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Review article

The human eccrine sweat gland: Structure, function and disorders

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ABSTRACT

The ability to thermoregulate is a key component in allowing humans to live and work in a variety of torrid environments. A key thermoregulatory component is the role the skin plays in dissipating heat, through vasodilation of skin blood vessels and its critical role in the secretion of sweat. The role of sweating has for a long time been regarded primarily as the main function of the human eccrine sweat gland, although it has been known for a considerable length of time that sweat, produced in response to heat and exercise, was more than just a salt solution and contained a variety of other substances in addition to electrolytes. Recent studies have shown that there is more to the human eccrine gland, such as manufacturing and releasing compounds that contribute to the defensive barrier of the skin, as well as stem cells present in the gland, having a role to play re-epithelialization of the skin in response to wound healing. Disorders of sweat glands and the resultant conditions, most often relate to defects in the secretion of sweat and its release on to the skin surface. This review concentrates on the processes that enable the production of human sweat.

Keywords: eccrine sweat glands, secretory mechanisms, re-absorptive function, sweat composition, hyperhidrosis, anhidrosis

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INTRODUCTION

The ability to regulate body temperature is a key component in allowing humans to live and work in a variety of torrid environments. A key component of this thermoregulation is the role the skin plays in dissipating heat. In addition to the vasodilation of skin blood vessels, the other mechanism most closely associated with thermoregulation to heat or exercise, is the role of sweat secretion. It has been calculated that about 1% of human body weight need be evaporated as sweat to prevent a 10°C rise in temperature.[1] Unfortunately, in some cases, people's ability to sweat in response to heat or exercise can be compromised, in that some may overproduce sweat or while others lack the ability to sweat, which can cause health and psycho-social problems. However, it is unclear why some people are affected and others not so. This review concentrates on the processes that enable the production of human sweat.

Heat is primarily lost from the body by the latent heat of evaporation of the sweat fluid from the skin surface, however the efficiency of evaporative heat loss is affected by the heat and humidity of the prevailing atmosphere. In hot climates, where there can be a lot of water vapour in the atmosphere, the ability to lose heat through the latent heat of evaporation will be compromised and so sweat fluid on the skin surface will not evaporate.

Curiously, while most mammals have sweat glands, it is only higher primates, horses and some breeds of cattle that use sweating for thermoregulation in response to heat or exercise.[1] It is generally thought that other mammals use secretions from sweat glands, in conjunction with other skin glands, for defense and conditioning of the skin, as well as lubricating contact surfaces, such as the palms and eyelids. It has recently become clear that human sweat gland functions are more diverse than first thought. Secretions from sweat glands have been shown to contain compounds that contribute to the human skin's innate immunity mechanisms that help defend us from many different types of pathogens.[2] Most recently, studies have identified that stem cells present in eccrine sweat glands are a major contributor to renewing epithelial cells after skin wounding.[3,4]

The formation of sweat, in response to heat and exercise, is the multifactorial creation of a fluid, by the eccrine type sweat gland within the skin, which empties on to the skin surface, to be evaporated. It is thought that there are approximately 2–4 million sweat glands dispersed throughout the human body[1] and that number does not increase with age, but the density of sweat glands per cm^2 will change as the skin stretches. Areas with the highest density per cm^2, are the palms of the hand, soles of the feet and forehead, although this has varied between published studies.[1,5] Glands appear to be absent from tissues, such as the lips and glans penis.

Two distinct types of sweat gland exist in the human body: the eccrine or atrichial type (not connected to a hair follicle) and the apocrine or epitrichial type (linked a hair follicle). A third sweat gland, the apoeccrine gland, has also been proposed and is thought to be an amalgam of both an apocrine and the eccrine gland in one structure with an increased ability to produce sweat. The existence of the apoeccrine gland is controversial and so is not discussed in this review.[6–8]

This review concentrates on the human eccrine gland, despite some of the quoted studies using simian palm, rat and mouse plantar glands, which have been shown to demonstrate structural and functional differences to the human gland (mice and rats lack a reabsorptive duct).

THE ECCRINE GLAND

The eccrine or atrichial gland is, by far, the most prevalent sweat gland and produces sweat in response to a hot environment or exercise, but can also produce sweat in response to emotional stimuli, such as fear, anxiety and pain.

Eccrine gland function is primarily controlled by the temperature-regulating centre of the hypothalamus, via a post-ganglionic cholinergic branch of the sympathetic nervous system. However, it has been shown that glands can be stimulated to secrete by other compounds.

The eccrine gland primarily consists of a coiled simple tubular structure that resides at the lower edge of the dermis of the skin and which connects to the skin surface via a straight intradermal portion and an intra-epidermal segment (Figure 1). The gland can range in length from 2–4 mm and with an outer diameter of between 30–60 μm, but this can vary substantially between individuals.[9] A connective tissue capsule surrounds the coiled portion of the gland and acts to separate it from the dermal tissue. A basal membrane surrounds the tubule cells and in turn is surrounded by a fibrocytic sheath.[10,11]

Eccrine glands consist of two functionally and structurally different zones. These are the secretory coil, which makes up most of the coiled part of the gland and elucidates the sweat fluid, and the duct,

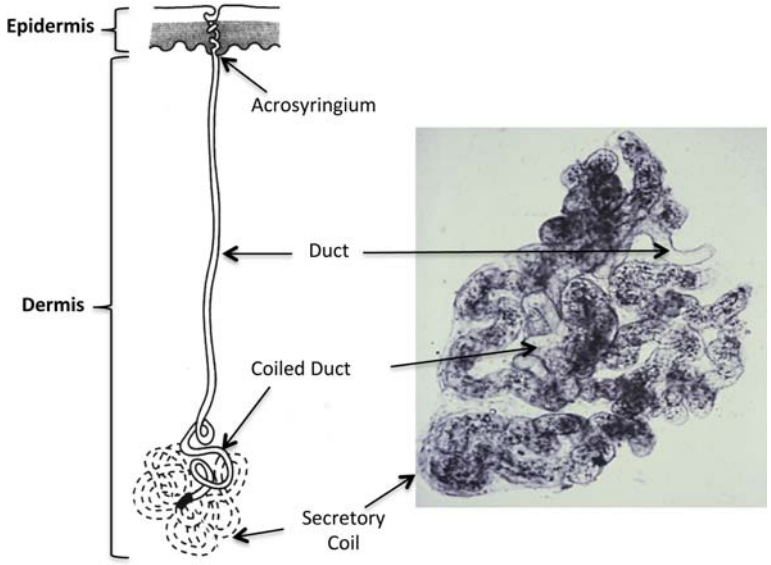

Figure 1. **The left side of the figure shows a diagrammatic representation of an eccrine gland and its position in the dermis, with the secretory coil in the lower part with the duct leading to the skin surface. The right side of the figure shows a micrograph of an eccrine gland isolated from the dermis. The secretory coil has a darker granular appearance, while the duct is appears thinner and translucent.**

which acts to reabsorb sodium and chloride from the primary sweat, thereby minimizing excessive salt loss and possible circulatory collapse. The duct has a small, coiled portion attached the secretory coil and then a straight segment that leads to the intra-epidermal segment known as the acrosyringium.

The secretory coil produces a secretion that flows towards the skin surface, by hydrostatic pressure, via the reabsorptive duct and the acrosyringium. The primary secretion mainly consists of a sodium chloride rich solution with some potassium, that is isotonic to plasma, but which also contains small amounts of other compounds.

The secretory coil consists of three cells types.[12] A tubule consisting of a single layer of epithelial cells, of which ~50% are termed granular or dark cells and ~50% termed clear/agranular cells, with a small amount being the third type the myoepithelial cells surrounding the tubule. The dark cells contain numerous osmiophilic granules that can be seen at the ultrastructural level[13] these give the cells their dark appearance. The granules are released from the cells on sweat production, however, not all the granules appear to be released during sweating.[10] The clear cells contain very few granules and have a much lighter appearance and are the cells most closely involved in the creation of the sodium-chloride

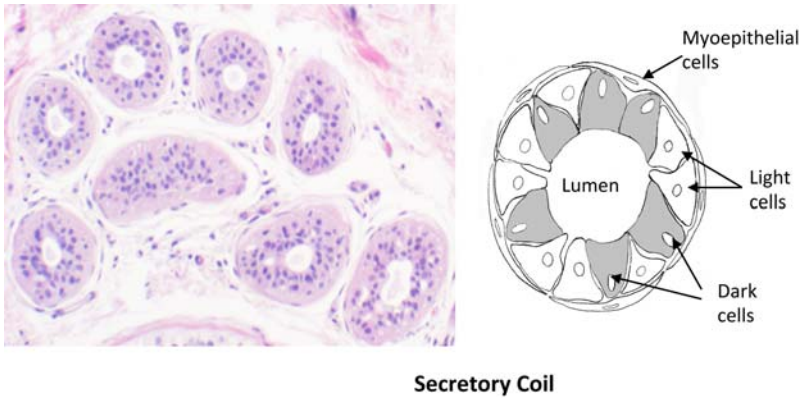

Figure 2. **The micrograph on the left shows a histological section of an eccrine gland secretory coil stained with haematoxylin and eosin. The right panel shows an artists impression of a cross section through the secretory coil demonstrating the different types of cells.**

rich sweat fluid. The myoepithelial cells contain intermediate filaments and are functionally like smooth muscle cells.

The structure of the tubule shows the dark cell membranes occupying the largest area of the luminal surface and the clear cells occupying a broad base connection with basal lamina[10] (see Figure 2). Most of the cell-to-cell connections at the luminal surface are between dark cells or between dark and clear cells, however, there doesn't appear to be any clear-to-clear cell connections.

When the eccrine gland is stimulated to secrete, numerous intercellular canaliculi form between adjacent clear cells, but not between dark cells. These canaliculi increase the luminal surface area of the clear cells, which is thought to accommodate increased secretion. The diameter of the secretory lumen can vary, depending on whether the gland was actively producing sweat fluid (Bovell unpublished data).

The myoepithelial cells form an outer layer of flattened cells in a co-axial pattern around the secretory tubule cells and are only seen in the secretory coil portion of the gland. When the gland is stimulated to secrete, the myoepithelial cells shorten and thicken, developing a tension on the gland,[10,14] which suggests that these cells function to maintain the structural integrity of the gland in the presence of increased hydrostatic pressure during increased output from the gland during sweating.

The glandular duct has two separate regions, a coiled segment and a straight segment that leads to the skin surface. The coiled duct follows on from the secretory coil and then merges into the straight duct. The duct's general dimensions are: overall width approximately 40–80 μm diameter and a luminal diameter smaller than the secretory coil, of 10–15 μm.

At the point where the secretory coil ends and the reabsorptive duct starts, the duct it is lined with a two layers of epithelial cells (Figure 3).[10,12] The epithelial cells are connected at numerous points, throughout the duct, by desmosomes and gap junctions, which allows electrical coupling between the inner and outer cells of the and allows the epithelial layer to function as a syncytium, but yet form a barrier between the extracellular and luminal compartments.

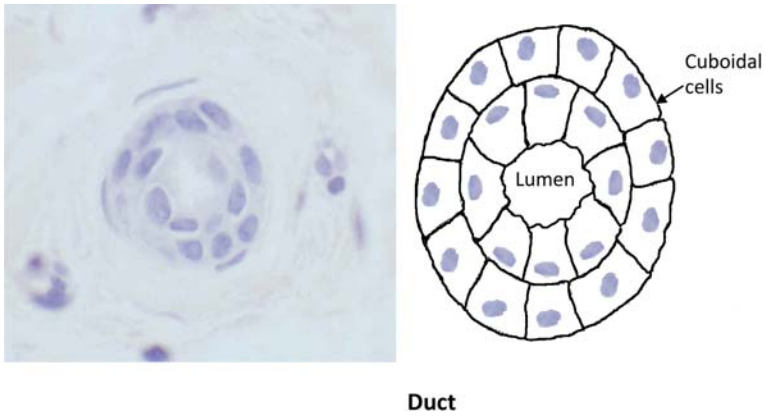

Figure 3. The panel shows a haematoxylin and eosin stained histological section through the duct segment of an eccrine gland. The right side panel shows an artist's impression of a cross section through the duct, highlighting the two layers of cells.

The gross structure of the straight duct is similar to that of the coiled duct. However, nearer the skin surface the number of cell layers present in the duct epithelial layer gradually increases from 2 in the coiled duct to 6 layers, so that by the time the duct becomes the acrosyringium, the number of cell layers can be as many as ten. Further characterization of the reabsorptive duct cells demonstrated that a novel human type II epithelial keratin was present in these cells, which was different to the keratins found in cells in other parts of the eccrine gland.[15]

While the function of the early part of the straight duct is similar to the coiled duct, the nearer the skin surface the more the make-up of this segment changes, which suggested, that at the higher levels, the tissue was less metabolically active and operated more as an outlet for the sweat fluid to exit on to the skin surface.[11] However, recent studies have shown that ductal cells are much more active than originally thought and they contribute to the re-epithelialization of the skin in response to damage[3,4] through the regular cell division that occurs in the basal cells of the duct layers.[16]

SECRETORY MECHANISMS

Clear/agranular cells

It is well documented that eccrine sweat glands are stimulated by cholinergic and adrenergic agonists, although α- and β-adrenergic agonists act via different intracellular second messengers.[1] However it has also been shown that a number of different neurotransmitters and humoral agents also influence sweat gland function, some of which include - aldosterone,[17,18] vasoactive intestinal peptide,[19] purines,[20,21] pyrimidines[20] oxytocin,[22] estrogen[23] and galanin.[24] The G-protein-coupled proteinase-activated-receptor-2 (PAR-2) has also been shown to be present in secretory coil cells. PAR-2 receptors are triggered when its N-terminal domain is cleaved by proteolytic agents to expose a tethered ligand domain of the receptor, which then binds to a segment of the receptor and initiates intracellular signaling oathways. Activation of the PAR-2 receptor by trypsin caused a rapid and transient transepithelial movement of Cl^- across a sweat gland secretory cell line, that was dependent on intracellular calcium.[25] After exposure to trypsin the secretory coil cells were unresponsive to further exposure to trypsin for a considerable time.

It is now clear that agonist binding to sweat gland cell plasma membrane receptors initiates a complex series of biochemical processes that result in alterations in the levels of intracellular second messengers, namely intracellular calcium $[Ca^{2+}]_i$ or cyclic adenosine monophosphate – cAMP. Cholinergic, α-adrenergic, purinergic, oxytocin and trypsin all bring about an increase in $[Ca^{2+}]_i$, whereas β-adrenergic and VIP generate increases in cAMP. These two intracellular messengers have been shown to have discrete functions, with changes in $[Ca^{2+}]_i$ having faster effects, whereas increases in cAMP bring about slower and longer lasting responses, however there is evidence that they can act synergistically.[26]

The ubiquitous Na^+/K^+-ATPase, located on the basolateral membrane of the epithelial cells, provides the driving force for fluid production.[27] This was confirmed by the finding that ouabain, a blocker of the Na^+/K^+ pump, inhibited sweat secretion.[28] The Na^+/K^+ pump keeps the Na^+ concentration inside the cells low, which creates a Na^+ gradient into the cells. Studies in the simian palm sweat gland demonstrated that sweat secretion could be blocked using loop diuretics, which suggested that a $Na^+/K^+/2\,Cl^-$ (NKCC) electro-neutral co-transporter, as seen in other secretory epithelia, was present in sweat glands. As Na^+ enters the cell via the co-transporter down its concentration gradient, it takes with it K^+ and $2\,Cl^-$ into the cell.[29] The Na^+ recycles through the Na^+/K^+-ATPase and the K^+ through basolateral membrane K^+ channels, while the Cl^- accumulates in the cell against an electrochemical gradient and against concentrations above its equilibrium potential.[30] Using immunofluorescence techniques, the sub-form of the $Na^+/K^+/2\,Cl^-$ -NKCC1, has localized to the basolateral membrane of clear cells, but not dark cell membranes of the human eccrine gland,[31] strengthening the view that this co-transporter has a fundamental role in sweat production and that the clear cells are involved in fluid production (Figure 4).

Evidence from human sweat glands is entirely consistent with that from many other epithelial tissues identifying the basic cell mechanisms of second messenger systems ($[Ca^{2+}]_i$ and cAMP), as being involved in secretion.[32,33] Agonists of sweat secretion which generate increases in $[Ca^{2+}]_i$ primarily activate the gland by acting through G protein-coupled receptors, which in turn activate a membrane bound enzyme phospholipase C that sets off a series of reactions to create inositol-1,4,5 inositol trisphosphate (IP_3). The IP_3 diffuses into the cytosol and attaches to receptors ($InsP_3$ R2) on the endoplasmic reticulum (ER) membrane, allowing Ca^{2+} stored inside the ER to be released into the cell cytoplasm.[34] The importance of these receptors and the subsequent release of Ca^{2+} from the ER stores, has been highlighted in a paper by Klar et al, where a patient with a congenital lack of $InsP_3$ R2, displayed inability a lack of sweat secretion and generalized anhidrosis and heat intolerance.

The released Ca^{2+}, amongst other things, rapidly binds to closed ion channels in the luminal and basolateral membranes. One of these is a luminal Cl^- ion channel, which opens when the Ca^{2+} binds to it, allowing Cl^- to diffuse rapidly down a concentration gradient into the gland lumen, while another is a K^+ channel located on both the luminal and basolateral membranes, which too is opened when Ca^{2+} binds to it.

The diffusion of Cl^- into the lumen depolarizes the cell and abolishes the driving force for secretion, which is dependent on the negative intracellular potential. However, this depolarization is countered by Ca^{2+} ions binding to calcium-activated K^+ channels, situated on the basolateral and luminal membranes, bringing about a simultaneous increased efflux of K^+ ions out of the cell. This efflux

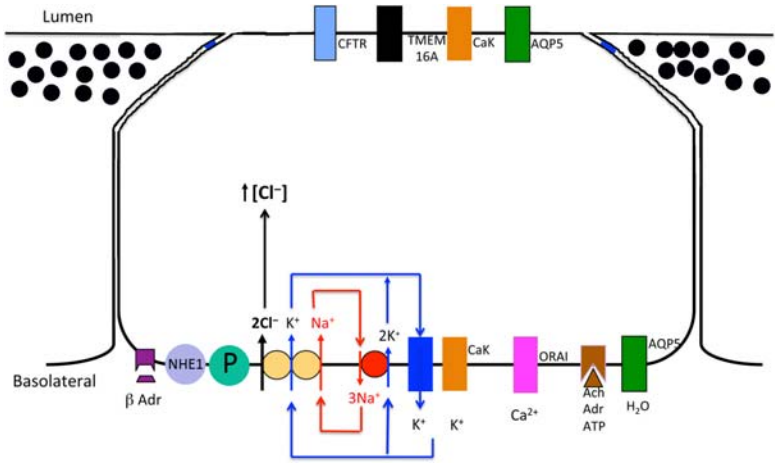

Figure 4. The figure shows a diagrammatic representation of the mechanisms involved in the formation of sweat from a clear cell. The coil consists of a single layer of interspersed dark and clear cells. In the diagram, the cell nucleus and the intracellular calcium stores are not shown. In the clear cells, the Na^+/K^+-ATPase on the basolateral (B/L) membrane is the driving force for secretion, as it moves 3 Na^+ ions out for 2 K^+ ions in to the cell. This creates a gradient for Na^+ to enter the cells via the electroneutral $Na^+/K^+/2\ Cl^-$ (NKCC1) co-transporter, carrying with it K^+ and 2 Cl^- ions, allowing Cl to accumulate inside the cells above its equilibrium potential. The K^+ ions entering the cell exit the cell via a K^+ channel on the B/L membrane. Also present on the B/L membrane are K^+ and Ca^{2+} channels that are activated by increases in Ca^{2+}- brought about by stimulation. Shown are agonist receptors for cholinergic, α & β adrenergic, purinergic and PAR-2 receptors. Included in the diagram is the V-H^+-ATPase (P) and the Na^+/H^+ exchanger number one (NHE1). Both of these have been shown to be present in the clear cells, but as yet it is unclear which membrane they are on and in which direction they function. Finally, the water channel aquaporin number 5 (AQP-5) is shown on the B/L membrane. Shown on the luminal membrane are two Cl^- channels, these are the Ca^{2+} activated Cl^- channel – TMEM16A and the cAMP activated Cystic Fibrosis Transmembrane conductance Regulator (CFTR) Cl^- channel, along with a Ca^{2+}-activated K^+ channel and the aquaporin channel AQP5.

hyperpolarizes the cell and permits sustained secretion. A secretion-induced K^+ ion efflux has been shown in both human eccrine[35] and simian palm glands[36] and the activation of these luminal K^+ channels may also contribute to the K^+ concentration of the forming sweat.

Two types of luminal Cl^- channels have been linked to the movement of Cl^- into the gland lumen. These are, a calcium-activated chloride ion channel (CaCC) and a cAMP-dependent chloride channel known as the Cystic Fibrosis Transmembrane Regulator (CFTR). While CFTR has been localized to the clear cells[37] its role in secretion is unclear. The CaCC play the greater role in sweat formation as they were found to exhibit two main features, 1), they were activated by increases in $[Ca^{2+}]_i$ and 2), they were outwardly rectifying (Review).[38] Recently, studies have identified two channels: an orphan protein, TMEM16A (Anoctamin)[39,40] and Bestrophin,[41] which are an anion-selective membrane proteins activated by increases in intracellular Ca^{2+}, allowing Cl^- to flow passively into the lumen of the gland. Although the presence of Bestrophin-2 has been demonstrated on the luminal membrane of mouse sweat glands,[42] TMEM16A has been identified in the luminal membrane of the human eccrine gland secretory coil.[43] Differences in function between these two channels have been discussed, elsewhere.[44] TMEM16A has been shown to be a CaCC[39] and suggests a fundamental role for this channel in allowing sweat to form in the lumen of the human eccrine gland. As there are differences between the mouse footpad sweat glands and the human eccrine gland, it remains to be seen whether Bestrophin-2 is present and functional in the human sweat gland.

The efflux of Cl^- ions through calcium-activated chloride channels causes the lumen to become electronegative with respect to the tubule cells, thus generating an electrochemical gradient to attract Na^+. As no Na^+ channels have been identified on the luminal membrane, Na^+ is presumed to pass paracellularly into the lumen to join the Cl^- ions. As the Na^+ arrives in the lumen of the gland it forms NaCl and creates an osmotic gradient for water to pass into the lumen to form a primary isotonic fluid. Initially it was thought that the movement of water was also paracellularly through the tight junctions between cells, however, the detection of water channels; Aquaporins, in the plasma membranes of many cell types, altered this viewpoint.[45,46]

It has been reported that the aquaporin water channel number five (AQP5) is expressed in both rat and human sweat gland secretory cells[47] and that this channel could allow water to enter the lumen, via the cells, to create the sweat secretion. It had been shown previously that the enzyme Carbonic Anhydrase II (CAII) was substantially present in the secretory coil clear cells.[48] Using this expression of CAII it has been possible to further localize AQP5 expression to the clear cells only and not the dark cells.[31] The role of AQP5 in sweat gland function has not been full characterized, however, the rapid translocation of AQP5 during sweating to the apical and intercellular canaliculi membranes of clear cells, suggests that it contributes to the secretion of sweat.[49] In addition, alterations in the expression of aquaporins have been reported in several diabetes related pathologies and could account for the reduction in sweat secretion seen in diabetes.[50–52]

The presence of the proton pump, vacuolar-H^+-ATPase (V-ATPase), has also been shown in the secretory coil[53] and more recently the V-ATPase has been localized solely to the clear cells.[31] The joint presence of CAII and V-H^+ -ATPase in the clear cells suggests that the formation of HCO_3^- and H^+ by CAII and the transport of H^+ by V-H^+-ATPase could have a role to play in the creation of the sweat fluid. The role of V-H^+-ATPase in sweat secretion is yet to be fully determined.

It is interesting to note that isolated eccrine glands failed to function properly in bicarbonate-free media,[54] suggesting that human eccrine gland secretion has an unusual requirement for bicarbonate.

Dark/granular cells

While the role of the clear cell has been fairly well documented, that of the dark cell is less well characterized. Investigations into human eccrine gland dark cell function have been hampered by the inability to isolate dark cells from glands. While enzymatic digestion of isolated monkey palm glands created viable cell suspensions, it has not been possible to replicate the same using human glands. This appears to be due to human cells becoming apoptotic very quickly after digestion (MT Clunes, unpublished work).

Dark cells express CGRP, as well as containing a prominent Golgi apparatus, several mitochondria and numerous granules distributed near the luminal membrane, which on stimulation are shed into the lumen, although complete degranulation of the cells does not occur.[10] The granules are thought to be the source of such things as dermcidin, sialomucin, glycoproteins and other compounds.

More recent findings from mouse sweat gland cells have suggested that dark cells may assist in sweat formation through an interaction with clear cells. A recent study showed that the Forkhead transcription factor, FoxA1, was required for sweat production from mouse sweat glands and its lack resulted in a consequent down-regulation of Best2, a Ca^{2+}-activated anion channel in clear cells.[42]

INTRACELLULAR SECOND MESSENGERS

Calcium ions [Ca^{2+}] and cAMP are key 'second messengers' in intracellular signaling pathways regulating exocrine gland secretory functions (Reviews).[55–59] These second messengers interact and between them they regulate the physiological responses of secretory cells. Both these messengers have been found to operate in simian and human sweat gland cells.[5,21,26,35,54,60–63] Experiments using intradermal microdialysis of acetylcholine with and without a calcium chelator present, in to the skin of volunteers, demonstrated that sweating did not occur when the calcium chelator was present,[64] further highlighting the role of extracellular calcium in sweat gland function.

Calcium

The basis of Ca^{2+} release from internal stores in exocrine cells is now fairly clear[65–69] and in sweat glands,[20,35,70] however, the mechanisms that regulate Ca^{2+} entry into the human eccrine gland are only now becoming better understood. It is commonly accepted that the amount of Ca^{2+} emptying out of the intracellular Ca^{2+}-stores, brought about by the IP_3 pathway, starts a signaling sequence that initiates Ca^{2+} entry across the plasma membrane from the interstitial fluid. This is known as store–operated Ca^{2+} entry (SOCE).[71,72] This Ca^{2+} entry sustains the increase in $[Ca^{2+}]_i$, which plays a key function in regulating the mechanism that supports secretion from these cells.

Despite the signaling processes underpinning SOCE having been researched over the last two decades, it is only fairly recently that the key molecular components been identified. These include the Stromal Interaction Molecule (STIM) family proteins (STIM1 and 2), that act as Ca^{2+} sensors at the ER membrane, and Orai family proteins (Orai1, 2 and 3) which act as Store Operated Ca^{2+} Channels

(SOCC) in the plasma membrane.[73–77] ER Ca^{2+} store depletion activates STIM 1, which in turns binds to Orai 1 causing the channel to open, allowing Ca^{2+} to enter the cell.[78] In addition to Orai 1, there are non-selective Transient Receptor Potential Cation (TRPC) channels that are permeable to Ca^{2+},[79] which are also regulated by store depletion and STIM 1 activation.[80] TRPC channels are triggered by a Phospholipase C pathway and store depletion.[81]

SOCE proteins have been demonstrated in a secretory cell line derived from the coils of human eccrine sweat glands – NCL-SG3 cells (Mohamed et al, 2015), however, the viral transformation of the eccrine cells to create the NCL-SG3 cells resulted in these cells being devoid of functional cholinergic and alpha-adrenergic receptors and so some caution is required in interpreting these results.
An investigation into SOCE proteins in intact human sweat glands is currently underway and preliminary results have shown that eccrine glands express key SOCE proteins. Defects in SOCE proteins have been found in sweat glands isolated from horses suffering from anhidrosis (dry coat),[82] which suggests that defects in these proteins has a major impact on sweat formation.

Failure of the calcium release and entry mechanisms will result in both a reduction in sweat output and release of secretory granules into the developing sweat fluid and their subsequent release on to the skin surface.

cAMP

It is now well documented that the binding of catecholamines to GCPR initiates a conformational change of a GPCR located on the plasma membrane, which activates the enzyme adenylate cyclase, subsequently catalyzing the generation of cAMP from ATP. The cAMP then diffuses inside the cell and acts as a messenger molecule facilitating the opening of ion channels or the release of granules from secretory cells.[83] Although eccrine sweat glands are primarily activated by cholinomimetics, catecholamines also activate sweat secretion[84] and adrenergic agonists acting via *g*-protein coupled

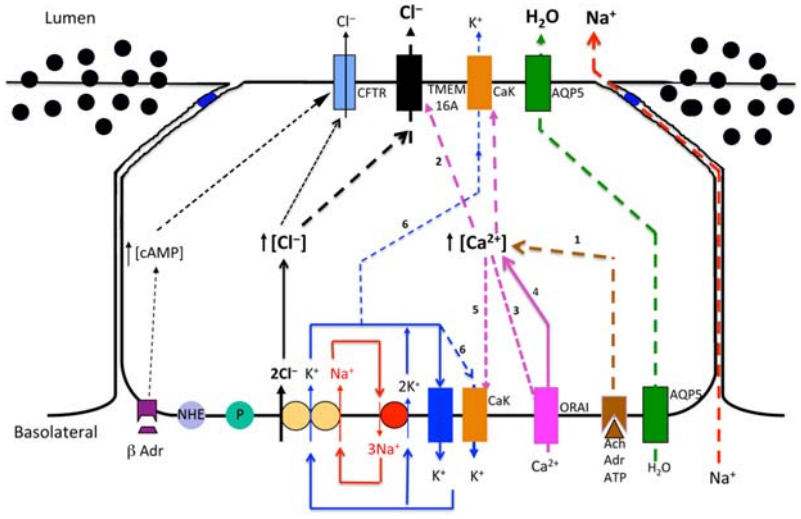

Figure 5. The diagram illustrates the sequence of events that follows activation of the clear cells by agonists. (1) Cholinergic, α-adrenergic and PAR-2 agonists binding to G-protein coupled receptors initiate intracellular mechanisms that result in Ca^{2+} being released from intracellular ER stores. (2) The increased $[Ca^{2+}]_i$, allows Ca^{2+} to bind to the TMEM16A Cl channel causing it to open, (3) allowing Cl^- ions, that had accumulated in the cell, to exit into the lumen. The exit of these Cl^- ions depolarizes the cell, which would otherwise abolish secretion if it were not for (4) the reduction of Ca^{2+} in the intracellular stores resulting in Store Operated Calcium Entry through the interaction of STIM and ORAI proteins at the plasma membrane. (5) This influx of Ca^{2+} maintains an increased $[Ca^{2+}]_i$ allowing (6) the simultaneous opening of the Ca^{2+}-activated K^+ channels on the B/L and apical membranes, (7) which allows K^+ ions to exit the cell, causing a hyperpolarization of the cell. This hyperpolarization maintains the electro-chemical gradient for Na^+ to continue to enter the cell. Once the Cl^- is in the lumen this creates an electrochemical gradient (8) for Na^+ to pass paracellularly into the gland lumen creating NaCl. The presence of NaCl in the lumen generates (9) an osmotic gradient for water to pass through the AQP5 channel into the lumen creating the primary sweat solution that is isotonic to plasma. The diagram also shows the action of β-adrenergic agonists in increasing intracellular levels of cAMP, which facilitates the opening of the CFTR channel, allowing Cl^- to enter the lumen. The diagram also shows the release of granules from dark cells, which even after continued sweating for several hours, are not completely depleted of granules.

receptors (GPCR's) generate cAMP, and so are also important.[26,62,63,85] It has been demonstrated that cAMP plays a vital part in the functioning of CFTR (see later) in sweat glands.[86,87]

Interactions between the $[Ca^{2+}]_i$ and cAMP signaling pathways have been shown to regulate the other's activity, generating stimulatory or inhibitory effects on cells, modifying the strength of response, or acting in tandem to synergistically define the cellular response.[55,58,88–90] Such active crosstalk between these two second messenger systems is evident in sweat gland cells, where increased levels of cAMP are potentiated by cholinergic-induced increases in $[Ca^{2+}]_i$[26,63] and highlights their inter-connectivity in functioning sweat glands (Figure 5).

REABSORPTIVE DUCT FUNCTION

The sweat initially formed in the secretory coil is isotonic to plasma with the concentration of $Na^+ \sim 145$ mmol/L and the concentration of $Cl^- \sim 115$ mmol/L, (with lactate and HCO_3^- being the residual anions). However, the sweat that exits on to the skin surface has approximately 70 mmol/L Na^+ and 80 mmol/L Cl^-, indicating that solute reabsorption takes place in the reabsorptive duct without water being reabsorbed. Essentially, the duct functions to reabsorb ions from the primary fluid formed in the secretory coil.

While the driving force for secretion is transepithelial passage of Cl^- ions into the lumen, the driving force for the reabsorption of Na^+ and Cl^- in the duct is the movement of Na^+ down a steep concentration gradient from the sweat fluid into the ductal cells and then into the interstitial fluid. The gradient is generated by the Na^+/K^+ pump on the basolateral membranes of both the luminal and peripheral cells (Figure 6). Sodium passes down the gradient into the cells via a channel on the luminal membrane that is conductive to Na^+ ions, called the Epithelial Sodium Channel (ENaC).[91] The declining Na^+ concentration in the sweat fluid creates a driving force for Cl^- to follow the Na^+ passively. The epithelium is highly permeable to Cl^- ions and this occurs through ATP-dependent and pH sensitive CFTR channels, which are expressed abundantly in the luminal and basolateral membranes of the ductal cells.[92] Studies have shown that there is a functional interaction between the ENaC and CFTR

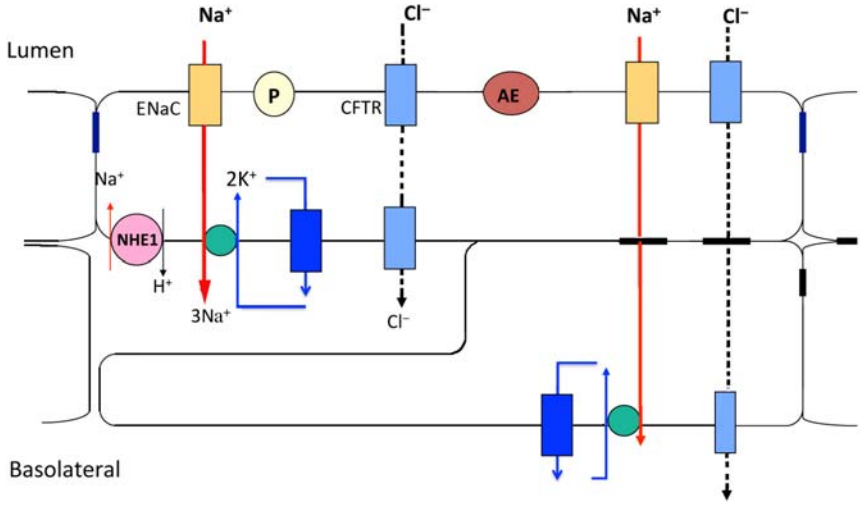

Figure 6. The figure is a diagrammatic representation of the reabsorptive duct with two epithelial layers that can function individually or as a syncytium, because of the gap junctions allowing the passage of small molecules and ions between the cells. The diagram shows the mechanisms involved in reabsorption in each of the luminal cells. The left hand side of the diagram shows how Na^+ and Cl^- can be moved into the extracellular fluid (ECF) between the luminal and basal cell layers. The mechanisms on the right hand side of the diagram show how Na^+ and Cl^- can move from the lumen to the basal layer of cells through gap junctions and then onto the ECF. Similar to the secretory coil, the driving force for reabsorption is a Na^+ gradient into the cell created by the Na^+/K^+ pump on the B/L membrane. Sodium ions move down their concentration gradient through the epithelial sodium channel (ENaC) into the cell and subsequently exit the cell via the Na^+/K^+ pump. The loss of Na^+ from the sweat creates a gradient for Cl^- to follow sodium and enter the cells through CFTR channels on the luminal membrane and to pass through the cell and into the ECF via B/L membrane CFTR channels. Also shown are a vacuolar proton pump (V-H^+-ATPase) and an anion exchanger (AE) on the luminal membrane and a sodium/hydrogen exchanger (NHE) on the B/L membrane, which function at slower flow rates.

in the sweat duct.[93] The K^+ transported into the duct cells by Na^+/K^+ pump exits out of the cells via basolateral K^+ channels.

Defects in the gene coding for CFTR, result in impaired or abolished Cl^- conductivity in epithelial cells and underlie the potentially lethal genetic condition Cystic Fibrosis (CF). In the sweat gland the impaired conductivity affects ductal reabsorption of Cl^- leading to an increased Cl^- concentration in the sweat that exits on to the skin surface. Analysis of sweat from newborns was used to identify raised Cl^- concentrations, which, for many years, was used as a diagnostic tool to identify possible CF sufferers.

As the sweat moves through the reabsorptive duct and NaCl is removed, this results in the sweat exiting on to the skin surface being hypotonic with respect to plasma. As this happens the electrochemical diffusion of Cl^- into the cell becomes limited, as the Cl^- concentration in the duct declines to ~50 mM.[94] However, at low secretory rates, the final sweat NaCl concentration may fall below 10–15 mM and the sweat can reach pH 4.5.[95] This low sweat Cl^- concentration, suggests that another method of Cl^- reabsorption must function that involves facilitates this change in sweat pH (Figure 7). As the sweat can be acidified in the duct, a proton-excreting mechanism must exist at the luminal membrane. A model was proposed to account for this discrepancy in which a system of proton driven Cl^- absorption took place. The model predicted that CA in the ductal cells forms H^+ and HCO_3^-, and that a V-ATPase secretes protons into the lumen of the gland, whilst HCO_3^- leaves the cell down its chemical gradient in exchange for absorbed Cl^- via a HCO_3^-/Cl^- exchanger. The demonstration of a HCO_3^-/Cl^- exchanger localized to the luminal cells of the eccrine duct,[96] coupled with the finding of luminal V-ATPase,[53,97,98] supports the hypothesis that Cl^- is absorbed against its electrochemical gradient using the energy of the V-ATPase proton pump.

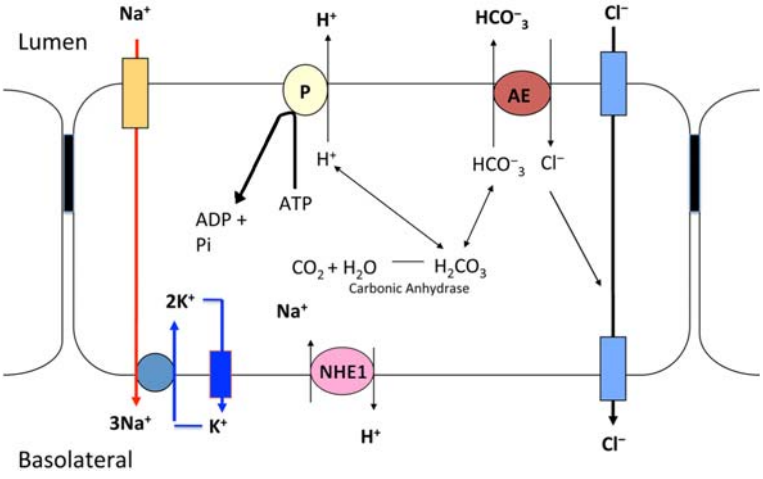

Figure 7. The figure shows a diagrammatic representation of the additional mechanisms that come into play at slow sweat flow rates, where sweat pH can become more acidic than at faster flow rates and the concentration of Cl^- ions decreases below expected levels. Reabsorptive duct cells have been shown to express carbonic anhydrase I (CAI) in the luminal cells. The CAI creates carbonic acid, which rapidly dissociates to H^+ and HCO_3^- ions. The H^+ ions are pumped into the lumen via the V-H^+-ATPase (P), which relies on the breakdown of ATP, while the HCO_3^- ions are exchanged for Cl^- by the AE. This facilitates further reabsorption of Cl^- ions. The NHE on the B/L membrane contributes to the removal of H^+ from the cell.

The demonstration that acid-base transport coupled to Na^+ movement occurred at the basolateral membrane of the ductal cells[99] led to the discovery that a Na^+/H^+ exchanger (NHE), is also involved in regulating cell pH and proton transport, which also exists at the basolateral membranes of the inner and outer cell layers of the duct.[100]

SWEAT COMPOSITION

The basis of the fluid from which sweat is derived is extracellular fluid, which has a composition of small solutes similar to that of plasma and many of these solutes are essentially lost to further use once the sweat empties on to the skin surface.

Inorganic electrolytes are the major solutes present in sweat and all of the main small ones found in plasma are also found in sweat. The concentrations of Na^+ and Cl^- hinge on the sweat rate and can vary from about 10–15 mEq/L at low flow rates, to 40–50 mEq/L at high rates.[101] The Na^+ concentration is influenced by the hormone aldosterone.[18] In contrast to Na^+ and Cl^-, K^+ levels tend to increase with decreasing sweat rate.[102] The K^+ concentration is marginally higher than in plasma, while concentrations of Calcium are usually 1–2 mmo/L, while magnesium, phosphate and sulphate are at micro-molar concentrations.

At increased sweat output, bicarbonate and lactate concentrations are around 20 mmol/L or slightly increased.[103] The concentration of both these solutes declines with lower flow rates. At low flow rates, HCO_3^- can virtually disappear and the sweat pH becomes acidic.[1]

The sweat fluid content was thought to primarily to provide thermoregulation through evaporative heat loss, while the other contents of sweat, such as proteins etc[1] contributed to the well-being of the skin, but were ancillary to the primary function of evaporative heat loss. However, the identification of antimicrobial peptides in sweat has altered that view (see below).

Although it had been known for sometime that sweat contained proteins, some of which had immune functions; immunoglobulins[104] and interleukins,[105] these proteins had not been fully characterized. The fairly recent discovery that eccrine glands constitutively produce an anti-microbial peptide AMP, referred to as dermcidin (DCD),[106] has resulted in a greater emphasis in obtaining a clearer understanding of the skin's defence mechanisms and the role that sweat secretions have in that function.

Schittek and co-workers[106–109] demonstrated that this AMP was manufactured and released into the secretory coil and transported to the skin surface by sweating. These studies showed that the AMP played an important role contributing to innate immunity by restricting bacterial growth on the skin and that its activity was effective across a wide range of pH and salt concentrations.

Additional AMP types are also expressed in eccrine gland sweat and which co-exist to form a barrier against infection on the skin surface through sweat secretion. These were the cathelicidin family of AMPs, LL-37,[110,111] and lactoferrin,[112] which demonstrated a potent antimicrobial activity.

Small organic solutes also appear in sweat. For example glucose levels in sweat are $<1\%$ of plasma levels, however that figure can vary according to blood glucose levels. Raised levels of blood glucose could lead to increased amounts of glucose being secreted by sweat on to the skin surface in diabetes and contribute to increased rates of skin infections.

Trace elements, such as, iron, copper, zinc, manganese and iodine can also be found in sweat.[2]

DISORDERS OF ECCRINE SWEAT GLANDS

While there are several non-neoplastic disorders of eccrine sweat glands, by far the most common conditions associated with these glands are the potentially lethal genetic disease Cystic Fibrosis (CF) and hyperhidrosis.

Cystic Fibrosis

In the USA there is an estimated 1000 new cases diagnosed each year, occurring at the highest rate amongst the Caucasian population of Northern European descent (Cystic Fibrosis Foundation, USA). Mutations in the gene coding for the CFTR channel cause a defective coding for the channel protein resulting in misfolding occurring in the channel or the absence of the channel. Over 1000 CFTR gene mutations have been detected, the most common type ($\sim 70\%$) being a 3-base deletion (ΔF508) resulting in the absence of a single amino acid. The defect results in the Cl^- channels (CFTR) in the reabsorptive duct segment of the gland being unable to properly reabsorb Cl^- ions. This results in an increased Cl^- concentration in the sweat, which creates an electrochemical attraction reducing the amount of Na^+ that diffuses from the sweat into the ductal cells, thereby increasing the NaCl concentration of the sweat. For many years the classic diagnostic test for CF was to check the salt concentration in the sweat, primarily in newborn babies.

It is presumed that the defect limits Cl^- movement through the CFTR channel in the clear cells, however as overall sweat output appears unaffected, there has been no work done to investigate it further.

Hyperhidrosis

Hyperhidrosis is an unremitting disorder typified by an uncontrolled and excessive secretion of sweat, which can be generalized on the body or localized to specific areas, such as the axillae or the hands and feet. A 2004 report[113] estimated that 2.8% (7.8million) of the American population endured this condition. The condition can be further classified as either primary hyperhidrosis, where the excessive sweating normally starts at puberty or secondary hyperhidrosis, where the uncontrolled sweating is due to, for example, thyroid disorders, tumors, diabetes etc. The cause is unclear and although it has been ascribed to an overactive sympathetic nervous system, this has not been proved. Previously, differences in the transepithelial transport of Cl^- and $[Ca^{2+}]_i$ levels in primary cultured sweat gland cells from hyperhidrotic and control glands were reported, as well as an increased distribution of P_2Y receptor sub-types and AQP5 in hyperhidrotic glands.[20,114,115] Recently, the cellular mechanisms involved in sweat secretion have come under greater scrutiny. The finding that TMEM16A splice variants in eccrine gland cells may influence $[Ca^{2+}]_i$,[43] and that differences exist in the proteins controlling the influx of calcium into cells between control and hyperhidrotic cells (Bovell unpublished) suggest that changes in the intracellular mechanisms of sweat secretion could underlie the excess secretion seen in hyperhidrosis. This is further supported by studies showing the key role of Orai1 protein in regulating Ca^{2+} in mammary gland secretion.[116] (*Note - amongst early sweat gland workers, mammary glands were thought to be modified apocrine sweat glands).

Idiopathic Anhidrosis

This is where previously functioning sweat glands undergo changes that result in a reduction in the volume of sweat that they can produce. This should not be confused with the lack of sweat on the skin surface due to heritable ectodermal dysplasia.

Idiopathic anhidrosis was often associated with people moving from temperate climates to living and working in hot humid climates, but nowadays with readily available air conditioned homes, offices and transport, it is very rare. However, it is interesting to note that horses, the other major mammal using sweating for thermoregulation, can develop this condition in hot and humid climates (a particular problem in the horse racing industry in the Far East (Hong Kong, Singapore, Philippines, Korea, North Australia) and the Southern States of America). The figures for the prevalence of this condition in humans are not readily available, however, as many as 22% of thoroughbred horses, from a population of 50 horses, were reported by Korean equine veterinarians.[117]

The causes of Idiopathic anhidrosis are not fully understood. Comparison of the expression of the proteins involved in controlling Ca^{2+} influx into control and anhidrotic equine sweat gland cells, demonstrated that anhidrotic cells had reduced expression of STIM1 protein and reduced stimulated increases in $[Ca^{2+}]_i$.[82] This suggests that poorly regulated $[Ca^{2+}]_i$ is a factor in anhidrosis, which ties in with the findings of Cui et al.[42] who reported that idiopathic anhidrosis in humans may be due to changes in Bestrophin-2 function.

SUMMARY

The physiology of sweating has intrigued scientists for decades, but its primary function was thought to be for thermoregulation. However, that view has changed since it became clear that the contents of sweat are much more than just adjuncts to a salt solution, and participate in an important role in the maintenance of the skin barrier and innate immune defense. Understanding in greater detail dark cell function and the production and release of the different compounds in sweat, offers an opportunity to improve the lives of many people suffering from debilitating skin conditions.

The increasing number of people suffering from the debilitating condition – hyperhidrosis, has also resulted in increased efforts to find a specific cause and ways of ameliorating the condition. That work involves looking in more detail at the complex intracellular signaling pathways involved in creating sweat.

UNRESOLVED ISSUES

There are a number of questions that still remain to be investigated:

(1) The fact that sweat output Increases in response to heat and exercise is well documented, but what is not clear is how the sweat glands achieve this increased output. Is the increase simply the cellular mechanisms working at faster rate, or is the number of component parts,

for example NKCC1, AQP5, STIM1 etc, increased to allow greater sweat production and salt reabsorption?

(2) It is also unclear what changes occur in sweat glands that result in the loss of the ability to secrete sweat (anhidrosis) or result in the excess production of sweat (hyperhidrosis).

(3) As sweat contains compounds that help maintain the skin as barrier, it is unclear whether the production of these compounds matches the increased fluid production in prolonged sweating? It is presumed that these components are in the granules present in some sweat gland cells, however it is not clear what happens to these granules on prolonged sweating?

(4) Does the production and release of the protective sweat components keep pace with high and prolonged output? If not, does prolonged sweating reduce the effectiveness of the protective agents on the skin and result in increased skin infections?

(5) How to get ductal stem cells to create new glands *in situ* in burn victims?

(6) Do duct stem cells re-epithelialize all wound types?

(7) There are now suggestions that the new textiles used in sports clothing, 'wicks' away sweat from the body surface. Is this new sports clothing absorbing the beneficial components, rather than leaving them on the skin surface, thereby increasing the risk of increased rates of skin infections?

CONCLUSIONS

For many decades the eccrine sweat gland was regarded as only having a key role in thermoregulation and little else, however, that has changed. New and exciting avenues of research are elucidating novel information on sweat gland structure and functions. This is providing knowledge and understanding that is helping address conditions such as CF, hyperhidrosis, anhidrosis, increased rates of skin infections in atopic dermatitis, as well as the utilization of sweat gland stem cells in improving wound healing (perhaps a key factor for diabetes) and being able to overcome temperature regulation issues in burns victims through the re-growing glands in the damaged skin.

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