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CYCLIC NUCLEOTIDES, PROSTAGLANDINS AND POLYAMINES IN PSORIASIS

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1. INTRODUCTION

Psoriasis is a complex, probably hereditary proliferative skin disease. Numerous studies done over the last years demonstrate an imbalance of a variety of intracellular mediators and related biochemical systems in the diseased skin, and it is probable that the interplay of these modulators may determine the final expression of the disease in the epidermal component of the tissue. Our aim is to review the state of these systems in psoriasis, to report the functions that these modulators can effect in the epidermis, and to discuss how the interplay of these systems may be involved in the modulation of epidermal cell homeostasis.

2. PSORIASIS

As suggested by Watson *et al.* (1971) and Farber *et al.* (1974) multifactorial heredity and the environment may be important determinants in the cutaneous expression of psoriasis. Individuals with this disease have increased frequencies of specific cell-surface products of the HLA antigen groups (Woodrow *et al.*, 1975, and Beckman *et al.*, 1977), develop the disease either spontaneously or in an area of skin damage, and show seemingly partially uncontrolled, nonmalignant growth of the epidermis. The involved epidermis is characterized by markedly increased proliferative rates (Weinstein and McCullough, 1973; Marks, 1978; Harper *et al.*, 1978), reduced epidermal cell differentiation (Voorhees *et al.*, 1974; Voorhees *et al.*, 1968) and an increased glycogen content (Voorhees *et al.*, 1974). The epidermal abnormality is not restricted to the lesional area since the normal appearing skin of the psoriatic patient has been reported to show a 48 per cent increase in DNA synthesis when compared to normal human skin (Marks, 1978).

The cause of the misregulated epidermal cell growth in psoriasis is unknown. Numerous investigations of psoriatic epidermis have pointed to the involvement of several mediators and their biochemical regulatory systems, possibly as critical or primary modulators, in epidermal cell regulation. These are the cyclic nucleotide, the arachidonic acid and prostaglandin, and diamine and polyamine systems.

3. THE CYCLIC NUCLEOTIDE SYSTEM

3.1. CYCLIC AMP

3.1.1. Metabolism

Cyclic AMP (adenosine 3',5'-monophosphate) is predominantly located in the cytoplasm of the basal cells of the epidermis (Verma *et al.*, 1976). In the skin, as in other tissues, the level of cyclic AMP is regulated by its rate of formation and degradation. The formation of cyclic AMP from ATP is catalyzed by the cell membrane bound enzyme adenylate cyclase (Duell *et al.*, 1971; Marks and Rebien, 1972a). Cyclic AMP is degra-

dated by hydrolysis to 5' AMP. Cyclic nucleotide phosphodiesterase(s) catalyze this reaction and may be present in multiple forms in the skin (Voorhees *et al.*, 1973; Marks and Raab, 1974; Rusin *et al.*, 1978; Murray and Rogers, 1978).

At present, all the effects of cyclic AMP on cell organ function are thought to be mediated by specific activation by cyclic AMP of protein kinase (see Corbin and Lincoln, 1978 for a complete discussion) which catalyzes the phosphorylation of a variety of protein acceptors by ATP (Kumar *et al.*, 1971; Voorhees *et al.*, 1972a). Cyclic AMP-dependent protein kinase exists as a dimer of associated regulatory (R) plus catalytic (C) subunits, i.e., (RC)₂. The regulatory subunit inhibits the enzymatic activity of the catalytic unit by binding to it. This inhibition of C by R is relieved by the binding of cyclic AMP to R and the subsequent dissociation of the R and C units. The free catalytic unit then phosphorylates various protein substrates resulting in the biological effect of cyclic AMP (see George and Ignarro, 1978 for a review of other tissues). Cyclic AMP-dependent protein kinase has been demonstrated to exist in multiple forms in monkey (Kumar *et al.*, 1973), adult and neonatal skin (Kumar *et al.*, 1972; Kumar *et al.*, 1976). The biological significance of these various forms in the skin is at present unknown although interaction of the cyclic AMP and polyamine systems in other tissues has been suggested to occur through activation and inhibition of specific types of protein kinase (Bachrach *et al.*, 1978; Byus *et al.*, 1978; Hochman *et al.*, 1978).

3.1.2. Assay

The epidermis contains approximately 0.2–0.4 pmol of cyclic AMP/ μ g DNA (see Table 1) and therefore no more than 16–20 pmol of cyclic AMP are present in the average epidermal biopsy obtained from a psoriatic or normal person. Two sensitive and specific radio-ligand assays have been used to measure cyclic AMP in the epidermis. One method is the protein binding assay reported by Gilman (1970) which utilizes rabbit muscle protein kinase. The competition of ³H-labeled cyclic AMP and unlabeled cyclic AMP in standards and samples for the R unit of the enzyme complex forms the basis of the assay. The second assay is a radioimmunoassay which involves competition of labeled (¹²⁵I) and unlabeled cyclic AMP for highly specific antibody (Steiner *et al.*, 1969). When studying epidermal tissue, it is necessary to remove interfering substances from the sample by Dowex column chromatography for accurate assay of cyclic AMP and of cyclic GMP (Marcelo *et al.*, 1979).

TABLE 1. Cyclic Nucleotide Values for Psoriatic and Normal Epidermis reported in the Literature

Reference	Psoriatic	Normal	
	pmol cyclic AMP/ μ g DNA ^a		
	Involved	Uninvolved	
Voorhees <i>et al.</i> , 1972b	0.40 \pm 0.10 (25)	0.70 \pm 0.10 (25)	0.50 \pm 0.10 (25)
Voorhees <i>et al.</i> , 1973	0.72 \pm 0.11 (25)	1.08 \pm 0.09 (25)	0.86 \pm 0.08 (19) ^b
Yoshikawa <i>et al.</i> , 1975a	0.196 \pm 0.025 (10)	0.186 \pm 0.034 (10)	not done
Marcelo <i>et al.</i> , 1979	0.198 \pm 0.04 (28)	0.148 \pm 0.02 (28)	0.367 \pm 0.05 (34)
	pmol cyclic AMP/mg wet weight		
Härkönen <i>et al.</i> , 1974	2.10 \pm 0.35 (14)	1.62 \pm 0.36 (13)	0.94 \pm 0.17 (11)
Yoshikawa <i>et al.</i> , 1975a	1.16 (10) ^c	0.91 (10) ^c	not done
Wadskov and Søndergaard, 1978	not done	not done	1.31 \pm 0.10 (8) ^c
Marcelo <i>et al.</i> , 1979	0.32 \pm 0.046 (28)	0.32 \pm 0.045 (28)	0.81 \pm 0.097 (34)
	fmol cyclic GMP/ μ g DNA ^a		
Voorhees <i>et al.</i> , 1973	11.8 \pm 3.4 (12)	6.1 \pm 1.0 (12)	not done
Marcelo <i>et al.</i> , 1979	11.4 \pm 2.2 (28)	4.9 \pm 0.7 (28)	6.5 \pm 1.08 (34)

^amean \pm SEM; N number is in parenthesis.

^bDuell, unpublished data.

^cConverted to wet weight from dry weight using a 1:3 ratio in the references.

3.1.3. Cyclic AMP and Hormonal Stimulation of the Epidermis

The concentration of cyclic AMP in the epidermis increases after exposure of the animal or tissue slices to β -adrenergic activation by epinephrine, norepinephrine or isoproterenol (Powell *et al.*, 1971; Marks and Grimm, 1972; Yoshikawa *et al.*, 1975b). Histamine, possibly by H_2 receptor activation (Iizuko *et al.*, 1977) and prostaglandin (PG) E_1 and E_2 (Adachi *et al.*, 1975), possibly by specific receptor binding (Lord *et al.*, 1978), stimulate cyclic AMP synthesis in pig and human epidermis, although not in mouse epidermis (Marks and Rebien, 1972). Duell *et al.* (1975) demonstrated stimulation of cyclic AMP synthesis by mammalian epidermal slices with adenosine and 5' AMP, again probably through specific receptor interaction. Adachi *et al.* (1977) have also observed the increase of cyclic AMP content of the epidermis by 5' AMP, and report specific refractoriness of epidermal slices to 5' AMP. These investigators also reported refractoriness to epinephrine in floating epidermal slices (Adachi *et al.*, 1977), and in an *in vivo* assay in which hormone was injected intradermally and the epidermis removed by keratome (Iizuka *et al.*, 1978a). Recovery from the epinephrine-induced refractoriness in the *in vivo* situation began only after 48 hours and was not complete until one week after the initial injection (Iizuka *et al.*, 1978a). Specific refractoriness to PGE and histamine have also been demonstrated in the epidermis (Adachi *et al.*, 1977). The physiological significance of the refractory state of the epidermis in response to those hormones is at present obscure although it may have serious implications in the design of hormone therapy to alleviate the epidermal abnormalities of psoriasis.

3.2. CYCLIC AMP AND PSORIASIS

Since the initial report that changes in the cyclic AMP levels of the epidermis could be involved in the altered state of psoriatic lesional tissue (Voorhees *et al.*, 1972b), several laboratories have examined the content and metabolism of cyclic AMP in lesional and uninvolved epidermis. The cyclic nucleotide values for psoriatic and normal frozen epidermal biopsies reported in the literature are presented in Table 1. Both pmol per μ g DNA and per mg wet weight are listed so that all the reported values can be compared. Approximately 100 psoriatic lesional, psoriatic uninvolved, and normal biopsy samples have been processed and assayed for cyclic AMP levels. Seventy to eighty of the assays have been done by this laboratory over the last 6 yr. In the final analysis, the data indicate an abnormality in the cyclic AMP system in psoriasis, with a decrease in the lesional tissue levels associated with the disease process, probably at some specific time point in the development of the lesion. The data also suggests that there are time periods during which normal basal, and increased cyclic AMP epidermal content occur, a particular level of cyclic AMP possibly associated with a specific state in lesion development.

In our view, the data presented in Table 1 suggest that both increases and decreases in psoriatic epidermal cyclic AMP levels could be involved in the exacerbation, maintenance and possibly, the remission of the lesion. The precise relationship of cyclic AMP levels with lesion state is as yet unknown, although, as will be later discussed, cyclic AMP can have both positive and negative effects on epidermal growth using *in vitro* systems.

Besides determining the levels of cyclic AMP in the psoriatic epidermis, investigations of biopsy tissue have suggested that there are changes in the cyclic AMP generating system of lesional epidermis. Involved psoriatic tissue has been reported to demonstrate decreased responsiveness to β -adrenergic stimuli (Hsia *et al.*, 1972; Yoshikawa *et al.*, 1975b; Iizuka *et al.*, 1978b) and to PGE₁ (Aso *et al.*, 1975) and increased responsiveness to histamine (Iizuka *et al.*, 1978b). In addition, Iizuka *et al.* (1978c) have reported that the 'pure' epidermis from lesional tissue showed increases in both high and low km cyclic AMP and cyclic GMP phosphodiesterase activity. These reports suggest that altered cyclic AMP levels in lesional epidermis may be secondary to changes in the cyclic AMP generating and degrading enzyme systems. However, we can not dismiss the possibility

that the observed changes in the response of whole lesional tissue to β -adrenergic, PGE and histamine stimulation of cyclic AMP synthesis and the altered phosphodiesterase activity actually reflects a changed cell population, i.e. a larger portion of germinative cells, and is not an actual change in per cell enzyme activity.

Other data implicating changes in cyclic AMP in the manifestation of psoriasis are found in a number of clinical observations. Topical use of papaverine, a phosphodiesterase inhibitor that elevates cyclic AMP in epidermal tissue strips, produces a statistically significant improvement of psoriatic lesions (Stawiski *et al.*, 1975). Topical application of propranolol, a β -receptor antagonist (Gaylarde *et al.*, 1978) has been reported to cause psoriasiform changes in guinea-pig skin. Intradermal injection of the drug induced a proliferative response in uninvolved psoriatic skin while having no effect on the epidermis from normal volunteers (Wiley and Weinstein, 1977). Psoriasis-like skin reactions have also been seen with practolol, a β_1 -receptor antagonist (Tegner, 1976). It is possible that the psoriasis-like skin changes seen with these β -antagonists reflects some β -adrenergic-cyclic AMP abnormality in psoriatic skin although other modes of action are possible, such as propranolol acting as a membrane stabilizer and influencing prostaglandin tissue effects (Manku *et al.*, 1977).

3.3. CYCLIC GMP

3.3.1. Metabolism and Control of Epidermal Levels

Cyclic GMP (guanosine 3',5'-monophosphate), the other cyclic nucleotide shown to have widespread biological function, is present in the epidermis. Cyclic GMP is synthesized from GTP by the enzyme guanylate cyclase. Marks (1973) has identified guanylate cyclase activity in mouse epidermis homogenate supernatant. The cyclase is not stimulated by several β -agonists, acetylcholine, serotonin, thyroxine or somatotropin. Our attempt to define agents that alter epidermal cyclic GMP levels indicate that norepinephrine, PGF_{2 α} , triamcinolone acetonide, the phosphodiesterase inhibitors 1-methyl-3-isobutylxanthine and papaverine, indomethacin and injury do not change epidermal cyclic GMP levels (Marcelo, unpublished data). In our hands, acetylcholine and the cholinergic agonist, carbachol, can increase the epidermal levels of cyclic GMP but the time-course variation, the failure to block the effect with atropine and the demonstration of only a partial block with hexamethonium (nicotinic receptor antagonist), may indicate a non-receptor-specific epidermal cell membrane action with these agents, or the presence of cell refractoriness due to release of cholinergic transmitters during removal of the epidermal tissue by keratome (Marcelo, unpublished data). The only consistently high cyclic GMP value we have observed in our mouse epidermal strip studies is a three- to four-fold increase in epidermis snap frozen immediately after keratoming when compared to epidermis frozen 1–30 min after removal (floating in buffer at 37°C). The increase in cyclic GMP is partially or completely blocked by either ether or carbon dioxide anesthesia of the animal prior to death and epidermal keratoming (Marcelo, unpublished data). Belman *et al.* (1978) have reported an increase in epidermal cyclic GMP 36 hr after application of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), a tumor promoter and epidermal irritant at high doses. This suggests to us that, while epidermal levels of cyclic GMP may be increased by receptor-mediated action of cholinergic hormones, the epidermal cyclic GMP system can be stimulated by other cell membrane perturbations, perhaps defining interaction of epidermal cell cyclic GMP with rapid membrane-associated phenomenon, i.e. Ca²⁺ or other ion influx, some oxidation-reduction phenomenon such as those reported by White *et al.* (1976) in a rat lung fraction or by Haddox *et al.* (1976) in the lymphocyte.

Marks and Raab (1974) have studied the cyclic GMP phosphodiesterase activity of mouse epidermis supernatants and have reported one soluble phosphodiesterase activity hydrolyzing cyclic GMP, separate from another soluble fraction that degrades cyclic AMP. Rusin *et al.* (1978) have demonstrated cyclic GMP phosphodiesterase activity in

mouse epidermal homogenates which was not inhibited by Ro 20-1724, an inhibitor of cyclic AMP in mouse epidermal slice and homogenate systems, suggesting the existence of a separate system for degrading epidermal cyclic GMP.

3.4. ASSAY

As with cyclic AMP, the levels of cyclic GMP are extremely small, ranging from 5-7 fmol per μg DNA. The RIA assay using a highly specific antibody generated to a succinylated derivative of cyclic GMP in rabbits and goats (Marcelo *et al.*, 1979), is used to assay for cyclic GMP. The sensitivity of the assay is increased to meet the requirements of the extremely low cyclic GMP tissue levels by acetylation as described by Harper and Brooker (1975). Using the acetylation technique, the standard curve for a cyclic GMP RIA runs from 1-200 fmol, and requires extensive purifications of the epidermal samples (Marcelo *et al.*, 1979).

3.4.1. Cyclic GMP in Psoriasis

As presented in Table 1, the levels of cyclic GMP have been determined in forty psoriatic lesional and uninvolved, and thirty four normal epidermal biopsies. The results indicate a statistically significant two-fold increase in the epidermal cyclic GMP levels of the lesional tissue. This increase in cyclic GMP has been postulated to be associated with the increased proliferative state of the psoriatic lesional epidermis (Voorhees *et al.*, 1973). However, the function or cause of the increased cyclic GMP levels in psoriasis is not as simple as postulated in 1973 since Marks (1978) has demonstrated increased proliferative activity in uninvolved psoriatic tissue that does not show any increase in assayable cyclic GMP levels (Table 1). Moreover, in non-psoriatic proliferative systems, i.e. TPA stimulated epidermal proliferation, the role of cyclic GMP remains undefined (Mufson *et al.*, 1977; Belman *et al.*, 1978).

3.5. FUNCTION OF CYCLIC NUCLEOTIDES IN *In Vitro* SYSTEMS

Investigations of the cyclic AMP system in the epidermis has shown association of several hormonal receptors with changes in epidermal levels of this cyclic nucleotide, and both cyclic AMP and cyclic GMP have been implicated in the hyperproliferative and abnormally functioning psoriatic epidermis. These investigations suggest that changes in epidermal cyclic nucleotides can influence epidermal cell function.

3.5.1. Cyclic AMP

Several *in vitro* studies designed to define the mode of cyclic AMP-related epidermal cell function(s) have been reported. Dibutyryl cyclic AMP and isoproterenol have been shown to inhibit the G₂ phase of the epidermal cell cycle in an *in vitro* 5-hr mouse ear assay (Voorhees *et al.*, 1972c). These results were confirmed and extended by Harper *et al.* (1974), Flaxman and Harper (1975) using organ and explant (Chopra, 1977) human skin cultures. In addition, Chopra (1977), employing the human explant culture system reported a 93 per cent inhibition of the labeling index 4 days after exposure of the cultures to 1 mM dibutyryl cyclic AMP plus the PDE inhibitor theophylline. Delescluse *et al.* (1974) have demonstrated inhibition of adult guinea-pig ear cultures by cyclic AMP elevating agents and analogues, an effect associated with increased differentiation.

Green, using a neonatal human keratinocyte system that employs a 3T3 feeder layer to support the proliferation and extensive differentiation of the cultures (Rheinwald and Green, 1977; Sun and Green, 1976; and Rice and Green, 1978) reports a positive correlation between four agents known to increase cellular cyclic AMP levels and increases in the growth of the colonies of cultured keratinocyte (Green, 1978).

We have investigated the effect of dibutyryl cyclic AMP, 8-bromo cyclic AMP and cholera toxin plus a phosphodiesterase inhibitor on a neonatal mouse primary keratinocyte system which initiate predominantly as basal cells and show progressive specialization (differentiation) over time (Marcelo *et al.*, 1978). The results indicate that the cyclic AMP elevating agents cause a dose- and time-dependent stimulation of keratinocyte proliferation (Marcelo, 1979). The treated cells show accelerated stratification, specialization and shedding of top cell layers. Moreover, the data indicated that cyclic GMP was not involved in the stimulation of proliferation observed with the cyclic AMP elevating agents. These results indicate that increased cyclic AMP levels are associated with keratinocyte culture proliferation and accelerated specialization in neonatal systems and can inhibit epidermal cell growth in adult human *in vitro* systems. The difference in response between neonatal and adult epidermal cell response to elevated cyclic AMP levels may result from differences between *in vitro* techniques, species differences or a different cyclic AMP control mechanism operating in adult versus neonatal cells. Our preliminary studies using adult keratinocyte cultures on collagen gels show that elevated levels of cyclic AMP can both stimulate and inhibit the cultures. The difference between the neonatal and adult system, at least *in vitro*, seems to be in both the dose- and time-response curves of the cells (Marcelo and Duell, 1979). It is possible that both the stimulatory and inhibitory effects of cyclic AMP on epidermal cell proliferation can be exploited to control the cutaneous symptoms of psoriasis.

3.5.2. Cyclic GMP

Using the neonatal mouse keratinocyte culture system, we have demonstrated that 10^{-7} M 8-bromo cyclic GMP enhances keratinocyte specialization without any effect on cell proliferation (Marcelo, 1979). This suggests a role for cyclic GMP in the specialization of the neonatal epidermis. The role of this cyclic nucleotide in adult systems is yet to be defined.

4. THE ARACHIDONIC ACID AND PROSTAGLANDIN SYSTEM

4.1. METABOLISM AND ASSAY

Prostaglandins are 20-carbon monocarboxylic acids with five membered cyclopentane rings and are synthesized in cells from essential polyunsaturated precursor fatty acids, i.e., arachidonic acid (20:4) and 8,11,14-eicosatrienoic acid (20:3). Arachidonic acid and its biotransformation products, and 20:3 and its products are in skin, although 20:3 and 20:4 occur in a 1–10 ratio (Wilkinson and Walsh, 1977). The precursor acids for prostaglandin synthesis arise from several intracellular lipid pools, one being the membrane phospholipids. The enzyme responsible for release of fatty acid precursors for prostaglandin biosynthesis is phospholipase A_2 (see Galli *et al.*, 1978 for detailed reviews). The precursor fatty acids can be introduced into membrane phospholipids by esterification. When the precursor fatty acids are in the 'free' non-membrane bound form, they are acted upon by several enzyme systems to form a group of biologically active molecules (Fig. 1). Arachidonic acid, the most abundant free fatty acid in the skin, is acted upon by two enzymes, each resulting in a separate series of products. One enzyme is fatty acid cyclooxygenase which forms the endoperoxides (PGG_2 and PGH_2) and the products thromboxane A_2 and B_2 , and PGE_2 , $PGF_{2\alpha}$, and PGD_2 (Samuelsson, 1976). The other enzyme, lipoxygenase results in the formation of the products HPETE (hydroperoxy-HETE) and HETE (12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (Samuelsson, 1976). Prostaglandins and their precursors are present in very small amounts in tissues. To assay for these compounds, mass spectrometric, gas chromatography-mass spectrometric and radioimmunoassays have been developed (Samuelsson and Paoletti, 1976; Lindgren *et al.*, 1974).

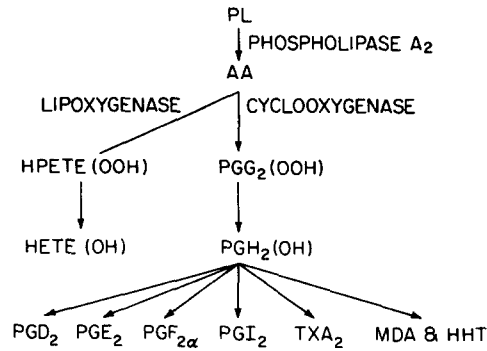


FIG. 1. Arachidonic acid and its major biotransformation products. PL: membrane phospholipids; AA: free arachidonic acid; HETE: 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid; HPETE: hydroperoxy-HETE; MDA: malondialdehyde. There is a 26-fold increase in free arachidonic acid and an 86-fold increase in HETE in psoriatic lesional epidermis. PGE₂ is increased 40 per cent while PGF_{2α} is elevated 80 per cent in psoriasis (Hammarström *et al.*, 1975).

4.2. PROSTAGLANDINS AND THEIR PRECURSORS IN THE SKIN

In rat skin, two major essential fatty acids are linoleic and arachidonic acids both found in the phospholipid component (Prottey, 1977). Arachidonic acid is the most important precursor of the prostaglandins (Prottey, 1977) and the prostaglandins produced by the microsomal fraction of skin are PGF_{2α}, PGE₂, and D₂ (20:4 or arachidonic acid the precursor) (Kingston and Greaves, 1976). However, Wilkinson and Walsh (1977) have reported that 20:3 is also converted to PGE₁, PGD₁, and PGF_{1α} by mouse skin homogenates.

PGE₂ is the major prostaglandin synthesized by human skin homogenates and arachidonic acid is the major 20-carbon acid esterified into the phospholipid fraction (Jonsson and Anggard, 1972). The enzyme activity is localized in the microsomal fraction (Ziboh, 1973). PGE₂ has been implicated in cutaneous inflammatory response to injury. Anggard and Jonsson (1972) demonstrated that PGE₂ is formed in the skin following a burn injury. Jørgensen and Søndergaard (1973) studying the response of human cutaneous blood vessels to intradermal injection of PGE₁, reported that this prostaglandin can mediate vascular permeability in sustained acute inflammation of the skin and thus may be responsible for sustained skin inflammation. Søndergaard *et al.* (1973) have reported a similar effect with PGE₁ on human skin.

The essential fatty acid-deficient rat (Ziboh and Blank, 1973) and hairless mouse (Lowe *et al.*, 1977a) systems have been used to study the functional significance of arachidonic and 20:3 fatty acids, and their products in the epidermis. The cutaneous features of the condition are thickening of the skin and scaling, acanthosis, hyperkeratosis and increased epidermal mitotic indices that are relieved by topical application of PGE₂ and linoleic acid (Ziboh *et al.*, 1972; Lowe and Stoughton, 1977a; Lowe, 1977). The decrease in the mitotic rate of EFA deficient epidermis by topical application of PGE₂ suggests control of epidermal proliferation by the cellular PGE₂ content. However, the direction of the proliferative response to PGE₂ varies since in normal hairless mice topical PGE₂ stimulates epidermal proliferation (Lowe and Stoughton, 1977b).

4.3. THE ARACHIDONIC ACID AND PROSTAGLANDIN SYSTEM IN PSORIASIS

The epidermal phospholipids are elevated in psoriasis, a change possibly connected with alterations of membrane-bound phospholipids (Tsambaos *et al.*, 1977). How these changes may be involved in lesional epidermal cell membrane structure and function is unknown. The changes in psoriatic lipid metabolism are not those seen in the EFA-deficient condition although the general symptoms, i.e., scaling, increased mitotic rate,

etc., are similar. In the EFA deficient state 20:3 and 20:4 fatty acids and their products are absent, while in psoriatic lesional tissue there is a 40 and 80 per cent increase in the lesional levels of PGE₂ and PGF_{2 α} , respectively (Hammarström *et al.*, 1975). These changes in prostaglandins are not as pronounced as the 26-fold increase in free arachidonic acid and 82-fold increase in HETE found in the lesional tissue. This increase in free arachidonic acid and HETE in psoriasis suggests some changes in the psoriatic epidermal cell control of the enzymes involved in arachidonic acid biotransformation. One possible causative mechanism for this change is the presence of an endogenous inhibitor of prostaglandin synthesis (Pennys *et al.*, 1977) that may be exaggerated in psoriasis (Simon *et al.*, 1977). How a comparatively decreased rate of prostaglandin synthesis in association with an accelerated free arachidonic acid metabolism to a predominant HPETE and HETE system can affect epidermal cell functions is unknown, although the biotransformation process itself may be involved in the changes observed in epidermal cell function. The fact that glucocorticoids, which reduce the levels of both arachidonic acid and HETE in the tissue (Hammarström *et al.*, 1977), is therapeutic in psoriasis supports the theory that these compounds may be involved in the disease process.

5. POLYAMINE SYSTEM

The diamine putrescine, and the polyamines spermidine and spermine are found in all living material (Raina and Jänne, 1975). These amines are polycationic in nature, are elevated in actively proliferating tissues and bind tightly to nucleic acids suggesting an interrelationship between them and the synthesis of nucleic acids and proteins (Tabor and Tabor, 1976; Knutson and Morris, 1978). A requirement for intracellular polyamine concentration during the G₁ to S transmission in CHO cells has been reported by Fuller *et al.*, (1977). This association of G₁ to S transition with induction of polyamine biosynthesis has been associated with enhanced cell cyclic AMP levels suggesting that cyclic AMP may regulate polyamine synthesis, at least in some systems (Hibasami *et al.*, 1977; Costa, 1978). Fluctuations in polyamine levels have been reported in a number of disease states including cancer (Russell, 1973), cystic fibrosis and psoriasis (Russell and Durie, 1978, and Russell *et al.*, 1978).

5.1. METABOLISM AND ASSAY IN THE SKIN

As diagrammed in Fig. 2, the diamine putrescine is synthesized from ornithine, the reaction catalyzed by ornithine decarboxylase (ODC). Putrescine stimulated S-adenosyl-L-methionine decarboxylase and spermidine stimulated S-adenosyl-L-methionine decarboxylase (SAMD) are involved in the synthesis of spermidine and spermine, respectively, from putrescine with ODC being the rate-limiting enzyme for the overall synthesis of these naturally occurring polyamines (Jänne *et al.*, 1978).

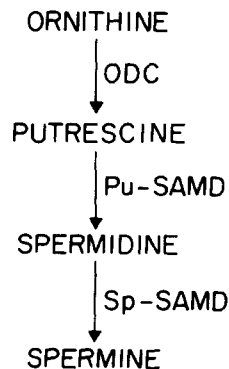


FIG. 2. The diamine and polyamine biosynthetic system. ODC: ornithine decarboxylase; Pu-SAMD: putrescine stimulated S-adenosyl-L-methionine decarboxylase, synthesis of spermidine; Sp-SAMD: Spermidine stimulated S-adenosyl-L-methionine decarboxylase, synthesis of spermine.

In cells, polyamines occur in the free form while polyamine conjugates and metabolites are found in urine and serum (Daves, 1978, contains extensive detailed reviews). Assay of polyamine involves fluorometric assay of dansyl derivatives (acetyl conjugates) separated by thin layer chromatography or mass spectrometric analyses of the conjugates (Bachrach, 1978). High pressure liquid chromatography of the dansyl derivatives has been described (Abdel-Monem *et al.*, 1978) and Russell (1973) has described an automated system using an amino acid analyzer.

5.2. POLYAMINES IN THE SKIN AND IN PSORIASIS

Ornithine decarboxylase has been characterized in rat skin and reported to be increased in stimulated follicular proliferation and to fluctuate in response to changes in dietary protein (Morrison and Goldsmith, 1978). Wound healing (Mizutani *et al.*, 1974) and ultraviolet irradiation (Lowe *et al.*, 1978) increase ODC activity in the skin and epidermal injury by topical application of a series of phorbol esters (i.e., TPA) is associated with induction of ODC and SAMD (O'Brien *et al.*, 1975). Another type of tumor promotion, wounding, also caused induction of ODC activity (Clark-Lewis and Murray, 1978) suggesting a correlation between enhanced polyamine biosynthesis and the epidermal growth involved in the tumor promoting process. This correlation may not be absolute since Murray (1978) reported enhancement of epidermal cell mitosis with TPA in Vitamin B₆-deficient mice where ODC activity is reduced due to lack of pyridoxal 5'-phosphate, an essential co-factor for ODC activity (Raine and Jänne, 1975). Murray (1978) interprets this data as not supporting a concrete cause-effect relationship between ODC and induction of proliferation in mouse epidermis.

The spermidine and spermine levels in blood samples from psoriatic patients were found to be two to three times those of control subjects (Proctor *et al.*, 1975). Grosshans and Henry (1978) reported that the levels of all the polyamines were significantly elevated in the lesional tissue while the uninvolved psoriatic tissue demonstrated an increase in only the putrescine levels. In a simultaneous study, Russell *et al.* (1978) found that the putrescine, spermidine and spermine levels were higher in lesional than in uninvolved psoriatic tissue, and that uninvolved tissue levels of these same three polyamines are higher than those of normal volunteer epidermis. The urine from psoriatic patients reflected the increased epidermal polyamine levels, and a six-fold increase in epidermal ODC and SAMD were associated with the increased putrescine, spermidine and spermine levels. The increased activity of polyamine biosynthetic enzymes were significantly decreased by application of glucocorticoids to the lesions for 24 hr, indicating that while elevated polyamine biosynthesis is associated with the active lesion, efficacious treatment reduces the activity of these enzymes in the epidermis. Russell *et al.*, (1978) suggest that use of ODC and SAMD inhibitors may be used as part of a therapeutic program to control the disease.

6. CHANGES IN EPIDERMAL CELL HOMEOSTASIS AND THE CYCLIC NUCLEOTIDE, PROSTAGLANDIN AND POLYAMINE SYSTEMS

Psoriasis is characterized by changes in a number of modulatory systems, any one or all of which may be involved in the cutaneous manifestation of the disease. The level of cyclic AMP seems to be decreased in the psoriatic epidermis and a decreased lesional cyclic AMP concentration when compared with the uninvolved epidermis from the same patient has been reported in several studies (Table 1). Cyclic GMP, a second biologically active cyclic nucleotide, is increased approximately two-fold in the lesional areas when compared to both uninvolved and normal volunteer epidermis.

A less sensitive β -adrenergic system may be responsible, in part, for the lowered levels of cyclic AMP in the lesional tissue. Moreover, this decrease in the total lesional cyclic AMP may contribute to the markedly increased proliferation of the lesional tissue. The

development of psoriasiform lesions and increased proliferation in the presence of β -adrenergic antagonists supports the theory of a malfunctioning β -adrenergic receptor system in psoriasis.

In vitro investigations of the effect of cyclic AMP on epidermal cells in culture has added a new dimension to the possible mechanism of effect of cyclic nucleotides in psoriasis. *In vitro* investigations have demonstrated that cyclic AMP can both stimulate (the neonatal mouse, and neonatal and adult human) and decrease the growth (adult human, guinea-pig ear) of the epidermal cell, suggesting a bidirectional effect by this cyclic nucleotide. A precise definition of the effects of cyclic AMP and cyclic GMP on psoriatic lesional and uninvolved tissue is still a missing part in the puzzle that must be completed in order to decipher the role of cyclic nucleotides in this cutaneous disorder.

The levels of putrescine, spermine and spermidine are elevated in the lesional epidermal tissue concomitant with an increase in ODC activity. The increased ODC and polyamine levels correlate with the enhanced proliferative activity of the tissue (Russell *et al.*, 1978) and, if the result of studies in other systems is applicable to the skin, should correlate with elevated cyclic AMP levels. Roseeuw *et al.* (1979) have demonstrated some correlation between increased ODC- and cyclic AMP-stimulated keratinocyte growth. However, the correlation occurs in only approximately 70 per cent of the experiments, suggesting a casual relationship, at best, between the cyclic nucleotide and polyamine systems, at least in the *in vitro* situation. However, these *in vitro* studies do not disprove the possible occurrence of a critical link between the cyclic nucleotide and polyamine system in the exacerbation of psoriasis. For example, it is possible that while cyclic AMP may be decreased when the bulk DNA or protein of a lesion is considered, this cyclic nucleotide may be localized at increased concentrations in key regulatory areas (basal cells). It is possible that a functional connection among cyclic AMP, polyamines and epidermal cell proliferation may occur only at key time points in psoriatic skin.

The activity and induction of the polyamine biosynthetic system has recently been linked, in a mandatory mode, with stimulated epidermal cell proliferation. Verma *et al.* (1977) have reported that TPA-induced ODC activity is blocked by indomethacin, an inhibitor of prostaglandin synthesis. This block of enzyme induction is overcome by addition of PGE₂ application. These results indicate that TPA induction of ODC activity and proliferation in the epidermis can be linked in a cause-effect series with some as yet unknown PGE₂-requiring step, at least in mouse epidermis.

To what extent the polyamine-prostaglandin-cyclic-nucleotide-proliferation model systems, both *in vivo* and *in vitro*, reflect the psoriatic epidermal cell state is unknown. However, investigations of the functional role of these mediators in model systems will help to decipher the functional interaction of these modulators in the epidermis, and possibly lead to formulation of safe and efficacious therapeutic programs to control this disease.

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