69-9625

HO, Chen-kung, 1937-

ELECTRON MICROSCOPIC LOCALIZATION OF SODIUM, CHLORIDE AND ADENOSINETRIPHOSPHATASE IN THE SWEAT GLAND IN CYSTIC FIBROSIS.

The University of Nebraska, Ph.D., 1968
Health Sciences, pathology

University Microfilms, Inc., Ann Arbor, Michigan

ELECTRON MICROSCOPIC LOCALIZATION OF SODIUM, CHLORIDE AND ADENOSINETRIPHOSPHATASE IN THE SWEAT GLAND IN CYSTIC FIBROSIS

By

Chen-kung Ho

A THESIS

Presented to the Faculty of

The Graduate College in the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Interdepartmental Area of Medical Sciences

Under the Supervision of Gordon E. Gibbs, Ph. D., M. D.

The University of Nebraska College of Medicine

Omaha, Nebraska

June, 1968

TITLE

ELECTRON MICROSCOPIC LOCALIZATION OF SODIUM, CHLORIDE AND ADENOSINETRIPHOSPHATASE IN THE SWEAT GLAND IN CYSTIC FIBROSIS

BY

Chen-kung Ho

APPROVED	DATE
Herbert P. Jacobi	July 8, 1968
Richard B. Wilson	July 8, 1968
Robert J. Ellingson	July 8, 1968
John S. Latta	July 8, 1968
Gordon E. Gibbs (Chairman)	July 8, 1968

SUPERVISORY COMMITTEE

GRADUATE COLLEGE UNIVERSITY OF NEBRASKA

GRAD 52002 300 5-66(T)

ACKNOWLEDGMENTS

I wish to express my deep appreciation to Dr. Gordon E. Gibbs, who, as my advisor, has introduced me to the interesting field of cystic fibrosis research. His guidance, assistance and full support have made this study possible.

I am also grateful to Dr. Richard B. Wilson and Dr. Arlene R. Seaman for their assistance in the electron microscopical aspects of the work, to Mr. James T. Smith for the preparation of electron micrographs and the personnel of the Cystic Fibrosis Research Center, Mrs. Doris Raasch, Mrs. Novelene Gibbons, and Mrs. Marvene Morse, whose arrangement for the skin biopsies was very helpful.

Thanks are extended to Dr. Herbert P. Jacobi who has read the manuscript, to Mr. and Mrs. Guy D. Griffin, Miss Sandra Funk and Miss Jan Markham who have made valuable assistance in the preparation of this manuscript.

TABLE OF CONTENTS

I. Introduction and Review of the Literature

1. History of Cystic Fibrosis Research

Cystic fibrosis is a hereditary disease transmitted as an autosomal recessive trait. In the United States, the incidence for homozygotes is about one in every 2,000 live births and that for heterozygotes is 2-5% of the general population. The disease is found predominantly in Caucasians and very rarely in other races (di Sant'Agnese and Talamo, 1967). Although a variety of symptoms may be encountered, exocrine pancreatic insufficiency, chronic pulmonary infections and elevated electrolytes in sweat are the main manifestations of the disease. The pathological changes in various organs, such as pancreas, lungs, liver, etc., are thought to be secondary to obstruction caused by accumulation of viscid mucus in the mucus-secreting structures, such as pancreatic ducts, bronchi, bile ductules, etc. (Farber, 1945; Bodian, 1953). Considering the disease as the generalized disorder of mucous glands, Farber (1945) suggested the term "mucoviscidosis" in place of "cystic fibrosis of the pancreas". However, after the sweat gland defect in this disease was discovered (di Sant'Agnese et al., 1953), it has been considered that the term "mucoviscidosis" is as improper as "cystic fibrosis of the pancreas".

Although the disease does not only affect the pancreas, the term "cystic fibrosis of the pancreas" or in short, "cystic fibrosis" is still being used commonly in a broad sense to designate the disease.

Before the recognition of cystic fibrosis as a disease entity, the disorder was described under various names, for example, celiac disease (Hess and Saphir, 1935), steatorrhea (Parmelee, 1935; Harper, 1938), vitamin A deficiency (Blackfan and Wolbach, 1933), intestinal infantilism (Huet, 1934), etc. Many case reports that appeared in the literature before the late 1930's, in retrospect, are cases of cystic fibrosis. The earliest autopsied report was from Landsteiner in 1905, who described a case of meconium ileus.

In 1936, Fanconi et al. in Switzerland reported two cases of celiac syndrome. The post-mortem examination revealed cystic fibrosis of the pancreas, bronchiectasis, and chronic pulmonary infection. In connection with many other cases they had seen before and similar cases described by other authors, they thought that association of pancreatic and pulmonary lesions was a significant feature of the disease and coined the term "Zystischer Pankreasfibromatose" (cystic fibrosis of the pancreas).

Harper (1938) in Australia reported eight cases of congenital steatorrhea, many of them, if not all, are now thought to be cases of cystic fibrosis.

In 1938, Andersen of New York analyzed forty-nine autopsies with clinical manifestations of celiac syndrome, twenty-seven of which were collected from the literature and the rest of which were her own series. The salient pathological findings common to the cases were cystic fibrosis of the pancreas and chronic pulmonary infection. She concluded that cystic fibrosis was a disease entity which was one of the disease entities causing celiac syndrome.

In the same year, Blackfan and May in Boston reported the pathological findings and clinical manifestations of thirty-five infants with cystic fibrosis of the pancreas.

Before the discovery of abnormal sweat electrolytes in cystic fibrosis, diagnosis of the disease depended largely on determination of the pancreatic enzymes in duodenal aspirations (Andersen, 1942). Duodenal secretions relatively free from contamination of gastric juice could be obtained with the use of bilumen gastroduodenal tube (Gibbs, 1948). In conjunction with the secretin test, this technique was able to give more reliable information for the diagnosis of cystic fibrosis (Gibbs, 1950a).

For many years, the pancreatic enzymes were thought to be totally absent in cystic fibrosis. However, there are many reported cases in which the enzymes are present in appreciable amounts (Gibbs et al., 1950b; di Sant'Agnese, 1955). It is estimated that 10% of the patients with cystic fibrosis do not have a pancreatic deficiency, in contrast to 80% of them who have lost all pancreatic enzymes, the remaining 10% having the enzyme activity between these two extremes (di Sant'Agnese and Talamo, 1967).

Kessler et al. in 1951 reported twelve cases of heat prostration in infants and children. Seven of these patients had cystic fibrosis. They suspected that the heat susceptibility in this disease was probably due to an abnormality in the sweat gland function. Inspired by this, di Sant'Agnese et al. (1953) carried out the determination of electrolyte concentrations in sweat from the patients with cystic fibrosis. As a result, the great discovery was made. They found that the mean sodium concentration of sweat from the patients with cystic fibrosis was 133 mEq./L. with a range of 80 to 190 mEq./L., the mean value for controls was 59 mEq./L. with a range of 10 to 120 mEq./L. The mean chloride level in the sweat of the patients was 106 mEq./L., with a range of 60 to 160 mEq./L., the corresponding values for the controls were 32 mEq./L. for the mean and 4 to 80 mEq./L. for the range.

The mean values of potassium in fibrocystics and controls were 18 mEq./L. and 12 mEq./L., respectively; the difference is statistically significant, although it is not as marked as sodium and chloride.

Because of the distinct difference in sweat sodium and chloride concentrations between cystic fibrosis and normal, the sweat test, in which either the chloride or sodium level is measured, has become a very useful and simple tool in the diagnosis of this disorder.

The sweat glands from patients with cystic fibrosis and those from normal subjects have a similar ultrastructure (Munger et al., 1961b). The finding by the authors that fibrocystic patients had a greater number of unidentified dense bodies in the cells of the secretory segment than did the normal controls cannot be confirmed (di Sant'Agnese and Talamo, 1967). The density of the sweat glands (Gibson and di Sant'Agnese, 1963) and the rate of sweat secretion (Emrich et al., 1967) in cystic fibrosis are not different from those of the normals. Concentrations of sodium, chloride and potassium ions in the serum of uncomplicated cases of cystic fibrosis are also normal (di Sant'Agnese et al., 1953). There is no defect in the adrenocortical functions (Chodos et al., 1965; Montalvo et al., 1967), and the sweat glands respond to exogenous aldosterone as do those in normal subjects (Grand et al., 1967), although the response is not as marked as in the latter group.

In normal subjects, the sodium concentrations rise with increasing sweating rates (Gordon and Cage, 1966). This has been cited as an indirect evidence to support the hypothesis that the precursor fluid of sweat is isotonic to plasma and subject to modification by the duct, which reabsorbs sodium and chloride and renders the final solution hypotonic. When the excretion of sodium ions exceeds the capacity of

reabsorption of the ions in the duct, the sodium levels in the sweat rise. The direct evidences which support the hypothesis have been obtained through techniques of microcryoscopy (Slegers, 1963) and micropuncture (Schulz et al., 1965). Slegers reported that the precursor fluid in the secretory tubule in patients with cystic fibrosis, as well as in normal subjects, was isotonic with blood plasma. The fluid in the duct was hypotonic in the normal controls, but had a higher tonicity in the patients. It seems apparent that the abnormal electrolyte contents of sweat in cystic fibrosis are due to a defect in the function of the duct. Emrich et al. (1967) reported that water reabsorption in the sweat glands of patients with cystic fibrosis was markedly decreased. Therefore, the high tonicity of the sweat from the patients cannot be attributed to the water reabsorption in excess of sodium reabsorption. They also reported that sodium reabsorption in the sweat duct of patients with cystic fibrosis was decreased by 50 to 75% in comparison with normals.

Sodium concentrations rise with increasing sweating rate in cystic fibrosis, as well as in normals (Gibson and di Sant'Agnese, 1963; Sibinga and Barbero, 1963). Inferring from these findings, the authors stated that the precursor fluid of the sweat gland was more concentrated in cystic fibrosis than in normals. Several other investigators also have expressed the opinion that the defect of sweat glands in cystic fibrosis is localized in the secretory segment rather than in the duct (Emrich et al., 1967).

Mangos and McSherry (1967a) found a factor (or factors) in the sweat and in the saliva (Mangos et al., 1967b) of patients with cystic fibrosis, which could inhibit sodium reabsorption in the striated duct of the rat parotid gland. The factor (s) was heat labile and non-dialyzable.

The authors suggested that it was probably this factor which inhibited retrieval of sodium in the ducts of the sweat gland as well as of the salivary gland in cystic fibrosis. The sodium transport inhibitory factor in fibrocystic saliva does not inhibit Na^+-K^+-activated ATPase (Mangos et al., 1967b).

Gibbs et al. (1963, 1964, 1965a, 1965b, 1967) determined various enzymes in the sweat gland, including alkaline phosphatase, acid phosphatase, cholinesterases, carbonic anhydrase, phosphatidic acid phosphatase, lactate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase and ouabain-sensitive ATPase, and concluded that there was no difference between cystic fibrosis and controls.

There are very few conditions other than cystic fibrosis, which have high concentrations of sweat electrolytes. The other conditions include adrenal insufficiency and a few reported cases of glycogen-storage disease with glucose-6-phosphatase deficiency, vasopressin-resistant diabetes insipidus, familial ectodermal dysplasia with sensori-neural deafness, and familial hypoparathyroidism associated with partial anodontia, mental deficiency, pernicious anemia, steatorrhea and/or adrenocortical insufficiency (di Sant'Agnese and Talamo, 1967).

Sodium and chloride concentrations are also elevated in mixed saliva from patients with cystic fibrosis. The mean value for sodium is 27 mEq./L., in contrast with 16 mEq./L. in normals. There is no abnormality in the potassium levels (di Sant'Agnese et al., 1958). Although there have been some controversies over the sodium and chloride levels in parotid and submaxillary saliva, it is now gener-

ally agreed that in both parotid (di Sant'Agnese et al., 1958) and submaxillary saliva (Mandel et al., 1967) from the patients with cystic fibrosis, sodium and chloride are slightly, but significantly increased. Since there is an overlap in values between cystic fibrosis and normal, determination of sodium or chloride levels in saliva is not valuable for diagnosis of the disease.

di Sant'Agnese et al. (1958) reported that sodium concentration in tears was elevated in cystic fibrosis; while Schwachman and Antonowicz (1962) reported that the electrolytes in tears were normal.

Concentrations of sodium, chloride and potassium ions in duodenal juice are normal in cystic fibrosis (di Sant'Agnese et al., 1958).

Sodium and chloride levels in tracheobronchial secretions are lower in cystic fibrosis than in bronchiectasis (Chernick and Barbero, 1963).

Extensive studies of glycoproteins have been carried out in recent years. Dische et al. (1959) first reported an increase in the ratio of fucose to sialic acid in the benzene-ethanol precipitated fraction of the duodenal juice from patients with cystic fibrosis. Subsequent studies have revealed that the fucose content or fucose to sialic acid ratio is also elevated in the glycoproteins in urine (Dische et al., 1961), submaxillary saliva (Chernick and Barbero, 1963), sweat (Pallavicini et al., 1963), tracheobronchial mucus (Johansen, 1963) and rectal mucus (Roelfs et al., 1967) from patients with cystic fibrosis.

Chernick et al., (1961) demonstrated that submaxillary saliva from patients with cystic fibrosis was turbid, in contrast with the saliva from normal individuals, which is clear. The mean calcium concentration and nitrogen content in reflex stimulated submaxillary saliva in cystic fibrosis are 12.43 mg.% and 0.480 mg./ml. respectively, and the corresponding values in normal are 7.51 mg.% and 0.219 mg./ml. respective-

ly (Chernick et al., 1961). There is a difference in the polyacrylamide-gel electrophoretic patterns of submaxillary saliva between cystic fibrosis and normal. The patterns can be changed to resemble each other by adding calcium to normal saliva or by decalcifying the fibrocystic saliva with EDTA (ethylenediaminetetra-acetic acid). There are no immunological differences between the various bands separated by electrophoresis or between the bands of fibrocystic and normal origin. Glycoproteins in submaxillary saliva from patients with cystic fibrosis and from normal subjects are probably identical. By interacting with calcium ions present in high concentration (as is the case in fibrocystic saliva), glycoproteins perhaps form the reversible precipitates of high molecular weight, which are different in physical properties from the glycoproteins of lower calcium contents as are found in normal submaxillary saliva (Gugler et al., 1967). Calcium content in tracheobronchial secretions is also elevated in cystic fibrosis (Chernick and Barbero, 1963).

Lowe et al. (1966) reported that they found a unique glycoprotein possessing antigenic activity in stool from patients with cystic fibrosis. This was not confirmed by Broglio et al. (1967).

Maxfield and Wolins (1962) reported that Tamm-Horsfall mucoprotein in the urine of patients with cystic fibrosis was abnormal in that the tetramer form of the mucoprotein was prone to aggregate and unstable, and that the smaller "fragments" of the mucoprotein molecules were present in the dispersed phase of fibrocystic urine. However, Friedmann and Johnson (1967) maintained that the structure of Tamm-Horsfall mucoprotein in the urine from patients with cystic fibrosis was not different from that of the controls.

Dysfunction of the autonomic nervous system has been related to the pathogenesis of cystic fibrosis (Roberts, 1959). When stimulated with methocholine chloride the submaxillary gland of normal children produces saliva which is turbid and has a composition similar to that found in children with cystic fibrosis (Chernick et al., 1961). When guanethidine is administered to the patients with cystic fibrosis, the turbid submaxillary saliva becomes clear, and the amylase and glycoprotein concentrations are decreased. The concentrations of sodium, potassium and calcium remain unchanged. Although guanethidine is an adrenergic blocking agent, its action on the composition of fibrocystic saliva is probably not due to its adrenergic blocking effect (Chernick and Barbero, 1967). Eyerman et al. (1961) reported that acetylcholine levels in sweat were elevated in cystic fibrosis. Dische et al. (1962) observed that the composition of glycoproteins in the dog submaxillary saliva could be altered by the administration of pilocarpine, or by stimulation of the chorda tympani nerve. In spite of the above mentioned observations and other similar findings, the role played by the autonomic nervous system in pathogenesis of the disease still remains undetermined.

Talamo et al. (1964) found that transferrin in the urine was absent or very low in concentration in cystic fibrosis, whereas it was present in most of the normal individuals studied. The transferrin levels in the serum were normal in the patients. However, Gugler found that the concentration of transferrin in the urine of patients with cystic fibrosis was normal (di Sant'Agnese and Talamo, 1967).

Autoimmune factors have been considered in pathogenesis of cystic fibrosis. Murray and Thal (1960) reported that circulating antibodies

against the human pancreas were present in six out of seven cases of cystic fibrosis, as were in chronic pancreatitis and carcinoma of the pancreas. However, Stein et al. (1964) failed to confirm the findings. In the bronchial mucus from fibrocystic patients, but not in that from non-fibrocystic patients, Stein et al. (1964) found the antibodies which could react with extracts of the lungs and pancreas from other patients with cystic fibrosis, but not with the extracts of non-fibrocystic lungs and pancreas.

Spock et al. (1967) discovered a serum factor in cystic fibrosis, which caused a dyskinesis of ciliary movement in explants of rabbit tracheal epithelium. The factor is also present in heterozygotes in a smaller concentration. The factor is a protein. It is heat labile, and is precipitated with the euglobulins. It is not known at present whether this factor is identical to the sodium transport inhibitory factor found in the sweat (Mangos and McSherry, 1967a) and saliva (Mangos et al., 1967b) of patients with cystic fibrosis.

Danes and Bearn (1968) reported that homozygotes and heterozygotes of cystic fibrosis had a marked cytoplasmic intravesicular metachromasia in the skin fibroblasts grown in cell culture. Such a finding was very rarely seen in control individuals. This discovery, in addition to its possible application in detection of cystic fibrosis carriers, might provide a clue to the primary defect of this disease.

Various aspects of the disease have been extensively studied. Yet, to date the fundamental defect which can account for the whole picture of the disease has not been found. Understanding of pathogenesis at the molecular level requires further studies in the future.

2. Eccrine Sweat Gland

A. Normal Structure

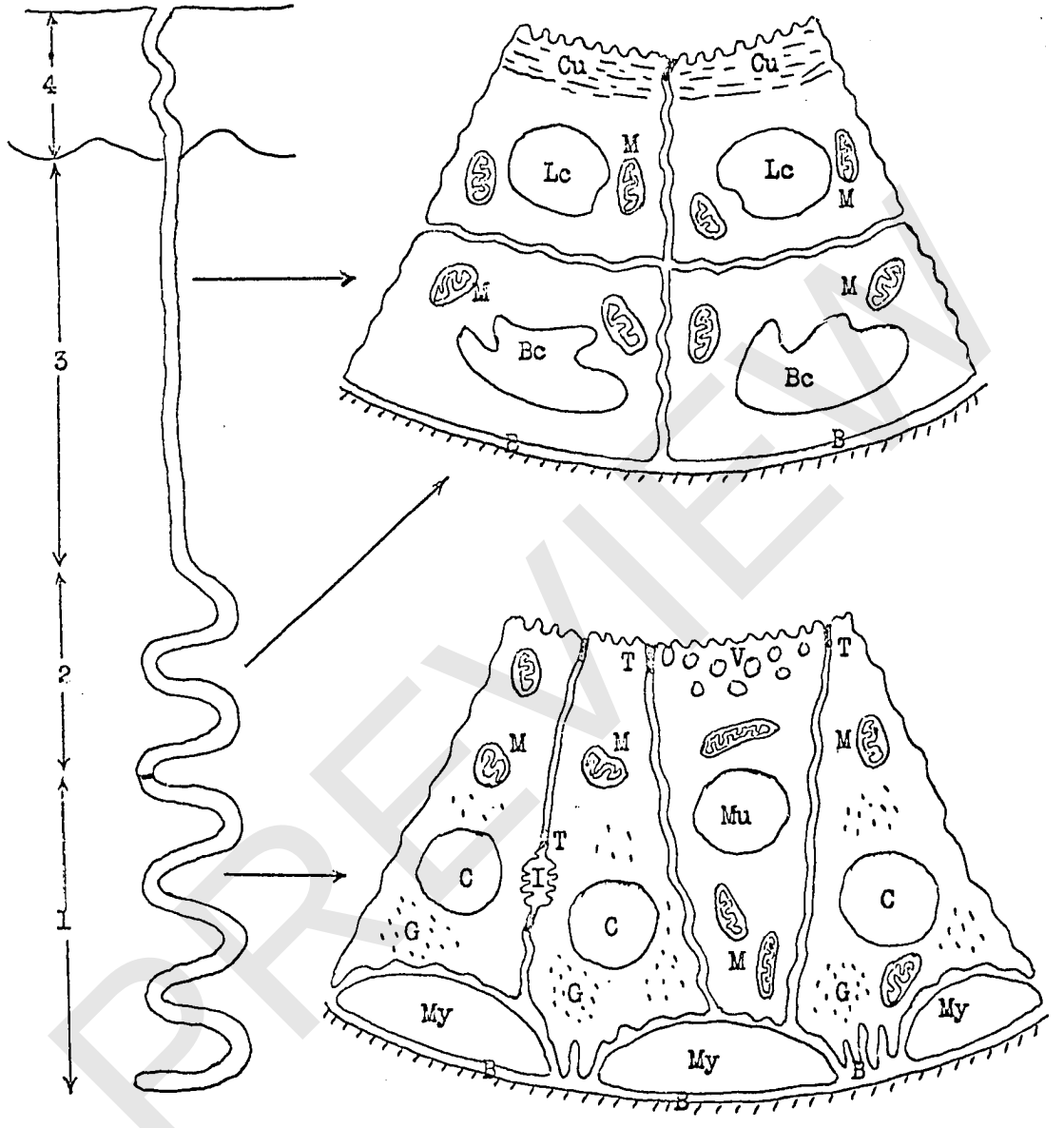
a. General Features

The eccrine sweat gland is a simple tubular gland which has two distinct segments. The first segment is composed of a single layer of secretory cells resting either directly on the basement membrane or on the myoepithelial cells. The second segment is composed of two layers of cells, and is referred to as the excretory duct. The secretory tubule and a part of the excretory duct constitute the coil portion of the sweat gland, the ratio of the length of secretory tubule to that of the duct in the coil being 1.3:1.0 (Kuno, 1956a). The coil is situated in the lower part of the dermis or at the dermosubcutaneous junction. That part of the duct which is embedded in the dermis is straight, and is termed straight duct. The epidermal portion of the duct runs a spiral course and opens onto the skin surface (Diagram 1.).

Both the coil and the duct are enveloped by the capillary network. The ratio of the surface of the secretory tubule to that of the capillaries is on the average 1:2.7. For the coiled duct, the value is 1:2.2 and for the straight duct, it is 1:1.7 (Kuno, 1956b).

b. The Secretory Tubule

There are two types of secretory cells. One is basophilic and another is acidophilic. Montagna *et al.* (1953) called the former dark cell and the latter clear cell, according to their stained appearances under light microscope. However, their appearances are reversed under electron microscope, *i.e.*, the basophilic cell appears light and the acidophilic cell appears dark (Munger, 1961a). The terminology of Munger (1961a) is



1. Secretory tubule
2. Coiled duct
3. Straight duct
4. Epidermal duct
B = Basal lamina
Bc = Basal cell
C = Clear cell
Cu = Cuticular border
G = Glycogen
I = Intercellular canaliculus
Lc = Luminal cell
M = Mitochondria
Mu = Mucoid cell
My = Myoepithelial cell
T = Terminal bar
V = Secretory vacuole

Diagram 1: The Structure of the Sweat Gland

used in this thesis; the basophilic cell is called the mucoid cell and the acidophilic cell the clear cell as defined by Montagna et al. (1953). The mucoid cell is pyramid-shaped; its luminal end is broader than the basal end which abuts on the basement membrane [The term "basement membrane" will be used in the same sense as has been used by light microscopists, namely, to refer to the amorphous layer underlying the epithelium plus a layer of closely associated collagenous fibrils. When the amorphous layer is to be specifically designated, the term "basal lamina" will be used (Fawcett, 1966)] or on the myoepithelial cell. Its luminal border has developed into many short microvilli. Its lateral membrane interdigitates with that of other cells. The nucleus is round or oval. The Golgi complex is usually situated at the apical pole of the cell. Many secretory vacuoles that contain acid mucopolysaccharide are also found in this region. Both smooth and rough-surfaced endoplasmic reticulum are present in the cytoplasm. Free ribosomes are numerous, and are in part responsible for the basophilia of this type of cells. Lipid droplets and a few glycogen granules are present in the cytoplasm. Mitochondria are filamentous. Terminal bars join adjacent cells at the luminal end. Intercellular canaliculi have never been found between two mucoid cells or between a mucoid cell and a clear cell (Ellis, 1962; Gordon and Cage, 1966).

The clear cell has a narrow surface lining the lumen and has a broader basal surface which abuts either on the myoepithelial cells or directly on the basement membrane. The nucleus is spherical or ovoid. The mitochondria are also spherical or ovoid. A scanty amount of lipid droplets, smooth-surfaced endoplasmic reticulum, rough-surfaced

endoplasmic reticulum, and the Golgi complex are present. Numerous glycogen granules, more or less gathered in groups, are characteristic of this type of cells. Intercellular canaliculi are found only between the clear cells. Microvilli protrude into the lumen of the intercellular canaliculus. Terminal bars seal off the intercellular space from communicating with the intercellular canaliculus. The basal plasma membrane of the clear cell abutting directly on the basement membrane is thrown into irregular infoldings. The lateral surfaces of the adjacent clear cells are deeply interdigitated (Ellis, 1962).

The myoepithelial cells are found in the secretory segment and the transititional zone, but not in the duct. They are elongated, spindle-shaped and sometimes branched. Their long axes are parallel to that of the secretory tubule, or they may take a spiral course around the tubule. The myoepithelial cells do not form a complete sealing around the secretory cells. Some secretory cells may directly rest upon the basement membrane. In some myoepithelial cells, two ends of the cell may attach to the basement membrane, while the rest of the cell surface is surrounded by secretory cells. The nucleus is usually situated in the side close to the secretory cells. Around the nucleus there is a small area of cytoplasm which contains mitochondria, smooth and rough-surfaced endoplasmic reticulum, the Golgi complex, fat droplets and glycogen granules. The major portion of the cytoplasm is comprised of parallel myofilaments, among which the cytoplasmic cores containing mitochondria, the endoplasmic reticulum and glycogen granules are scattered. A few microtubules run among the myofilaments. The basal plasma membrane of the myoepithelial cell is slightly folded. The crevice thus formed is filled with basal lamina. The apical plasma

membrane is lined with numerous pinocytotic vesicles. With control of their tension, myoepithelial cells might regulate the size of ports, through which the extracellular fluid gains access to the secretory cells, and thus control the sweating rate. Their role in compressing sweat out of the lumen is probably a minor one (Ellis, 1965).

c. The Duct

The coiled duct and the straight duct have the similar structures. They are composed of two layers of epithelial cells. The luminal cells have short microvilli along the luminal border. Their apical cytoplasm is specialized into cuticular border which is comprised of numerous tonofilaments, vesicles, granules and a few mitochondria. The basal cytoplasm of the luminal cell contains mitochondria, some smooth-surfaced and rough-surfaced endoplasmic reticulum, and granules of glycogen. The Golgi complex is seen only infrequently. Desmosomes join the apposing plasma membranes together. They are particularly numerous in the cuticular border. The nuclei of the luminal cells occasionally assume irregular shapes.

The basal cell has numerous round or short-rod-shaped mitochondria. The nuclei are elongated and irregular in shape. Many of them have deep indentations. The intercellular boundaries are highly convoluted. The thickness of basal lamina is not uniform in all sections. In some sections, it is well developed, while in others it is poorly developed.

The intraepidermal duct has a very narrow lumen. The ductal cells below the level of the stratum granulosum of the epidermis contain keratohyalin granules (Ellis, 1962).

B. Mechanism of Secretion

Sweat contains many chemical components, of which sodium and potassium are the major cations, chloride and lactate are the major anions, and urea is the principal non-ionic solute (Gordon and Cage, 1966). Sodium and chloride concentrations rise with an increase in the sweat rate, while the concentrations of potassium, lactate and urea decline with an increase in the sweat rate. The sodium and chloride levels in sweat are much lower than those in the plasma, whereas the potassium, lactate and urea concentrations are much higher than those in the plasma (Emrich et al., 1967). The low concentrations of sodium and chloride in sweat are due to reabsorption from the precursor fluid of these electrolytes by the duct. Potassium ions, in addition to those derived from the precursor fluid, are probably secreted into the sweat by the duct (Slegers, 1967). Lactate ions are not mainly derived from the blood, but possibly from glycogenolysis and anaerobic glycolysis occurring in the clear cell of the secretory tubule (Gordon and Cage, 1966). That the sweat/plasma ratio of urea is greater than one is interpreted as the result of reabsorption of water by the duct (Emrich et al., 1967). There is, however, evidence showing that urea may be derived from non-plasma sources (Synthesis of urea probably occurs in the sweat gland.). If this is the case, the inference that water is reabosrbed in the sweat gland cannot be made on the basis of the finding that the sweat/plasma ratio of urea is greater than one (Brusilow, 1967).

The hypothesis that the secretory tubule of sweat glands secretes an isotonic solution and that the duct reabsorbs sodium, chloride and other ions in excess of water, so that the final sweat is hypotonic

with plasma has now been widely accepted. Evidence supporting this hypothesis has been obtained from microcryoscopy (Slegers, 1963), micropuncture (Schulz et al., 1965) and clearance work (Emrich et al., 1967).

In micropuncture studies, Schulz et al. stated that the precursor fluid in the sweat coil of the normal adult had a value of 318 mOsm./L. with a Na^+ concentration of 147 mEq./L. and a Cl^- concentration of 123 mEq./L. The final sweat was hypotonic, with a value of 154 mOsm./L., in which Na^+ concentration was 22 mEq./L. and Cl^- concentration was 28 mEq./L. The potential difference between the duct lumen and the interstitium was measured to be 40 mV., with the lumen negative to the interstitium. The potential difference dropped and the sodium concentration of the final sweat was increased from 22 mEq./L. to 90-107 mEq./L., following subcutaneous injection of ouabain. This clearly indicates the role of the duct in elaboration of hypotonic sweat.

The precursor fluid in the secretory coil is not the ultrafiltrate of the plasma, since the components in the precursor fluid are not the same as in the plasma, for example, the sweat is essentially free from bicarbonate (Gordon and Cage, 1966). Moreover, sweat secretion is not affected for an appreciable period of time after the blood supply is cut off (Collins et al., 1959). The mechanism of formation of the precursor fluid have not been elucidated. Gordon and Cage (1966) have presented a hypothesis. According to them, lactate is secreted into the intercellular canaliculus by the clear cells. This is accompanied by passive diffusion of potassium ions from the cytoplasm to the intercellular canaliculus. Part of the potassium ions, when reaching the principal lumen of the secretory tubule, diffuse into the mucoid cells.