46).

Quaternary surface-active agents are widely used in antimicrobial, cleansing, and cosmetic preparations, e.g., cetylpyridinium chloride and benzylalkonium chloride. Biological toxicity of these agents is related to the surfactant characteristics and metabolism of the quaternary ammonium group. Bodor et al. (47) designed a new class of soft quaternary agents which cleave via chemical and/or enzymatic hydrolysis to nontoxic (non-quaternary and non-surface active) moieties after they exert their biological effects. The main feature of this class of compounds (Fig. 10) is the close structural analogy to the corresponding normal quaternary ammonium salts. The hydrolytic sensitivity of the ester group leads to the simultaneous destruction of the quaternary group and the surfactant properties.

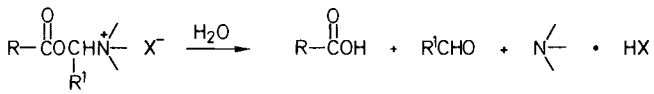


Fig. 10 General structure of soft quaternary agents

A new class of antimuscarinic drugs was designed and synthesized as selective, local agents, particularly as inhibitors of eccrine sweating (48). These compounds are soft quaternary ammonium esters in which there is only one carbon atom separating the ester oxygen and the quaternary head. Hydrolysis results in the destruction of the quaternary head. Their fast deactivation upon absorption prevents any systemic effects.

Pro-soft drugs. Many endogenous substances (e.g., steroid hormones such as hydrocortisone, testosterone, and estradiol) can be considered soft drugs since they are readily metabolized by the body when their concentrations are close to their natural levels. At physiologic levels, there are no toxicities associated with their use; however, cutaneous metabolism of these endogenous compounds is so fast and efficient that this level cannot be used clinically. The solution for this problem is the design of specific chemical protecting techniques for their sustained release, or a prodrug-soft drug combination. Work along these lines is under development by Bodor et al. (49). They have designed and synthesized ethyl ester thiazolidine derivatives of progesterone that yielded more than twice the radiolabeled steroid concentration in the skin after topical application compared to topical application of progesterone itself (50).

Conclusion

The main barrier to the percutaneous absorption of topically applied drugs is the stratum corneum. The structure of the stratum corneum can be described by a multilayer matrix of hydrophobic and hydrophilic components. This barrier results from the death of the differentiated epidermal keratinocytes. The stratum corneum may act as a reservoir from which substances can be absorbed over long periods of time.

The skin is capable of phase 1 functionalization reactions (oxidation, reduction, and hydrolysis) and phase 2 conjugation reactions (glucuronide, sulfate, and glutathione formation). The enzymes involved in these reactions include those with relatively high substrate specificity which form metabolites directly acting or modifying the action of other hormones at their skin targets, and those with lower substrate specificity responsible for the metabolism of xenobiotics.

The actions of skin metabolism are important in the design of rational cutaneous therapies. Every molecule which passes the stratum corneum comes in contact with the skin's enzyme systems. Drug metabolism in the skin can increase or decrease the pharmacological activity of an xenobiotic and also influence systemic effects, depending on whether the metabolites are active or not. Our increased awareness of the potential of using these cutaneous enzyme systems in the design of rational drug therapy has led to the development of several novel prodrug and soft drug designs.

References

(1) Voorhees, J. J., Duell, E. A., Stawiski, M. (1974) Adv. Cyclic Nucl. Res. 4, 117–162.

(2) Aoyagi, T., Umeda, K., Iizuka, H., Miura, Y. (1981) Br. J. Dermatol. 105, 257–266.

(3) Royer, E., Chaintreul, J., Meynadier, J., Michel, B., Guilhou, J. J., Crastes de Paulet, A. (1982) Dermatologica 165, 533–543.

(4) Hammerstrom, S., Hamberg, M., Samuelsson, B., Duell, E., Stawiski, M., Voorhess, J. (1975) Proc. Nat. Acad. Sci. USA 72, 5130–5134.

(5) Hammerstrom, S., Lindgras, J., Marcelo, C., Duell, E., Anderson, T., Voorhess, J. (1979) J. Invest. Dermatol. 72, 268.
(6) Penneys, N. S., Eaglstein, W., Ziboh, V. (1980) Br. J. Dermatol. 103, 257–262.
(7) Russel, D. H., Stawiski, M. A., Duell, E. A. (1976) Pharmacologist 18, 157.
(8) Voorhees, J. J. (1979) Archs. Derm. 115, 943–944.
(9) Rheinwald, J. G., Green, H. (1977) Nature 265, 421–424.
(10) Frati, L., D'Armiento, M., Gulletta, E., Verna, R., Covelli, I. (1977) Pharm. Res. Commun. 9, 815–822.
(11) Carpenter, G. (1979) Ann. Rev. Biochem. 48, 193–216.
(12) Schalla, W., Schaefer, H. (1982) in: Dermal and Transdermal Absorption (Brandau, R., and B. H. Lippoid, eds.) pp. 41–72, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany.
(13) Wester, R. C., Maibach, H. I. (1983) Drug Met. Rev. 14, 169–205.
(14) Tauber, U. (1981) in: Dermal and Transdermal Absorption (Brandau, R., and Lippoid, B. H., eds.) pp. 134–151, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany.
(15) Feldmann, R. J., Maibach, H. I., (1967) J. Invest. Dermatol. 48, 181–183.
(16) Bartek, M. J., La Budde (1975) in: Animal Models in Dermatology (Maibach, H. I., ed.) pp. 103–120, Churchill Livingstone, New York.
(17) Leung, L. C., Ando. H. Y. (1979) J. Pharm. Sci. 68, 571–574.
(18) Smith, L. H., Holland, J. M. (1981) Toxicol. 21, 47–57.
(19) Koa, J., Hall, J., Holland, J. M. (1982) Toxicol. Appl. Pharmacol. 68, 206–217.
(20) Holland, J. M., Kao, J. Y., Whitaker, M. J. (1984) Toxicol. Appl. Pharmacol. 72, 272–280.
(21) Bundgaard, H., Hoelgaard, A., Mollgaard, B. (1983) Int. J. Pharm. 15, 285–292.
(22) Morsches, B., Holzmann, H. (1982) Arzneim.-Forsch. 32, 298–300.
(23) Wester, R. C., Noonan, P. K., Smeach, S., Kosobud, L. (1983) J. Pharm. Sci. 72, 745–748.
(24) Rongone, E. L. (1977) in: Advances in Modern Toxicology Vol. 4: Dermatology and Pharmacology (Marzulli, F. N., and Maibach, H. I., eds.) pp. 93–137, Hemisphere Publishing Corp., Washington D.C.
(25) Pannatier, A., Jenner, P., Testa, B., Etter, C. (1978) Drug Met. Rev. 8, 319–343.
(26) Noonan, P. K., Wester, R. C. (1982) in: Dermatotoxicology, second edition (Marzulli, F. N., and Maibach, H. I., eds.) pp. 71–90, Hemisphere Publishing Corp., Washington D.C.
(27) Pohl, R., Philpot, R., Fouts, J. (1976) Drug Metab. Dispos. 4, 442–450.
(28) Bowden, G., Slaga, T., Shapas, B., Boutwill, R. (1974) Cancer Res. 34, 2634–2642.
(29) Cox, J. S. G., Beach, J. E., Blair, A. M. J. N., King, J., Lee, T. B., Loveday, D. E. E., Moss, G. F., Orr, T. S. C., Ritche, J. T., Sheard, P. (1970) in: Advances in Drug Research, Vol. 5 (Harper, N. J., and Simmons, A. B., eds.) pp. 115, John Wiley, New York and London.
(30) Haider, S. A. (1977) Br. Med. J. 1, 1570–1572.
(31) Bodor, N., Zupan, J., Selk, S. (1980) Int. J. Pharm. 7, 63–75.
(32) Weirich, E. G., Lonfauer, J. K., Kirkwood, A. K. (1976) Dermatologica 152, 87–99.
(33) Loftsson, T., Kaminski, J. J., Bodor, N. (1981) J. Pharm. Sci. 70, 743–749.
(34) Loftsson, T., Kaminski, J. J., Bodor, N. (1981) J. Pharm. Sci. 70, 756–758.
(35) Sloan, K. B., Bodor, N. (1982) J. Pharm. Sci. 12, 299–313.
(36) Mollgaard, B., Annie, H., Bundgaard, H. (1982) J. Pharm. Sci. 71, 153–162.
(37) Fondak, A. A., Friedman, A. E., Klein, R. J. (1975) Abstract from a symposium at Stanford University, Stanford, Calif., Aug. 26–29.
(38) Lieberman, M., Schafer, T. W., Putt, S. R. (1973) J. Invest. Dermatol. 60, 203–206.
(39) Yu, C. D., Fox, J. L., Ho, N. F. H., Higuchi, W. I. (1979) J. Pharm. Sci. 68, 1341–1346.
(40) Yu, C. D., Fox. J. L., Ho, N. F. H., Higuchi, W. I. (1979) J. Pharm. Sci. 68, 1347–1357.
(41) Shannon, W. M., Baker, D. C., Higuchi, W. I. (1983) 185th National Meeting of the American Chemical Society, March 20–25, Seattle, Washington.
(42) Sloan, K. B., Hashida, M., Alexander, J., Bodor, N., Higuchi, T. (1983) J. Pharm. Sci. 72, 372–378.
(43) Bodor, N. (1977) in: Design of Biopharmaceutical Properties by Prodrug and Analogs (Roche, E. B., ed.) pp. 98, Academy of Pharmaceutical Sciences, Washington D.C.
(44) Kapp. J. F., Koch, H., Topert, H., Kessler, J., Gerhards, E. (1977) Arzneim.-Forsch. 27, 2191–2204.
(45) Yeung, D., Nacht, S., Bucks, D., Maibach, H. (1982) Clin. Res. 30, 616A.
(46) Maibach, H. I., Robertson, D. B. (1982) in: Basic and Clinical Pharmacology (Katzung, B. G., ed.) pp. 708–722, Lange Medical Publications, Calif.
(47) Bodor, N., Kaminski, J. J., Selk, S. (1980) J. Med. Chem. 23, 469–474.
(48) Bodor, N., Woods, R., Kaminski, J. J. (1980) J. Med. Chem. 23, 474–480.
(49) Bodor, N., Sloan, K. B., Little, R. J., Selk, S. H., Caldwell, L. (1982) Int. J. Pharm. 10, 307–321.
(50) Bodor, N., Sloan, K. B. (1980) U.S. Patent 4, 213, 978.