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THE HUMAN SKIN: FRAGRANCES AND PHEROMONES

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Summary—Non-human mammalian pheromones are commonly used as perfumery ingredients. The actual purpose for using these compounds is as a fixative or carrier for the odor effects of the other ingredients as well as a contributor, in part, to the over-all scent of the perfume. Although such materials are used for their fixative and odor qualities rather than their pheromonal effects, perfumes are generally marketed as having the ability to enhance sexual attractiveness. While providing a scent may elicit a positive pleasant response, this should not be confused with a pheromone reponse. The attractive effect of perfumes is principally related to the effect of the pleasant scent. A more logical approach would be to use human pheromones which, for humans, are both more natural and more effective as true sensual attractants. It seems likely that implementation of this approach will constitute an important paradigm in the perfume industry as perfumery moves from the realm of art to that of science.

INTRODUCTION

Many species use pheromones as a means of intraspecies communication. However, the mechanisms of delivery vary for each species. To approach the problem of identifying human pheromones we must try to understand the mechanism by which they might work in humans. Human skin is a multi-function organ which may play a role in pheromone delivery. Desquamating skin may provide an airborne vehicle for pheromone communication. These possibilities are discussed.

"The female who so assiduously washes off her own biological scent, then proceeds to replace it with commercial 'sexy' perfumes which in reality are no more than diluted forms of the products of the scent glands of other, totally unrelated mammalian species." (Desmond Morris, "The Naked Ape")

We know that many, perhaps all, mammalian species use pheromones as a means of inducing sex-related behavior in potential mates. For example, mammalian pheromones have been identified in deer, hamsters, hyenas, marmosets, rabbits, pigs, dogs, cats and rhesus monkeys. We also know that for hundreds of years non-human, mammalian pheromones have been used in perfumes. In fact, the vast majority of perfumes on the market today utilize some form of a mammalian pheromone or a synthetic analog as a significant component. However, since pheromones are species specific, they do not produce a physiological pheromone effect in humans. The most notable of these ingredients are muscone, produced by the musk deer (*Moschus moschiferus*), civetone and skatole produced by the civet cat (*Viverra civetta*), and castoreum produced by the Canadian beaver (*Castor fiber*). These substances were originally obtained from the anal sacs of these animals. This practice is now substituted by chemical synthetics and is ubiquitous in the perfume industry. The number of major perfumes [1] utilizing such materials or their synthetic equivalents as a significant component are listed in Table 1.

The purpose of using these compounds in perfumes is as a fixative or carrier for the fragrance of the other ingredients, and as a contributor, in part, to the over-all scent of the perfume. Although such materials are used in perfumes for their fixative and odorous qualities rather than any known physiological pheromone effect, perfumes are generally marketed as having the ability to enhance sexual attractiveness. This is implicit in the names of many perfumes, such as "Intimate", "Le Temps d'Aimer", "Sex Appeal", "Moment Supreme", "L'Aimant", "Aphrodisia" and "Pheromone". This is also apparent from the sexy advertising campaigns that usually accompany the marketing strategies of the perfume industry.

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Table 1

Animal	No. of Female fragrances utilizing animal pheromones (samplings = 400)		No. of Male fragrances utilizing animal pheromones (samplings = 350)	
	No.	%	No.	%
Musk Deer	340	85	328	94
Civet "cat"	156	39	21	6
Beaver	26	7	47	13
Pig	2+		2+	

The reason that the total number of animal pheromones is higher than the samplings is because some fragrances utilize more than one animal pheromone.

It is important to understand what commercial perfumes can and cannot do. It may be that providing a scent elicits a pleasant psychological response. However, this should not be confused with a true pheromone effect. A true pheromone provides chemical communication between members of the same species and elicits a neurophysiological response resulting in an alteration in sensual behavior. The human behavior expected from pheromone stimulation is an enhancement of libido. While a true pheromone will elicit an enhancement of libido, a scented perfume may to some extent be pleasant to the senses. It would be expected, however, that the effect of pheromones would be more profound and irresistible.

If the receptors for fragrances and pheromones were different, pheromone effects could be distinguished from fragrance effects by measuring differences in receptor binding or stimulation. Fragrance *per se* is experienced by stimulation of olfactory epithelium. In mammals, pheromone receptors are located in the vomeronasal organ (VNO) [2]. While this organ has been thought to be vestigial in human adults, data recently presented [3, 4] indicates that this organ could be functional in humans and therefore provide a likely location for human pheromone receptors. The olfactory epithelium is both physically and functionally distinct from the VNO. Thus, measurement and comparison of the VNO and olfactory stimulation can be used to distinguish and compare the activity of compounds which produce a potentially pleasant effect from those which elicit a true pheromone response. Of course a pheromone may also be odorous, and the behaviour induced by an odorous pheromone may therefore have a psychological component. By these criteria, any perceived attractive effect of commercial perfumes which are presently available is attributable primarily to a pleasant scent.

A more logical approach to perfumery would be to use human pheromones which, for humans, are both more natural and more effective as true attractants. For perfumes, these human-appropriate pheromones could be combined with an attractive fragrance thereby exploiting aspects of sensual attraction. It seems likely that implementation of this approach will constitute an important paradigm in the perfume industry as perfumery moves from the realm of art to that of science.

MECHANISMS OF HUMAN PHEROMONE DELIVERY

We have approached the problem of identifying human pheromones by first trying to understand the likely mechanism of pheromone communication between individuals. Many species utilize pheromones as a means of sexual communication. However the mechanism of delivery varies for each species. Several mammalian species exhibit distinct behaviors which facilitate the introduction of volatile pheromones into the air. For example, many mammals "mark territory" by urination; while male pigs produce a pheromone in their saliva which is aerosolized by mastication. Humans do not exhibit any specific behavior which has been associated with pheromone communication, however, various sexually dimorphic odors are present on the skin, and it has been suggested that a putative pheromone would be introduced into the environment from the skin.

At least two mechanisms can be envisaged by which pheromones can be liberated into the environment from the skin. One possibility assumes that putative pheromones are highly volatile. Odorous molecules present on the skin would volatilize and the molecules would be inhaled by another in close proximity. A more interesting possibility is that the skin itself provides a natural delivery system for relatively non-volatile pheromones present on, or associated with, the skin surface. This intriguing possibility will now be considered in detail.

THE SKIN AS A POSSIBLE PHEROMONE DELIVERY VEHICLE

The skin is the largest organ of the body, encompassing over 1.7 m^2 for a 70 kg man [5]. The primary function of the skin is to wrap the

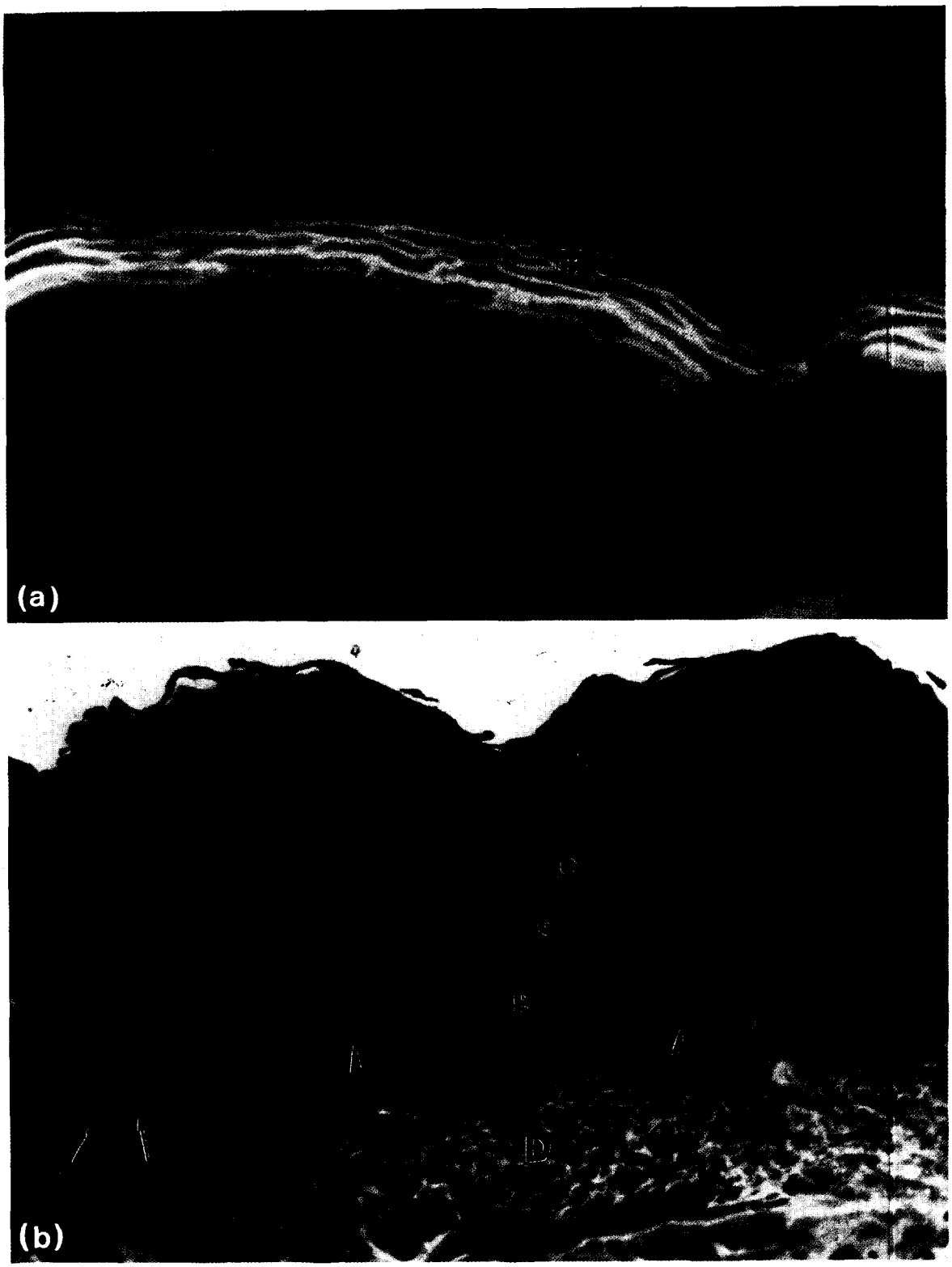

Fig. 1. (a) Frozen section of the epidermis stained with a florescent dye to visualize the stratum corneum (SC), which consists of numerous layers of flattened, highly compacted horny cells (HC). (b) One micron plastic section of a portion of the epidermis consisting of basal (B), spinous (S), granular (G), and horny (H) cells. Basal keratinocytes situated at the tips of the epidermal downgrowths (arrows) have a relatively smooth dermal–epidermal junction and are thought to represent stem cells. Remaining basal keratinocytes are characterized by a "serrated" dermal–epidermal interface (arrow heads). Red staining area just above the granular cells is the portion of stratum corneum where horny cells are extremely cohesive. D, dermis.

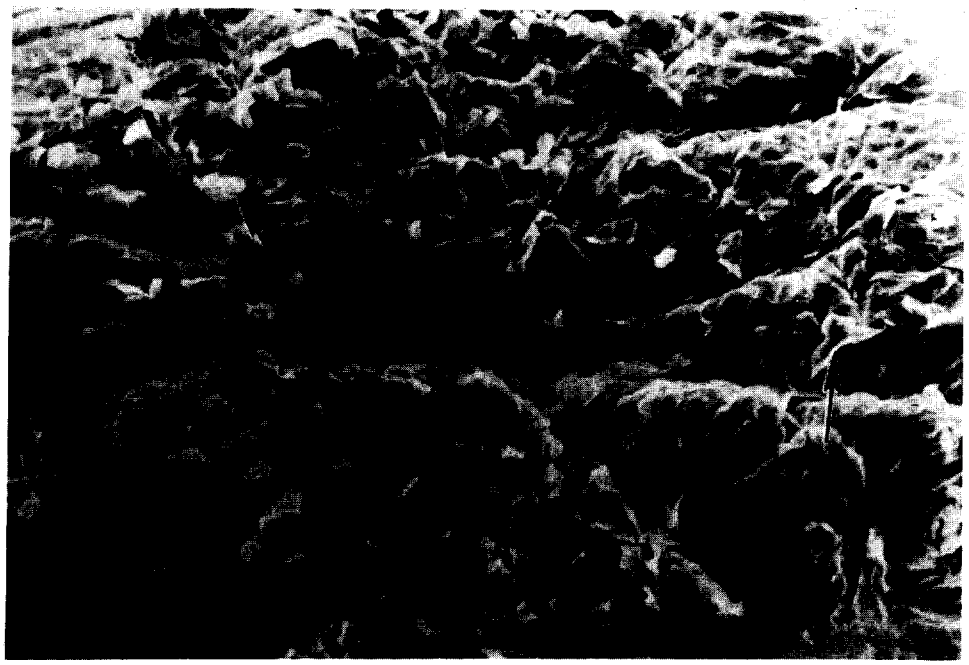

Fig. 2. Scanning electron micrograph of the surface of the skin. Single or small clusters of cells are continuously shed or desquamated (arrows).

organism and separate it from the environment. As a protective organ, the skin must lessen the effects of physical and mechanical traumas, prevent the penetration of noxious agents, and ensure against excessive loss of fluids. The epidermis, the outermost tissue of the skin, is the first line of defense in this system. The epidermis is composed of living cells (keratinocytes)

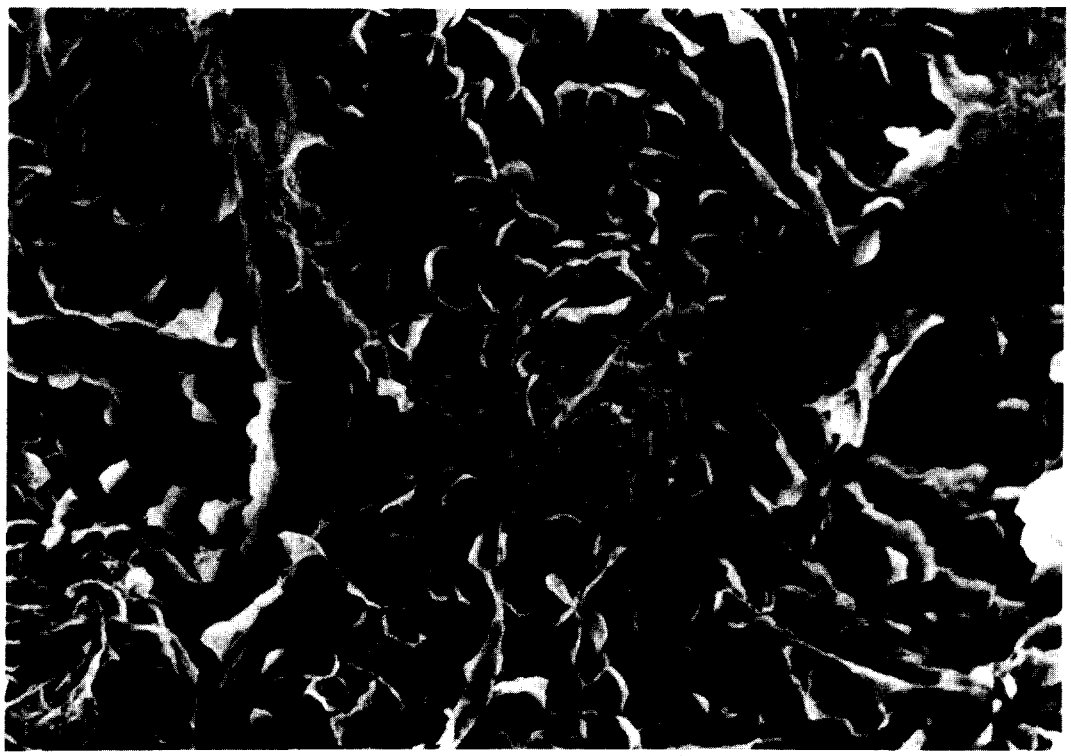

Fig. 3. High magnification scanning electron micrograph of the skin surface showing outermost cells in the process of desquamation.

and a tough outer wrapping known as the stratum corneum. The stratum corneum is a two-component region consisting of multi-layers of flattened, highly compacted, overlapping "horny cells", in a matrix of lipid-rich intercellular material [6–8]. Horny cells are extremely cohesive in the lower region of the stratum corneum closest to the viable keratinocytes, and more loosely organized at the surface [Figs 1(a) and (b)]. From the surface, horny cells and their intercellular material eventually slough or desquamate into the environment (Figs 2 and 3) [9, 10]. The cells of the stratum corneum develop from keratinocytes through a specialized program of differentiation known as keratinization [11]. Three types of stage-specific keratinocytes have been identified: the basal

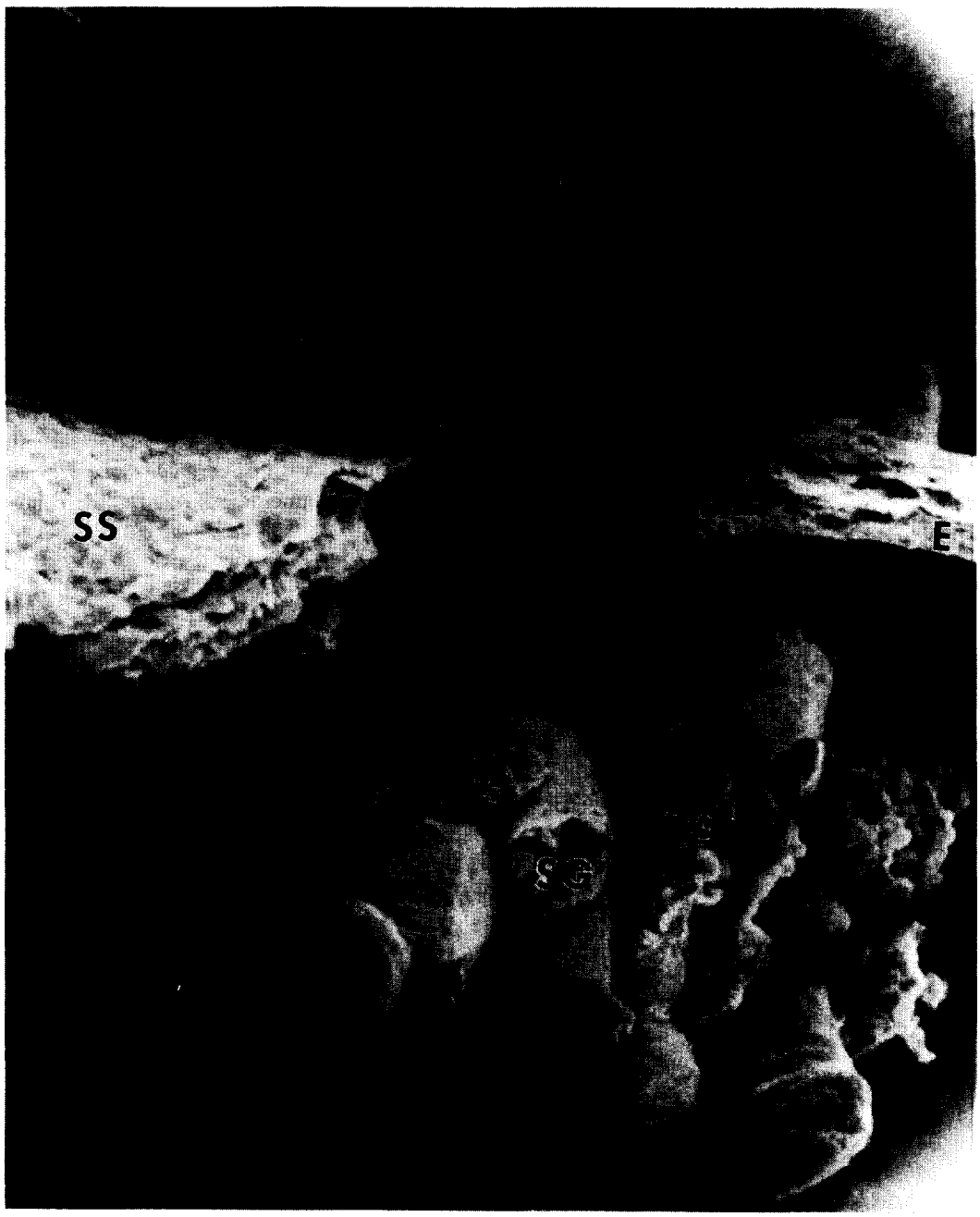

Fig. 4. Low magnification scanning electron micrograph depicting the relationship of some underlying appendages. Major appendage visualized is the pilosebaceous unit consisting of the follicular canal (FC) and the multilobulated sebaceous glands (SG). Arrows point to a portion of the coiled duct of a sweat gland.

keratinocytes (which provide a stem cell function) [12, 13], spinous keratinocytes, and granular cells [Fig. 1(b)]. This is a dynamic system in which horny cells are constantly being sloughed from the skin surface and the desquamated horny cells are being replaced by differentiating keratinocytes [14]. This process of keratinization is both spatially and temporally regulated. As cells mature from basal keratinocytes to desquamating horny cells they move vertically from the innermost layers of the epidermis, nearest the dermal capillaries, through the outer epidermal layer, the stratum corneum and finally they desquamate from the skin surface [11]. This process takes from 14 to 21 days. Keratinization can be divided into two phases: a synthetic phase and a transformation phase. In the synthetic phase keratinocytes sequentially elaborate a series of stage-specific differentiation products. Late in this process, during the transformation phase, granular cells undergo a series of consolidation and condensation changes which result in the formation of the compact stratum corneum [14]. During the synthetic phase, keratinocytes metabolize a variety of nutrients and other components provided by the blood from the dermal capillaries. They also produce stage-specific metabolites, and sub-cellular structures which include keratin filaments, membrane bound granules, keratohyalin granules, and a distinctive, thickened cell membrane. It is during this synthetic phase that the maturing keratinocytes may produce or play a role in the processing of pheromone or pheromone precursors. For pheromones may be produced *de novo* by keratinocytes. Alternatively, keratinocytes may process pheromone precursors supplied by the blood exudiate. The pheromones might then be sequestered within the keratinocytes, incorporated into the intracellular structures characteristic of these cells or secreted into the intercellular material.

The major known differentiation product elaborated by keratinocytes is the keratin filament (Fig. 5). Keratin filaments are a member of the class of intermediate filaments and are composed of keratins; a heterogeneous family of acidic and neutral-to-basic proteins. Keratins are usually produced in the basal layer and during the course of stratification and differentiation, more complex patterns of keratin expression are manifested. Keratin filaments are present in basal, spinous, granular and horny cells and represent the major structural component of the keratinocyte (reviewed in [5]). These filaments exist as bundles and are

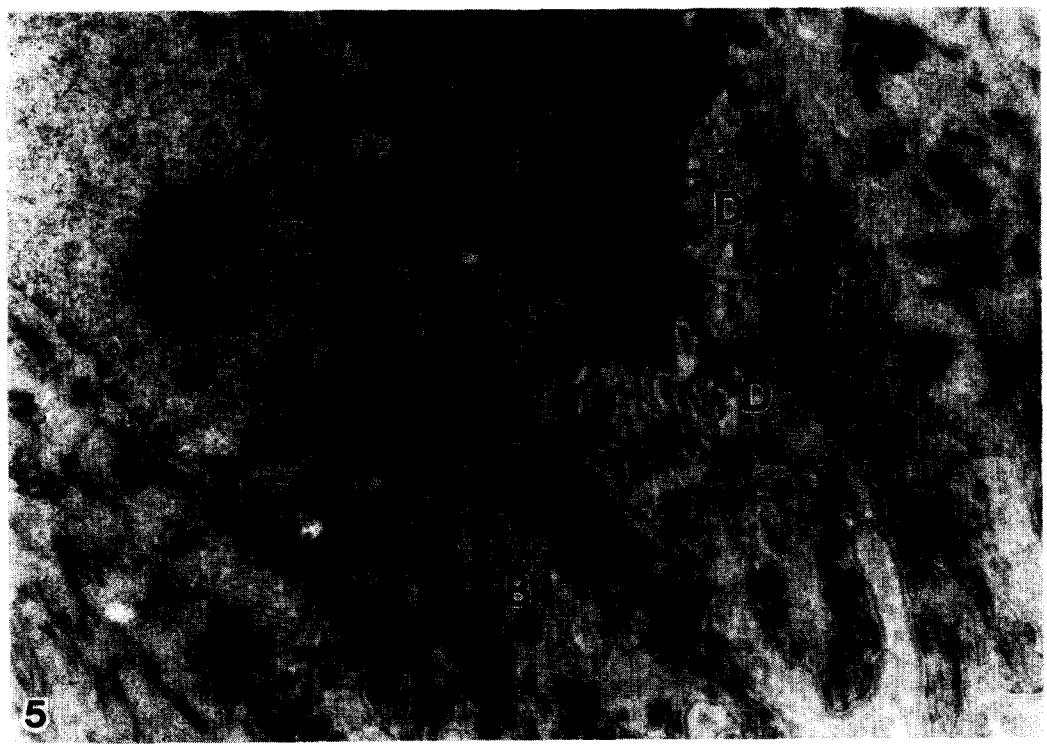

Fig. 5. Transmission electron micrograph showing a portion of basal keratinocyte (B) filled with numerous keratin filaments (F). Filaments exist in bundles and are connected to desmosomes (D). N, nucleus.

connected to desmosomes, which connect the filament systems of individual neighboring cells and help maintain cell shape. Another known differentiation product elaborated is a small, round to ovoid membrane bound granule, 0.1–0.4 m in size. These granules have a highly periodic internal structure consisting of alternating dense and less dense lamellae, and are known as lamellar bodies or membrane coating granules (Fig. 6) [6, 15]. They first appear in spinous cells and are preferentially located at the apical portion of the membrane. They are the only differentiation product not retained by the cell. During the transformation phase, they fuse with the cell membrane and discharge or secrete their contents into the intercellular spaces [6, 7]. These lamellae mix with the intercellular material and form broad stacks of lipid-rich, membrane-like sheets between the adjacent horny cells (Figs 7 and 8) [6, 7, 15]. This extensive system of membrane-like figures, which comprises the intercellular component of the stratum corneum, is thought to govern permeability and plays an important role in the barrier function of the stratum corneum [6, 7, 16]. A third differentiation product can be seen at the light microscope level within granular cells as round specks, or coarse irregular lumps. These dense bodies are known as keratohyalin granules and appear as electron dense bodies that progressively grow in size, forming large masses within the uppermost granular (transitional) cells. They are often surrounded by ribosomes and are always in close association with keratin filaments [17]. Biochemically, these granules are primarily a globular protein (as distinguished from the helical keratin proteins) rich in proline, histidine and cystine [18]. Recently it has been shown that the histidine-rich basic protein in the keratohyalin granule was a high molecular weight, phosphorylated, polymeric precursor called profilaggrin [19]. This had led to the concept that the keratohyalin granule is a source of profilaggrin which is processed to filaggrin during the transformation phase [20]. Within the horny cell, filaggrin may function as an interfilamentous matrix protein that aligns the keratin filaments [20]. The fourth and possibly most important differentiation product is the modified membrane that surrounds the horny

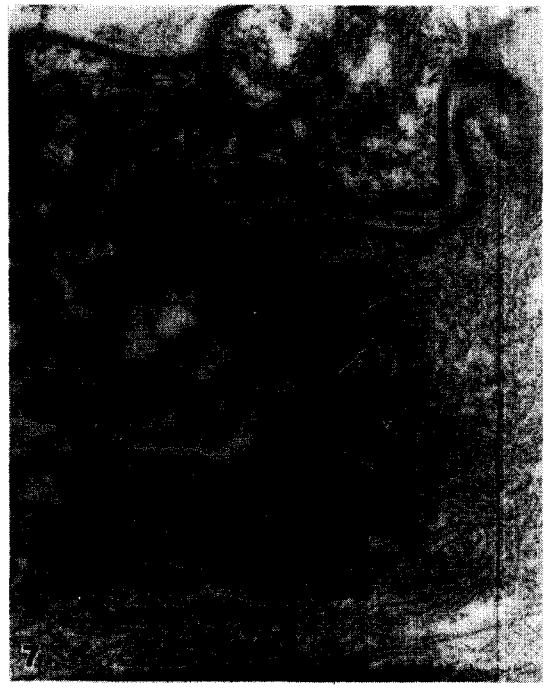

Fig. 7. Transmission electron micrograph of a portion of two adjacent horny cells (HC). Membrane-like figures or bilayers (arrows) are seen within the intercellular spaces. The thickened electron-dense inner layer of the horny cell membrane (arrow heads) is an extremely resistant structure which provides the "first line" of defense for the organism.

Fig. 6. Transmission electron micrograph showing membrane-bound granules (mcg) with a highly organized internal structure closely associated with the apical portion of the granular cell membrane. These granules fuse with the plasma membrane (asterisk) and discharge their contents into the interfollicular spaces between adjacent cells (arrows).

Fig. 8. Transmission electron micrograph of the upper portion of the epidermis consisting of granular (G) cells and horny (H) cells. Granular cells are seen in various stages of transformation (asterisk), which results in the formation of the flattened, consolidated, enucleated horny cells. Horny cells are surrounded by spaces filled with lipid-rich material (arrows) forming the two-component stratum corneum.

cell. Plasma membranes surrounding most cells are approximately 90–100 nm in thickness. This is in contrast to the horny cell membrane which is 200–240 nm thick [6]. Morphologically this is because the inner leaflet of the horny cell membrane is markedly thickened, consisting of a dense 120 nm thick band [6]. This dense band is formed by the cross-linking of loricrin, a precursor of the modified horny cell envelope, by γ-glutamyl-lysyl-isopeptide bonds [13, 21]. The presence of these cross-linked proteins as well as the unique bonds results in a highly resistant structure which encases the filament/matrix complex of the horny cell and thus, provides the "first line" of defense against environmental insults [22]. The presence of pheromones in keratinocytes does not preclude the possibility that some pheromones may be glandularly produced. The skin surface also accumulates glandular secretions. Many of these secretions come from the eccrine, apocrine, sebaceous (Fig. 4) and ceruminous glands. These skin glands are most numerous in the areas of the axilla, face and scalp, scrotum, mons pubis, anus and external ear. Many of these secretions change their chemical composition under the influence of bacteria present on the outer layer of the skin. It is fundamental to the arrangement and function of the stratum corneum that the horny cells are tightly bound together until they reach the skin surface and are desquamated as single cells or small clusters of cells, enmeshed in intercellular material. The fundamental cause of cell desquamation is loss of intercellular cohesion at the surface of the skin, but how this loss is regulated so that the overall integrity of the barrier is maintained is not presently known.

Horny cells and their accumulated metabolites, along with the intercellular material of the stratum corneum, are sloughed into the environment during the process of desquamation. We believe that this sloughed material contains pheromones produced as keratinocyte metabolites as well as pheromones produced as glandular secretions. It has been estimated that approximately 1000 horny cells per square centimeter are lost per hour [23]. This value represents an average from various body sites, some of which lose cells at greater rates than others. The forearm has been estimated to lose approx. 1300 cells/h whereas the abdomen loses only 650 cells/h. Another factor influencing the rate of cell loss is the effect of the external

environment. Exposed regions of the body would be more susceptible to cell loss due to mechanical contact. The mass of cells and intercellular material introduced into the environment as a result of desquamation is generally not appreciated. A person of average size is estimated to shed 4×10^7 cells per day. It is the magnitude of this cloud of "invisible" airborne particles that could suggest its role as a pheromone delivery system. What makes this mechanism intriguing is that since pheromones are delivered as part of a solid substrate there is no need to limit the search for pheromones of volatile or odorous compounds. It is commonly assumed that since pheromones are received in the nose they must have an odor. This might be true if pheromone receptors were the same as odor receptors. However, since pheromone reception may perhaps occur in the VNO, the pheromones may elicit no sensory awareness in the recipient—in other words, pheromones could also be odorless and non-volatile.

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