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Biology of sweat glands and their disorders. I. Normal sweat gland function

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The basic mechanisms of sweat gland function and an updated review of some relatively common disorders of sweat secretion, are presented. Although sweat secretion and ductal absorption are basically biophysical and biologic cellular processes, a detailed description of the basic biophysical principles of membrane transport has been avoided to make the discussion more readable. The cited references will, however, help those readers primarily interested in the basic details of sweat gland function. Part I of this article includes a discussion of morphologic characteristics, central and peripheral nervous control of sweat secretion, neurotransmitters, intracellular mediators and stimulus secretion coupling, Na-K-Cl cotransport model for the ionic mechanism of sweat secretion, ingredients of sweat, ductal function, the pathogenesis of abnormal sweat gland function in cystic fibrosis, and the discovery of the apoeccrine sweat gland. Part II, to be published in the May issue of the JOURNAL, reviews reports of all those major disorders of hyperhidrosis and hypohidrosis that have appeared in the literature during the past 10 years. It is hoped that this review will serve as a resource for clinicians who encounter puzzling disorders of sweating in their patients, as well as for investigators who wish to obtain a quick update on sweat gland function. (J AM ACAD DERMATOL 1989;20:537-63.)

The eccrine sweat gland is one of the major cutaneous appendages. Its principal function is thermoregulation during exposure to a hot environment or during physical exercise. Failure to regulate body temperature under either of these conditions leads to hyperthermia and/or heat stroke. During the summer heat wave of 1980, deaths in the United States from heat stroke were estimated at 1265[1]; most of the victims were either elderly persons or young children. Heat intolerance of patients with anhidrotic ectodermal dysplasia and other anhidroses is well known.

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The eccrine sweat gland is a secretory as well as an excretory organ. Sweat is a dilute electrolyte solution that contains mainly sodium chloride (NaCl), potassium (K), and bicarbonate (HCO_3). In addition, it contains inorganic compounds such as lactate, urea, and ammonia.[2-4] Lactate and urea are most germane to this discussion because they both are used in topical preparations for control of retention hyperkeratoses. The excretory function of the sweat gland can be instrumental in delivering drugs to the stratum corneum. For example, orally administered griseofulvin and ketoconazole are secreted into sweat, thereby quickly reaching the stratum corneum where dermatophytes are present, bypassing the slow diffusive pathway across the epidermal cell layer.[5]

The role of the eccrine sweat gland in various skin diseases is still poorly understood. The presence in sweat of various proteolytic enzymes,[6-8] however, raises a possibility that eccrine sweat is potentially proinflammatory and can modify vari-

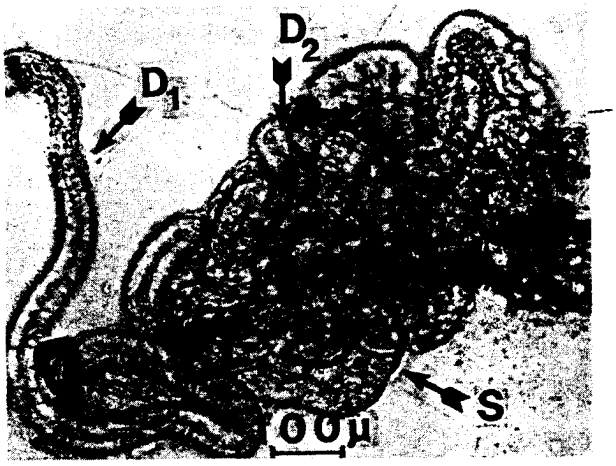

Fig. 1. An isolated human eccrine sweat gland showing both the duct and the secretory portion *(S)*. D_1, Distal or straight duct; D_2, proximal or coiled duct (*Bar* = 100 μm.)

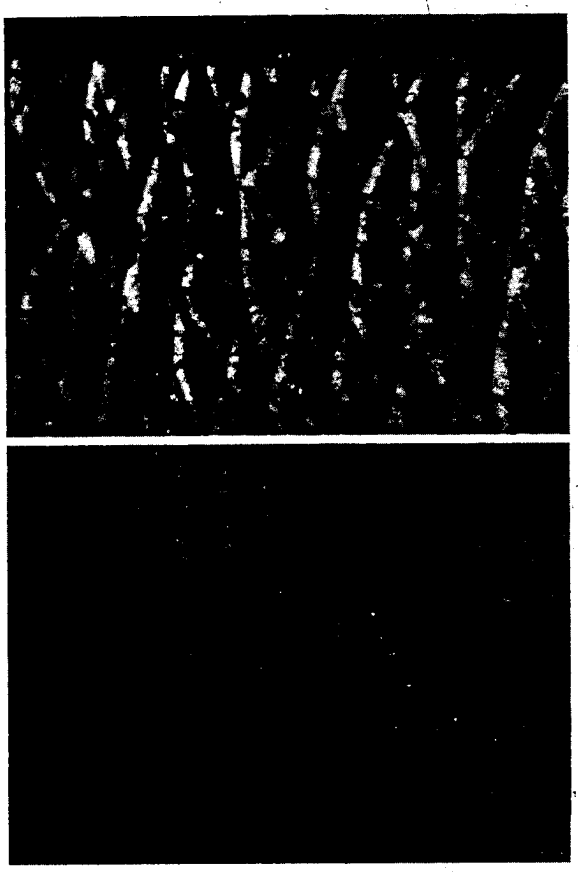

Fig. 2. Localization of sweat pores relative to skin creases and ridges on the forearm (**A**) and the pulp of finger (**B**) as visualized with plastic dental silicone. Note that the image is the negative replica of the skin surface. Sweat droplets are visualized as holes in the silicone mold. On the hairy skin (**A**) the pore usually opens where creases converge whereas on the palms and the soles the pores open at the ridge (**B**). The grid on the upper portion of **A** is 1 mm. A few of the holes may be air bubbles trapped in the resin during mixing. Knowledge of the exact site of a ductal opening on the skin surface will help differentiate miliaria from other erythematous papules such as viral rash, vasculitis, drug eruption, folliculitis, and dermatitic papules.

ous dermatoses if sweat migrates into the epidermis as a result of poral occlusion.

The eccrine sweat gland is one of the target organs in cystic fibrosis, a semilethal, recessive disease that is prevalent among white persons. In fact, increased NaCl concentration in sweat still is regarded as one of the most reliable diagnostic criteria for cystic fibrosis. During the past several years two new observations concerning the sweat glands of persons with cystic fibrosis, namely chloride impermeability of the sweat duct[9] and defective β-adrenergic function of the secretory coil,[10] have accelerated overall research interest in cystic fibrosis, resulting in the discovery of abnormal β-adrenergic regulation of chlorine (Cl) channels in tracheal cell membranes.[11,12] Sweat gland research is still an arcane field, and the progress in our understanding of the detailed mechanism of sweat gland function is rather slow. In Part I of this article we provide an update on the mechanism of eccrine sweat secretion; Part II (to be published in the May issue of the JOURNAL) reviews the recent articles on some of the clinical problems of eccrine secretion, such as hyperhidrosis and hypohidrosis.

MORPHOLOGY OF THE ECCRINE SWEAT GLAND

The eccrine sweat gland is a simple tubular epithelium consisting of the duct and the secretory coil (Fig. 1); 1.6 to 4.0 million glands are distributed over nearly the entire body surface, including the glans penis and the foreskin but not the lips, external ear canal, clitoris, or labia minora.[2,13] The average density of the glands varies in different persons and anatomic sites; for example, 64 glands/cm² on the back, 108/cm² on the forearm, 181/cm² on the forehead, and 600 to 700/cm² on the palms and soles[2,3] (Fig. 2).

Embryologically, sweat glands begin to develop at 3 months as a cord of epithelial cells growing from the epidermal ridge on the palms and soles and at about 5 months on the rest of the body. By

the eighth fetal month, they resemble adult sweat glands.[2, 13-15] The coiled portion of the sweat gland is made up of the secretory coil and the proximal duct. The distal duct is relatively straight and connects the coil with the epidermis. The duct again forms a spiral in the epidermis and the stratum corneum (forming the acrosyringium or intraepidermal sweat duct unit), and it opens directly onto the skin surface. The size of the sweat gland varies as much as fivefold among different persons, which largely correlates with the individual (and perhaps also regional) difference in the rate of sweating (maximal sweat rate ranges from 2 to 20 nl/min/gland).[16] The dimension of the secretory coil is about 60 to 80 μm in diameter and 2 to 5 mm in length.[16] Although the duct has a slightly smaller diameter, it has approximately the same tubular length as the secretory coil in each gland. At the light microscopic level, three types of cells are found in the secretory coil: dark, clear (or secretory), and myoepithelial cells (Fig. 3, *A*). In contrast, the duct consists of two layers of cells: the luminal and the basal ductal cells. The fine morphologic structure of the secretory (clear) cell is characterized by the presence of intricate basal infoldings[14, 17] and the intercellular canaliculi. The basal infoldings stain densely with paranitrophenyl phosphatase[18] (which reflects the catalytic activity of Na-K-ATPase) (Fig. 3, *B*), and they bind labeled ouabain.[19] In contrast, the intracellular canaliculus, which is actually a pouch made up of the luminal membrane opening into the lumen, is free of both ouabain binding[19] and paranitrophenyl phosphatase activity.[18] This supports the notion that the basolateral membrane, but not the luminal membrane, is the sodium (Na)-pump site and is the site for active transport of ions for sweat secretion.

The presence of many mitochondria in clear cells is consistent with the thesis that clear cells are mainly responsible for sweat secretion.[3] The dark cell is easily discernible because of the presence in the cytoplasm of many electron-dense "dark cell granules." Myoepithelial cells are located at the periphery of the secretory tubule and are filled with dense myofilaments. The morphologic differences between these three cell types are readily illustrated when these cells are dissociated by collagenase digestion.[20] Typical secretory (clear) cells contain many mitochondria and membrane villi, whereas typical dark cells show a smaller number of

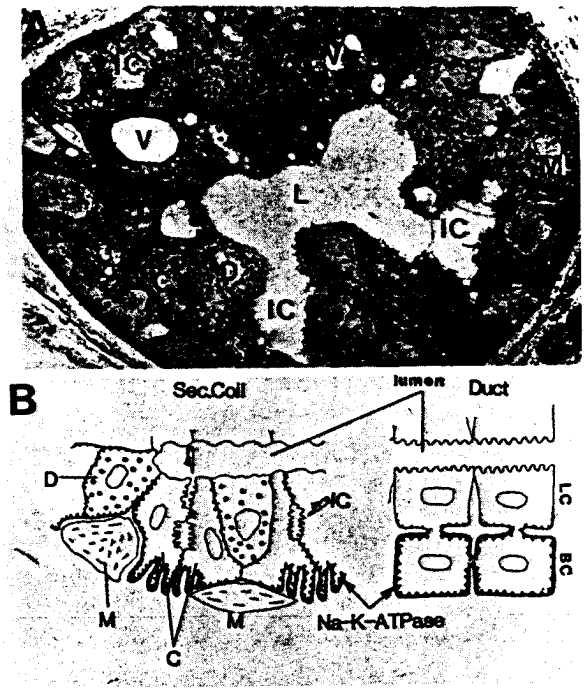

Fig. 3. A, Cross-section of the secretory coil of the monkey palm eccrine sweat gland (model of human sweat gland) after stimulation with methacholine. The dilated lumen *(L)* is seen here, but it is not a constant finding. *V,* Vacuole; *LC,* intercellular canaliculi; *M,* myoepithelial cell; *C,* clear or secretory cell; *D,* dark cell. **B,** Schematic illustration of the ultrastructure of the eccine sweat gland and the localization of paranitrophenyl phosphatase (equivalent of the catalytic activity of Na-K-ATPase; indicated by a thick serrated line). *LC,* Luminal ductal cell; *BC,* basal ductal cell; *C,* clear cell; finger sign, intercellular canaliculi.

membrane villi and mitochondria (Fig. 4). Some dark cells, however, also have intricate membrane villi and many mitochondria (Fig. 5). Likewise, some clear cells contain a few dark cell granules. This observation suggests either that some dark cells are actively involved in membrane transport or that dark cells develop from clear cells or vice versa. The function of the dark cells is still unknown; however, secretion of sweat glycoproteins that react positively to periodic acid–Schiff may be one of their functions.[21] The myoepithelial cell is spindle shaped and contracts in response to cholinergic but not adrenergic stimulations[22] (Fig. 6). Its cytoplasm is filled with myofilaments and a small number of mitochondria. The function of the myoepithelium may be to provide mechanical support for the secretory coil wall against the increase in luminal hydrostatic pressure rather than simply to pump out preformed sweat.[22] Again,

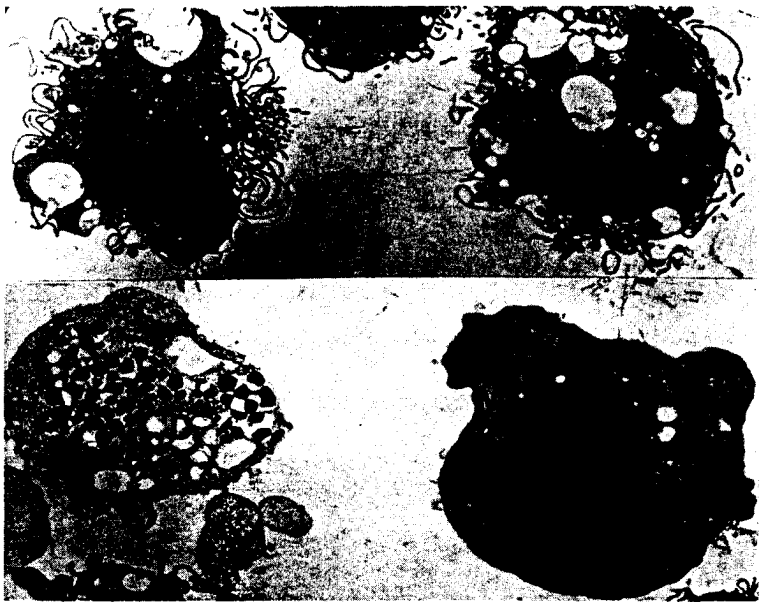

Fig. 4. Electron photomicrograph of dissociated cells of the rhesus monkey palm secretory coils. *C,* Secretory cell; *D,* dark cell; *M,* myoepithelial cell. (×5500.)

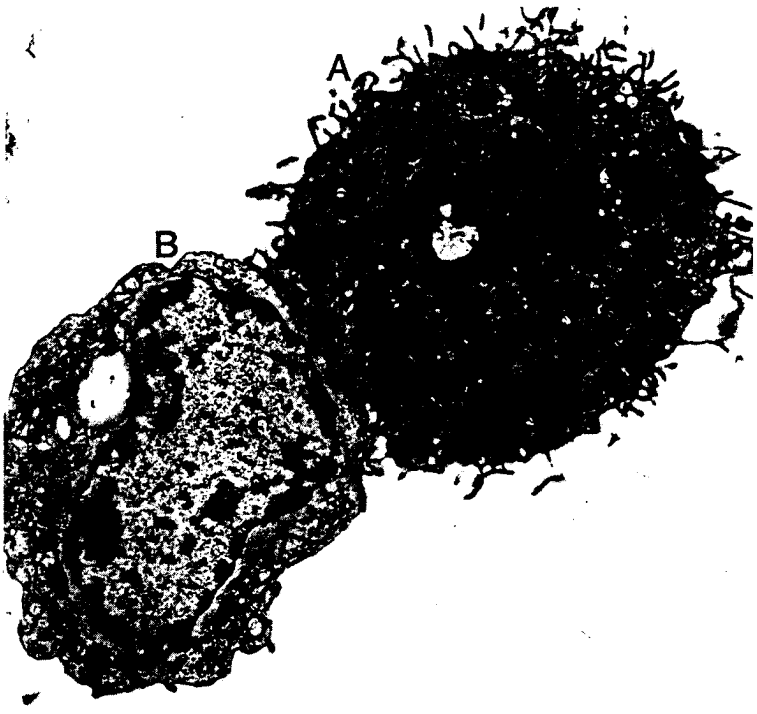

Fig. 5. Atypical dissociated cells from monkey palm eccrine sweat glands. **A,** Dark cells with intricate membrane villi. **B,** Atypical cell with a small amount of myofilaments in the perinuclear region and many mitochondria.

some of the dissociated cells show a small amount of myofilaments in the perinuclear region and relatively large number of mitochondria (Fig. 5), which suggests the interchangeability of different cell types or the presence of undifferentiated cells. The interchangeability of different cell types also is suggested because in cell culture of sweat gland cells, different types of cells quickly dedifferentiate

within 10 days and lose their original morphologic characteristics (unpublished data).

The duct is composed of two layers of cells, the basal and luminal ductal cells. The basal ductal cell is replete with mitochondria, and the entire circumference of its cell membrane shows a strong paranitrophenyl phosphatase activity (Fig. 3),[18] suggesting that Na-pumping occurs in the entire cell membrane. In contrast to the basal ductal cell the luminal ductal cell has fewer mitochondria, much less paranitrophenyl phosphatase activity, and a dense layer of tonofilaments near the luminal membrane[14] (which often is referred to as the cuticular border because of its resemblance to cuticle at the light microscopic level).

NERVOUS AND NONNERVOUS CONTROL OF ECCRINE SWEATING

Central control of sweating

Regulation of internal temperature is one of the most fundamental functions of the body. When body temperature is gradually increased, sweating occurs at and above a certain core temperature (temperature setpoint [T_{se}]), which suggests the presence of a sweat control center that senses the change in body temperature. Boulant[23] reported that the preoptic area and anterior hypothalamus contain many thermosensitive neurons that sense changes in internal temperature and initiate appropriate thermoregulatory responses to maintain constant internal temperature. In experimental animals, local heating of the preoptic region and hypothalamus activates sweating, panting, vasodilation, and various behaviors that enhance heat loss such as skin wetting and movement to a cooler environment.[24,25] In contrast, preoptic cooling induces shivering as well as nonshivering metabolic thermogenesis. Microelectrode studies have revealed that many preoptic neurons are thermosensitive and can be grouped into two basic types: warm-sensitive and cold-sensitive neurons. The firing rate of warm-sensitive neurons increases not only with an increase in the local preoptic temperature (T_{po}) but also with an increase in afferent impulses from the cutaneous and spinal thermoreceptors caused by an elevation of the skin temperature.[23] Although various warm-sensitive neurons fire at different rates at a given T_{po}, those neurons that showed the slowest rate of firing below T_{po} of 37° C were found to precipitously increase their firing rates with further increases in T_{po} between 37° and 42° C. Because of their high thermosensitivity at this temperature range, Boulant[23] speculated that these preoptic neurons may be involved in the control of thermal sweating.[2] Conversely, the firing rate of preoptic cold-sensitive neurons is generally facilitated by extrahypothalamic cooling and inhibited by extrahypothalamic warming, although hypothalamic cooling itself has relatively little effect on the firing rate of cold-sensitive neurons.

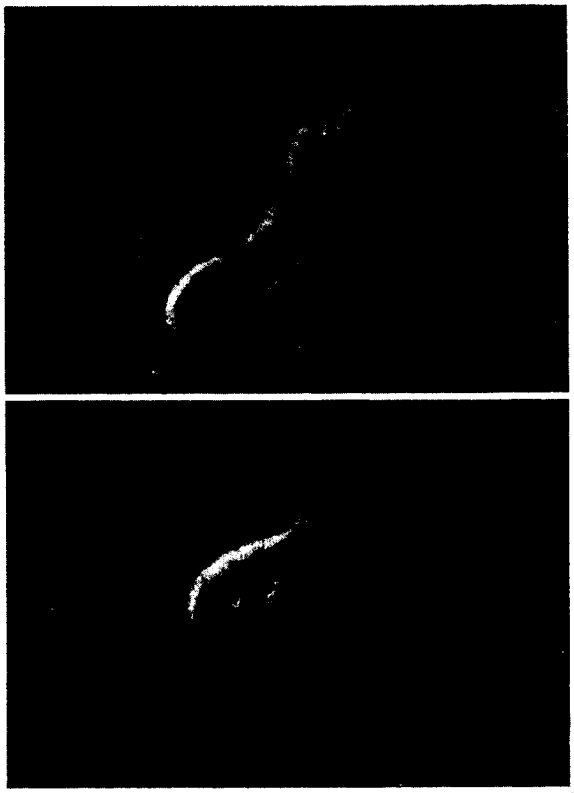

Fig. 6. Photomicrographs show contraction of dissociated myoepithelial cell of the monkey palm eccrine sweat gland in vitro. **A,** Freshly prepared unstimulated cell bathed in Ringer's solution. **B,** After stimulation with 10^{-6} mol/L methacholine. (×240.)

How, then, are these thermal and nervous stimuli integrated in control of sweating? Nadel et al.[26-28] observed that the sweating process appears to respond as if controlled by a weighted sum of central (or core) and peripheral (or skin) temperatures. If skin temperature is relatively constant, sweating rate is linearly related to core temperature above T_{se}. As skin temperature rises, the sweating-to-core temperature relation shifts in the direction of lower core temperature (i.e., sweating is inducible at a lower core temperature as though T_{se} has been lowered). It was further observed that on a

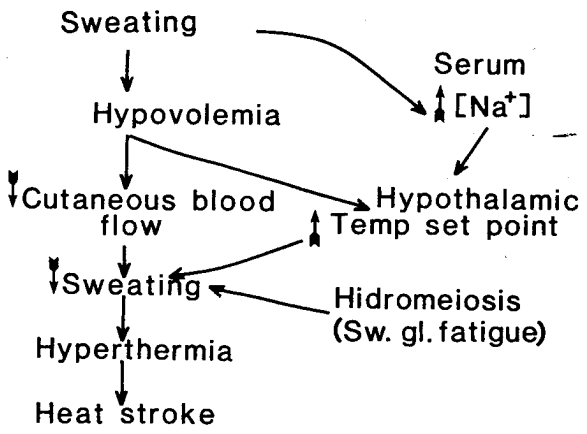

Fig. 7. Hypothetic sequence of events resulting in heat stroke during prolonged profuse sweating without replacement of salt water.

degree-to-degree basis an increase in core temperature is about nine times more efficient than that of the mean skin temperature in stimulating the sweat center[26-28] and thus the sweating rate. Therefore the increase in core temperature is a major determinant of T_{po} whereas the skin temperature effect, which is mediated by afferent impulses (originated from the cutaneous thermoreceptors) to the preoptic region of the hypothalamus, plays a relatively minor role in activation of sudomotor activity.

Temperature set-point (T_{se})

As already discussed, T_{se} plays a critical role in regulation of body temperature. The importance of T_{se} is best illustrated in episodic hypothermia with hyperhidrosis[29] in which a pathologic episodic decrease of T_{se} precedes a decrease in body temperature and the onset of profuse sweating. In normal subjects T_{se} also changes during acclimatization, according to the homeostatic state, and during exercise or heat exhaustion.

Then what are the mechanisms by which T_{se} is regulated under physiologic conditions? It is well known that sweating is drastically decreased or absent during heat exhaustion despite marked elevations of body temperature. In attempting to determine whether the change in plasma electrolyte concentrations might be involved as a regulator of T_{se}, investigators infused hypertonic (by 15 to 20 mM) NaCl into the brain of experimental animals or employed other procedures that produce hypernatremia and observed that the body temperature indeed increases by about 1° C.[30] Likewise, during exercise at 40% volume of oxygen utilization $(VO_2)_{max}$, the selective elevation of plasma Na concentration in human beings slows the onset of sweating and raises the plateau level of core temperature, whereas increasing plasma Ca concentration had an opposite effect.[31] Fortney et al.[32] found that isotonic hypovolemia reduces the sensitivity of the sweating response, increases the threshold core temperature for the onset of sweating response, and increases the threshold core temperature for the onset of cutaneous vasodilation. Thus these observations may be relevant to the aberrant regulation of sweat gland function during prolonged heat stress.

Fig. 7 schematizes the possible sequence of events that might lead to heat exhaustion on the basis of the aforementioned ionic theory for control of the sweat center. For example, because sweat secreted onto the skin surface is hypotonic (a result of ductal Na absorption), prolonged profuse sweating without fluid and salt intake could cause both hypovolemia and hypernatremia, both of which elevate T_{se}. Furthermore, hypovolemia also may decrease cutaneous blood flow. These factors together with hidromeiosis (a decrease of sweating resulting from poral occlusion by hyperhydration of the stratum corneum and/or sweat gland fatigue[33,34] may lead to decreased sweating, then hyperthermia, dysfunction of multiple organ systems, and finally heat stroke.

Innervation of the sweat gland

The nerves surrounding the sweat glands are the sympathetic postganglionic fibers, which consist of nonmyelinated class C nerve fibers. In contrast to the ordinary sympathetic innervation, however, acetylcholine is the principal terminal neurotransmitter. The possibility of dual cholinergic and adrenergic innervation had been cause for speculation and debate for three decades[3] until Uno and Montagna[35,36] settled the issue by directly demonstrating catecholamine fluorescence and the characteristic adrenergic and cholinergic nerve and organs in the periglandular nerves of both human and monkey eccrine sweat glands.

Review of the literature fails to reveal recent articles that significantly modify our conventional knowledge of the gross anatomy of the central and peripheral sympathetic nervous system innervating the eccrine sweat gland and other cutaneous

organs.[2,3] The efferent central sudomotor pathway consits of (1) cerebral cortex to hypothalamus, (2) hypothalamus to the medulla, (3) the medulla, mostly crossed, to the lateral horn of the spinal cord, (4) the lateral horn to sympathetic ganglia, and (5) sympathetic ganglia to sweat glands as postganglionic C fibers. The cerebral cortical influence of hypothalamus has been suggested by the increased or decreased sweating on the contralateral side of the body in patients with cerebral lesions or during stimulation of cortex with methacholine[38] or by the occurrence of hyperhidrosis or hypohidrosis in patients with brain lesions. It also has been suggested that in human beings the sensory stimuli arising from the frontal and premotor areas (or area 6 of Brodmann) may be responsible for emotional sweating.[37] The sympathetic fibers arising from the hypothalamus cross mostly at the levels of pons, and the crossing is almost complete in the medulla so that the lesions in the medulla or the spinal tracts, such as trauma, thrombosis, and tumors, should in theory cause ipsilateral anhidrosis.[37,39]

That Horner's syndrome often is associated with anhidrosis of the head and neck is well known, although there are instances in which lesions of the cervical and thoracic sympathetic systems cause only miosis and ptosis but no abnormal sweating.[39] In the spinal cord, new neurons start in the lateral horn, pass through the white ramus communicans, and synapse in the sympathetic ganglia, where new postganglionic nonmyelinated C fibers originate. These postganglionic fibers exit the spinal cord, pass through the gray ramus communicans, join the peripheral mixed nerves, and finally reach the sweat glands. Thus the transverse spinal lesions should cause ipsilateral anhydrosis in the segments below the lesion.

In reality, however, the so-called "sympathetic dermatomes" are less accurate than the sensory dermatomes because of a larger overlap[38]; for example, one preganglionic sympathetic fiber synapses with multiple postganglionic cells, enabling a lesion located in one sympathetic ganglia to affect at least six dermatomal levels. According to the classic studies by List and Peet and others,[40-42] the preganglionic sweat fibers innervating the head, neck, and chest are supplied by C8 to T4, the arms by T4 to T7, the trunk by T5 to T12, and the legs by T9 to L3. The fact that resection of the stellate ganglion (made up mainly by fusion of the inferior cervical ganglion and the first thoracic ganglion) is more likely to cause Horner's syndrome than resection of T2 and T3 ganglions alone suggests the relative importance of C8 to T1 for sympathetic innervation of the face and neck.[42] Resection of T2 and T3 alone is often adequate for control of palmar hyperhidrosis, which indicates that the arms are innervated mainly by T2 and T3[43] rather than by T4 to T7, as indicated by the classic study by List and Peet.[4] Damage to the peripheral (postganglionic) fibers may cause relatively well-defined areas of anhidrosis, as in localized anhidrosis after such events as trauma, surgery of the skin, and leprosy.

Sweat test as part of neurologic examinations of sweating disorders

To test the intactness of sympathetic innervation it is essential that thermal sweating be induced in a sauna or by appropriate heating devices and that anhidrotic or hyperhidrotic areas be mapped. Any disruption of sympathetic nervous transmission should cause abnormal sweating responses. The modified iodinated starch method[43] or other appropriate color indicators for sweat may be used for visualization of sweating. A local pharmacologic sweat test (e.g., by intradermal injection of iontophoretic application of 0.1% pilocarpine or methacholine) then may be used to determine cholinergic receptor function of the sweat glands in the anhidrotic skin areas under thermal stimulation. In theory, differentiating anhidrosis as a result of preganglionic denervation from that of postganglionic lesions is possible, because after severance of sympathetic preganglionic fibers, cholinergic responsiveness of the sweat glands is preserved for several months to 2 years, whereas after damage to the postganglionic fibers, pharmacologic sweating tapers within a few weeks to a few months.[44] Thus it is sometimes essential to follow the change in pharmacologic responsiveness for a period of several weeks to several months by repeated intradermal sweat tests. Examination of other autonomic and neurologic signs is also essential (see "Hyperhidrosis" and "Hypohidrosis" in Part II of this article, to be published in the May issue of the JOURNAL). Unilateral hyperhidrosis or anhidrosis in dermatomal distribution is suggestive of the abnormality of innervation and warrants extensive neurologic examination.

After complete history and physical examina-

tions, including sensory and motor function tests, autonomic function tests must be included wherever appropriate. These include examinations for Horner's signs (to rule out the disturbance of cervical and thoracic sympathetic train), pupillary responses to light, accommodation, 2.5% methacholine and 1% phenylephrine (to rule out tonic pupil), Schirmer's test for lacrimation (to rule out familial dysautonomia and sensory neuropathy with anhidrosis), salivation test, blood pressure responses to upright position, cold immersion of hands, apneic face immersion in cold water and Valsalva's maneuver (to rule out orthostatic hypotension and/or systematic autonomic dysfunction), and intradermal histamine test and nerve conduction tests (to examine peripheral sensory and motor neuropathy).[45]

It is also important to ascertain that a lack of sweating is not due to other causes such as poral occlusion, absence or atrophy of the sweat glands, or direct damage to the glands by trauma or inflammation of the skin. The increase in the number of patent pores after adhesive tape stripping to remove poral plugging (stripping must be repeated 20 times) is evidence for involvement of superficial poral occlusion as the mechanism of anhidrosis. Skin biopsy specimens will help determine the absence or atrophy of the glands, although in many reported cases of anhidrosis,[46,47] sweat glands are noted to be morphologically intact. To definitively rule out the deep poral occlusion or altered pharmacologic receptor function, in vitro sweat induction from isolated sweat glands[48] may be necessary. The importance of nerve biopsy has been emphasized in congenital sensory neuropathy with anhidrosis.[47] In reality, however, interpretation of the neurologic examination of sweating disorders is extremely perplexing and confusing. The difficulty is caused in part by our insufficient knowledge of the adaptive mechanism of the nervous system after damage, such as degeneration and regeneration of severed autonomic fibers, sprouting of new neurons and the misdirected reinnervation of new fibers, and postdenervation hypersensitivity of pharmacologic receptors in the sweat glands, among others.

Periglandular neurotransmitters

We have already discussed the involvement of both acetylcholine and norepinephrine as periglandular neurotransmitters in the eccrine sweat glands.[35,36] More recently the presence of vasoactive intestinal peptide has been observed in the periglandular nerves of the cat paw sweat glands,[49] as well as in the human eccrine glands.[50] The same laboratory also reported the immunoreactivity for atrial natriuretic peptide, calcitonin gene-related peptide, and galanin (but not for substance P) in the periglandular nerves of human axillary eccrine sweat glands.[51] The role of these peptides on the sweat gland function is unknown. Inasmuch as the immunoreactivity for atrial natriuretic peptide, calcitonin gene-related peptide, and galanin also were localized in the cutaneous sensory nerves, however, their function, if any, may not be specific for the sweat gland. In our laboratory, evidence was obtained indicating that vasoactive intestinal peptide not only stimulates sweat secretion in a cAMP-mediated fashion but also synergistically amplifies the augmentative effect of methacholine on isoproterenol-stimulated cAMP accumulation in isolated eccrine sweat glands.[52] Vasoactive intestinal peptide–induced sweat secretion is as profuse as the maximal cholinergic sweat rate but is rather short-lived, presumably because of desensitization of their receptors.[52] Thus it is not certain that vasoactive intestinal peptide also is involved as a stimulant of sweat secretion unless it is available periglandularly in the order of 10^{-7} mol/L, which is an unlikely possibility. If the periglandular concentrations of vasoactive intestinal peptide are in the range of 10^{-9} mol/L, it can still amplify isoproterenol-stimulated cAMP accumulation in the presence of acetylcholine.

Functional significance of periglandular norepinephrine is also unknown. Norepinephrine, however, may have a function similar to vasoactive intestinal peptide because it is only 20% to 50% as effective as acetylcholine as a stimulant of sweat secretion[3,10,53] (and thus may not be important as a sudorific agent) but increases intracellular cAMP especially in the presence of acetylcholine. Neither vasoactive intestinal peptide nor norepinephrine amplifies cholinergic sweat secretion.[52,53] Thus it appears that the only salient biochemical consequence of multiple innervation of the sweat gland known to date is maximal augmentation of cAMP accumulation in the secretory cells. If so, understanding the function of augmentatively increased cAMP in the sweat gland will offer a clue to the ultimate functional significance of multiple innervation.

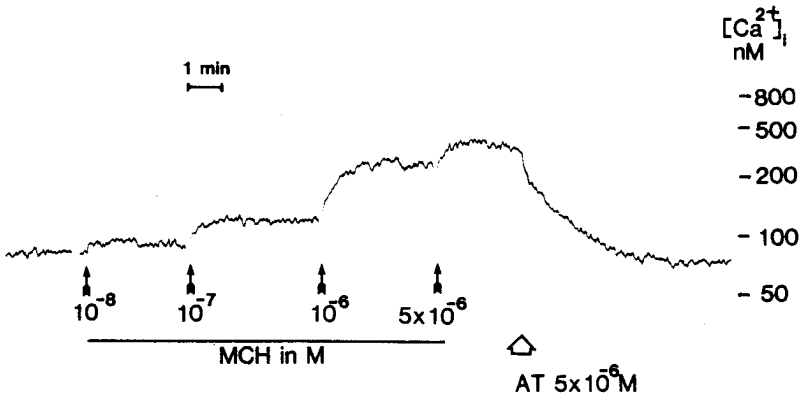

Fig. 8. Effect of methacholine *(MCh)* on intracellular Ca concentration [Ca^{2+}], in partially purified dissociation sweat secretory cells as determined by quin 2 method. Incremental doses of MCh were added to quin 2–loaded cells, and fluorescence of quin 2-Ca was continuously monitored. Also note the decrease of MCh-stimulated Ca concentration to the prestimulation level after addition of atropine *(AT)*. Ca concentration is expressed in nanomoles.

MECHANISM OF SWEAT SECRETION

Role of intracellular calcium in cholinergic sweat secretion

Eccrine sweating is induced in vivo and in vitro by intradermal injection of cholinergic agents, epinephrine, phenylephrine, or isoproterenol.[2, 3, 10, 53] Furthermore, the sudorific effects of these agents are blocked by their respective pharmacologic inhibitors. Thus the eccrine secretory cells may be endowed with cholinergic, α-adrenergic, and β-adrenergic receptors although direct demonstration of these receptors remains to be reported. Activation of different receptors leads to secretion of nearly isotonic primary fluid by the secretory coil. Nevertheless, the maximal secretory rate differs markedly in different agonists; 4:1:2 for cholinergic (methacholine), α-adrenergic (phenylephrine), and β-adrenergic (isoproterenol) stimulations, respectively, in the monkey palm secretory coils in vitro[53] and approximately 5:1:1 in human sweat glands in vivo and in vitro.[10]

We observed in experiments with isolated monkey sweat glands that both metacholine- and phenylephrine-induced sweat secretion depend strictly on the presence of extracellular calcium ions whereas isoproterenol-induced sweating is relatively insensitive to removal of Ca from the bathing medium.[53a] Because strontium (Sr), which nonspecifically enters the secretory cells in the absence of stimulants and Ca in the medium, induces copious sweat secretion in vitro,[54, 55] and because Sr is known to substitute for Ca in other tissues, we surmised that the increase in intracellular Ca may be the intracellular mediator of cholinergic or α-adrenergic stimulations and that metacholine somehow stimulates Ca influx into the cell. A Ca ionophore (A23187), a carrier of Ca across the cell membrane, also stimulates sweat secretion in vitro, which lends further support to the postulate on the role of intracellular Ca.

In a more recent study, intracellular Ca concentrations were directly measured in isolated, partially purified, sweat secretory cells[20] (Fig. 8) with the use of a fluorescent probe quin 2. (The quin 2 method is based on the principle that its acetoxymethyl derivative, quin 2 A/M, easily enters the cell but is trapped in the cytoplasm when hydrolyzed to become impermeant quin 2 by endogenous esterase. Thus cells can be easily loaded with the desired amount of quin 2 by briefly incubating with quin 2 A/M. The quin 2 then forms a complex with intracellular Ca and emits fluorescence in proportion to the cellular Ca concentration. However, not all the observations on the mechanism of cholinergic sweat secretion are readily explained by the Ca hypothesis. For example, although high concentrations of A23187 (i.e., higher than 10^{-6} mol/L) induce sweat secretion in the Ca-dependent manner after various latent periods, lower concentrations of the ionophore (e.g., 10^{-7} mol/L) does not evoke sweat secretion despite the fact that the intracellular concentration increases to a level that is higher than attainable by the highest concentration of metacholine.[20] In other words, the

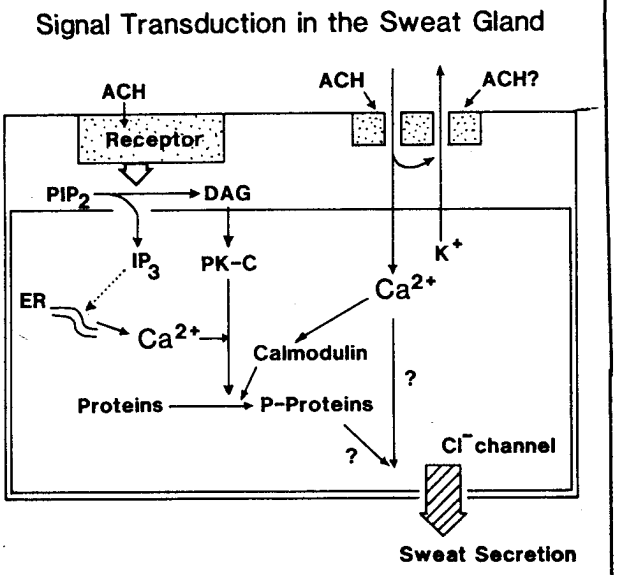

Fig. 9. Signal transduction that may be partially involved in eccrine sweat secretion. Evidence indicates that in the sweat gland binding of acetylcholine *(ACH)* to its receptors stimulates Ca influx, potassium *(K^+)* release, degradation of phosphatidylinositol phosphates, and production of inositol triphosphate *(IP_3)*. *PIP_2*, Phosphatidylinositol 4,5-bisphosphate, *PK-C*, protein kinase C; *ER*, endoplastic reticulum; *P-proteins*, phosphorylated proteins. Note that in the sweat secretory cells, Ca of exogenous origin is more important than that of endogenous stores.

increase in intracellular Ca concentration alone may not suffice to maximally induce stimulus secretion coupling, suggesting that a missing factor (or factors) also is needed. If the missing factor is involved in cholinergic sweat secretion, one of the likely candidates for such a factor would be metabolites of phosphatidylinositol, including diacylglycerol, a stimulant of protein kinase C.

In other tissues the phosphatidylinositol–protein kinase cascade also has been implicated as an intracellular regulator of a variety of cellular functions.[57,58] It is generally believed that inositol triphosphate, one of the metabolites of phosphatidylinositol, is involved in the release into the cytoplasm of free Ca from the internal Ca stores, usually endoplasmic reticulum. We recently demonstrated that during stimulation of isolated secretory coils, phosphatidylinositol is indeed hydrolyzed and inositol triphosphate accumulated in the glands.[59,60] If the phosphatidylinositol–protein kinase system is involved in the eccrine sweat gland (Fig. 9), then the combination of a phorbol ester (12-*O*-tetradecanoyl phorbol-13-acetate, a strong stimulant of PKC) and the increased intracellular Ca concentration should induce sweat secretion. Indeed, when 10^{-7}M A23187, which markedly increases Ca concentration but by itself fails to induce sweat secretion,[20] is combined with 5×10^{-8}M 12-*O*-tetradecanoyl phorbol-13-acetate a small amount of sweat can be induced. Thus at present we can conclude only that both intracellular Ca and the phosphatidylinositol–protein kinase cascade are involved in stimulus secretion coupling of cholinergic eccrine sweat secretion, but an additional unknown factor or factors still may be involved.

Tissue level of cyclic guanosine monophosphate (cGMP) also increases in the sweat gland but only during the transient period of cholinergic stimulation.[61] Furthermore, exogenous cGMP does not stimulate sweat secretion, suggesting that cGMP may not play a critical role as an intracellular regulator of cholinergic mechanism of sweat secretion.

Cyclic adenosine monophosphate (cAMP) as a second messenger of β-adrenergic sweating

Stimulation of the sweat gland with the maximal dose of isoproterenol in the presence of a phosphodiesterase inhibitor, theophylline or 3-isobutyl-1-methylxanthine, induces only 20% to 40% of the maximal cholinergic sweating rate in vivo and in vitro.[3,11,53] Furthermore, neither isoproterenol nor norepinephrine synergistically enhances cholinergic sweating[52] (their effects are only additive to cholinergic stimulation at best) in vitro. Therefore, even though norepinephrine actually is present periglandularly, its role may not be simply as another stimulant of sweat secretion or as a synergistic enhancer of cholinergic sweating in vivo. Nevertheless, isoproterenol does induce sweat secretion in normal sweat glands but not in those affected by cystic fibrosis.[11]

In a variety of tissues, the role of cAMP has been established as an intracellular mediator of β-adrenergic agonists and other cAMP-elevating agents (such as vasoactive intestinal peptide, cholecystokinin, secretin, and prostaglandins). This general thesis also appears valid in isoproterenol-induced sweating[3,62,63] in which (1) the time course and dose response of isoproterenol-induced cAMP accumulation in the sweat gland is consistent with those of isoproterenol-induced sweating, (2) exogenous cAMP induces a small amount of sweat

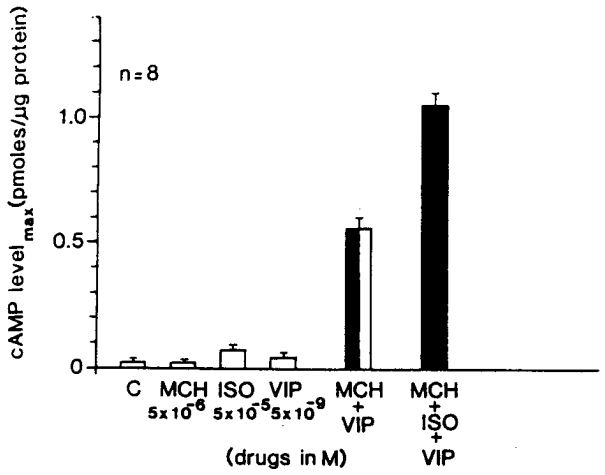

Fig. 10. Synergistic effect of methacholine *(MCH)* and isoproterenol *(ISO)* on vasoactive intestinal peptide–stimulated cAMP production. (Modified from Sato K, Sato F. Am J Physiol 1987;253:935-41. Reprinted with permission.)

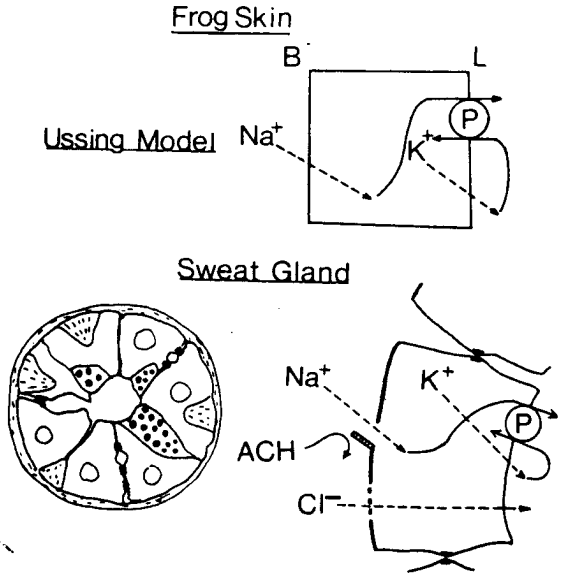

Fig. 11. Ussing's leak-pump model originally proposed for the ionic mechanism of sweat secretion but replaced by the modified cotransport models shown in Figs. 12 and 15. *P,* Na pump; *ACH,* acetylcholine.

secretion in vitro, (3) theophylline alone induces small amount of sweating and also enhances isoproterenol-induced sweating, (4) both isoproterenol-induced sweating and isoproterenol-induced cAMP accumulation are relatively insensitive to removal of extracellular Ca, and (5) cholinergic stimulation by itself fails to enhance cellular cAMP. Observations 4 and 5 further illustrate the difference in the mechanism of sweat secretion between cAMP-dependent (as in isoproterenol stimulation) and predominantly Ca-dependent mechanisms (as in cholinergic stimulation). It is widely held that the effect of intracellular cAMP is mediated by cAMP-dependent protein phosphorylation, and in fact a number of sweat glandular proteins undergo phosphorylation by cAMP.[59] Unfortunately, as in many other tissues, functions and characteristics of individual phosphorylated proteins remain to be identified.

As briefly discussed in a previous section, vasoactive intestinal peptide–induced sweating also is mediated by cAMP accumulation except that it requires only 100 times lower concentrations to achieve the same effect as isoproterenol.[52] We also have remarked that neither vasoactive intestinal peptide nor norepinephrine may be primarily involved as sudorific agents in physiologic sweating, but these agents may be important in maximization of cAMP accumulation, together with acetylcholine[52] (Fig. 10). Thus, even though physiologic eccrine sweating (i.e., perspiration in daily life) is predominantly cholinergic, and cholinergic stimulation alone does not accumulate cAMP, it always may be accompanied by considerable accumulation of cellular cAMP because of the simultaneous presence of vasoactive intestinal peptide and norepinephrine. The physiologic function of the concomitant cellular cAMP accumulation during cholinergic sweat secretion is not clear at present; however, it may be involved in promoting glandular growth[3] and in protecting the cells from the abnormal increase in Ca concentration.[20]

Ionic mechanism of cholinergic sweat secretion: Leak-pump model versus Na-K-Cl cotransport model

The principal function of the eccrine sweat gland is secretion of fluid and electrolytes. Sweat is formed by two steps: secretion of nearly isotonic primary sweat by the secretory coil in response to mainly cholinergic stimulation[64] and partial reabsorption of NaCl by duct, resulting in delivery of hypotonic sweat onto the skin surface. Until the mid 1970s it generally had been assumed that movement of ions during cholinergic sweat secretion might be explained on the basis of the Ussing's leak-pump model (Fig. 11), originally developed in 1948 to describe the ionic movement in the frog skin and subsequently in the toad urinary bladder.[65] According to this model (1) first, cholinergic

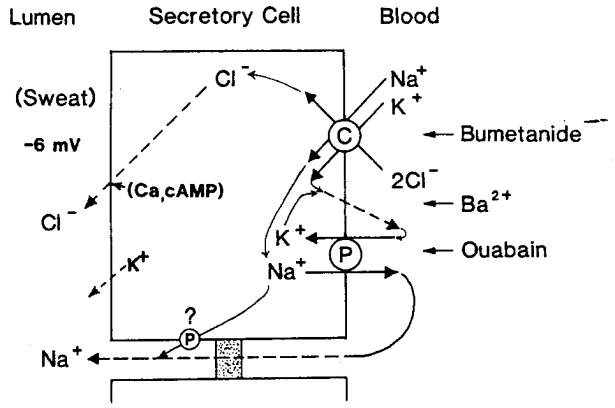

Fig. 12. Na-K-Cl cotransport model for ionic mechanism of eccrine sweat secretion. *Bumetanide* is the inhibitor of the cotransporter; *Ba^{2+}*, the inhibitor of K-channels; and *ouabain*, the inhibitor of Na-K exchange pump. Acetylcholine increases Ca entry into the cell (not indicated). *Finger signs*, probable sites of Ca action. *C*, Cotransporter; *P*, pump. *Interrupted lines* indicate the rheogenic diffusion of respective ions. In cystic fibrosis sweat glands, cAMP-regulated Cl channels may be abnormal.[62]

stimulation increases the permeability to Na of the basal cell membrane of the secretory cell, causing influx of Na into the cell, (2) Na is then pumped out from the cell interior to the secretory coil lumen by the Na pump across the luminal membrane, increasing the osmolarity and the positive electrical potential of the tubular lumen, and (3) finally, Cl enters the lumen passively, according the lumen positive electrical potential gradient while water follows according to the osmotic gradient. Indeed, sweat secretion could be inhibited by ouabain, an inhibitor of Na pump and Na-K-ATPase, and the ouabain-sensitive Na-K-ATPase is present in the tissue homogenates of the secretory coil.[66,67] When the electrical potential difference of the secretory coil lumen was actually measured, however, it was about 0 mV at rest and −6 mV during metacholine stimulation.[3,55,68] Such an electrical profile was difficult to reconcile with the leak-pump model because the model predicted a positive luminal potential.

At about the same time, a number of investigators studying the transport mechanisms of rectal glands, tracheal epithelium, thick ascending limb of Henle's loop, and intestine came to realize that the Na-K-Cl cotransport model originally postulated in Ehrlich ascites tumor cells[69] may best explain the transport mechanisms in these and other secretory epithelia (for review see reference 70).

Some of the common features of the Na-K-Cl cotransport system include (1) Na-K-ATPase and thus Na-pump site in the basolateral membrane, (2) presence of an electroneutral Na-K-2Cl (and/or Na-Cl) cotransporters in the basolateral membrane, (3) K channels in the basolateral membrane, (4) Cl channels in the luminal membrane, (5) inhibition of transport by "loop diuretics" such as furosemide, piretanide, and bumetanide, and (6) interdependency of ion fluxes; that is, if one of these ions is omitted, transport of remaining ions also is blocked in a stoichiometric manner. Of these, criteria 1, 3, 4, 5, and 6 are at least in part supported by our recent studies of the eccrine sweat gland[18,71-74] (Fig. 12), thereby suggesting that the ionic movement during cholinergic sweat secretion may be adequately explained by the cotransport model.

According to the Na-K-Cl cotransport model, movement of an ion is generally coupled with those of others. For example, during stimulation of membrane transport, K movement out of the cell across the basolateral membrane is coupled with the movement of Cl into the lumen across the luminal membrane (see the upper half of Fig. 12) so as to negate the net change of electrical charges. The resultant decrease in cellular KCl concentration provides a favorable chemical potential gradient for the influx of K and Cl from the basal medium via a Na-K-Cl cotransporter that carries into the cell one Na, one K, and two Cl ions in an electrically neutral fashion. The Na carried into the cell by the cotransporter is then pumped out across the basolateral membrane in exchange for K (usually in a ratio of 3 Na:2 K) in such a way as to keep the cellular Na concentration from increasing too high. K diffusion out of the cell, via the K channel across the basolateral membrane, also generates the K diffusion potential, which helps keep the membrane potential always negative. The negative cell potential in turn facilitates cellular Cl to cross the luminal membrane through the Cl channels by providing the driving force. Diffusion of Cl across the luminal membrane then depolarizes the luminal membrane and generates the lumen negative transepithelial potential, which attracts Na. Thus after each cycle of ionic movement, two

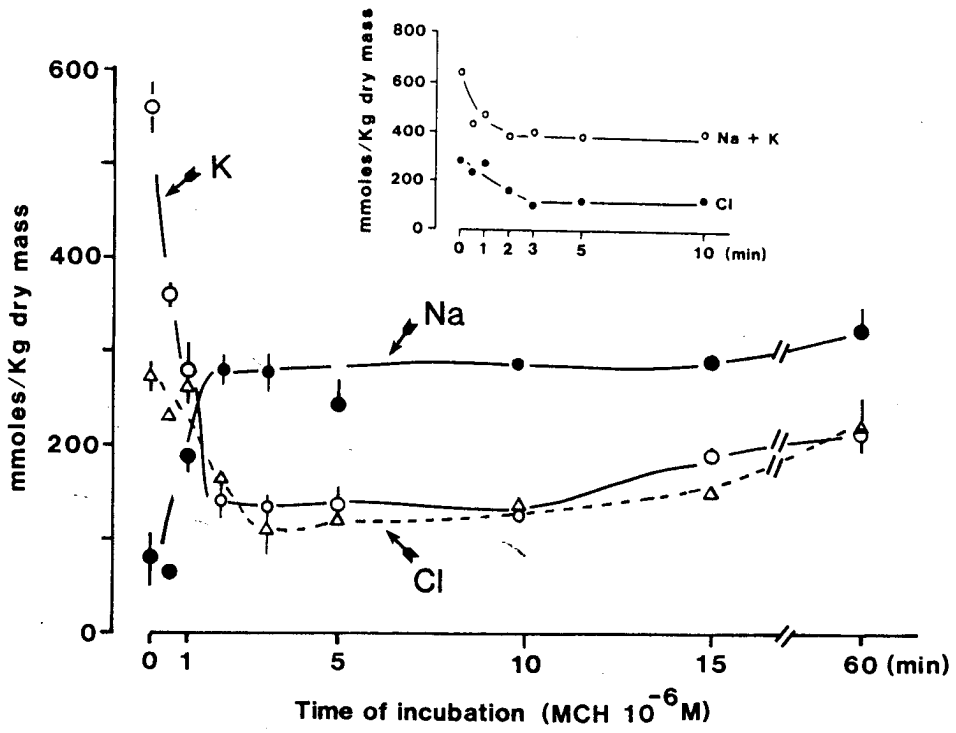

Fig. 13. Electron photomicrograph of x-ray microanalysis of cellular concentrations of Na, K, Cl in secretory cells of isolated monkey sweat glands after stimulation with methacholine. The concentrations are conveniently expressed on a dry mass basis. The decrease in the sum of Na and K shown in the insert may reflect the decrease in cellular volume (and thus the increase of the dry mass per unit area of tissue section) rather than the actual decrease in cation concentrations. Methacholine was added at time zero. (From Saga K, Sato K. J Membr Biol [In press.] Reprinted with permission.)

Cl ions are ultimately transported into the lumen traversing the cell while both Na and K are recycled across the basolateral membrane. Because the basolateral cell membrane is not readily permeable to Na ions, Na travels across the intercellular junction (i.e., paracellular pathway) into the lumen to join Cl there. Water can readily enter the lumen so as to maintain the near isotonicity of the luminal fluid (i.e., primary sweat).

Although this Na-K-Cl model is instrumental in explaining the electrically silent transport processes in these Cl-transporting epithelia, the question remains as to how the membrane channels, cotransporters, and Na-pumps are regulated. In the salivary glands, Ca appears to stimulate K channels[77] and in tracheal epithelium, cAMP may be involved in regulating Cl channels,[70,75] which is possibly mediated by cAMP-dependent protein phosphorylation. Unfortunately, the mechanism by which the cotransporter is regulated is totally unknown.

Movement of cellular ions during sweat secretion

Although the Na-K-Cl cotransport system has been speculated to occur in the sweat[72] and the salivary glands,[76,77] as well as in the exocrine pancreas,[78] much remains to be done to prove such a hypothesis concerning the eccrine sweat gland. For example, the original cotransport model calls for complete recycling of both Na and K across the basolateral membrane so that no drastic change of intracellular electrolytes needs to occur, as long as the cellular electrochemical potential profiles for Na, K, and Cl are favorable for movement of respective ions and that the respective membrane channels are opened during stimulation. To our surprise, however, when we studied the metacholine-induced change in intracellular ion concentrations of the secretory cells with the use of x-ray microanalysis and the microtitration method, both cellular K and Cl concentrations decreased to about 50% of the unstimulated values whereas Na

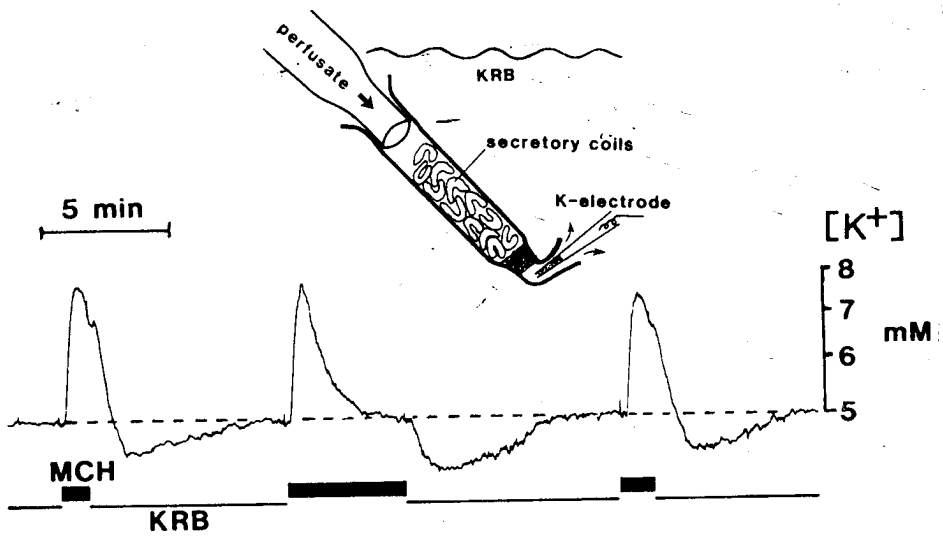

Fig. 14. K efflux from the continuously superfused secretory coils placed in a microcapillary and stimulated by methacholine containing superfusate. *KRB*, Krebs' Ringer's solution. As shown in the insert, K concentration in the effluent was continuously measured with a K-sensitive electrode. (Modified from Saga K, Sato F, Sato K. J Physiol 1988;405:205-17. Reprinted with permission.)

concentration doubled[79] (Fig. 13). This indicates that metacholine caused a massive KCl efflux from the cell and Na influx into the cell. Furthermore, we found that dark cells also show a similar change in cellular ion concentrations,[79] suggesting either that dark cells are also endowed with cholinergic receptors or that they are functionally coupled with secretory (clear) cells so that both types of cell behave like a syncytium.

Given the large magnitude of K movement, we then studied the exact time course of K efflux, with the use of the continuous superfusion method in which the isolated secretory coils placed in a glass microcapillary were superfused with metacholine, and the K concentration in the effluent was continuously monitored with a K-sensitive electrode. As shown in Fig. 14, K efflux commences immediately on cholinergic stimulation, peaking within seconds, but it rapidly tapers to zero within 2 minutes of continuous metacholine stimulation, during which time the cell loses about half of its cellular K.[74,79] When metacholine stimulation was stopped, K was taken up by the cell (Fig. 14), most likely by the Na-K exchange pump because it is completely inhibited by ouabain. It appears that the efflux of K and Cl from the cell may be mediated by a metacholine-induced increase in cellular Ca concentration, because in the absence of Ca in the medium, KCl efflux is drastically inhibited.[79] Similar K movement also has been observed in other exocrine cells.[73]

What, then, is the functional significance of the observed drastic decrease in cellular K and Cl concentrations in relation to the ionic mechanism of sweat secretion? Although the definitive answer is not yet available, this decrease appears to be the grand strategy adopted by many exocrine secretory cells to trigger and sustain ionic movements for exocrine secretion (i.e., the drastic decrease in cellular K and Cl concentrations may maximally stimulate the Na-K-Cl cotransporters by creating the most favorable chemical gradient). Movement of Na, K, and two Cl ions into the cell by the cotransporter causes the cellular Na concentration to increase, which subsequently stimulates the Na pump, as discussed earlier. This thesis is partially supported because the inhibitor of the cotransporter, bumetanide, inhibits the increase in cellular Na concentration in the sweat gland.[79]

All the observations, however, are not explained on the basis of the Na-K-Cl cotransport model in the sweat gland. For example, amiloride at 0.1 mM, the inhibitor of Na-H exchange, and DIDS (4,4′-didsothio-cyanostilbene-2,2′-disulfonic acid) at 0.1 mM, the inhibitor of HCO_3-Cl exchange, reduce sweating rate by about 20% in vitro[72] and partially reduce metacholine-induced K efflux,[80] suggesting that these parallel ion exchangers also

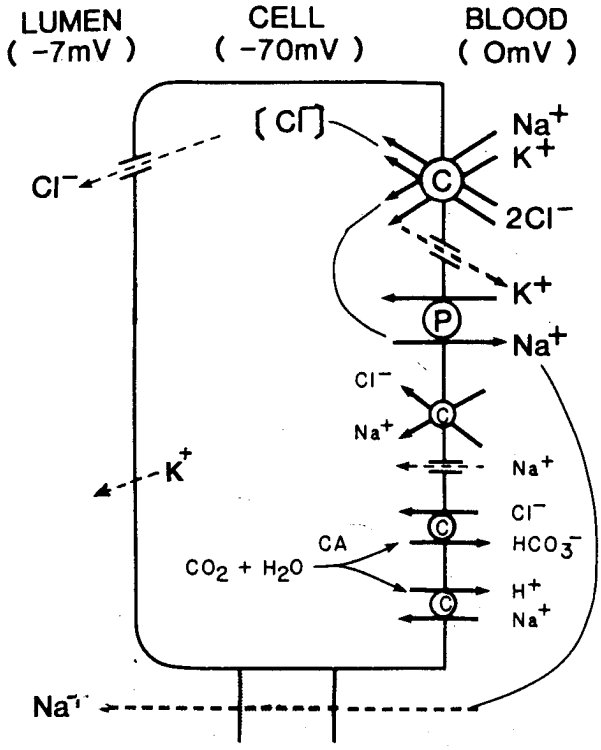

Fig. 15. A modified model consisting of Na-K-Cl cotransporter and parallel antiporters for the mechanism of ion transport during cholinergic sweat secretion. *Larger circles* indicate the major transport mechanism. *Interrupted lines* indicate the rheogenic passive movement of ions (ionic conductance). The electrophysiologic study suggests a small increase in Na permeability across the basal membrane. *C*, Cotransporter or parallel exchangers; *P*, pump; *CA*, carbonic anhydrase.

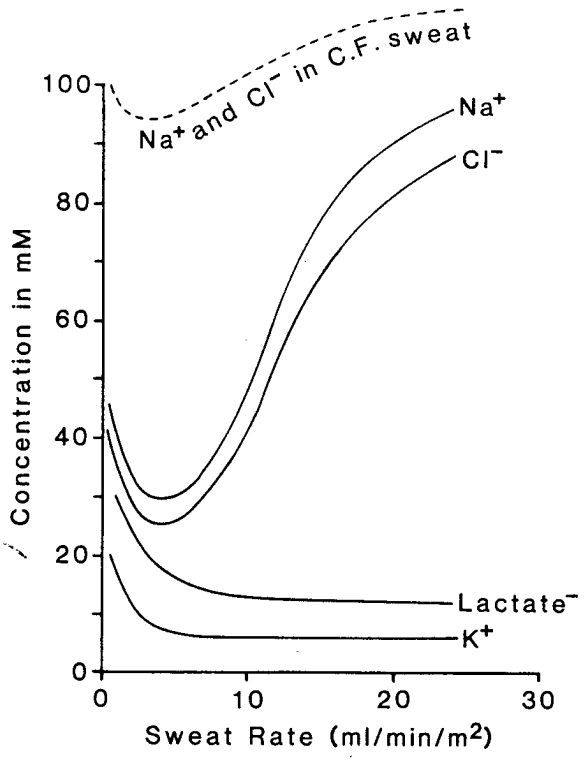

Fig. 16. Schematic illustration of the relationship between sweat solute concentrations versus sweat rates. Note that sweat in cystic fibrosis contains high NaCl concentration at all sweat rates because of decreased ductal absorption.

are involved in the control of ionic movement during cholinergic sweat secretion, which constitutes a small departure from the original cotransport model (Fig. 15). The drastic inhibition of K efflux by bumetanide also is difficult to explain[74] unless bumetanide has other unknown effects such as nonspecific inhibition of Ca transport. Furthermore, in contrast to the original cotransporter model, K permeability across the luminal membrane also may increase during metacholine, but not during isoproterenol, stimulation (Fig. 12), as suggested by the fact that during the transient stage of metacholine stimulation, the luminal K concentration rises to as much as 9 mM.[64]

SOLUTE CONCENTRATIONS IN HUMAN SWEAT

As indicated earlier, sweat solute concentrations are determined by secretion of solutes into the secretory coil and by the subsequent ductal modification (either secretion or absorption).[81] Thus they are usually influenced by the sweat rate because the ductal modification often is a function of the flow rate. Furthermore, extreme caution should be exercised to ensure that the observed solute concentrations are not affected by evaporative water loss and/or epidermal contamination.[82,83]

Sweat Na concentration

Of all the sweat solutes, sweat Na concentration is perhaps the most extensively studied and the easiest to understand. In the primary fluid it is nearly isotonic to plasma Na concentration irrespective of the sweat rate.[64,84] Thus the hypotonicity of sweat Na concentration depends strictly on ductal Na absorption. Because the ductal capacity to absorb Na is both limited and dependent on the duration of time, luminal fluid is exposed to the ductal wall (which is inversely related to the sweat rate); sweat Na concentration increases with increasing sweat rate (Fig. 16).[3] At very low sweat rates, however, sweat Na concentration again rises

slightly, for unknown reasons, both in vivo and in vitro.[84] Its normal range is therefore between 10 to 20 mM at the relatively low sweat rate range, but it can reach 100 mM near the maximal sweat rate.

Regulation of ductal Na absorption is still poorly understood. Although it is known that aldosterone can increase ductal Na absorption[3] and a relatively high sweat Na concentration (e.g., 70 to 80 mM) has been reported in Addison's disease,[85] it is not certain that aldosterone is the principal regulator of ductal absorption. Likewise, it is not established that the mechanism for the increased ductal Na absorption during salt deprivation[86] is entirely due to increased aldosterone secretion. The individual difference in ductal Na absorption also may be influenced by the degree of glandular hypertrophy, because the sweat ducts also undergo hypertrophy in parallel with the secretory portion, and the long hypertrophic ducts may absorb more sodium at a given sweat rate.[16,87] It remains to be studied whether vasopressin (antidiuretic hormone [ADH]), adrenergic agonists, acetylcholine, epidermal growth factor, vasoactive intestinal peptide, and other periglandular neurotransmitters have any regulatory roles in ductal Na absorption.

A study by Ratner and Dobson[88] suggests the absence of an ADH effect on sweat osmolarity; however, their study is still inconclusive because free water clearance was not determined. Local injection of ADH reduced the sweating rate to about 50% that of the control,[89] but it is not clear whether the observed decrease in the sweat rate is due to ductal water absorption or to inhibition of sweat secretion because intradermal injection of ADH causes vasoconstriction.

Sweat K concentration

The values of K concentration in the pooled primary sweat were nearly isotonic to plasma, that is, 5 to 6 mm,[48] but a more recent study has revealed that K concentration in the primary fluid can rise transiently to as high as 9 mM immediately after metacholine stimulation in an isolated monkey secretory coil. K concentration, however, then tapers to 6 to 7 mM during the steady state of sweat secretion, which is still higher than 5 mM in the bath.[64] Thus K concentration higher than 6 to 7 mM in the final sweat, which is seen in some but not in all persons, may be due to K secretion by the duct. K secretion by the duct may be partially controlled by aldosterone,[90] but other mechanisms such as K permeability of the ductal wall and luminal negative electrical potential also may influence sweat K concentration.

Sweat pH and HCO_3 and Cl concentrations

Cl concentration in the skin surface sweat also increases with sweat rate in proportion to sweat Na concentration, except that Cl concentration is usually 20 to 25 mM lower than that of Na in both the final sweat (Fig. 16) and the primary fluid. The anion deficit (concentrations of $Na + K - Cl$) of 25 to 30 mM usually consists of HCO_3 concentration from 15 to 20 mM (in the human primary sweat; unpublished data) and lactate concentration of approximately 10 mM. Because sweat pH is controlled by the HCO_3-CO_2 buffer system, both pH and HCO_3 concentration must be determined under a constant carbon dioxide pressure (P_{CO_2}). When measured at 5% P_{CO_2}, sweat pH is approximately 7.2 to 7.3[83] in the primary sweat collected from the isolated human secretory coils in vitro irrespective of the sweat rate.[87] In contrast, the pH of the final sweat is about 5.0 at the lower sweat rate range and 6.5 to 7.0 at the higher sweat rate range,[3] indicating that the duct is capable of acidifying sweat, presumably by reabsorbing HCO_3 (or secreting H^+). Thus the ductal acidification helps preserve HCO_3 for the body during prolonged profuse sweating. Needless to say, sweat pH determined in the air (<0.04% P_{CO_2}) is about 6.5 to 7.0.

Sweat lactate concentration

Plasma lactate concentration is usually less than 2 mM whereas sweat lactate concentration ranges from 10 to 15 mM, indicating that sweat lactate is derived mainly from the sweat gland as an end product of glycolysis.[91,92] Sweat lactate may be instrumental in controlling desquamation of stratum corneum as evidenced by the well-known beneficial effects of topical lactate preparations against retention hyperkeratosis.

Sweat urea concentration

Because urea can readily cross the glandular wall and cell membrane, sweat urea concentration is approximately the same as, or slightly higher than, that of plasma. This is best illustrated by the occurrence of urea frost on the skin of patients with uremia. Average urea concentrations in healthy subjects, as determined in our laboratory, range

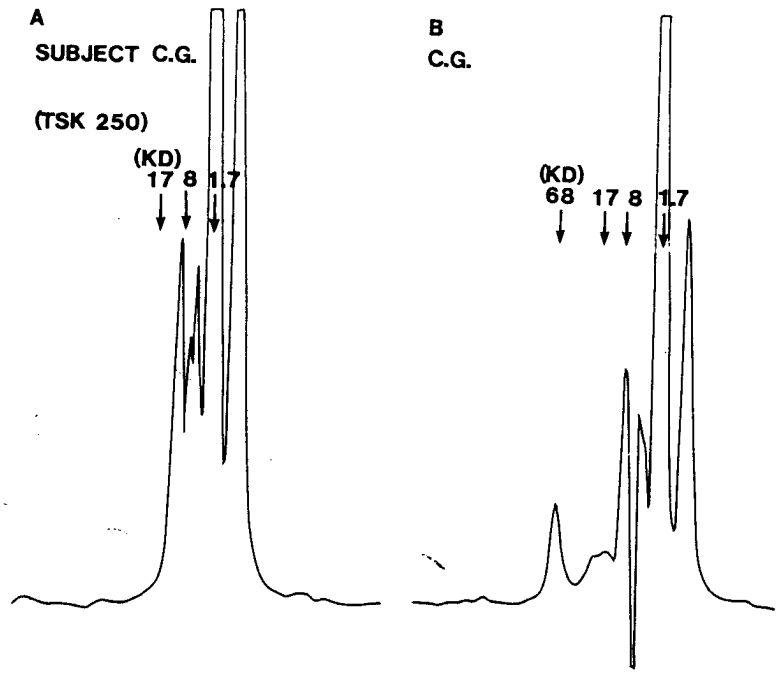

Fig. 17. Difference in high-pressure liquid chromatography (with the use of a TSK 250 size discrimination column) elution profile of sweat proteins and peptides in sweat samples from different sweat rates. **A,** Sweat sample from the low sweat rate; **B,** from the maximal sweat rate in the same subject. Note that a 68 kD protein peak is evident only in sample **B.** Sweat was collected over a petroleum jelly barrier under a plastic sweat collector to minimize epidermal contamination.

from 15 to 25 mg/dl.[82] Whether urea also is produced by the sweat gland remains to be studied. Like lactate, sweat urea may function as a natural skin moisturizer.

Sweat ammonia concentration

Sweat contains 0.5 to 8 mM total ammonia, which is 20 to 50 times higher than the plasma ammonia concentration.[93,94] The accumulation of ammonia in sweat may be explained mainly by free nonionic diffusion of ammonia free base (NH_3) from plasma to the more acid ductal sweat and by entrapment of ammonia in sweat as poorly permeable NH_4^+. In other words, if sweat pH is 1 or 2 pH units more acid than plasma (which is usually the case), sweat can accumulate 10 or 100 times more NH_4^+, respectively, by the diffusion-entrapment mechanism alone.

Amino acids in sweat

The presence of amino acids in human sweat has been reported, but their concentrations vary in different studies.[3] Furthermore, very few studies have directly compared the sweat-to-plasma ratio for each amino acid.[95] Thus it is not certain that sweat amino acid concentrations are correlated with their serum concentrations. The pitfalls in determining sweat amino acid concentrations include the fact that amino acids are abundantly present in the stratum corneum. Consequently, those of epidermal origin can easily contaminate sweat, probably because aminopeptidase activity (which releases terminal amino acids from proteins or polypeptides) is very high in the epidermis as well as in sweat.[83] It is not certain that the very high sweat amino acid concentrations (6 to 10 times higher than those in plasma) reported by Gitlitz et al.[95] truly represent those actually secreted by the secretory coil. For example, the sweat-to-bath ratio of H^3-glycine was about 1.0 in an isolated secretory coil in vitro,[96] whereas a sweat-to-plasma value of 6.7 was reported by Gitlitz et al.[95] in sweat collected from the skin surface.

Proteins in sweat

Although total protein concentrations in sweat, as determined by Lowry's method,[97] range from 15 to 25 mg/dl,[82] those measured by the Bradford dye-binding method (using a kit from Bio-Rad, Richmond, Calif.) in the same sweat samples yield

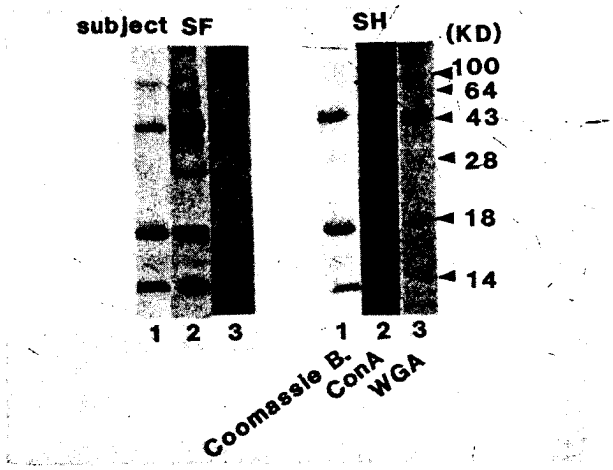

Fig. 18. Lectin-binding proteins in human sweat. 15 μg of protein concentrated by a YM-10 membrane was separated in each lane by an SDS-PAGE (sodium dodecylsulfate-polyacrylamide gel electrophoresis), transferred to a nitrocellulose membrane, incubated with lectin-biotin, and appropriately stained. *ConA,* Concanavalin A; *WGA,* wheat germ agglutinin.

only 5 to 8 mg/dl. Because the latter method mainly determines large-molecular-weight proteins, whereas Lowry's method determines the total tyrosine hydroxy groups (of both proteins and small peptides), the observed disagreement between the two methods suggests the abundance in sweat of small-molecular-weight peptides. This was confirmed when fresh, concentrated ×10 (by lyophilization) sweat samples were subjected to a size fractionation high-performance liquid chromatography (HPLC) column (TSK 250), which disclosed that the majority of sweat peptides are in the small-molecular-weight range (Fig. 17). Furthermore, the composition of sweat proteins and peptides is influenced by the sweat rate in each person.

Of particular interest is the fact that the concentration of high-molecular-weight (>10 kD, but especially 68 kD peak) proteins increases with increasing sweat rate and decreases again when sweat rate is tapered by lowering the temperature in a sauna (Fig. 17). The mechanism for this differential secretion of sweat proteins is not clear. Possible mechanisms include the differing degree of proteolytic degradation during outflow of sweat, secretion of proteins from different intracellular storage sites, and differing protein synthesis with increasing pharmacologic stimulation (because of increased sudomotor activation). Although considerable individual differences exist in the HPLC elution pattern of large-molecular-weight sweat protein peaks (as well as small peptides),[98] one-dimensional sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of SDS-denatured sweat proteins (>10 kD) shows two major bands at 43 and 18 kD, and four less prominent bands at 100, 64, 28, and 14 kD in all the subjects (Fig. 18), suggesting that sweat proteins are made up of these protein subunits. All these protein subunits react to concanavalin A (ConA) indicating that they contain α-D-mannose and/or α-D-glucose terminal residues. Some of these bands also bind wheat germ agglutinin (WGA), which binds *N*-acetyl-D-glucosamine or *N*-acetylneuraminic acid. The 43 and 18 kD bands are predominantly PAS reactive.[99] Evidence indicates that the PAS-positive or ConA binding sweat proteins may be derived mainly from the dark cell granules[98, 99] and may be the same PAS-positive protein involved in poral occlusion in miliaria.[100]

In addition to these glycoproteins (Fig. 19, spots *b* and *c*), sweat contains varying number of protein spots when analyzed by two-dimensional electrophoresis. Most of these small protein spots are not consistently present in different subjects. Thus it is not certain whether these additional small protein spots are the fragments of major sweat glycoproteins, epidermal contaminants, or protein fragments of glandular debris. The same precautions may apply to the various sweat proteins reported by other authors.[101, 102] Although the presence of immunoglobulins and other serum components has been reported in the past,[103, 104] these findings may have to be reexamined with the use of uncontaminated human sweat. A recent study of Yanagawa et al.[99] failed to confirm the presence of serum components other than albumin in human sweat collected over a white petrolatum (Vaseline) barrier on the skin to minimize epidermal contamination.

Presence of proteolytic enzymes in sweat

The presence in sweat of kallikrein (kinin-producing or kiniogenase)-like activity has been suggested by Fräki et al.,[105] who used nonspecific substrates and a simple method of sweat collection (without rigorous precautions to minimize epidermal contamination). Hibino et al.[106] recently provided compelling evidence that human sweat indeed contains kallikrein of a glandular type.

Furthermore, presence in sweat of kininase has led Hibino et al. to suggest that a kallikrein-kinin system may be functionally present in the sweat gland. Sweat also contains a cathepsin B–like enzyme[7, 107] (the endogenous inhibitor of cysteine proteinase[108]), urokinase,[109] and perhaps more proteases.[6] Although the functional significance of these proteolytic enzymes is not known, glandular kallikrein is speculated to be involved in cleavage of bioactive precursor peptides of nerve growth factor and/or epidermal growth factor.[110] Kinin (a fragment of kininogen cleaved by kallikrein) is also a well-known vasodilator and a natriuretic agent, suggesting that the kallikrein-kinin system could be playing a similar role in the sweat gland. The proinflammatory nature of proteolytic enzyme is widely recognized. It is easy to speculate therefore that migration of sweat into the epidermis in the presence of ductal blockade may aggravate or modify various skin diseases such as psoriasis, atopic dermatitis, and other papulosquamous diseases.

Epidermal growth factor in sweat

The presence in human sweat of epidermal growth factor has been reported by Pesonen et al.[111] In light of our own recent observation that epidermal growth factor is abundantly present in the epidermis and the stratum corneum (and thus can easily contaminate into sweat), we undertook to reexamine whether epidermal growth factor is indeed present in human sweat and, if so, how its secretion is regulated. With precautions to minimize epidermal contamination during sweat collection, we confirmed that epidermal growth factor is indeed present in eccrine sweat and that its concentration (i.e., epidermal growth factor/unit sweat volume) increases in proportion to the increase in sweat rate[112] (and thus the concentration of large molecular proteins as discussed earlier). Of greater interest is that axillary sweat contained about 50 times more epidermal growth factor than back sweat, suggesting that apocrine or apoeccrine glands may be responsible for the high concentrations of epidermal growth factor in the axillae.

The function of epidermal growth factor in sweat and its mechanism of secretion in sweat is totally unknown. Recently, however, Arita et al.[116] observed that immunohistochemical staining of endogenous epidermal growth factor occurs predominantly in the eccrine dark cells. Nanney et al.[114] localized the immunoreactive epidermal growth factor receptors predominantly in the myoepithelium of the eccrine and apocrine glands and in the luminal membrane of the eccrine ductal cells. With the autoradiography technique, Nanney and colleagues also observed the binding sites of the epidermal growth factor to occur predominantly in the eccrine sweat duct cells. Both immunoreactive receptors and radioactive binding sites of epidermal growth factor were noted in the epidermal basal and squamous cells, sebaceous glands, and hair follicles.[114]

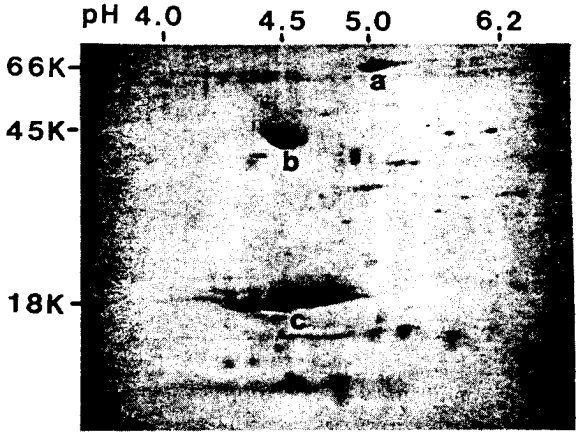

Fig. 19. Two-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of SDS-denatured sweat protein. Spot *a* is presumably albumin; spots *b* and *c* are glycoproteins, 43 and 18 kD respectively. (Modified from Yanagawa S, Yokozeki H, Sato K. J Appl Physiol 1986;60:1615-22. Reprinted with permission.)

Because the apocrine and apoeccrine cells and the eccrine dark cells are characterized by the abundance of electron-dense "dark cell granules," it is possible that epidermal growth factor in sweat is derived from these granules and that it may be involved in regulation of the ductal and myoepithelial cell function. Our most recent observation (unpublished data) that epidermal growth factor, but not vasoactive intestinal peptide and isoproterenol, stimulates protein phosphorylation of the eccrine ductal cells is consistent with this thesis.

Other ingredients of sweat

—Whether glucose is present in sweat has long been a controversial issue. A study by Boysen et al.[82] has shown that although 0.2 to 0.5 mg/dl glucose is present in sweat when blood glucose was 80 mg/dl, it increased to 0.6 to 1.2 mg/dl when

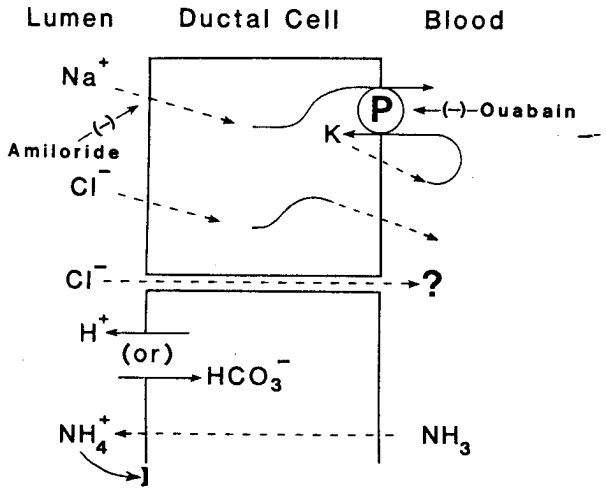

Fig. 20. Schematic illustration for the ionic mechanism of ductal NaCl and HCO_3 absorption. *P,* Na pump inhibitable by ouabain. *Solid lines,* Active transport; *interrupted lines,* passive movement of ions. Amiloride is the inhibitor of Na channels. HCO_3 absorption is achieved either by direct absorption or by H ion secretion. Free diffusion of NH_3 into the lumen and its accumulation in sweat as impermeant NH_4^+ resulting from the acid pH of ductal sweat also is depicted.

blood glucose was transiently increased to 200 to 250 mg/dl by oral and intravenous glucose bolus. Thus patients with uncontrolled diabetes may excrete more glucose in sweat, creating a favorable environment for bacterial growth in the skin. The mechanism of glucose secretion in sweat is unknown. Pyruvate concentration is sweat ranges from 0.2 to 1.6 mM.[115] Also reported to be present in (or excreted into) sweat are sulfaguanidine, sulfadiazine, antipyrine and aminopyrine,[116] histamine,[117] prostaglandin-like activity,[118] cAMP,[82] amphetamine-like compounds,[119] iodide,[120] phenytoin, phenobarbital, carbamazepine,[121] and ethanol.[122] The presence in sweat of *trans*-3-methyl-2-hexenoic acid, the purported odorous substance in patients with schizophrenia, has been reported by Smith et al.[123]; however, the observation has not been confirmed by other investigators.[124, 125]

Secretion of griseofulvin and ketoconazole in sweat

After a single oral dose of griseofulvin the highest concentration of the drug is observed in the stratum corneum within several hours.[126] This was partially explained when Shah et al.[5] observed that the drug actually is secreted into sweat and binds to the stratum corneum. As expected, when sweat secretion was blocked by topical antiperspirants, accumulation of griseofulvin in the stratum corneum did not occur.[5] More recently, Harris et al.[127] also observed that orally administered ketoconazole is delivered to the stratum corneum in a similar manner. Topical application of griseofulvin is not effective, suggesting that the active ingredients are the metabolic byproducts of the drug. It follows therefore that the best way to improve the antifungal effect of these agents is to obtain maximal perspiration while the patient is taking the drugs and to spread sweat onto the affected skin because the infected skin area is often anhidrotic because of poral occlusion.[128] Because ketoconazole (and perhaps also griseofulvin) preferentially binds to the stratum corneum in an aqueous medium–like sweat,[127] minimum cleansing of the skin (e.g., daily shower) may not significantly reduce the antifungal potency of these drugs.

REABSORPTION OF NaCl BY THE DUCT

The principal function of the sweat duct is to reabsorb Na, Cl, and HCO_3 from the plasmalike primary fluid and to conserve these vital electrolytes.[3] The duct consists of the proximal segment, which coils intimately with the secretory coil, and the distal duct, which is relatively straight or slightly helical as it ascends to reach the acrosyringium. Indirect evidence suggests that Na-K-ATPase (Na pump) is involved in ductal absorption of NaCl.[3] Namely, ouabain-sensitive Na-K-ATPase is present in the duct, and ductal NaCl absorption is inhibited by ouabain. The proximal duct contains about 10 times higher Na-K-ATPase activity than does the distal duct in the monkey palm sweat gland,[67] suggesting that the proximal duct may be more active in reabsorbing NaCl.

The duct is made up of two layers of cells. The basal (or peripheral) ductal cells may be involved primarily in ductal NaCl absorption because these cells are rich in Na-K-ATPase activity[18] (Fig. 3, *B*), mitochondria, and membrane villi. That the entire cell membrane of the basal cell is equipped with Na-K-ATPase (and thus Na pumps) appears to be an elaborate mechanism designed to maximize the Na-pumping sites. The luminal cell may be involved by allowing Na and Cl ions to enter passively into its cytoplasm and transferring them to the basal cells. Such functional partition of the

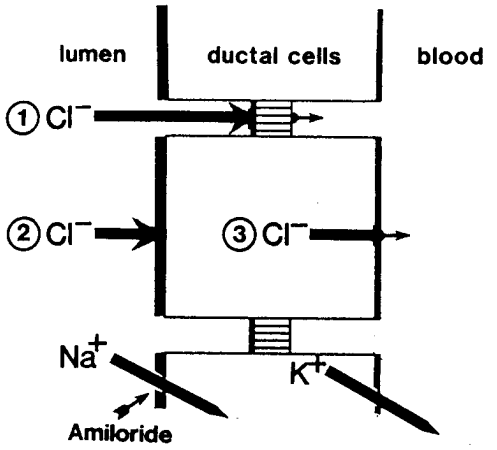

Fig. 21. Schematic representation of possible sites of abnormal Cl^- permeability in cystic fibrosis sweat duct on the basis of electrophysiologic analysis. *1,* Cell junction (paracellular shunt pathway); *2,* Luminal membrane; *3,* basolateral membrane. Increased amiloride-sensitive luminal Na^+ conductance and the basolateral K conductance also is shown. (Modified from Sato K, Saga K, Sato F. In: Mastella G, Quinton PM, eds. Cellular and molecular basis of cystic fibrosis. San Francisco: San Francisco Press, 1988:171-85. Reprinted with permission.)

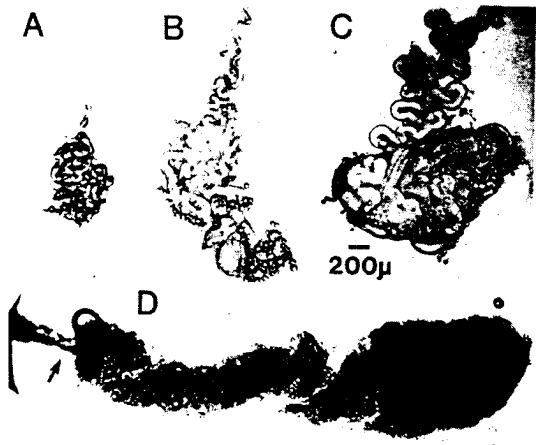

Fig. 22. Examples of apoeccrine glands (**B** and **C**) in the axillary skin of an 18-year-old man. **A,** Typical eccrine gland; *C,* typical apocrine gland. Note the short thick duct of the apocrine gland. (Modified from Sato K, Sato F. Am J Physiol 1987;251:R181-7. Reprinted with permission.)

two layers of cells, however, requires the cells to behave like a syncytium through intimate intercellular communication, for example, through gap junctions. To our knowledge the presence of gap junctions between ductal cells has not been reported.

Recent electrophysiologic studies, however, indicate that ductal cells are electrically coupled.[59] The ionic mechanism of ductal NaCl absorption has been modeled after the Ussing's leak-pump model originally developed in the frog skin,[65] as illustrated in Fig. 20. The minimum components of the model include the presence of Na-K exchange pumps (see also Fig. 3, *B*) and K conductance at the basolateral membrane, amiloride-sensitive Na channels across the luminal membrane, and Cl conductance either across the cell membranes or across the cell junctions (paracellular pathway) or both. The presence of amiloride-sensitive Na channels at the luminal membrane is suggested because 10^{-5} mol/L amiloride inhibits the ductal NaCl absorption,[84,129] depolarizes the ductal luminal potential (luminal negative potential of less than 20 mV changes to positive values), and increases the membrane resistance of the luminal membrane.[59] The duct is also highly permeable to Cl ions because reduction of luminal Cl by replacement with impermeant anions increases the ductal resistance and hyperpolarizes the luminal potential.[59,129]

In brief, ductal NaCl absorption proceeds as follows. Na passively enters the cell from the ductal lumen through amiloride-sensitive Na channels at the luminal membrane and is pumped out across the basal membrane in exchange for K (Fig. 20). Cellular K leaks out passively through K channels, generating a membrane potential.[59] K then recycles, being pumped in, in exchange for cellular Na. Cl follows passively down the gradient through Cl channels at both the luminal and basolateral membranes and across intercellular junctions (paracellular pathways).

In addition to NaCl absorption, the sweat duct also may absorb HCO_3 either directly or via H ion secretion.[64] As discussed earlier, the capacity for the duct to absorb NaCl is limited so that sweat NaCl concentration increases with increasing sweat flow rate (Fig. 16). The overall regulatory mechanism of ductal absorption function is still poorly understood.

ABNORMAL SWEAT GLAND FUNCTION IN CYSTIC FIBROSIS

The elevated sweat NaCl concentration (e.g., 65 to 120 mM in cystic fibrosis vs <65 mM in normal sweat) is one of the diagnostic criteria for cystic

Table I. Morphologic and functional characteristics of glands in human axillae

	Eccrine	Apocrine	Apoeccrine
Glandular size	Small	Large	Intermediate and variable
Duct	Long and thin, made up of proximal coiled and distal straight or tortous portion	Very short and thick	Comparable to eccrine duct
Ductal opening	Skin surface (many ducts open near the hair)	Directly into upper portion of follicular canal	Comparable to eccrine duct
Secretory coil (live tissue under a light or stereomicroscope)	Uniform small outside diameter and very narrow lumen; individual live cells not visible by light microscopic examination	Uniform large outside diameter and wide lumin; hexagonal secretory cells clearly visible by light microscopic examination; tubule tightly packed and often angular at curve in adult	Luminal and outside diameter often irregular; some tubules show segmental dilation; tubules show slow meander and curve not angular in shape
Cell type	Secretory (clear), dark, and myoepithelial	Columnar secretory cell; myoepithelial	Dilated segment resembles apocrine gland; nondilated segment often retains eccrinelike morphology; eccrinelike thin tubules sometimes lack typical dark cells
Intercellular cannaliculi	Present	Absent	Present only in the partially dilated or nondilated segment
Development	Present at birth	Present at birth	Not present before adolescence
Pharmacologic finding of secretion	C >> B >> A	C = A	C > B > A
Sweat secretory rate	Continuous; high per-unit glandular size	Transient or intermittent; variable	Continuous; high per-unit glandular size
Secretory product	Serous fluid	Milky, protein-rich fluid	Serous fluid

A, α-Adrenergic; *B*, β-adrenergic; *C*, cholinergic.

fibrosis. It has been known since the mid 1960s that the elevated sweat NaCl concentration in cystic fibrosis sweat is due to the decreased ductal NaCl absorption, because the NaCl concentration in the primary sweat is nearly isotonic to plasma in both control and cystic fibrosis.[130] Nevertheless, the mechanism of abnormal ductal absorptive function in cystic fibrosis had remained unknown. In 1983 Quinton and Bijman[131] first observed that the electrical potential of the sweat ductal lumen is about twice as negative in cystic fibrosis ducts (−60 mV) as in normal ducts (−30 mV) both in vivo and in vitro.[10, 131] It also was observed that the lumen of the normal duct, but not of the cystic

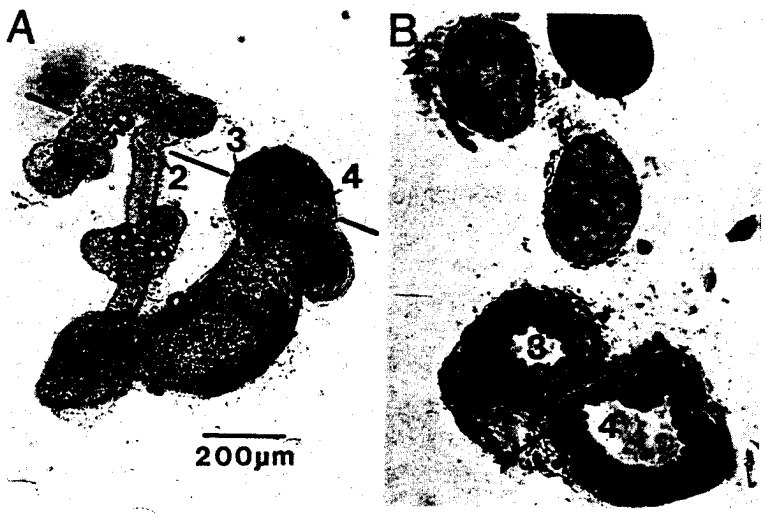

Fig. 23. Segmentally dilated apoeccrine secretory portion. Only a short segment was removed from the large apoeccrine secretory coil for ultrastructural study. For detailed ultrastructure of cross-sections *1* and *4*, see reference 133. **A,** Isolated segment photographed before embedding. **B,** Embedded tissue, 1 μm thick, cut along the line shown in **A.**

fibrosis duct, hyperpolarizes (to the level of cystic fibrosis ductal potentials) when luminal Cl is replaced by impermeant anions such as gluconate or sulfate.[131] Quinton and Bijman[131] thus speculated that the impermeability of Cl across the luminal membrane may be the basic pathogenesis for abnormal ductal NaCl absorption in the sweat duct affected by cystic fibrosis.

More recent electrophysiologic analysis of the membrane permeability of the isolated duct in cystic fibrosis has suggested that Cl permeability is abnormally low, not only across the luminal membrane but across the cellular junction and across the basolateral membrane[59] (Fig. 21). Interestingly, both luminal Na permeability and basolateral K permeability appear to be increased in the duct affected by cystic fibrosis, presumably as a compensatory mechanism (Fig. 21).

The secretory portion of the sweat gland in cystic fibrosis appears to be normal as far as its cholinergic responsiveness is concerned. When its β-adrenergic sweating response was studied in cystic fibrosis, however, Sato and Sato[10] discovered that the secretory portion failed to show sweating responses to a combination of isoproterenol and theophylline in vivo as well as in vitro despite the fact that the sweat gland affected by cystic fibrosis accumulated cAMP normally in response to the β-adrenergic agents. They speculated therefore that the pathogenesis of the defective β-adrenergic responsiveness of the sweat secretory cells in cystic fibrosis may be distal to cAMP production in the cAMP cascade.

More recently, investigators in two different laboratories, using patch clamp techniques, have simultaneously found that cultured tracheal cells in cystic fibrosis failed to show Cl-channel activities when stimulated with isoproterenol in intact cells. When the membrane patches were excised and exposed to the Ca-containing bathing media, however, Cl channels were activated in the affected cells as well as in normal cells, suggesting that Cl channels are present but may be abnormally regulated in tracheal cells of cystic fibrosis.[11,12] In the eccrine secretory cells, Cl channels[132] are assumed to be present in the luminal membrane (see Figs. 12 and 15), but, as in tracheal cells affected by cystic fibrosis, they also may be abnormally regulated by the cAMP cascade.

DISCOVERY OF APOECCRINE SWEAT GLAND

Until very recently, only two types of sweat glands were thought to exist in humans and mammals, that is, eccrine and apocrine. These two types of glands are distinct from one another structurally, developmentally, and functionally. The eccrine glands of the general skin surface serve for thermoregulation through continuous secretion of serous fluid, whereas human apocrine glands are

present in certain anatomic locations, made up of a single layer of tall columnar secretory cells, always associated with hair follicles (thus also referred to as epitrichial glands), and secrete a rather viscous fluid rich in precursors of odoriferous substances (Table I).[2,3,13] Discovery of a third type of human glands, the apoeccrine gland, was rather coincidental. When dissecting sweat glands from a piece of axillary skin obtained from the surgery for hyperhidrosis, we were puzzled by the fact that most of the sweat glands we saw under the dissection microscope were unclassifiable, that is, neither eccrine nor apocrine glands, although typical eccrine and apocrine glands also were present[133] (Fig. 22). The atypical glands varied in size but were larger than typical eccrine glands and smaller than typical apocrine glands dissected from the same skin specimen. Isolation of more axillary glands in a number of other subjects (who were nonusers of topical antiperspirants) not only confirmed the initial observation but revealed the consistent morphologic, developmental, and functional characteristics, which allowed us to conclude that these atypical glands represented a new type of gland. We decided to call them *apoeccrine* because these glands share some of the morphologic and functional features with both eccrine and apocrine glands (Table I).[133]

The apoeccrine gland has a long duct that opens directly onto the skin surface like the eccrine sweat duct but is distinct from the apocrine duct, which is very short and opens directly into the upper portion of the hair follicle.[133] The apoeccrine secretory portion is irregularly dilated. In some glands only a short segment is dilated like a balloon, but sometimes the entire length of the secretory tubule is irregularly dilated. Of most interest is that the dilated segment was found to consist of an apocrine-like single layer of epithelium whereas the undilated segment tended to show the ultrastructural features typical of the eccrine secretory portion[133] (Fig. 23).

The apoeccrine glands appear to develop during puberty from the eccrine glands, or eccrinelike precursor glands, and account for as much as 45% of all the axillary glands[133] in 16- to 18-year-old persons. In some persons studied after publication of the article,[133] however, the apoeccrine glands represented less than 10% of the axillary glands. When the dilated segments of the apoeccrine glands were cannulated and stimulated in vitro, they yielded an extremely copious serous sweat secretion in response to both methacholine and epinephrine, suggesting that apoeccrine glands may significantly contribute to overall axillary sweating in the adult human being.[134]

Apoeccrine glands may be seen only in the adult axillae. We have thus far failed to detect similar glands in biopsy skin specimens from the arm, scalp, or groin. The development of an apocrine-like structure from the eccrinelike secretory epithelium may suggest the plasticity of glandular development and may help us understand the occurrence of apocrine-like epithelial structures in some pathologic conditions such as adenoma sebaceous and other appendageal tumors of the skin.

REFERENCES

1. Centers for Disease Control. MMWR 1981;30:277-9.
2. Kuno Y. Human perspiration. Springfield, Ill.; Charles C Thomas, 1956.
3. Sato K. The physiology, pharmacology, and biochemistry of the eccrine sweat gland. Rev Physiol Biochem Pharmacol 1977;79:51-131.
4. VanScott EJ, Yu R. Control of keratinization with α-hydroxy acids and related compounds. I. Topical treatment of ichthyotic disorders. Arch Dermatol 1974; 110:586-90.
5. Shah VP, Epstein WL, Riegelman S. Role of sweat in accumulation of orally administered griseofulvin in skin. J Clin Invest 1974;53:1673-8.
6. Horie N, Yokozeki H, Sato K. Proteolytic enzymes in human eccrine sweat: a screening study. Am J Physiol 1986;250(4 Pt 2):R691-8.
7. Yokozeki H, Hibino T, Sato K. Partial purification and characterization of cysteine proteinases in eccrine sweat. Am J Physiol 1987;252(6 Pt 2):R1119-29.
8. Hibino T, Takemura T, Sato K. Evidence that human eccrine sweat contains kallikrein and kininase [Abstract]. Clin Res 1987;35:923A.
9. Quinton PM. Chloride impermeability in cystic fibrosis. Nature 1983;301:421-2.
10. Sato K, Sato F. Defective beta adrenergic response of cystic fibrosis sweat glands in vivo and in vitro. J Clin Invest 1984;73:1763-71.
11. Welsh MJ, Liedtke CM. Chloride and potassium channels in cystic fibrosis airway epithelia. Nature 1986; 3222:467-70.
12. Frizzell RA, Rechkemmer G, Shoemaker RL. Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. Science 1986;233:558-60.
13. Montagna W, Parakkal PF. The structure and function of skin. 3rd ed. New York: Academic Press, 1974:376-96.
14. Ellis R. Eccrine sweat glands: electron microscopy cytochemistry and anatomy. In: Jadassohn J, ed. Handbuch der Haut- und Geshlechtskrankheiten; vol 1. Normale und pathologische Anatomie der Haut. Berlin: Springer-Verlag, 1967:223-66.
15. Hashimoto K, Gross BG, Lever WF. The ultrastructure of the skin of human embryos. I. Intraepidermal eccrine sweat duct. J Invest Dermatol 1965;45:139-51.

16. Sato K, Sato F. Individual variations in structure and function of human eccrine sweat gland. Am J Physiol 1983;245:R203-8.
17. Kurosumi K, Shibasaki S, Ito T. Cytology of the secretion in mammalian sweat glands. Int Rev Cytol 1984;87:253-329.
18. Saga K, Sato K. Ultrastructural localization of ouabain-sensitive, K-dependent *p*-nitrophenyl phosphatase activity in monkey eccrine sweat gland. J Histochem Cytochem 1988;36:1023-30.
19. Quinton PM, Tormey JM. Localization of Na-K-ATPase sites in the secretory and reabsorptive epithelia of perfused eccrine sweat glands: a question to the role of the enzyme in secretion. J Membr Biol 1976;29:383-99.
20. Sato K, Sato F. Relationship between quin 2–determined cytosolic [Ca^{++}] and sweat secretion. Am J Physiol 1988;254:C310-7.
21. Yanagawa S, Yokozeki H, Sato K. Origin of periodic acid–Schiff–reactive glycoprotein in human eccrine sweat. J Appl Physiol 1986;60:1615-22.
22. Sato K, Nishiyama A, Kobayashi M. Mechanical properties and functions of the myoepithelium in the eccrine sweat gland. Am J Physiol 1986;237(3):C177-84.
23. Boulant JA. Hypothalamic mechanisms in thermoregulation. Fed Proc 1981;40:2843-50.
24. Smiles KA, Elizondo RS, Barney CC. Sweating responses during changes of hypothalamic temperature in the rhesus monkey. J Appl Physiol 1976;40:653-7.
25. Adair ER. Skin, preoptic and core temperatures influence behavioral thermoregulation. J Appl Physiol 1977;42:559-64.
26. Nadel ER, Bullard RW, Stolwijk JAJ. Importance of skin temperature in the regulation of sweating. J Appl Physiol 1971;31:80-7.
27. Nadel ER, Mitchel JW, Saltin B, Stolwijk JAJ. Peripheral modifications to the central drive for sweating. J Appl Physiol 1971;31:828-33.
28. Nadel ER, Pandolf B, Roberts MF, Stolwijk JAJ. Mechanisms of thermal acclimation to exercise and heat. J Appl Physiol 1974;37:515-20.
29. LeWitt PA, Newman RP, Greenberg HS, Rocher LL, Calne DB, Ehrenkranz JRL. Episodic hyperhidrosis, hypothermia, and agenesis of corpus callosum. Neurology 1983;33:1122-9.
30. Greenleaf JE. Hyperthermia and exercise. Int Rev Physiol 1979;20:157-208.
31. Nielsen B. Effect of changes in plasma Na and Ca ion concentration on body temperature during exercise. Acta Physiol Scand 1974;91:123-9.
32. Fortney SM, Nadel ER, Wegner CB, Bove JR. Effect of blood volume on sweating rate and body fluids in exercising humans. J Appl Physiol 1981;51:1594-1600.
33. Collins KJ, Weiner JS. Observation on arm-bag suppression of sweating and its relationship to thermal sweat-gland "fatigue." J Physiol 1962;161:538-56.
34. Candas V, Libert JP, Vogt JJ. Effect of hidromeiosis on sweat drippage during acclimation to humid heat. Eur J Appl Physiol 1980;44:123-33.
35. Uno H. Sympathetic innervation of the sweat glands and piloerector muscle of macaques and human beings. J Invest Dermatol 1977;69:112-30.
36. Uno H, Montagna W. Catecholamine-containing nerve terminals of the eccrine sweat glands of macaques. Cell Tissue Res 1975;158:1-13.
Rothman S. Physiology and biochemistry of the skin. Chicago: The University of Chicago Press, 1954:168-80.
38. List CF, Pimentana ADeM. Sweat secretion in man: spinal reflex sweating. Arch Neurol Psychiatry 1944; 51:501-7.
39. Nathan PW, Smith MC. The location of descending fibres to sympathetic neurons supplying the eye and sudomotor neurons supplying the head and neck. J Neurol Neurosurg Psychiatry 1986;49:187-94.
40. List CF, Peet MM. Sweat secretion in man. II. Anatomic distribution of disturbances in sweating associated with lesions of the sympathetic nervous system. Arch Neurol Psychiatry 1938;40:27-43.
41. Netter FG. The Ciba collection of medical illustrations; vol 1. Nervous system. Summit, N.J.: Ciba, 1953: 80-7.
42. Welch E, Geary J. Current status of thoracic dorsal sympathectomy. J Vasc Surg 1984;1:202-12.
43. Sato KT, Richardson A, Sato K. One step iodine starch method for direct visualization of sweating. Am J Med Sci 1988;295:528-31.
44. Faden AI, Chan P, Mendoza E. Progressive isolated segmental anhidrosis. Arch Neurol 1982;39:172-5.
45. Johnson RH, Spaulding JMK. Disorders of the autonomic nervous system. Philadelphia: FA Davis, 1974.
46. Itoh Y, Yagishita S, Nakajima S, Nakano T. Congenital insensitivity to pain with anhidrosis: morphological and morphometrical studies on the skin and peripheral nerves. Neuropediatrics 1986;17:103-10.
47. Sulzburger MB, Griffin TB. Induced miliaria, postmiliarial hypohidrosis, and some potential sequelae. Arch Dermatol 1969;99:145-51.
48. Sato K. Sweat induction from an isolated eccrine sweat gland. Am J Physiol 1973;225:1147-51.
49. Lundberg JM, Hokfeit T, Schultzberg M, Uvnas-Wallensten K, Kohler C, Said SI. Occurrence of vasoactive intestinal polypeptide (VIP)–like immunoreactivity in certain cholinergic neurons of the cat: evidence from combined immuno-histochemistry and acetylcholinesterase staining. Neuroscience 1979;4:1539-59.
50. Vaalassti A, Tainio H, Rechardt L. Vasoactive intestinal polypeptide (VIP)–like immunoreactivity in the nerves of human axillary sweat glands. J Invest Dermatol 1985; 85:246-8.
51. Tainino H, Vaalasti A, Rechardt L. The distribution of substance P-, CGRP-, galanin- and ANP-like immunoreactive nerves in human sweat glands. Histochem J 1987;19:375-80.
52. Sato K, Sato F. Effect of VIP on sweat secretion and cAMP accumulation in isolated simian eccrine glands. Am J Physiol 1987;253:R935-41.
53. Sato K, Sato F. Pharmacologic responsiveness of isolated single eccrine sweat glands. Am J Physiol 1981;240:R44-51.
53a. Sato F, Sato F. Role of calcium in cholinergic and adrenergic mechanisms of eccrine sweat secretion. Am J Physiol 1981;241:C113-20.
54. Sato K, Sato F. Spontaneous sweat secretion in calcium-free strontium medium. Am J Physiol 1982;242: C353-9.
55. Sato K, Sato F. Transepithelial potential during Sr-induced spontaneous sweating. Am J Physiol 1982; 242:C360-5.
56. Tsien RY, Pozzan T, Rink TJ. Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored

with a new intracellularly trapped fluorescent indicator. J Cell Biol 1984;94:325-34.
57. Berridge MJ, Irvine RF. Inositol triphosphate, a novel second messenger in cellular signal transduction. Nature 1984;312:315-21.
58. Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature 1984; 308:693-7.
59. Sato K, Saga K, Sato F. Membrane transport and intracellular events in control and cystic fibrosis eccrine sweat glands. In: Mastella G, Quinton PM, eds. Cellular and molecular basis of cystic fibrosis. San Francisco: San Francisco Press, 1988:171-85.
60. Sato K, Sato F. Mechanism of signal transduction in cholinergic eccrine sweating [Abstract]. Clin Res 1986; 34:778A.
61. Sato K, Sato F. Cyclic GMP accumulation during cholinergic stimulation of eccrine sweat glands. Am J Physiol 1984;247:C234-9.
62. Sato K, Sato F. Cyclic AMP accumulation in the beta adrenergic mechanism of eccrine sweat secretion. Eur J Physiol 1981;390:49-53.
63. Sato K, Yokozeki H, Saga K, Sato F. Some characteristics of beta adrenergic dysfunction in cystic fibrosis sweat glands. In: Martinez JR, ed. Epithelial ion transport in cystic fibrosis. Proceedings of the Conference at the University of Missouri School of Medicine. Columbia: Univ Mo Press, 1986:45-60.
64. Sato K, Sato F. Nonisotonicity of simian eccrine primary sweat induced in vitro. Am J Physiol 1987;252: R1099-105.
65. Ussing HH. Transport of electrolytes and water across the epithelia. Harvey Lecture Series 1965;59:1-30.
66. Slegers JFG, Van't Hot-Grootenboer AE. The localization of sodium transport sites in a forward pumping system. Pflugers Arch 1971;327:167-85.
67. Sato K, Dobson RL, Mali JWH. Enzymatic basis for the active transport of sodium in the eccrine sweat gland. Localization and characterization of Na-K-ATPase. J Invest Dermatol 1971;57:10-6.
67a. Sato K, Taylor JR, Dobson RL. The effect of ouabain on eccrine sweat gland function. J Invest Dermatol 1969;53:275-82.
68. Sato K. Differing luminal potential difference of cystic fibrosis and control sweat secretory coils in vitro. Am J Physiol 1984;247:R646-9.
69. Geck P, Pietrzyk C, Burckhardt BC, Pfeiffer B, Heinz E. Electrically silent cotransport of Na^+, K^+, and Cl^- in Ehrlich cells. Acta Biochim Biophys Hung 1980;600:432-47.
70. O'Grady SM, Palfrey HC, Field M. Characteristics and functions of Na-K-Cl cotransport in epithelial tissues. Am J Physiol 1987;253:C177-92.
71. Sato K. Effect of methacholine on ionic permeability of basal membrane of the eccrine secretory cell. Pflugers Arch 1986;407(suppl 2):S100-6.
72. Sato K, Sato F. Effect of periglandular ionic composition and transport inhibitors on rhesus monkey eccrine sweat gland function in vitro. J Physiol (Lond) 1987;393:195-212.
73. Petersen OH, Maruyama Y. Calcium-activated potassium channels and their role in secretion. Nature 1984; 307:693-6.
74. Saga K, Sato F, Sato K. K^+ efflux from the monkey eccrine secretory cell during the transient of stimulation with agonists. J Physiol 1988;405:205-17.
75. Frizzell RA, Rechkemmer G, Shoemaker RL. Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. Science 1986;233:558-60.
76. Case RM, Hunter M, Novak I, Young JA. The anionic basis of fluid secretion by the rabbit mandibular salivary gland. J Physiol 1984;349:619-30.
77. Martinez JR, Cassity N. Cl^- requirement for saliva secretion in the isolated, perfused rat submandibular gland. Am J Physiol 1985;249(4 Pt 1):G464-9.
78. Hootman SR, De Lisle RC. Ionic mechanisms of exocrine pancreatic secretion In: Quinton PM, Martinez JR, Hopfer U, eds. Fluid and electrolyte abnormalities in exocrine glands in cystic fibrosis. San Francisco: San Francisco Press, 1982;107-22.
79. Saga K, Sato K. Electronprobe x-ray microanalysis of cellular ions in the eccrine secretory coil cells during methacholine stimulation. J Memb Biol [In press.]
80. Kang WH, Saga K, Sato K. Effects of transport inhibitors and ions on K efflux from monkey eccrine secretory coils during cholinergic stimulation [Abstract]. Clin Res 1988;36:660A.
81. Mangos JA. Transductal fluxes of Na, K and water in the human eccrine sweat glands. Am J Physiol 1973; 224:1235-40.
82. Boysen TS, Yanagawa S, Sato F, Sato K. A modified anaerobic method of sweat collection. J Appl Physiol 1984;56:1302-7.
83. Yokozeki H, Sato K. Thiol-dependent aminopeptidase as a marker of epidermal contamination in sweat. J Appl Physiol 1987;63(3):R1040-8.
84. Sato K. Mechanism of eccrine sweat secretion. In: Quinton PM, Martinez JR, Hopfer U, eds. Fluid and electrolyte abnormalities in exocrine glands in cystic fibrosis. San Francisco: San Francisco Press, 1982;35-52.
85. Conn JJ, Louis LH, Johnston MW, Johnson BJ. The electrolyte content of thermal sweat as an index of adrenal cortical function. J Clin Invest 1948;27:529-30.
86. Sigal CB, Dobson RL. The effect of salt intake on sweat gland function. J Invest Dermatol 1968;50:451-5.
87. Sato K, Dobson RL. Regional and individual variations in the function of the human eccrine sweat gland. J Invest Dermatol 1970;54:443-9.
88. Ratner AC, Dobson RL. The effect of antidiuretic hormone on sweating. J Invest Dermatol 1964;43:379-81.
89. Fasciolo JC, Totel GL, Johnson RE. Antidiuretic hormone and human eccrine sweating. J Appl Physiol 1969;27:303-7.
90. Sato K, Dobson RL. The effect of intracutaneous D-aldosterone and hydrocortisone on the human eccrine sweat gland function. J Invest Dermatol 1970;54: 450-9.
91. Sato K, Dobson RL. Glucose metabolism of the isolated eccrine sweat gland. I. The effects of mecholyl, epinephrine and ouabain. J Invest Dermatol 1971;56:272-80.
92. Sato K, Dobson RL. Glucose metabolism of the isolated eccrine sweat gland. II. The relation between glucose metabolism and sodium transport. J Clin Invest 1973;52:2166-74.
93. Brusilow SW, Gordes WH. Ammonia secretion in sweat. Am J Physiol 1967;214:513-7.
94. Emrich HM, Oelert H. pH-Wert und Gesamtammoniak im menschlichen Schwiss. Pflügers Arch 1966;290: 311-4.
95. Gitlitz PH, Sunderman FW, Hohnadel DC. Ion-exchange chromatography of amino acids in sweat

collected from healthy subjects during sauna bathing. Clin Chem 1974;20:1305-12.
96. Sato K. Biology of eccrine sweat glands. In: Fitzpatrick TB, et al., eds. Dermatology in general medicine. 3rd ed. New York: McGraw-Hill, 1987:195-209.
97. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
98. Sato F, Takemura T, Hibino T, Sato K. Lectin-binding glycoproteins in human eccrine sweat [Abstract]. Clin Res 1987;35:714A.
99. Yanagawa S, Yokozeki H, Sato K. Origin of periodic acid–Schiff–reactive glycoprotein in human eccrine sweat. J Appl Physiol 1986;60:1615-22.
100. Lobitz WC Jr, Dobson RL. Miliaria. Arch Environ Health 1965;11:460-4.
101. Rubin RW, Penneys NS. Subpicoprogram analysis of sweat proteins. Anal Biochem 1983;131:520-4.
102. Sens DA, Simmons MA, Spicer SS. The analysis of human sweat proteins by isoelectric focusing. I. Sweat collection utilizing the macroduct system demonstrates the presence of previously unrecognized sex-related proteins. Pediatr Res 1985;19:873-8.
103. Page CO, Remington JS. Immunologic studies in normal human sweat. J Lab Clin Med 1967;69:634-50.
104. Förström L, Goldyne ME, Winkleman RK. IgE in human eccrine sweat. J Invest Dermatol 1975;64: 156-7.
105. Fräki JE, Jansen CT, Hopsu-Havu VK. Human sweat kallikrein: biochemical demonstration and chromatographic separation from several other esteropeptidases in the sweat. Acta Derm Venereol (Stockh) 1970;50: 321-6.
106. Hibino T, Takemura T, Sato K. Demonstration of glandular kallikrein and angiotensin-converting enzyme (kininase-II) in human sweat [Abstract]. Clin Res 1988;36:655A.
107. Fräki JE. Human skin proteases. Separation and characterization of two acid proteases resembling cathepsin B_1. Arch Dermatol Res 1976;255:317-30.
108. Yokozeki H, Hibino T, Sato K. Cysteine proteinase inhibitor in human eccrine sweat [Abstract]. Clin Res 1986;34:993A.
109. Takemura T, Hibino T, Sato K. Plasminogen activator in human eccrine sweat is a urokinase-type [Abstract]. 1988;36:698A.
110. Isackson PJ, Dunbar JC, Bradshaw RA. Role of glandular kallikreins as growth factor processing enzymes: structural and evolutionary considerations. J Cell Biochem 1987;33:65-75.
111. Pesonen K, Viinikka L, Koskimies A, Banks AR, Nicolson M, Perheentupa J. Size heterogeneity of epidermal growth factor in human body fluids. Life Sci 1987;40(26):2489-94.
112. Mangal P, Sato F, Sato K. Secretion of epidermal growth factor (EGF) in human control subjects and patients with cystic fibrosis [Abstract]. Clin Res 1988;36:671A.
113. Arita T, Kishimoto S, Hirata S, Koishi K. Immunohistochemical localization of human epidermal growth factor receptor in skin of both normal and patients with tumors of eccrine sweat gland. Proc Jap Soc Invest Dermatol 1987;11:135-6.
114. Nanney LB, Magid M, Stoscheck CM, King LE. Comparison of epidermal growth factor binding and receptor distribution in normal human epidermal appendages. J Invest Dermatol 1984;83:385-93.
115. Emrich HM, Zweibel RKH. Veränderungen des Lactat-Pyruvat-Quotienten im menschlichen Schweiss bei verschieden starkem Schwiezen. Pflugers Arch 1966; 290:315-9.
116. Johnson H, Maibach HI. Drug excretion in human eccrine sweat. J Invest Dermatol 1971;56:182-8.
117. Garden JW. Plasma and sweat histamine concentration after heat exposure and physical exercise. J Appl Physiol 1966;21:631-5.
118. Frewin DB, Eakin KE, Downey JA, Bhattachejee P. Prostaglandin-like activity in human eccrine sweat. Aust J Exp Biol Med Sci 1973;51:701-2.
119. Vree TB, Th A, Muskens JM, Rossum JM. Excretion of amphetamines in human sweat. Arch Int Pharmacodyn Ther 1972;199:311-7.
120. Harden RM, Alexander WD. Quantitative aspects of iodide excretion in human thermal sweat. Clin Sci 1963;25:79-87.
121. Parnas J, Flachs H, Gram L, Wurtz-Jorgensin A. Excretion of antiepileptic drugs in sweat. Acta Neurol Scand 1978;58(3):197-204.
122. Phillips M, McAloon MH. A sweat patch test for alcohol consumption: evaluation in continuous and episodic drinkers. Alcoholism. Clin Exp Res 1980;4(4): 391-5.
123. Smith K, Thompson GF, Koster HD. Sweat in schizophrenia patients: identification of odorous substance. Science 1969;166:398-9.
124. Perry TL, Melancon SB, Lesk D, Hansen S. Failure to detect trans-3-methyl-2-hexenoic acid in the sweat of schizophrenic patients. Clin Chim Acta 1970;30:721-5.
125. Gordon SG, Smith K, Rabinowitz JL, Vagelos PR. Studies of *trans*-3-methyl-2-hexenoic acid in normal and schizophrenic humans. J Lipid Res 1973;14:495-503.
126. Epstein WL, Shah VP, Riegelman S. Griseofulvin levels in stratum corneum. Study after oral administration in man. Arch Dermatol 1972;106:344-8.
127. Harris R, Jones HE, Artis WM. Orally administered ketoconazole: route of delivery to the human stratum corneum. Antimicrob Agents Chemother 1983;24(6): 876-82.
128. Hatzix J, Tosca A, Varelzidis A, Stratigos J. The role of sweat in the pharmacokinetics of ketoconazole and griseofulvin. Br J Dermatol 1987;117:797-800.
129. Quinton PM. Effect of some ion transport inhibitors on secretion and reabsorption in intact and perfused single human sweat glands. Pflugers Arch 1981;391:309-13.
130. Di Sant'Agnese PA, Davis PB. Research in cystic fibrosis. N Engl J Med 1976;295:481-5, 534-41, 597-602.
131. Quinton PM, Bijman J. Higher bioelectric potentials due to decreased chloride absorption in the sweat glands of patients with cystic fibrosis. N Engl J Med 1983; 308:1185-9.
132. Sato K, Sato F. Presence of K and C channels in the isolated secretory cells of the rhesus monkey palm eccrine sweat gland as studied by the patch clamp techniques [Abstract]. Clin Res 1988;36:908A.
133. Sato K, Leidal R, Sato F. Morphology and development of an apoeccrine sweat gland in the human axillae. Am J Physiol 1987;252:R166-80.
134. Sato K, Sato F. Sweat secretion by human axillary apoeccrine sweat gland in vitro. Am J Physiol 1987; 251:R181-7.